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(54) Title: METHOD OF TREATING OPIOID OR OPIATE ADDICTION

(57) Abstract: The present disclosure provides methods, pharmaceutical compostions, and medical kits for treating an opioid and/or opiate addiction in a subject comprising administering a pharmaceutical composition comprising a therapeutically effective amount of an inhibitor of FYN activity or expression, or a pharmaceutically composition comprising same.

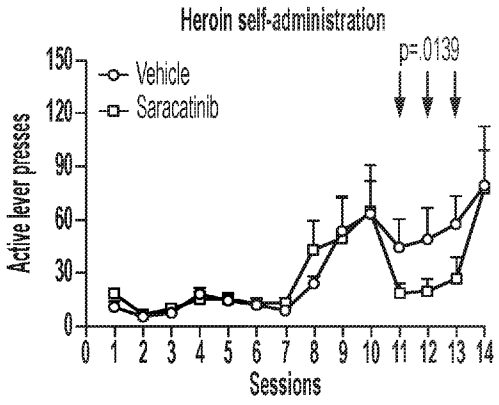


FIG. 9A

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METHOD OF TREATING OPIOID OR OPIATE ADDICTION

RELATED APPLICATION

[0001] This application claims priority to to U.S. Provisional Patent Application Serial No. 62/627,568, filed on February 7, 2018, entitled "METHOD FOR TREATING OPIOID ABUSE," the contents of which are hereby incorporated by reference.

STATEMENT REGARDING FEDERALLY FUNDED RESEARCH

[0002] This invention was made with government support under grant number NIH (NIDA) R01-DAO15446, as issued by the National Institutes of Health. The government has certain rights in the invention.

FIELD OF THE INVENTION

[0003] The present disclosure provides methods of treating an addiction. In particular, the methods of the present disclosure relate to treating, preventing, and/or mitigating at least one symptom or behavior of opiate addiction.

BACKGROUND

[0004] Opioid and opiate dependence is one of the most serious chronic and relapsing medical disorders of our time. Abuse of opiates (alkaloids derived from the opium poppy), such as heroin, morphine, and codeine, and illegal use of prescription opioids (synthetic or semi-synthetic pain medications that work in a similar way to opiates) have been skyrocketing with an unprecedented epidemic in recent years. For example, with an 80% increase in the number of heroin abusing or dependent individuals over the past few years, opiates are currently the second most prevalent class of abused drugs in the United States and opiate overdose is now the leading cause of death among drug users (Bryant *et al.*, 2004; Centers for Disease and Prevention, 2007; Muazzam *et al.*, 2012). As such, opioid addiction is a major medical and societal problem with 2 million Americans abusing or addicted to opioid, half of which are using heroin. Opioid abuse contributes to over 50,000 deaths per year in the United States, surpassing that of motor vehicle accidents. In fact, overdose deaths from opioids in the United States has increased from 6.1 per 100,000 in 1999 to 16.3 per 100,000 in 2015 with the percentage of fatal heroin overdoses more than tripling from 8% to 25%. This is due to the extremely high addictive potential of heroin as well as to the steeply increasing availability of both heroin and prescription opioids

(Inciardi et al., 2009a; Inciardi *et al.*, 2009b; Schneider *et al.*, 2009; Pollini *et al.*, 2011; Peavy *et al.*, 2012).

[0005] Addiction vulnerability is determined by a complex interaction of genetics, epigenetics, cellular and molecular impairments, as well as behavioral traits. Substance use disorders (SUDs) are chronic, relapsing disorders characterized by a pathological and debilitating drive to obtain and consume drugs despite the associated physical, emotional and social negative consequences (Koob and Volkow, 2010). A great deal is known about the neurobiological underpinnings of substance use disorders primarily based on animal studies.

[0006] Indeed, a major characteristic of substance use disorders is that behaviors aimed at obtaining and consuming the abused substances predominate at the expense of more adaptive behaviors. This is in part driven by the "hi-jacking" of the mesocorticolimbic reward system that in non-addicted subjects is normally excited by natural rewards such as food and sex. Abused drugs, though chemically diverse, can affect different components of this system and are therefore extremely reinforcing.

[0007] Midbrain dopaminergic neurons form a central part of the mesocorticolimbic reward system and are activated by all classes of abused substances (Johnson and North, 1992; Jones et al., 1998; Tapper et al., 2004; Waldhoer et al., 2004; Justinova et al., 2005). These dopaminergic neurons are located in the ventral tegmental area (VTA) and substantia nigra pars compacta (SNpc). In general, projections to the striatum and ventral pallidum (VP) underlie reinforcing effects of abused substances, while additional effects to other brain regions including the amygdala, the hippocampus and the prefrontal cortex are involved in affective and learning aspects of addiction (Fuchs et al., 2005; See, 2005). The common characteristic of natural and "unnatural" rewards is that they enhance dopaminergic transmission in the mesocorticolimbic reward system, leading to increased synaptic dopamine levels in the striatum (Johnson and North, 1992; Jones et al., 1998; Tapper et al., 2004; Waldhoer et al., 2004; Justinova et al., 2005). Drugs of abuse can achieve this effect by different mechanisms. Cocaine, for example, inhibits dopamine reuptake, MDMA leads to the release of this neurotransmitter from synaptic vesicles, while opioids inhibit GABAergic interneurons in the VTA leading to the dysinhibition of dopaminergic projection neurons.

[0008] While much has been studied and is known about the neurobiological underpinnings of substance use disorders, there remains a significant need for more effective treatments to inhibit addiction behavior. Current treatment of opioid abuse

employs a combination of opioid replacement therapy (ORT) under medical supervision. For example, methadone is one type of ORT provided to patients; however, the success depends on the patient and the overall failure rate is significant. Methadone is a synthetic opioid prescribed for moderate to severe pain. It is also commonly used to treat opiate addictions, especially addiction to heroin. Methadone acts on the same opioid receptors as morphine and heroin to stabilize patients and minimize withdrawal symptoms in the case of an addiction. Methadone is a federally designated Schedule II drug, meaning it has a legitimate legal use but also a high likelihood of its users developing a dependence. Because methadone is used as a way to curb addiction and reduce cravings, it is not as heavily regulated as other drugs. However, it is still a powerful opiate with potentially addictive qualities. People who start using methadone to overcome their heroin addiction are at a higher risk of abuse because they already have a history of opioid dependency.

[0009] Buprenorphine/naloxone (SUBOXONE®) is another example of ORT. The success rate is somewhat higher than methadone but there is a high rate of relapse once the patient discontinues the treatment. Naltrexone can be additionally administered to help prevent or control relapses. All of the ORT methods benefit significantly with a concomitant counseling regimen, peer or group support meetings and/or counseling by a mental health professional.

[0010] Today's trends of increased opioid-related addiction and opioid-related deaths have led to an increased burden on society, our government, and our health care system and will continue to do so (Hansen *et al.*, 2011). These trends and the limitations of current opioid replacement therapies highlight the critical need to develop new, more effective strategies for treating opiate and/or opioid addiction. The present disclosure has identified a novel approach for opioid addiction and has demonstrated their effectiveness in treating opioid and/or opiate addiction.

SUMMARY

[0011] The inventors, through direct study of the brains of human heroin abusers, discovered the surprising and unexpected association between increased expression and/or activity of FYN (a 59 kDa protein belonging to the Src family of nonreceptor tyrosine kinases, which also includes Src, Yes, Yrk, Blk, Fgr, Hck, Lck, and Lyn) and opioid and/or opiate usage. Moreover, the inventors have demonstrated that inhibiting FYN expression and/or activity ameliorates, mitigates and/or alleviates symptoms and behaviors associated with opioid and/or opiate addiction (*e.g.*, decreased drug-seeking and self-administration

behavior). In other words, the inventors have identified FYN as a new therapeutic target to treat opioid and/or opiate abuse. In one embodiment, the inventors have demonstrated the effectiveness of FYN as a therapeutic target for opioid/opiate abuse by administering saracatinib—a small molecule FYN inhibitor originally developed as a cancer drug (by ASTRAZENCA®) and which is disclosed in WO 01/94341, which is incorporated herein by reference in its entirety. Up until the time of this application, saracatinib was being investigated for use in treating Alzheimer's and alcoholism; however, the link to opioid and/or opiate treatment was not previously recognized or contemplated. The disclosure is not, however, limited to the use of saracatinib. Rather, the disclosure embraces any suitable means by which to inhibit FYN expression and/or activity, including administering saracatinib derivatives or other FYN small molecule inhibitors, as well as administering other suitable means to inhibit FYN expression and/or activity, such as administering anti-FYN antibodies or inhibitory nucleic acid molecules (*e.g.*, siRNA).

[0012] Accordingly, one aspect of the present disclosure relates to a method of treating, preventing, mitigating and/or ameliorating at least one symptom or behavior of opioid and/or opiate addiction in a subject. The method comprises administering to a subject in need thereof an effective amount of an agent that inhibits the expression and/or activity of at least one tyrosine kinase FYN. In one embodiment, the agent can be a small molecule inhibitor of FYN, including for example, saracatinib or a derivative thereof. In other embodiments, the agent can be a peptide or polypeptide inhibitor (*e.g.*, an anti-FYN antibody) or a nucleic acid-based inhibitor (*e.g.*, siRNA) of FYN expression and/or activity.

[0013] In any aspect or embodiment described herein, the tyrosine kinase FYN inhibitor can include at least one small molecule tyrosine kinase FYN inhibitor (*e.g.*, saracatinib or a derivative thereof); at least one polypeptide tyrosine kinase FYN inhibitor (e.g., an anti-FYN antibody); at least one tyrosine kinase FYN inhibitory nucleic acid (*e.g.*, siRNA that silences an mRNA transcript of a gene encoding FYN); or a combination thereof. It is envisioned that genome-editing tools (*e.g.*, CRISPR-Cas9) available in the art may also be used to edit the genome of a patient, and specifically, to edit the genome of a patient at the FYN gene in a manner that reduces or eliminates its expression.

[0014] In any aspect or embodiment described herein, the small molecule tyrosine kinase FYN inhibitor may include saracatinib or a derivative thereof, dasatinib (BRISTOL-MYERS SQUIBB) or a derivative thereof, bosutinib (PFIZER) or a derivative thereof, A 419259 trihydrochloride or a derivative thereof, herbimycin A or a derivative thereof, 1-

naphthyl PP1 or a derivative thereof, PP 1 or a derivative thereof, PP 2 or a derivative thereof, SU6656 or a derivative thereof, or a combination of any of the above.

[0015] In other aspects, the inhibitor agents (*e.g.*, small molecule inhibitors, polypeptide inhibitors, or nucleic acid inhibitors) can be formulated as pharmaceutical compositions comprising one or more inhibitor agents, and at least one pharmaceutically acceptable carrier.

[0016] In any aspect or embodiment described herein, the inhibitor agents (e.g., small molecule inhibitors, polypeptide inhibitors, or nucleic acid inhibitors) and pharmaceutical compositions comprising said agents are effective at mitigating, inhibiting, and/or alleviating opioid/opiate seeking behavior in the subject.

[0017] In any aspect or embodiment described herein, the pharmaceutical composition and/or agent(s) are effective at mitigating, inhibiting, and/or alleviating the subject's physiological dependence upon opioids/opiates.

[0018] In any aspect or embodiment described herein, the pharmaceutical compositions further comprise a pharmaceutically effective amount of a carrier, additive, or excipient.

[0019] In another aspect, the pharmaceutical compositions and/or agents described herein can be provided in the form of a pharmaceutical kit or package, that may further comprise instructions for administering said composition and/or agents to a subject in need, *e.g.*, a subject addicted to heroin.

[0020] In addition, the methods disclosed herein may also comprise administering a therapeutically effective amount of another treatment for opioid and/or opiate abuse. Said another treatment may include administering one or more opioid/opiate replacement therapies (ORT), such as, methodone or buprenorphine/naloxone (SUBOXONE®) or naltrexone or a combination thereof. Said another treatment may also include some form of therapy, including peer-to-peer support groups and professional drug rehabilitation therapy. Administering the other treatment may occur before or after the FYN-based treatment, or at or substantially at the same time as the FYN-based treatment (*i.e.*, co-administered).

[0021] In a particular embodiment, the disclosure provides a method of treating an opioid and/or opiate addiction in a subject comprising administering a therapeutically effective amount of saracatinib or a derivative of saracatinib, or a pharmaceutically acceptable salt thereof.

[0022] In another embodiment, the disclosure provides a method of treating an opioid and/or opiate addiction in a subject comprising administering a pharmaceutical composition

comprising a therapeutically effective amount of saracatinib or a derivative of saracatinib, or a pharmaceutically acceptable salt thereof.

[0023] In still another embodiment, the disclosure provides a method of treating an opioid and/or opiate addiction in a subject comprising administering a therapeutically effective amount of an inhibitor of FYN activity or expression, or a pharmaceutically composition comprising same.

[0024] In various embodiments, the subject being treated can have a tolerance, dependence, or an addiction to at least one opioid and/or opiate drug, which can include heroin, and also which can include oxycodone, hydrocodone, fentanyl, or naloxone.

[0025] The methods also contemplate further co-administering an opioid replacement therapy, which can include methodone therapy or buprenorphine/naloxone (SUBOXONE®) and/or naltrexone therapy.

[0026] The method further can comprise co-administering a histone acetylation inhibitor and/or a bromodomain inhibitor.

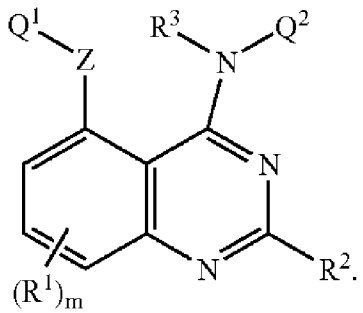
[0027] In certain embodiments, that the histone acetylation inhibitor can be at least one of anacardic acid or a derivative thereof, C 646 or a derivative thereof, EML 425 or a derivative thereof, PU139 or a derivative thereof, PU141 or a derivative thereof, MB-3 or a derivative thereof, CPTH2 or a derivative thereof, ISOX DUAL or a derivative thereof, L002 or a derivative thereof, Lys-CoA or a derivative thereof, NU 9056 or a derivative thereof, curcumin or a derivative thereof, garcinol or a derivative thereof, or a combination thereof.

[0028] In other embodiments, the bromodomain inhibitor can be at least one of JQ1 or a derivative thereof, ISOX DUAL or a derivative thereof, I-CBP 112 or a derivative thereof, SCG-CBP30 or a derivative thereof, I-BET151 (GSK1210151A) or a derivative thereof, I-BET762 (GSK525762) or a derivative thereof, OXF BD 02 or a derivative thereof, MS 436 or a derivative thereof, XMD 8-92 or a derivative thereof, PFI 1 or a derivative thereof, PF1 4 or a derivative thereof, PF CBP1 or a derivative thereof, XD 14 or a derivative thereof, bromosporine or a derivative thereof, RVX-208 or a derivative thereof, BMS-986158 or a derivative thereof, OTX-015 or a derivative thereof, ischemin sodium salt or a derivative thereof, TEN-010 or a derivative thereof, CPI-0610 or a derivative thereof, PLX-51107 or a derivative thereof, INCB054329 or a derivative thereof, GSK2820151 or a derivative thereof, BAY 1238097 or a derivative thereof, or a combination thereof.

[0029] In other embodiments, the FYN inhibitors (*e.g.*, saracatinib) can be administered orally, sublingually, intramuscularly, subcutaneously, intravenously, or by inhalation.

[0030] In still other embodiments, the small molecule FYN inhibitor is saracatinib or derivative thereof, dasatinib or derivative thereof, bosutinib or derivative thereof, A 419259 trihydrochloride or derivative thereof, herbimycin A or derivative thereof, 1-naphthyl PP1 or derivative thereof, PP 1 or derivative thereof, PP 2 or derivative thereof, SU6656 or derivative thereof, or combinations thereof.

[0031] In yet other embodiments, the small molecule FYN inhibitor is a compound of Formula IV



(IV),

wherein each of Q^1, Z, m, R^1, R^2, R^3, and Q^2 have any of the meanings as defined or claimed in PCT/GB2001/002424 (published as WO/2001/094341), which is incorporated herein by reference.

[0032] In still other embodiments, the inhibitor of FYN activity or expression is a polypeptide inhibitor, such as an anti-FYN antibody.

[0033] In yet other embodiments, the inhibitor of FYN activity or expression is a nucleic acid inhibitor, e.g., an siRNA inhibitor of a mRNA encoding FYN.

[0034] The preceding general areas of utility are given by way of example only and are not intended to be limiting on the scope of the present disclosure and appended claims. Additional objects and advantages associated with the methods, and processes of the present disclosure will be appreciated by one of ordinary skill in the art in light of the instant claims, description, and examples. For example, the various aspects and embodiments of the disclosure may be utilized in numerous combinations, all of which are expressly contemplated by the present description. These additional aspects and embodiments are expressly included within the scope of the present disclosure. The publications and other materials used herein to illuminate the background of the disclosure, and in particular cases, to provide additional details respecting the practice, are expressly incorporated herein by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] The accompanying drawings, which are incorporated into and form a part of the specification, illustrate several embodiments of the present disclosure and, together with the description, serve to explain the principles of the present disclosure. The drawings are only for the purpose of illustrating an embodiment of the present disclosure and are not to be construed as limiting the present disclosure. Further objects, features and advantages of the disclosure will become apparent from the following detailed description taken in conjunction with the accompanying figures showing illustrative embodiments of the disclosure, in which:

[0036] **FIG. 1**. Human striatum tissue was obtained from the ventral and dorsal striatum (putamen) at the level of the nucleus accumbens (NAc) for examination as described herein.

[0037] **FIG. 2A and FIG. 2B**. Stereotaxic surgeries included (FIG. 2A) guide cannulae implanted at a 10° angle at AP+1.7mm, ML+3.5mm and DV+2mm from bregma, and (FIG. 2B) For JQ1 injections (a bromodomain inhibitor), 28 gauge needle inserted through the guide cannulae to AP+17mm, ML+3.5mm and DV+5.2mm from bregma for JQ1 injections. Two (2) ml of 20uM JQ1 was injected over 1 minute in both hemispheres.

[0038] **FIG. 3A and FIG. 3B**. Gene ontology analysis of differentially accessible regions. (FIG. 3A) Enriched categories for neuronal ATAC peaks that are less accessible in heroin users. (FIG. 3B) Enriched categories for neuronal ATAC peaks that are more accessible in heroin users.

[0039] **FIG. 4**. Factors contributing to ATAC signal variability. Violin plots showing the percentage of ATAC signal variance explained by cell type (neuron vs. glia), diagnosis (heroin vs. control; shown separately in neuron and glia), gender, individual variability, post-mortem interval and residual factors. Top genes are indicated next to plot. Dx: diagnosis, PMI: post-mortem interval.

[0040] **FIG. 5A and FIG. 5B**. Heroin increases chromatin accessibility of the FYN promoter in neurons. ATAC signal indicates that chromatin accessibility within the top FYN peak in NeuN-positive neuronal (FIG. 5A) and NeuN-negative glial (FIG. 5B) cell populations. Data is represented as mean ± SEM.

[0041] **FIG. 6A, FIG. 6B, FIG. 6C, and FIG. 6D**. Heroin-related transcriptional impairments of FYN in the post-mortem human brain. FIG. 6A illustrates real-time quantitative PCR data showing FYN mRNA levels in the putamen of human heroin users and matched controls. Data is represented as mean ± SEM. FIG. 6B illustrates Pearson correlation between FYN mRNA and acetylated histone H3. R and P values correspond to

the heroin group. FIG. 6C and FIG. 6D illustrates Pearson correlations between normalized FYN mRNA levels and years of previous drug use (FIG. 6C) and urine morphine [ng/ul] toxicology at the time of death (FIG. 6D).

[0042] FIG. 7A, FIG. 7B, FIG. 7C, and FIG. 7D. Differential correlations between FYN and post-synaptic density components in human heroin users. Pearson correlations between normalized FYN mRNA levels and various members of the glutamatergic post-synaptic density (i.e., DLG5, GRIA1, GRM5, and HOMER1, respectively) in the putamen of human heroin users and matched controls. R and P values corresponding to correlations in the heroin group are shown.

[0043] FIG. 8A, FIG. 8B, and FIG. 8C. *Fyn* mRNA levels are affected by heroin *in vivo* and *in vitro*. (FIG. 8A) *Fyn* mRNA levels in the caudatoputamen of heroin self-administering adult male Long-Evans rats. (FIG. 8B) *Fyn* mRNA levels in primary striatal cells treated with 10 uM or 100 uM morphine. (FIG. 8C) *Fyn* mRNA in heroin self-administering adult male Long-Evans rats treated with aCSF or JQ1. Data is represented as mean ± SEM. P values from one-way ANOVA are shown. Cpu: caudatoputamen. aCSF: artificial cerebrospinal fluid.

[0044] FIG. 9A and FIG. 9B. FYN inhibitor saracatinib attenuates heroin-self administration behavior and drug-seeking behavior *in vivo*. (FIG. 9A) Active lever presses during 3 hours long heroin self-administration session in vehicle and saracatinib treated Long-Evans rats. Arrows represent days of saracatinib administration. Bar graphs represent mean ± SEM. Two-way ANOVA revealed significant group effect ($F_{1,29}=6.852$, $p=0.0139$). (FIG. 9B) Active lever presses during a 1 hour non-reinforced cue-induced drug-seeking session in vehicle and saracatinib treated Long-Evans rats. Data is represented as mean ± SEM. Two-way ANOVA revealed significant group ($F_{1,132}=7.376$, $p=0.0075$) and time ($F_{11,132}=2.685$, $p=0.0038$) effects.

DETAILED DESCRIPTION

[0045] The following is a detailed description provided to aid those skilled in the art in practicing the present disclosure. Those of ordinary skill in the art may make modifications and variations in the embodiments described herein without departing from the spirit or scope of the present disclosure. All publications, patent applications, patents, figures and other references mentioned herein are expressly incorporated by reference in their entirety.

[0046] Presently described are methods and compositions of matter that relate to the surprising and unexpected discovery that tyrosine kinase FYN is overexpressed and/or has

induced or increased activity in the neurons of subjects that are taking opioids and/or opiates (*e.g.*, opioid/opiate addicted subjects). Accordingly, the present disclosure provides methods of treating, preventing, ameliorating and/or mitigating symptoms and/or behaviors of opioid/opiate addiction by inhibiting the expression and/or activity of tyrosine kinase FYN in individuals that are taking and/or are addicted to opioids and/or opiates.

[0047] In one embodiment, the inventors have demonstrated the effectiveness of FYN as a therapeutic target for opioid/opiate abuse by administering saracatinib—a small molecule Fyn inhibitor originally developed as a cancer drug (by ASTRAZENCA®) and which is disclosed in WO 01/94341 (incorporated herein by reference). At the time of this filing, saracatinib is being investigated for use in treating Alzheimer's and alcoholism; however, the link to opioid and/or opiate treatment has not previously been recognized or contemplated in the art. However, the methods and compositions disclosed herein are not limited to the use of saracatinib to inhibit FYN activity or reduce its expression. Rather, the disclosure embraces any suitable means by which to inhibit FYN expression and/or activity, including saracatinib derivatives, other FYN small molecule inhibitors, or other suitables means to inhibit FYN expression and/or activity, such as polypeptide and/or peptide FYN inhibitors (*e.g.*, anti-FYN antibodies) or inhibitory nucleic acid molecules (*e.g.*, siRNA) which inhibit FYN expression, for example, by inhibiting the transcriptional activity of the gene encoding FYN, silence the encoded mRNA transcripts (*e.g.*, si RNA), or inhibition translational processes.

[0048] Accordingly, one aspect of the present disclosure relates to a method of treating, preventing, mitigating and/or ameliorating at least one symptom or behavior of opioid/opiate addiction in a subject. The method comprises administering to a subject in need thereof an effective amount of an agent that inhibits the expression and/or activity of at least one tyrosine kinase FYN. In one embodiment, the agent can be a small molecule inhibitor of FYN, including for example, saracatinib or a derivative thereof. In other embodiments, the agent can be a peptide or polypeptide inhibitor (*e.g.,* an anti-FYN antibody) or a nucleic acid-based inhibitor (*e.g.,* siRNA) of FYN expression and/or activity.

[0049] In any aspect or embodiment described herein, the composition, agent, or agents of the present disclosure are effective at mitigating, inhibiting, and/or alleviating opioid seeking behavior in the subject. For example, the composition, agent or agents can be effective at mitigating, inhibiting, and/or alleviating the subject's physiological dependence upon opioids.

[0050] In any aspect or embodiment described herein, the composition further comprises a pharmaceutically effective amount of a carrier, additive, or excipient.

[0051] In certain aspects, the present disclosure utilizes a small molecule (*i.e.*, having a molecular weight of below 2,000, 1,000, 900, 500, or 200 Daltons), which is a tyrosine kinase FYN inhibitor.

[0052] **Definitions**

[0053] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the present disclosure belongs. The terminology used in the description is for describing particular embodiments only and is not intended to be limiting of the disclosure.

[0054] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the disclosure. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges is also encompassed within the disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either both of those included limits are also included in the disclosure.

[0055] The following terms are used to describe the present disclosure. In instances where a term is not specifically defined herein, that term is given an art-recognized meaning by those of ordinary skill applying that term in context to its use in describing the present disclosure.

[0056] The articles "a" and "an" as used herein and in the appended claims are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article unless the context clearly indicates otherwise. By way of example, "an element" means one element or more than one element.

[0057] The phrase "and/or," as used herein in the specification and in the claims, should be understood to mean "either or both" of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with "and/or" should be construed in the same fashion, i.e., "one or more" of the elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the "and/or" clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to "A and/or B", when used in conjunction with open-ended language such as "comprising" can refer, in

one embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

[0058] As used herein in the specification and in the claims, "or" should be understood to have the same meaning as "and/or" as defined above. For example, when separating items in a list, "or" or "and/or" shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as "only one of" or "exactly one of," or, when used in the claims, "consisting of," will refer to the inclusion of exactly one element of a number or list of elements. In general, the term "or" as used herein shall only be interpreted as indicating exclusive alternatives (i.e., "one or the other but not both") when preceded by terms of exclusivity, such as "either," "one of," "only one of," or "exactly one of."

[0059] In the claims, as well as in the specification above, all transitional phrases such as "comprising," "including," "carrying," "having," "containing," "involving," "holding," "composed of," and the like are to be understood to be open-ended, i.e., to mean including but not limited to. Only the transitional phrases "consisting of' and "consisting essentially of' shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111.03.

[0060] As used herein in the specification and in the claims, the phrase "at least one," in reference to a list of one or more elements, should be understood to mean at least one element selected from anyone or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase "at least one" refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, "at least one of A and B" (or, equivalently, "at least one of A or B," or, equivalently "at least one of A and/or B") can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

[0061] It should also be understood that, in certain methods described herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited unless the context indicates otherwise.

[0062] The terms "co-administration" and "co-administering" or "combination therapy" refer to both concurrent administration (administration of two or more therapeutic agents at the same time) and time varied administration (administration of one or more therapeutic agents at a time different from that of the administration of an additional therapeutic agent or agents), as long as the therapeutic agents are present in the patient/subject to some extent, preferably at effective amounts, at the same time. In certain preferred aspects, one or more of the agents described herein, are coadministered in combination with at least one additional bioactive agent.

[0063] The term "agent" or "compound", as used herein, unless otherwise indicated, refers to any specific chemical compound or agent disclosed herein and includes tautomers, regioisomers, geometric isomers, and where applicable, stereoisomers, including optical isomers (enantiomers) and other stereoisomers (diastereomers) thereof, as well as pharmaceutically acceptable salts and derivatives, including prodrug and/or deuterated forms thereof where applicable, in context. Deuterated small molecules contemplated are those in which one or more of the hydrogen atoms contained in the drug molecule have been replaced by deuterium.

[0064] Within its use in context, the term compound or agent generally refers to a single compound or agent, but also may include other compounds, such as stereoisomers, regioisomers and/or optical isomers (including racemic mixtures) as well as specific enantiomers or enantiomerically enriched mixtures of disclosed compounds, or agents. The term also refers, in context to prodrug forms of compounds which have been modified to facilitate the administration and delivery of compounds to a site of activity.

[0065] The term "patient" or "subject" is used throughout the specification to describe an animal, preferably a human or a domesticated animal (*e.g.,* a dog, rat, mouse, or cat, or a farm animal such as a horse, cow, sheep, etc.), to whom treatment, including prophylactic treatment, with the agents/compounds according to the present disclosure is provided, *e.g.,* a person who is addicted to one or more opioids and/or opiates, such as heroin.

[0066] "Ameliorate", "ameliorating", "amelioration", "mitigate", mitigation", "mitigating", or the like, refers to decreasing, suppressing, attenuating, diminishing, arresting, or stabilizing the development or progression of a disease (*e.g.,* opioid or opiate

addiction), at least one symptom of the disease, and/or at least one behavior associated with the disease.

[0067] "Treat", "treatment", or "treating" as used herein, is defined as the application or administration of an agent or a composition described herein to a patient or subject, who has a disease or disorder (*e.g.,* opioid or opiate addiction) with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease or disorder, symptoms of the disease or disorder, or a behavior associated with the disease or disorder. The term "treatment" or "treating" is also used herein in the context of administering agents or a composition prophylactically. In the context of the present disclosure, the symptoms that may be alleviated can include, but are not limited to, any symptom associated with opioid addiction. In the context of the present disclosure, the behavior that may be alleviated, ameliorated, or mitigated with the agents or composition of the present disclosure include drug taking and/or drug seeking behavior. In some instances, treatment will prevent opioid addiction.

[0068] The term "prevent", "preventing", or "prevention" refers to the prevention of opioid addiction or prevents at least one symptom opioid addiction, and/or at least one behavior associated with opioid addiction, which means to prophylactically interfere with a pathological mechanism that results in the opioid addiction, a symptom of opioid addiction, or a behavior associated with opioid addiction.

[0069] The term "effective" is used to describe an amount of a compound, composition, agent, or component which, when used within the context of its intended use, effects an intended result. The term effective subsumes all other effective amount or effective concentration terms, which are otherwise described or used in the present application.

[0070] As used herein, the term "opioids" refers to substances that act on opioid receptors to produce morphine-like effects. Side effects of opioids may include itchiness, sedation, nausea, respiratory depression, constipation, and euphoria. Tolerance and dependence will develop with continuous use, requiring increasing doses and leading to a withdrawal syndrome upon abrupt discontinuation. The euphoria attracts recreational use, and frequent, escalating recreational use of opioids typically results in addiction. Examples include semi-synthetic and synthetic drugs , such as hydrocodone, oxycodone, and fentanyl, and naloxone. Opioids include and/or encompass "opiates," which typically refers to the natural alkaloids found in the resin of the opium poppy although some include semi-synthetic derivatives. As used herein, an "addiction" in the context of a drug such as an opioid is a brain disorder characterized by compulsive engagement in rewarding stimuli

despite adverse consequences. Further, addiction can mean a primary, chronic disease of brain reward, motivation, memory and related circuitry. Dysfunction in these circuits leads to characteristic biological, psychological, social and spiritual manifestations. This is reflected in an individual pathologically pursuing reward and/or relief by substance use and other behaviours. Addiction is characterized by inability to consistently abstain, impairment in behavioural control, craving, diminished recognition of significant problems with one's behaviours and interpersonal relationships, and a dysfunctional emotional response.

[0071] As used herein, an "addictive behavior" in the context of a drug such as an opioid is a behavior that is both rewarding and reinforcing.

[0072] As used in herein, an "addictive drug," such as an opioid or opiate, is a drug that is both rewarding and reinforcing.

[0073] As used herein, "dependence" on a particular drug is an adaptive physiological state that occurs with regular drug use and results in a withdrawal syndrome when drug use is stopped. It is usually associated with increased tolerance. Physical dependence tends to be a characteristic of substance use disorders, but alones does not imply addiction.

[0074] As used herein, "tolerance" of a drug is a condition in which higher doses of a drug are required to produce the same effect experienced during initial use. It is often associated with physical dependence.

[0075] As used herein, "withdrawal" of a drug is a characteristic of substance use disorders, but does not necessarily imply addiction. With regular use of a substance, biochemical and structural adaptations take place in the brain. Withdrawal is the group of symptoms and signs that a person experiences after a period of regular use, when the quantity of the substance in the brain is reduced.

[0076] As used herein, the agents and methods disclosed herein are useful in the treatment of "opioid/opiate abuse" which can encompass treatment of related concepts such as addition, dependence, tolerance, and withdrawal of an opioid or opiate drug.

[0077] If a compound of the present invention is depicted in form of a chemical name and as a formula in case of any discrepancy the formula shall prevail.

[0078] As used herein, "pharmaceutically acceptable salts" refer to derivatives of the disclosed compounds wherein the parent compound is modified by making acid or base salts thereof. Examples of pharmacologically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. For example, such salts include salts

from ammonia, L-arginine, betaine, benethamine, benzathine, calcium hydroxide, choline, deanol, diethanolamine (2,2'-iminobis(ethanol)), diethylamine, 2-(diethylamino)-ethanol, 2-aminoethanol, ethylenediamine, N-ethyl-glucamine, hydrabamine, 1H-imidazole, lysine, magnesium hydroxide, 4-(2-hydroxyethyl)-morpholine, piperazine, potassium hydroxide, 1-(2-hydroxyethyl)-pyrrolidine, sodium hydroxide, triethanolamine (2,2',2''-nitrilotris(ethanol)), tromethamine, zinc hydroxide, acetic acid, 2.2-dichloro-acetic acid, adipic acid, alginic acid, ascorbic acid, L-aspartic acid, benzenesulfonic acid, benzoic acid, 2,5-dihydroxybenzoic acid, 4-acetamido-benzoic acid, (+)camphoric acid, (+)-camphor-10-sulfonic acid, carbonic acid, cinnamic acid, citric acid, cyclamic acid, decanoic acid, dodecylsulfuric acid, ethane-1,2-disulfonic acid, ethanesulfonic acid, 2-hydroxy-ethanesulfonic acid, ethylenediaminetetraacetic acid, formic acid, fumaric acid, galactaric acid, gentisic acid, D-glucoheptonic acid, D-gluconic acid, D-glucuronic acid, glutamic acid, glutaric acid, 2-oxo-glutaric acid, glycerophosphoric acid, glycine, glycolic acid, hexanoic acid, hippuric acid, hydrobromic acid, hydrochloric acid, isobutyric acid, DL-lactic acid, lactobionic acid, lauric acid, lysine, maleic acid, (-)-L-malic acid, malonic acid, DL-mandelic acid, methanesulfonic acid, galactaric acid, naphthalene-1,5-disulfonic acid, naphthalene-2-sulfonic acid, 1-hydroxy-2-naphthoic acid, nicotinic acid, nitric acid, octanoic acid, oleic acid, orotic acid, oxalic acid, palmitic acid, pamoic acid (embonic acid), phosphoric acid, propionic acid, (-)-L-pyroglutamic acid, salicylic acid, 4-amino-salicylic acid, sebacic acid, stearic acid, succinic acid, sulfuric acid, tannic acid, (+)-L-tartaric acid, thiocyanic acid, p-toluenesulfonic acid and undecylenic acid. Further pharmaceutically acceptable salts can be formed with cations from metals like aluminium, calcium, lithium, magnesium, potassium, sodium, zinc and the like. (also see Pharmaceutical salts, Berge, S. M. et al., J. Pharm. Sci., (1977), 66, 1-19).

[0079] The pharmaceutically acceptable salts of the present invention can be synthesized from the parent compound which contains a cationic group and optionally an additional basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting other salt forms of these compounds with a sufficient amount of the appropriate base or acid in water or in an organic diluent like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile, or a mixture thereof. Moreover, counterions can generally be exchanged by ion exchange chromatography.

FYN Protein

[0080] The methods and compositions of the present disclosure relate to inhibiting the expression and/or activity of the FYN protein, including any isoform thereof. As will be

appreciated, a protein isoform, or "protein variant" is a member of a set of highly similar proteins that perform the same or similar biological roles. A set of protein isoforms may be formed from alternative splicings or other post-translational modifications of a single gene. Through RNA splicing mechanisms, mRNA has the ability to select different protein-coding segments (exons) of a gene, or even different parts of exons from RNA to form different mRNA sequences. Each unique sequence produces a specific form of a protein. A set of protein isoforms may also result from a set of closely related genes that evolved from a single gene in the past.

[0081] FYN is a 59-kDa member of the Src family of kinases typically associated with T-cell and neuronal signaling in development and normal cell physiology and exists naturally in several isoforms. Disruptions in these signaling pathways often have implications in the formation of a variety of cancers. By definition as a proto-oncogene, *FYN* codes for proteins that help regulate cell growth. Changes in its DNA sequence transform it into an oncogene that leads to the formation of a different protein with implications for normal cell regulation.

[0082] More specifically, FYN is a member of the protein-tyrosine kinase oncogene family. It encodes a membrane-associated tyrosine kinase that has been implicated in the control of cell growth. The protein associates with the p85 subunit of phosphatidylinositol 3-kinase and interacts with the FYN-binding protein. Alternatively spliced transcript variants encoding distinct isoforms exist in nature.

[0083] FYN is located downstream of several cell surface receptors, commonly associated with neuronal development and T-cell signaling. When FYN is activated it causes downstream activation of molecular signals that drive processes crucial to growth and motility of cells. FYN is primarily localized to the cytoplasmic leaflet of the plasma membrane, where it phosphorylates tyrosine residues on key targets involved in a variety of different signaling pathways. Tyrosine phosphorylation of target proteins by FYN serves to either regulate target protein activity, and/or to generate a binding site on the target protein that recruits other signaling molecules. Interestingly, FYN also has the ability to serve as a tumor suppressor. When this normal biology is compromised, the altered FYN becomes involved in the neoplastic transformation of normal cells to cancerous ones following the pathway from pre-invasive, to invasive, and ultimately metastasis.

[0084] Aliases to FYN include SLK, SYN, p59-FYN proto-oncogene, and Src family tyrosine kinase.

[0085] Publicly disclosed isoform polypeptide sequences include the following exemplary FYN sequences:

[0086] GenBank No.: NP_002028.1 (537 amino acids, human, isoform "A", and its corresponding mRNA sequence NM_002037, and its corresponding mRNA sequence NM_153047), having the following amino acid sequence:

MGCVQCKDKE ATKLTEERDG SLNQSSGYRY GTDPTPQHYP SFGVTSIPNY NNFHAAGGQG
LTVFGGVNSS SHTGTLRTRG GTGVTLFVAL YDYEARTEDD LSFHKGEKFQ ILNSSEGDWW
EARSLTTGET GYIPSNYVAP VDSIQAEEWY FGKLGRKDAE RQLLSFGNPR GTFLIRESET
TKGAYSLSIR DWDDMKGDHV KHYKIRKLDN GGYYITTRAQ FETLQQLVQH YSERAAGLCC
RLVVPCHKGM PRLTDLSVKT KDVWEIPRES LQLIKRLGNG QFGEVWMGTW NGNTKVAIKT
LKPGTMSPES FLEEAQIMKK LKHDKLVQLY AVVSEEPIYI VTEYMNKGSL LDFLKDGEGR
ALKLPNLVDM AAQVAAGMAY IERMNYIHRD LRSANILVGN GLICKIADFG LARLIEDNEY
TARQGAKFPI KWTAPEAALY GRFTIKSDVW SFGILLTELV TKGRVPYPGM NNREVLEQVE
RGYRMPCPQD CPISLHELMI HCWKKDPEER PTFEYLQSFL EDYFTATEPQ YQPGENL
(SEQ ID NO: 1);

[0087] GenBank No.: NP_694592 (534 amino acids, human, isoform "B", and its corresponding mRNA sequence NM_153047.3), having the following amino acid sequence:

MGCVQCKDKE ATKLTEERDG SLNQSSGYRY GTDPTPQHYP SFGVTSIPNY NNFHAAGGQG
LTVFGGVNSS SHTGTLRTRG GTGVTLFVAL YDYEARTEDD LSFHKGEKFQ ILNSSEGDWW
EARSLTTGET GYIPSNYVAP VDSIQAEEWY FGKLGRKDAE RQLLSFGNPR GTFLIRESE
TKGAYSLSIR DWDDMKGDHV KHYKIRKLDN GGYYITTRAQ FETLQQLVQH YSEKADGLCF
NLTVIASSCT PQTSGLAKDA WEVARRSLCL EKKLGQGCFA EVWLGTWNGN TKVAIKTLKP
GTMSPESFLE EAQIMKKLKH DKLVQLYAVV SEEPIYIVTE YMNKGSLLDF LKDGEGRALK
LPNLVDMAAQ VAAGMAYIER MNYIHRDLRS ANILVGNGLI CKIADFGLAR LIEDNEYTAR
QGAKFPIKWT APEAALYGRF TIKSDVWSFG ILLTELVTKG RVPYPGMNNR EVLEQVERGY
RMPCPQDCPI SLHELMIHCW KKDPEERPTF EYLQSFLEDY FTATEPQYQP GENL
(SEQ ID NO: 2);

[0088] GenBank No.: NP_694593 (482 amino acids, human, isoform "C" and its corresponding mRNA sequence NM_153048.3), having the following amino acid sequence:

MGCVQCKDKE ATKLTEERDG SLNQSSGYRY GTDPTPQHYP SFGVTSIPNY NNFHAAGGQG
LTVFGGVNSS SHTGTLRTRG GTGVTLFVAL YDYEARTEDD LSFHKGEKFQ ILNSSEGDWW
EARSLTTGET GYIPSNYVAP VDSIQAEEWY FGKLGRKDAE RQLLSFGNPR GTFLIRESET
TKGAYSLSIR DWDDMKGDHV KHYKIRKLDN GGYYITTRAQ FETLQQLVQH YSGTWNGNTK
VAIKTLKPGT MSPESFLEEA QIMKKLKHDK LVQLYAVVSE EPIYIVTEYM NKGSLLDFLK

DGEGRALKLP NLVDMAAQVA AGMAYIERMN YIHRDLRSAN ILVGNGLICK IADFGLARLI EDNEYTARQG AKFPIKWTAP EAALYGRFTI KSDVWSFGIL LTELVTKGRV PYPGMNNREV LEQVERGYRM PCPQDCPISL HELMIHCWKK DPEERPTFEY LQSFLEDYFT ATEPQYQPGE NL (SEQ ID NO: 3);

[0089] GenBank No.: NP_001116364 (534 amino acids, mouse, isoform "C", and its corresponding mRNA sequence NM_001122892.1), having the following amino acid sequence:

MGCVQCKDKE AAKLTEERDG SLNQSSGYRY GTDPTPQHYP SFGVTSIPNY NNFHAAGGQG LTVFGGVNSS SHTGTLRTRG GTGVTLFVAL YDYEARTEDD LSFHKGEKFQ ILNSSEGDWW EARSLTTGET GYIPSNYVAP VDSIQAEEWY FGKLGRKDAE RQLLSFGNPR GTFLIRESET TKGAYSLSIR DWDDMKGDHV KHYKIRKLDN GGYYITTRAQ FETLQQLVQH YSEKADGLCF NLTVVSSSCT PQTSGLAKDA WEVARDSLFL EKKLGQGCFA EVWLGTWNGN TKVAIKTLKP GTMSPESFLE EAQIMKKLKH DKLVQLYAVV SEEPIYIVTE YMSKGSLLDF LKDGEGRALK LPNLVDMAAQ VAAGMAYIER MNYIHRDLRS ANILVGNGLI CKIADFGLAR LIEDNEYTAR QGAKFPIKWT APEAALYGRF TIKSDVWSFG ILLTELVTKG RVPYPGMNNR EVLEQVERGY RMPCPQDCPI SLHELMIHCW KKDPEERPTF EYLQGFLEDY FTATEPQYQP GENL (SEQ ID NO: 4);

[0090] GenBank No.: NP_001116365 (537 amino acids, mouse, isoform "A" and its corresponding mRNA sequence NM_001122893.1), having the following amino acid sequence:

MGCVQCKDKE AAKLTEERDG SLNQSSGYRY GTDPTPQHYP SFGVTSIPNY NNFHAAGGQG LTVFGGVNSS SHTGTLRTRG GTGVTLFVAL YDYEARTEDD LSFHKGEKFQ ILNSSEGDWW EARSLTTGET GYIPSNYVAP VDSIQAEEWY FGKLGRKDAE RQLLSFGNPR GTFLIRESET TKGAYSLSIR DWDDMKGDHV KHYKIRKLDN GGYYITTRAQ FETLQQLVQH YSERAAGLCC RLVVPCHKGM PRLTDLSVKT KDVWEIPRES LQLIKRLGNG QFGEVWMGTW NGNTKVAIKT LKPGTMSPES FLEEAQIMKK LKHDKLVQLY AVVSEEPIYI VTEYMSKGSL LDFLKDGEGR ALKLPNLVDM AAQVAAGMAY IERMNYIHRD LRSANILVGN GLICKIADFG LARLIEDNEY TARQGAKFPI KWTAPEAALY GRFTIKSDVW SFGILLTELV TKGRVPYPGM NNREVLEQVE RGYRMPCPQD CPISLHELMI HCWKKDPEER PTFEYLQGFL EDYFTATEPQ YQPGENL (SEQ ID NO: 5); and

[0091] GenBank No.: NP_032080 (534 amino acids, mouse, isoform "B" and its corresponding mRNA sequence NM_008054.2) , having the following amino acid sequence:

MGCVQCKDKE AAKLTEERDG SLNQSSGYRY GTDPTPQHYP SFGVTSIPNY NNFHAAGGQG LTVFGGVNSS SHTGTLRTRG GTGVTLFVAL YDYEARTEDD LSFHKGEKFQ ILNSSEGDWW EARSLTTGET GYIPSNYVAP VDSIQAEEWY FGKLGRKDAE RQLLSFGNPR GTFLIRESET

TKGAYSLSIR DWDDMKGDHV KHYKIRKLDN GGYYITTRAQ FETLQQLVQH YSEKADGLCF NLTVVSSSCT PQTSGLAKDA WEVARDSLFL EKKLGQGCFA EVWLGTWNGN TKVAIKTLKP GTMSPESFLE EAQIMKKLKH DKLVQLYAVV SEEPIYIVTE YMSKGSLLDF LKDGEGRALK LPNLVDMAAQ VAAGMAYIER MNYIHRDLRS ANILVGNGLI CKIADFGLAR LIEDNEYTAR QGAKFPIKWT APEAALYGRF TIKSDVWSFG ILLTELVTKG RVPYPGMNNR EVLEQVERGY RMPCPQDCPI SLHELMIHCW KKDPEERPTF EYLQGFLEDY FTATEPQYQP GENL (SEQ ID NO: 6), each of which are publicly available amino acid and nucleotide sequences and are incorporated herein by reference. See Mkaddem SB et al., "Lyn and Fyn function as molecular switches that control immunoreceptors to direct homeostasis or inflammation," Nat Commun 8 (1), 246 (2017), which is incorporated herein by reference.

[0092] With regard any specific biomolecules herein disclosed, *e.g.,* specific FYN amino acid sequences, such as SEQ ID Nos: 1-6, the disclosure is not limited to such exemplary sequences but instead encompasses biomolecules that are similar thereto with regard to their sequences (*e.g.,* amino acid or nucleotide sequences) and/or function. In one embodiment, the present disclosure contemplates nucleic acid molecules or polypeptides (*e.g.,* FYN polypeptides) having a sequence identity of at least 70%, or at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 95%, or at least 99% of any disclosed sequence, *e.g.,* the FYN polypeptides of SEQ ID Nos: 1-6.

[0093] Without wishing to be limited by theory, the inventors discovered the surprising and unexpected association between increased expression and/or activity of FYN and opioid and/or opiate usage. Moreover, the inventors have demonstrated that inhibiting FYN expression and/or activity ameliorates, mitigates and/or alleviates symptoms and behaviors associated with opioid and/or opiate addiction (*e.g.,* decreased drug-seeking and self-administration behavior). In other words, the inventors have identified FYN as a new therapeutic target to treat opioid and/or opiate abuse. In one embodiment, the inventors have demonstrated the effectiveness of FYN as a therapeutic target for opioid and/or opiate abuse by administering saracatinib—a small molecule FYN inhibitor originally developed as a cancer drug (by ASTRAZENCA®) and which is disclosed in WO 01/94341 (incorporated herein by reference). At the time of this application, saracatinib was being investigated for use in treating Alzheimer's and alcoholism; however, the link to opioid and/or opiate treatment had not previously been recognized or contemplated in the art. The present disclosure is not limited to the use of saracatinib. Rather, the disclosure embraces any suitable means by which to inhibit FYN expression and/or activity, including saracatinib derivatives, other FYN small molecule inhibitors, or other suitables means to

inhibit FYN expression and/or activity, such as anti-FYN antibodies or inhibitory nucleic acid molecules (*e.g.,* siRNA).

[0094] The inhibitors disclosed and utilized herein are capable of inhibiting the expression and/or activity of at least one isoform of FYN, and preferably all FYN isoforms equally or substantially equally. The expression "inhibit FYN" or the like contemplates and/or means inhibition of at least one FYN isoform, and preferably, all FYN isoforms equally or substantially equally.

Small Molecule FYN Inhibitors

[0095] In any aspect or embodiment described herein, the agent can include as least one (*e.g.,* 1, 2, 3, 4, 5, 6, 7, or more) small molecule tyrosine kinase FYN inhibitor. For example, the agent can include at least one (*e.g.,* 1, 2, 3, 4, 5, 6, 7, or more) small molecule tyrosine kinase FYN inhibitor selected from the group consisting of:

[0096] saracatinib (4-(6-chloro-2,3-methylenedioxyanilino)-7-[2-(4-methylpiperazin-1-yl)- ethoxy]-5-tetrahydropyran-4-yloxyquinazoline) or a derivative thereof;

[0097] dasatinib (N-(2-chloro-6-methylphenyl)-2-{6-[4-(2-hydroxyethyl)piperazin-1-yl]-2-met- hylpyrimidin-4-ylamino}thiazole-5-carboxamide) or a derivative thereof;

[0098] bosutinib (4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[3-(4-methyl-1-piperazinyl)propoxy]-3-quinolinecarbonitrile) or a derivative thereof;

[0099] A 419259 trihydrochloride (7-[*trans*-4-(4-Methyl-1-piperazinyl)cyclohexyl]-5-(4-phenoxyphenyl)-7*H*-Pyrrolo[2,3-*d*]pyrimidin-4-amine trihydrochloride) or a derivative thereof;

[00100] herbimycin A ((15*R*)-17-demethoxy-15-methoxy-11-*O*-methyl-geldanamycin) or a derivative thereof;

[00101] 1-naphthyl PP1 (1-(1,1-Dimethylethyl)-3-(1-naphthalenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine) or a derivative thereof;

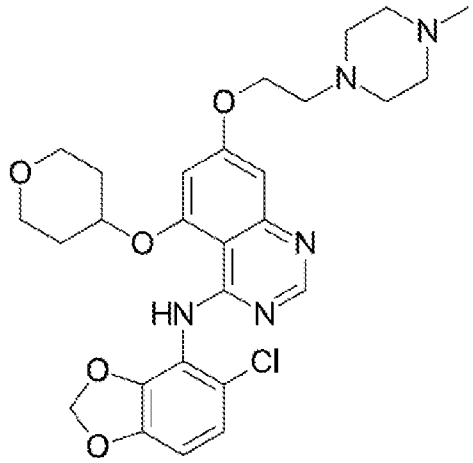
[00102] PP 1 (1-(1,1-Dimethylethyl)-1-(4-methylphenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine) or a derivative thereof;

[00103] PP 2 (3-(4-chlorophenyl) 1-(1,1-dimethylethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine) or a derivative thereof; and

[00104] SU6656 ((3*Z*)-*N*,*N*-Dimethyl-2-oxo-3-(4,5,6,7-tetrahydro-1H-indol-2-ylmethylidene)-2,3-dihydro-1H-indole-5-sulfonamide) or a derivative thereof, or a combination thereof, and any pharmaceutically acceptable salts thereof.

[00105] In a particular embodiment, the tyrosine kinase FYN inhibitor is saracatinib, having the IUPAC name N-(5-chloro-1,3-benzodioxol-4-yl)-7-[2-(4-methyl-1-

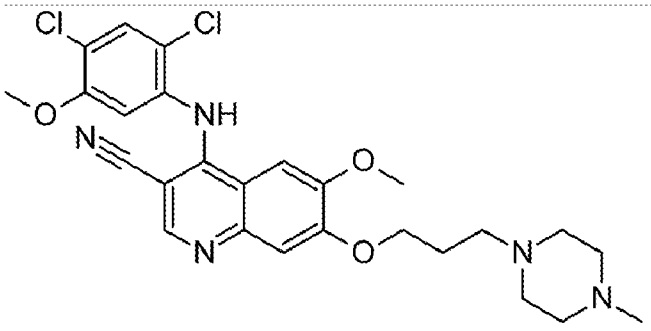
piperazinyl)ethoxy]-5-[(tetrahydro-2H-pyran-4-yl)oxy]-4-quinazolinamine and having the structure of Formula I, as follows:



(I), or a pharmaceutically acceptible salt thereof.

[00106] In another embodiment, the tyrosine kinase FYN inhibitor is a derivative of saracatinib or one or more of the related C-5 substituted anilinoquinazolines or pharmaceutically acceptable salts thereof which are described and shown in Hennequin LF et al., "N-(5-chloro-1,3-benzodioxol-4-yl)-7-[2-(4-methylpiperazin-1-yl)ethoxy]-5-(tetrahydro-2H-pyran-4-yloxy)quinazolin-4-amine, a novel, highly selective, orally available, dual-specific c-Src/Abl kinase inhibitor," J. Med. Chem. (2006), Vol. 49, No. 22, pages 6465-88, which is incorporated herein by reference in its entirety.

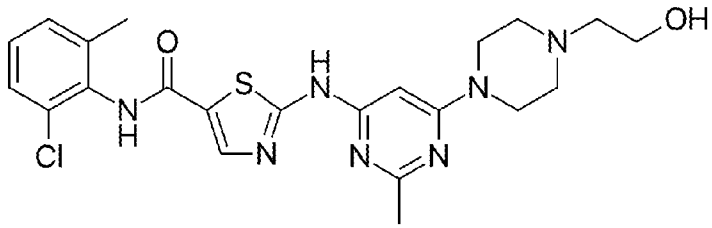
[00107] In another particular embodiment, the tyrosine kinase FYN inhibitor is bosutinib, having the IUPAC name 4-[(2,4-dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[3-(4-methylpiperazin-1-yl)propoxy]quinoline-3-carbonitrile and having the structure of Formula II, as follows:



(II), or a pharmaceutically acceptible salt thereof, and as described in Puttini, M. et al., "In vitro and in vivo activity of SKI-606, a novel Src-Abl inhibitor, against imatinib-resistant Bcr-Abl+ neoplastic cells," Cancer Res. (2006), Vol. 66, No. 23, pages: 11314–22, whch is incorporated herein by reference.

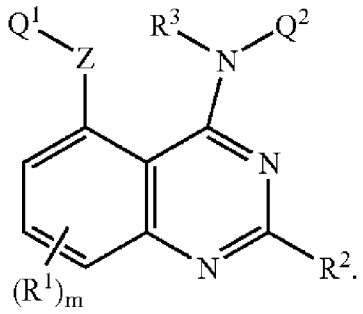
[00108] In still another particular embodiment, the tyrosine kinase FYN inhibitor is dasatinib, having the IUPAC name N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-

1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazole carboxamide monohydrate and having the structure of Formula III, as follows:



(III), or a pharmaceutically acceptible salt thereof, and as described in Puttini, M. et al., "In vitro and in vivo activity of SKI-606, a novel Src-Abl inhibitor, against imatinib-resistant Bcr-Abl+ neoplastic cells," Cancer Res. (2006), Vol. 66, No. 23, pages: 11314–22, whch is incorporated herein by reference.

[00109] In yet another embodiment, the tyrosine kinase FYN inhibitor is a quinazoline derivative of Formula IV,



(IV),

wherein Formular IV and each of Q^1, Z, m, R^1, R^2, R^3, and Q^2 have any of the meanings as defined or claimed in PCT/GB2001/002424 (published as WO/2001/094341, which is incorporated herein by reference), or wherein Formula IV and each of Q^1, Z, m, R^1, R^2, R^3, and Q^2 have any of the meanings as defined or claimed in an equivalent application or patent based on a national stage of PCT/GB2001/002424, including U.S. Patent Nos. 7,049,438 and 7,696,214, or a pharmaceutically acceptable salts thereof. These references are each incorporated herein by reference in their entireties.

[00110] In still another embodiment, the tyrosine kinase FYN inhibitor is the compound 4-(6-chloro-2,3-methylenedioxyanilino)-7-[2-(4-methylpiperazin-1-yl)ethox-y]-5-tetrahydropyran-4-yloxyquinazoline as described and claimed in U.S. 7,696,214, which is incorporated herein by reference. In yet another embodiment, the tyrosine kinase FYN inhibitor is defined based on having a similar gene expression profile as saracatinib based on datasets collated from multiple *in vitro* assays showing down-regulation of FYN-related gene targets, and includes one or more of the following compounds: mitomycin-c; irinotecan;

ochratoxin-a; vincristine; cephaeline; 5- iodotubercidin; clofarabine; apigenin; sarmentogenin; topotecan; rosiglitazone; podophyllotoxin; triamterene; fludarabine; dactinomycin; cytochalasin-b; etoposide; staurosporine; troglitazone; and proscillaridin.

[00111] The amount of each small molecule inhibitor administered and/or present in a pharmaceutical composition as described herein that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host and disease being treated, the particular mode of administration. Preferably, the compositions should be formulated to contain between about 0.05 milligram to about 750 milligrams or more, about 1 milligram to about 600 milligrams, or about 10 milligrams to about 500 milligrams of at least one small molecule described herein, alone or in combination with at least one other agent described herein. For example, about 0.05 mg to about 750 mg, about 100 mg to about 750 mg, about 200 mg to about 750 mg, about 300 mg to about 750 mg, about 400 mg to about 750 mg, about 500 mg to about 750 mg, about 600 mg to about 750 mg, about 0.05 mg to about 650 mg, about 100 mg to about 650 mg, about 200 mg to about 650 mg, about 300 mg to about 650 mg, about 400 mg to about 650 mg, about 500 mg to about 650 mg, about 0.05 mg to about 550 mg, about 100 mg to about 550 mg, about 200 mg to about 550 mg, about 300 mg to about 550 mg, about 400 mg to about 550 mg, about 0.05 mg to about 450 mg, about 100 mg to about 450 mg, about 200 mg to about 450 mg, about 300 mg to about 450 mg, about 0.05 mg to about 350 mg, about 100 mg to about 350 mg, about 200 mg to about 350 mg, about 0.05 mg to about 250 mg, about 100 mg to about 250 mg, or about 0.05 mg to about 150 mg of at least one small molecule described herein, alone or in combination with at least one other agent described herein, is administered to the subject.

[00112] The following methods are suitable for preparing the above compounds. The compounds according to the disclosure may be obtained using methods of synthesis which are known to the one skilled in the art and described in the literature of organic synthesis. General methods for functional groups protection and deprotection steps are described in the available literature in the state of the art, *e.g.*, see Greene, T. W. and Wuts, P. G. M. (eds.): Protective Groups in Organic Synthesis, third edition 1999; John Wiley and Sons, Inc. Preferably, the compounds are obtained analogously to the methods of preparation explained more fully hereinafter, in particular, as described in the experimental section.

[00113] The compounds above, as defined hereinbefore, may contain groups that may be further converted into the salts thereof, for pharmaceutical use particularly into pharmaceutically acceptable salts with inorganic or organic acids and bases. Acids which may be used for this purpose include for example hydrochloric acid, hydrobromic acid,

sulphuric acid, methanesulphonic acid, phosphoric acid, fumaric acid, succinic acid, lactic acid, citric acid, tartaric acid or maleic acid. Corresponding processes are known to the skilled person.

[00114] Moreover, where one or more stereoisomers may exist, the compounds above or intermediates in the synthesis of compounds above may be obtained as mixtures and then resolved into their stereoisomers, *e.g.*, enantiomers and/or diastereomers. Thus, for example, cis/trans mixtures may be resolved into their cis and trans isomers, and racemic compounds may be separated into their enantiomers.

[00115] Thus, for example, the cis/trans mixtures may be resolved by chromatography into the *cis* and *trans* isomers thereof. The compounds of general formula (I) or intermediates in the synthesis of compounds of general formula (I), which occur as racemates may be separated by methods known per se (cf. Allinger N. L. and Eliel E. L. in "Topics in Stereochemistry", Vol. 6, Wiley Interscience, 1971) into their optical antipodes and compounds of general formula (I) or intermediates in the synthesis of compounds of general formula (I) with at least 2 asymmetric carbon atoms may be resolved into their diastereomers on the basis of their physical-chemical differences using methods known per se, *e.g.*, by chromatography and/or fractional crystallization, and, if these compounds are obtained in racemic form, they may subsequently be resolved into the enantiomers as mentioned above.

[00116] The racemates are preferably resolved by column chromatography on chiral phases or by crystallization from an optically active solvent or by reacting with an optically active substance which forms salts or derivatives such as esters or amides with the racemic compound. Salts may be formed with enantiomerically pure acids for basic compounds and with enantiomerically pure bases for acidic compounds. Diastereomeric derivatives are formed with enantiomerically pure auxiliary compounds, *e.g.,* acids, their activated derivatives, or alcohols. Separation of the diastereomeric mixture of salts or derivatives thus obtained may be achieved by taking advantage of their different physico-chemical properties, *e.g.*, differences in solubility; the free antipodes may be released from the pure diastereomeric salts or derivatives by the action of suitable agents. Optically active acids in common use for such a purpose include, for example, the D- and L-forms of tartaric acid, dibenzoyltartaric acid, ditoluoyltartaric acid, malic acid, mandelic acid, camphorsulfonic acid, glutamic acid, aspartic acid, or quinic acid. Optically active alcohols applicable as auxiliary residues may be, for example, (+) or (-)-menthol and optically active acyl groups in amides may be, for example, (+) or (-)-menthyloxycarbonyl.

[00117] The substances according to the disclosure can be isolated and purified in a manner known per se, for example by distilling off the solvent under reduced pressure and recrystallizing the residue obtained from a suitable solvent or subjecting it to one of the customary purification methods, such as, for example, column chromatography on a suitable support material.

Polypeptide FYN Inhibitors

[00118] In any aspect or embodiment described herein, the agent can include at least one (*e.g.,* 1, 2, 3, 4, 5, 6, 7, or more) polypeptide tyrosine kinase FYN inhibitor, such as an anti-FYN antibody.

[00119] Anti-FYN antibodies may bind to or recognize a specific epitope on FYN, i.e., an "FYN epitope" or "FYN antigen epitope," although, knowledge of the specific epitope is not a prerequisite for using anti-FYN antibodies to inhibit FYN.

[00120] The epitopes are most commonly proteins (or portions thereof), short oligopeptides, oligopeptide mimics (i.e., organic compounds that mimic antibody binding properties of FYN), or combinations thereof. The minimum size of a peptide or polypeptide epitope for an antibody is thought to be about four to five amino acids. Peptide or polypeptide epitopes contain for example at least seven amino acids or for example at least nine amino acids or for example between about 15 to about 20 amino acids. In addition, since an antibody can recognize an antigenic peptide or polypeptide in its tertiary form, the amino acids comprising an epitope need not be contiguous, and in some cases, may not even be on the same peptide chain. Epitopes may be determined by various epitope mapping techniques known in the art, such as X-ray crystallography, Hydrogen/Deuterium Exchange Mass Spectrometry (HXMS), site-directed mutagenesis, alanine scanning mutagenesis, and peptide screening methods.

[00121] The generalized structure of antibodies or immunoglobulins is well known to those of skill in the art. These molecules are heterotetrameric glycoproteins, typically of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains and are typically referred to as full length antibodies. Each light chain is covalently linked to a heavy chain by one disulfide bond to form a heterodimer, and the heterotrameric molecule is formed through a covalent disulfide linkage between the two identical heavy chains of the heterodimers. Although the light and heavy chains are linked together by one disulfide bond, the number of disulfide linkages between the two heavy chains varies by immunoglobulin isotype. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at the amino-terminus a variable

domain (V_H), followed by three or four constant domains (C_{H1}, C_{H2}, C_{H3}, and C_{H4}), as well as a hinge region between C_{H1} and C_{H2}. Each light chain has two domains, an amino-terminal variable domain (V_L) and a carboxy-terminal constant domain (C_L). The V_L domain associates non-covalently with the V_H domain, whereas the C_L domain is commonly covalently linked to the C_{H1} domain via a disulfide bond. Particular amino acid residues are believed to form an interface between the light and heavy chain variable domains (Chothia et al., 1985, J. Mol. Biol. 186:651-663). Variable domains are also referred herein as variable regions.

[00122] Certain domains within the variable domains differ extensively between different antibodies, *i.e.*, are "hypervariable." These hypervariable domains contain residues that are directly involved in the binding and specificity of each particular antibody for its specific antigenic determinant. Hypervariability, both in the light chain and the heavy chain variable domains, is concentrated in three segments known as complementarity determining regions (CDRs) or hypervariable loops (HVLs). CDRs are defined by sequence comparison in Kabat et al., 1991, In: Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., whereas HVLs (also referred herein as CDRs) are structurally defined according to the three-dimensional structure of the variable domain, as described by Chothia and Lesk, 1987, J. Mol. Biol. 196: 901-917. These two methods result in slightly different identifications of a CDR. As defined by Kabat, CDR-L1 is positioned at about residues 24-34, CDR-L2, at about residues 50-56, and CDR-L3, at about residues 89-97 in the light chain variable domain; CDR-H1 is positioned at about residues 31-35, CDR-H2 at about residues 50-65, and CDR-H3 at about residues 95-102 in the heavy chain variable domain. The exact residue numbers that encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely determine which residues comprise a particular CDR given the variable region amino acid sequence of the antibody. The CDR1, CDR2, CDR3 of the heavy and light chains therefore define the unique and functional properties specific for a given antibody.

[00123] The three CDRs within each of the heavy and light chains are separated by framework regions (FR), which contain sequences that tend to be less variable. From the amino terminus to the carboxy terminus of the heavy and light chain variable domains, the FRs and CDRs are arranged in the order: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. The largely β-sheet configuration of the FRs brings the CDRs within each of the chains into close proximity to each other as well as to the CDRs from the other chain. The resulting conformation contributes to the antigen binding site (see Kabat et al., 1991, NIH Publ. No.

91-3242, Vol. I, pages 647-669), although not all CDR residues are necessarily directly involved in antigen binding.

[00124] FR residues and Ig constant domains are not directly involved in antigen binding, but contribute to antigen binding and/or mediate antibody effector function. Some FR residues are thought to have a significant effect on antigen binding in at least three ways: by noncovalently binding directly to an epitope, by interacting with one or more CDR residues, and by affecting the interface between the heavy and light chains. The constant domains are not directly involved in antigen binding but mediate various Ig effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC), complement dependent cytotoxicity (CDC) and antibody dependent cellular phagocytosis (ADCP).

[00125] The light chains of vertebrate immunoglobulins are assigned to one of two clearly distinct classes, kappa (κ) and lambda (γ), based on the amino acid sequence of the constant domain. By comparison, the heavy chains of mammalian immunoglobulins are assigned to one of five major classes, according to the sequence of the constant domains: IgA, IgD, IgE, IgG, and IgM. IgG and IgA are further divided into subclasses (isotypes), *e.g.,* IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of the classes of native immunoglobulins are well known.

[00126] The terms, "antibody," "anti-FYN antibody," "humanized anti-FYN antibody," and the like, specifically encompass monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (*e.g.,* bispecific antibodies), and antibody fragments, such as variable domains and other portions of antibodies that exhibit a desired biological activity, *e.g.*, FYN binding. The term "monoclonal antibody" (mAb) refers to an antibody that is highly specific, being directed against a single antigenic determinant, an "epitope." Therefore, the modifier "monoclonal" is indicative of antibodies directed to the identical epitope and is not to be construed as requiring production of the antibody by any particular method. It should be understood that monoclonal antibodies can be made by any technique or methodology known in the art; including *e.g.,* the hybridoma method (Kohler et al., 1975, Nature 256:495), or recombinant DNA methods known in the art (see, *e.g.*, U.S. Pat. No. 4,816,567), or methods of isolation of monoclonal recombinantly produced using phage antibody libraries, using techniques described in Clackson et al., 1991, Nature 352: 624-628, and Marks et al., 1991, J. Mol. Biol. 222: 581-597.

[00127] The term "monomer" refers to a homogenous form of an antibody. For example, for a full-length antibody, monomer means a monomeric antibody having two identical heavy chains and two identical light chains.

[00128] Chimeric antibodies consist of the heavy and light chain variable regions of an antibody from one species (*e.g.*, a non-human mammal such as a mouse) and the heavy and light chain constant regions of another species (*e.g.*, human) antibody and can be obtained by linking the DNA sequences encoding the variable regions of the antibody from the first species (*e.g.*, mouse) to the DNA sequences for the constant regions of the antibody from the second (*e.g.*, human) species and transforming a host with an expression vector containing the linked sequences to allow it to produce a chimeric antibody. Alternatively, the chimeric antibody also could be one in which one or more regions or domains of the heavy and/or light chain is identical with, homologous to, or a variant of the corresponding sequence in a monoclonal antibody from another immunoglobulin class or isotype, or from a consensus or germline sequence. Chimeric antibodies can include fragments of such antibodies, provided that the antibody fragment exhibits the desired biological activity of its parent antibody, for example binding to the same epitope (see, *e.g.*, U.S. Pat. No. 4,816,567; and Morrison et al., 1984, Proc. Natl. Acad. Sci. USA 81: 6851-6855).

[00129] The terms, "antibody fragment," "anti-FYN antibody fragment," "anti-FYN epitope antibody fragment," "humanized anti-FYN antibody fragment," "humanized anti-FYN epitope antibody fragment, and " "variant humanized anti-FYN epitope antibody fragment," refer to a portion of a full length anti-FYN antibody, in which a variable region or a functional capability is retained, for example, specific FYN epitope binding. Examples of antibody fragments include, but are not limited to, a Fab, Fab', F(ab')2, Fd, Fv, scFv and scFv-Fc fragment, a diabody, a linear antibody, a single-chain antibody, a minibody, a diabody formed from antibody fragments, and multispecific antibodies formed from antibody fragments.

[00130] Full length antibodies can be treated with enzymes such as papain or pepsin to generate useful antibody fragments. Papain digestion is used to produces two identical antigen-binding antibody fragments called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment. The Fab fragment also contains the constant domain of the light chain and the CH, domain of the heavy chain. Pepsin treatment yields a F(ab')2 fragment that has two antigen-binding sites and is still capable of cross-linking antigen.

[00131] Fab' fragments differ from Fab fragments by the presence of additional residues including one or more cysteines from the antibody hinge region at the C-terminus of the CH, domain. F(ab')2 antibody fragments are pairs of Fab' fragments linked by cysteine residues in the hinge region. Other chemical couplings of antibody fragments are also known.

[00132] "Fv" fragment contains a complete antigen-recognition and binding site consisting of a dimer of one heavy and one light chain variable domain in tight, non-covalent association. In this configuration, the three CDRs of each variable domain interact to define an antigen-biding site on the surface of the VH-VL dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody.

[00133] A "single-chain Fv" or "scFv" antibody fragment is a single chain Fv variant comprising the VH and VL domains of an antibody where the domains are present in a single polypeptide chain. The single chain Fv is capable of recognizing and binding antigen. The scFv polypeptide may optionally also contain a polypeptide linker positioned between the VH and VL domains in order to facilitate formation of a desired three-dimensional structure for antigen binding by the scFv (see, *e.g.*, Pluckthun, 1994, In The Pharmacology of monoclonal Antibodies, Vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315).

[00134] A "diabody" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy chain variable domain (VH) connected to a light chain variable domain (VL) in the same polypeptide chain (VH-VL or VL-VH). Diabodies are described more fully in, *e.g.*, Holliger et al. (1993) Proc. Natl. Acad. Sci. USA 90: 6444-6448.

[00135] Other recognized antibody fragments include those that comprise a pair of tandem Fd segments (VH-CH1-VH-CH1) to form a pair of antigen binding regions. These "linear antibodies" can be bispecific or monospecific as described in, for example, Zapata et al. 1995, Protein Eng. 8(10):1057-1062.

[00136] A "humanized antibody" or a "humanized antibody fragment" is a specific type of chimeric antibody which includes an immunoglobulin amino acid sequence variant, or fragment thereof, which is capable of binding to a predetermined antigen and which, comprises one or more FRs having substantially the amino acid sequence of a human immunoglobulin and one or more CDRs having substantially the amino acid sequence of a non-human immunoglobulin. This non-human amino acid sequence often referred to as an "import" sequence is typically taken from an "import" antibody domain, particularly a variable domain. In general, a humanized antibody includes at least the CDRs or HVLs of a non-human antibody, inserted between the FRs of a human heavy or light chain variable domain. The present invention contemplates specific humanized antibodies which contain

CDRs derived from mouse monoclonal antibodies or humanized CDRs inserted between the FRs of human germline sequence heavy and light chain variable domains. It will be understood that certain mouse FR residues may be important to the function of the humanized antibodies and therefore certain of the human germline sequence heavy and light chain variable domains residues are modified to be the same as those of the corresponding mouse sequence.

[00137] The polypeptide FYN inhibitors are not limited to anti-FYN antibodies, but also embrace any peptides or polypeptides which bind to the FYN protein and inhibit its activity, or alternatively, which inhibit the expression of the FYN gene.

Inhibitory FYN Nucleic Acids

[00138] In any aspect or embodiment described herein, the agent can include at least one (*e.g.*, 1, 2, 3, 4, 5, 6, 7, or more) tyrosine kinase FYN inhibitory nucleic acids.

[00139] An "inhibitory nucleic acid" is meant to refer to a single or double-stranded RNA, siRNA (short interfering RNA), shRNA (short hairpin RNA), or antisense RNA, or a portion thereof, or a mimetic thereof, that when administered to a cell results in a decrease (*e.g.*, by at least about 10%, at least about 25%, at least about 35%, at least about 50%, at least about 65%, at least about 75%, at least about 90%, about 10% to about 100%, about 10% to about 90%, about 10% to about 75%, about 10% to about 65%, about 10% to about 50%, about 10% to about 25%, about 25% to about 100%, about 25% to about 90%, about 25% to about 75%, about 25% to about 65%, about 25% to about 50%, about 10% to about 25%, about 35% to about 100%, about 35% to about 90%, about 35% to about 75%, about 35% to about 65%, about 50% to about 100%, about 50% to about 90%, about 50% to about 75%, about 65% to about 100%, about 65% to about 90%, about 70% to about 100%, or about 70% to about 90%) in the expression of a target gene (*e.g.*, tyrosine kinase FYN). A nucleic acid inhibitor may comprise or correspond to at least a portion of a target nucleic acid molecule, or an ortholog thereof, or comprises at least a portion of the complementary strand of a target nucleic acid molecule.

[00140] For example, the agent can include at least one (*e.g.*, 1, 2, 3, 4, 5, 6, 7, or more) tyrosine kinase FYN inhibitory nucleic acid that specifically targets tyrosine kinase FYN, thereby inhibiting the expression of tyrosine kinase FYN.

[00141] The inhibitory nucleic acid molecules of the present disclosure are essentially nucleobase oligomers that may be employed as single-stranded or double-stranded nucleic acid molecule to decrease the expression of the target proteins (*e.g.,* tyrosine kinase FYN). In one approach, the inhibitory nucleic acid molecule is a double-stranded RNA used for RNA

interference (RNAi)-mediated knock-down the gene expression of tyrosine kinase FYN, a histone acetyltransferase, or a protein having a bromodomain. In one embodiment, a double-stranded RNA (dsRNA) molecule is made that includes between eight and twenty-five (*e.g.*, 8, 10, 12, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25) consecutive nucleobases of a nucleobase oligomer of the target gene/protein. The dsRNA can be two complementary strands of RNA that have duplexed, or a single RNA strand that has self-duplexed (small hairpin (sh)RNA). Typically, dsRNAs are about 21 or 22 base pairs, but may be shorter or longer (up to about 29 nucleobases) if desired. Double stranded RNA can be made using standard techniques (*e.g.*, chemical synthesis or in vitro transcription). Kits to accomplish this are available, for example, from Ambion (Austin, Tex.) and Epicentre (Madison, Wis.). Methods for expressing dsRNA in mammalian cells are described in Brummelkamp et al. Science 296:550-553, 2002; Paddison et al. Genes & Devel. 16:948-958, 2002. Paul et al. Nature Biotechnol. 20:505-508, 2002; Sui et al. Proc. Natl. Acad. Sci. USA 99:5515-5520, 2002; Yu et al. Proc. Natl. Acad. Sci. USA 99:6047-6052, 2002; Miyagishi et al. Nature Biotechnol. 20:497-500, 2002; and Lee et al. Nature Biotechnol. 20:500-505 2002, each of which is hereby incorporated by reference.

[00142] An inhibitory nucleic acid molecule that "corresponds" to tyrosine kinase FYN comprises at least a fragment of the double-stranded gene, such that each strand of the double-stranded inhibitory nucleic acid molecule is capable of binding to the complementary strand of the target gene (*e.g.*, tyrosine kinase FYN gene). The inhibitory nucleic acid molecule of the present disclosure need not have perfect correspondence to the reference gene sequence. In one embodiment, an siRNA has at least about 85%, 90%, 95%, 96%, 97%, 98%, or even 99% sequence identity with the target nucleic acid. For example, a 19 base pair duplex having 1-2 base pair mismatch is considered useful in the methods of the disclosure. In other embodiments, the nucleobase sequence of the inhibitory nucleic acid molecule exhibits 1, 2, 3, 4, 5 or more mismatches.

[00143] The term "siRNA" refers to a small interfering RNA, which is a double stranded RNA that corresponds to or matches a reference or target gene sequence (*e.g.*, the gene of a tyrosine kinase FYN, a histone acetyltransferase, and/or a protein having a bromodomain). This matching need not be perfect so long as each strand of the siRNA is capable of binding to at least a portion of the target sequence. siRNA can be used to inhibit gene expression, see for example Bass, 2001, Nature, 411, 428 429; Elbashir et al., 2001, Nature, 411, 494 498; and Zamore et al., Cell 101:25-33 (2000).

[00144] The inhibitory nucleic acid molecules of the present disclosure are not limited to siRNAs, but include any nucleic acid molecule sufficient to decrease the expression of a the target nucleic acid molecule or polypeptide (such as, tyrosine kinase FYN).

[00145] An "antisense nucleic acid" is meant to refer to a non-enzymatic nucleic acid molecule that binds to target RNA by means of RNA--RNA or RNA-DNA interactions and alters the activity of the target RNA (for a review, see Stein et al. 1993; Woolf et al., U.S. Pat. No. 5, 849,902). Typically, antisense molecules are complementary to a target sequence along a single contiguous sequence of the antisense molecule. However, in certain embodiments, an antisense molecule can bind to a substrate such that the substrate molecule forms a loop, and/or an antisense molecule can bind such that the antisense molecule forms a loop. Thus, the antisense molecule can be complementary to two (or even more) non-contiguous substrate sequences or two (or even more) non-contiguous sequence portions of an antisense molecule can be complementary to a target sequence or both. For a review of current antisense strategies, see Schmajuk NA et al., 1999; Delihas N et al., 1997; Aboul-Fadl T, 2005.)

[00146] Each of the nucleic acid sequences encoding FYN protein noted above herein may be used, for example, in the discovery and development of therapeutic antisense nucleic acid molecule to decrease the expression of tyrosine kinase FYN.

[00147] The present disclosure further provides catalytic RNA molecules or ribozymes. Such catalytic RNA molecules can be used to inhibit expression of a target nucleic acid molecule (*e.g.*, tyrosine kinase FYN) *in vivo*. The inclusion of ribozyme sequences within an antisense RNA confers RNA-cleaving activity upon the molecule, thereby increasing the activity of the constructs. The design and use of target RNA-specific ribozymes is described in Haseloff et al., Nature 334:585-591 (1988), and U.S. Patent Application Publication No. 2003/0003469 A1, each of which is incorporated herein by reference. In any aspect or embodiment described herein, the catalytic nucleic acid molecule is formed in a hammerhead or hairpin motif. Examples of such hammerhead motifs are described by Rossi et al., Aids Research and Human Retroviruses, 8:183, 1992. Example of hairpin motifs are described by Hampel et al., "RNA Catalyst for Cleaving Specific RNA Sequences," filed Sep. 20, 1989, which is a continuation-in-part of U.S. Ser. No. 07/247,100 filed Sep. 20, 1988, Hampel and Tritz, Biochemistry, 28:4929, 1989, and Hampel et al., Nucleic Acids Research, 18: 299, 1990. These specific motifs are not limiting on the present disclosure and those skilled in the art will recognize that all that is important in an enzymatic nucleic acid molecule of the disclosure is that it has a specific substrate binding site which is complementary to one or

more of the target gene RNA regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart an RNA cleaving activity to the molecule.

[00148] In any aspect or embodiment described herein, the inhibitory nucleic acid molecules of the present disclosure are administered systemically in dosages between about 1 and about 100 mg/kg (*e.g.,* at least about 1 mg/kg, at least about 5 mg/kg, at least about 10 mg/kg, at least about 20 mg/kg, at least about 25 mg/kg, at least about 50 mg/kg, at least about 75 mg/kg, about 1 to about 100 mg/kg, about 1 to about 75 mg/kg, about 1 to about 50 mg/kg, about 1 to about 25 mg/kg, about 25 to about 100 mg/kg, about 25 to about 75 mg/kg, about 25 to about 50 mg/kg, about 50 to about 100 mg/kg, about 50 to about 75 mg/kg, or about 75 to about 100 mg/kg).

Co-Administered Therapies

[00149] In another embodiment, the methods and composition disclosed herein for inhibiting FYN activity and/or expression may be used in combination with another therapy, *e.g.*, an opioid replacement therapy (ORT).

[00150] For example, methadone is one type of ORT provided to patients; however, the success depends on the patient and the overall failure rate is significant. Methadone is a synthetic opioid prescribed for moderate to severe pain. It is also commonly used to treat opiate addictions, especially addiction to heroin. Methadone acts on the same opioid receptors as morphine and heroin to stabilize patients and minimize withdrawal symptoms in the case of an addiction. Methadone is a federally designated Schedule II drug, meaning it has a legitimate legal use but also a high likelihood of its users developing a dependence. Because methadone is used as a way to curb addiction and reduce cravings, it isn't as heavily regulated as other drugs. However, it is still a powerful opiate with potentially addictive qualities. People who start using methadone to overcome their heroin addiction are at a higher risk of abuse because they already have a history of opioid dependency.

[00151] Buprenorphine/naloxone (SUBOXONE®) is another example of ORT that may be administered or co-administered with the FYN inhibitors described herein. The success rate is somewhat higher than methadone but there is a high rate of relapse once the patient discontinues the treatment. Naltrexone can be additional administered to help prevent or control relapses. All of the ORT methods benefit significantly with a concomitant counseling regimen, peer or group support meetings and/or counseling by a mental health professional.

[00152] In addition, the FYN inhibitors described herein can be administered or co-administered with a standard of care treatment that may include group or peer-to-peer support groups as well as professional substance-abuse counseling.

[00153] In other aspects, the inhibitor agents (*e.g.,* small molecule inhibitors, polypeptide inhibitors, or nucleic acid inhibitors) of the disclosure can be combined (either directly in the same composition, or in a separate composition, and where administration is at the same time, or before or after) with a histone acetylation inhibitor.

[00154] In still other aspects, the inhibitor agents (*e.g.*, small molecule inhibitors, polypeptide inhibitors, or nucleic acid inhibitors) of the disclosure can be combined (either directly in the same composition, or in a separate composition, and where administration is at the same time, or before or after) with a bromodomain inhibitor.

[00155] In any aspect or embodiment described herein, the histone acetyltransferase inhibitor can include at least one of anacardic acid or a derivative thereof, C 646 or a derivative thereof, EML 425 or a derivative thereof, PU139 or a derivative thereof, PU141 or a derivative thereof, MB-3 or a derivative thereof, CPTH2 or a derivative thereof, ISOX DUAL or a derivative thereof, L002 or a derivative thereof, Lys-CoA or a derivative thereof, NU 9056 or a derivative thereof, curcumin or a derivative thereof, garcinol or a derivative thereof, or a combination thereof. In addition, the histone acetyltransferase inhibitory agent can be a nucleic acid target comprising at least one of GCN5, p300, PCAF, CBP, TAFII250, or a combination thereof; or a combination thereof.

[00156] In any aspect or embodiment described herein, the bromodomain inhibitor used in combination is at least one of: at least one small molecule bromodomain inhibitor; at least one polypeptide bromodomain inhibitor; at least one bromodomain inhibitory nucleic acid; or a combination thereof. In any aspect or embodiment described herein, the bromodomain inhibitor can include at least one of JQ1 or a derivative thereof, ISOX DUAL or a derivative thereof, I-CBP 112 or a derivative thereof, SCG-CBP30 or a derivative thereof, I-BET151 (GSK1210151A) or a derivative thereof, I-BET762 (GSK525762) or a derivative thereof, OXF BD 02 or a derivative thereof, MS 436 or a derivative thereof, XMD 8-92 or a derivative thereof, PFI 1 or a derivative thereof, PF1 4 or a derivative thereof, PF CBP1 or a derivative thereof, XD 14 or a derivative thereof, bromosporine or a derivative thereof, RVX-208 or a derivative thereof, BMS-986158 or a derivative thereof, OTX-015 or a derivative thereof, ischemin sodium salt or a derivative thereof, TEN-010 or a derivative thereof, CPI203 or a derivative thereof, CPI-0610 or a derivative thereof, PLX-51107 or a derivative thereof, INCB054329 or a derivative thereof, GSK2820151 or a derivative thereof, BAY

1238097 or a derivative thereof, or a combination thereof. The bromodomain inhibitory nucleic acids can include targets of at least one of p300, BRD2, BRD3, BRD4, PCAF, TAFII250, CBP, GCN5, or a combination thereof.

Therapeutic/Pharmaceutical Compositions

[00157] In any aspect or embodiment described herein, the agent may be administered in a pharmaceutical composition comprising an effective amount of at least one agent as described herein, each in an effective amounts, in combination with a pharmaceutically effective amount of a carrier, additive or excipient.

[00158] The agents as described herein may, in accordance with the disclosure, be administered in single or divided doses by the oral, parenteral or topical routes. Administration of the agent or agents may range from continuous (intravenous drip) to several oral administrations per day (for example, Q.I.D.) and may include oral, topical, parenteral, intramuscular, intravenous, sub-cutaneous, transdermal (which may include a penetration enhancement agent), buccal, sublingual and suppository administration, among other routes of administration. Enteric coated oral tablets may also be used to enhance bioavailability of the agents from an oral route of administration. The most effective dosage form will depend upon the pharmacokinetics of the particular agent chosen as well as the severity of disease in the patient. Administration of agent or agents according to the present disclosure as sprays, mists, or aerosols for intra-nasal, intra-tracheal or pulmonary administration may also be used. The present disclosure therefore also is directed to pharmaceutical compositions comprising an effective amount of an agent or agents as described herein, optionally in combination with a pharmaceutically acceptable carrier, additive or excipient. Agents according to the present disclosure may be administered in immediate release, intermediate release or sustained or controlled release forms. Sustained or controlled release forms are preferably administered orally, but also in suppository and transdermal or other topical forms. Intramuscular injections in liposomal form may also be used to control or sustain the release of agent(s) at an injection site.

[00159] The compositions as described herein may be formulated in a conventional manner using one or more pharmaceutically acceptable carriers and may also be administered in controlled-release formulations. Pharmaceutically acceptable carriers that may be used in these pharmaceutical compositions include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as prolamine sulfate,

disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

[00160] The compositions as described herein may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques. Preferably, the compositions are administered orally, intraperitoneally or intravenously.

[00161] Sterile injectable forms of the compositions as described herein may be aqueous or oleaginous suspension. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1, 3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or di-glycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such as Ph. Helv or similar alcohol.

[00162] The pharmaceutical compositions as described herein may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening, flavoring or coloring agents may also be added.

[00163] Alternatively, the pharmaceutical compositions as described herein may be administered in the form of suppositories for rectal administration. These can be prepared by mixing the agent with a suitable non-irritating excipient, which is solid at room temperature

but liquid at rectal temperature and therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols.

[00164] The pharmaceutical compositions as described herein may also be administered topically. Suitable topical formulations are readily prepared for each of these areas or organs. Topical application for the lower intestinal tract can be effected in a rectal suppository formulation (see above) or in a suitable enema formulation. Topically-acceptable transdermal patches may also be used.

[00165] For topical applications, the pharmaceutical compositions may be formulated in a suitable ointment containing the active component suspended or dissolved in one or more carriers. Carriers for topical administration of the agents of this disclosure include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. In certain preferred aspects of the disclosure, the agents may be coated onto a stent which is to be surgically implanted into a patient in order to inhibit or reduce the likelihood of occlusion occurring in the stent in the patient.

[00166] Alternatively, the pharmaceutical compositions can be formulated in a suitable lotion or cream containing the active components suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

[00167] For ophthalmic use, the pharmaceutical compositions may be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions in isotonic, pH adjusted sterile saline, either with our without a preservative such as benzylalkonium chloride. Alternatively, for ophthalmic uses, the pharmaceutical compositions may be formulated in an ointment such as petrolatum.

[00168] The pharmaceutical compositions as described herein may also be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other conventional solubilizing or dispersing agents.

[00169] It should also be understood that a specific dosage and treatment regimen for any particular patient will depend upon a variety of factors, including the activity of the specific agent employed, the age, body weight, general health, sex, diet, time of administration, rate

of excretion, drug combination, and the judgment of the treating physician and the severity of the particular disease or condition being treated.

[00170] These agents of the present disclosure can be administered by any appropriate route, for example, orally, parenterally, intravenously, intradermally, subcutaneously, or topically, including transdermally, in liquid, cream, gel, or solid form, or by aerosol form.

[00171] The concentration of each agent in the drug composition will depend on absorption, distribution, inactivation, and excretion rates of each agent as well as other factors known to those of skill in the art. It is to be noted that dosage values will also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed methods and compositions. The agent or agents may be administered at once, or may be divided into a number of smaller doses to be administered at varying intervals of time.

[00172] Oral compositions will generally include an inert diluent or an edible carrier. They may be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the agent or its derivative can be incorporated with excipients and used in the form of tablets, troches, or capsules. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition.

[00173] The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a dispersing agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring. When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil. In addition, dosage unit forms can contain various other materials which modify the physical form of the dosage unit, for example, coatings of sugar, shellac, or enteric agents.

[00174] The agent(s) or pharmaceutically acceptable salt(s) thereof can be administered as a component of an elixir, suspension, syrup, wafer, chewing gum or the like. A syrup may contain, in addition to the agent or agents, sucrose as a sweetening agent and certain preservatives, dyes and colorings and flavors.

[00175] The agent(s) or pharmaceutically acceptable salts thereof can also be mixed with other active materials that do not impair the desired action, or with materials that supplement the desired action. In certain preferred aspects of the disclosure, one or more (*e.g.,* 1, 2, 3, 4, 5, 6, 7, or more) agents according to the present disclosure are coadministered.

[00176] Solutions or suspensions used for parenteral, intradermal, subcutaneous, or topical application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parental preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[00177] If administered intravenously, preferred carriers are physiological saline or phosphate buffered saline (PBS).

[00178] Transdermal patches have the added advantage of providing controlled delivery of an agent(s) to the body. Such dosage forms can be made by dissolving or dispersing the agent in the proper medium. Absorption enhancers can also be used to increase the flux of the active ingredient across the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the active ingredient(s) (*i.e.*, agent(s)) in a polymer matrix or gel.

[00179] In one embodiment, the agent(s) are prepared with carriers that will protect the agent or agents against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art.

[00180] Liposomal suspensions may also be pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811 (which is incorporated herein by reference in its entirety). For example, liposome formulations may be prepared by dissolving appropriate lipid(s) (such as stearoyl phosphatidyl ethanolamine, stearoyl phosphatidyl choline, arachadoyl phosphatidyl choline, and cholesterol) in an inorganic solvent that is then evaporated, leaving behind a thin film of dried lipid on the surface of the container. An

aqueous solution of the agent or agents described herein are then introduced into the container. The container is then swirled by hand to free lipid material from the sides of the container and to disperse lipid aggregates, thereby forming the liposomal suspension.

Pharmaceutical Kits

[00181] The disclosure also provides in other aspects pharmaceutical tool kits or "kits" for the treatment, prevention, mitigation, and/or amelioration of at least one symptom or behavior of opioid addiction in a subject. In certain embodiments, the pharmaceutical tool kit can be prepared in a non-injectable form (*e.g.,* dissolution of dried powder agent of the disclosure in a liquid, drinkable vehicle) for self-administration by a subject in need.

[00182] In one embodiment, the kit includes a therapeutic or prophylactic composition as described herein. For example, the kit may comprise an effective amount of at least one agent or a composition having an effective amount of at least one agent, wherein the agent or agents is at least one tyrosine kinase FYN inhibitor in unit dosage form. In certain embodiment, the kits may include one or more dosage unit forms of one or more additional therapeutic agents for treating an opioid/opiate addiction (*e.g.,* an opioid replacement therapy, a histone acetylation inhibitor, or a bromodomain inhibitor). In some embodiments, the kit comprises a sterile container which contains a therapeutic or prophylactic composition or agent(s); such containers can be boxes, ampoules, bottles, vials, tubes, bags, pouches, blister-packs, or other suitable container forms known in the art. Such containers can be made of plastic, glass, laminated paper, metal foil, or other materials suitable for holding medicaments.

[00183] If desired, an agent or composition of the disclosure is provided together with instructions for administering it to a subject addicted to opioids or at risk of being addicted to opioids. The instructions will generally include information about the use of the composition or agent(s) for the treatment, prevention, mitigation, and/or amelioration of opioid addiction in a subject. In other embodiments, the instructions include at least one of the following: description of the composition and/or agent(s); dosage schedule and administration for treatment, prevention, mitigation, and/or amelioration of opioid addiction; precautions; warnings; indications; counter-indications; overdosage information; adverse reactions; animal pharmacology; clinical studies; and/or references. The instructions may be printed directly on the container (when present), or as a label applied to the container, or as a separate sheet, pamphlet, card, or folder supplied in or with the container.

[00184] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the therapeutic agents (*e.g.,* the small molecule, polypeptide, and nucleic acid inhibitors of FYN activity and/or

expression disclosed herein), pharmaceutical kits and therapeutic methods of the disclosure, and are not intended to limit the scope of the present disclosure.

EXAMPLES

Introduction.

[00185] As described above, heroin use continues to impose tremendous suffering and financial costs on society in the United States and worldwide. As such, a better understanding of neuronal maladaptations contributing to this disease is imperative to alleviate this burden. Historically, the field has focused on animal models of addiction, and it is surprising how little direct information there is about the addicted human brain. Therefore, the present disclosure utilized a unique collection of post-mortem human brains to study molecular alterations in long-term heroin users compared to matched controls. The study focuses on the striatum due to its essential role in regulating reward, motivation and goal-directed behaviors, impairments of which play a crucial role in SUDs.

[00186] Epigenetic mechanisms are dynamic processes regulating gene expression, which have recently emerged as key contributors to molecular impairments caused by exposure to environmental stimuli including abused substances. The inventors have shown that that chronic heroin use leads to hyperacetylation of specific lysine residues of histone H3 (H3K27ac) at specific genomic locations. These alterations contributed to transcriptional changes related to glutamatergic neurotransmission and played a significant role in mediating addiction-like behaviors such as drug taking and drug seeking.

[00187] Nonetheless, epigenetic regulation is extremely complex and studying a single post-translational histone modification gives limited insight into heroin-related alterations. Rather, transcriptional activity is influenced by a combination of epigenetic marks, and direct assessment of chromatin state might be the most informative measure of gene regulation.

[00188] To this end, the chromatin state was directly studied with the assay for transposase accessible chromatin coupled with high-throughput sequencing (ATAC-seq). Specifically, chromatin accessibility between heroin users and matched controls were compared genome-wide. Such studies are of critical importance for the addiction field as they significantly advance the biological understanding addiction in an unbiased manner and have the potential to discover novel therapeutic targets.

[00189] As a result, the tyrosine kinase FYN was identified as the most differentially accessible region in heroin users. It is demonstrated below that in line with this observation, FYN expression was significantly induced by heroin in both the human and rodent striatum, as well as in primary neurons *in vitro*. FYN is an especially intriguing therapeutic target for

opioid addition as it is localized in the post-synaptic density and is activated by glutamate signaling (Nygaard *et al*., 2014).

[00190] It is demonstrated below that a FYN inhibitor, e.g. saracatinib or a functional derivative thereof that is currently in phase I clinical trials for Alzheimer's disease, is able to significantly attenuate drug taking and drug seeking behavior in heroin self-administering rats. Overall, the findings below demonstrate the ability to utilize a FYN inhibitor (e.g., saracatinib) as a therapeutic for opioid addiction.

Material and Methods.

Human Heroin Abuse Population.

[00191] Human brains from apparent heroin overdose and normal control Caucasian subjects (determined by self-report and ancestral informative marker analysis) without head trauma were collected at autopsy within 24 hours at the Department of Forensic and Insurance Medicine, Semmelweis University, Hungary, as described previously (Drakenberg *et al*., 2006). Cause of death was determined by the forensic pathologist performing the autopsy. Inclusion criteria for the heroin group were documented history of heroin use and/or positive toxicology at the time of death, physical evidence of intravenous (*i.v.*) drug use (needle tracks); while multi-drug users (as determined by history and blood/urine toxicology at autopsy), subjects receiving methadone/buprenorphine treatment, and subjects with comorbid psychiatric diagnoses were excluded. For the control group, inclusion criteria were negative toxicology, no history or physical evidence of opiate or other drug use, and no documented psychiatric disorders. Table 1 provides a summary of the demographic information of the post-mortem human brain populations used for each molecular experiment. Univariate correlations for demographic variables (age, pH, etc.) were explored and significant variables were included in the final model. Ventral and dorsal striatal (putamen) tissue punches were obtained at the level of the nucleus accumbens (FIG. 1).

Table 1: Population Demographics.

	Control	Heroin
Number	n=37	n=48
Age	31.57 ± 1.86	26.19 ± 0.70
Race	Caucasian	Caucasian
Gender	m=30, f=7	m=40, f=8
PMI	< 24 hours	< 24 hours
brain pH	6.69 ± 0.04	6.58 ± 0.03
Ethanol toxicology	n=3	n=8
Blood ethanol	0.23 ± 0.13	0.13 ± 0.10

Data represent mean ± SEM

PMI: post-mortem interval

m: male

f: female

Rodent Heroin Self-Administration.

[00192] Adult male Long-Evans rats were maintained on reversed 12 hour dark/light cycle and underwent jugular vein catheterization. The rats were subsequently food-restricted and trained to self-administer heroin via the jugular catheter. The animals were placed in an operant chamber (29.5cm x 32.5cm x 23.5cm) housed in sound-attenuating boxes (MED Associates Inc., St. Albans, VT) with two levers; depression of one (designated the active lever) resulted in the delivery of 30 μg/kg heroin under a fixed-ratio 1 (FR1) schedule of reinforcement, whereas depression of the other (designated the inactive lever) had no programmed consequences. Each self-administration session was 3 hours.

[00193] Following 7 FR1 sessions, animals underwent 4 additional FR5 sessions and were fed ad libitum for the remainder of the study. For the JQ1 experiments, only rats that differentiated between active and inactive levers and showed an escalation of correct responses under FR5 schedule were selected for study. Two guide cannulae (C317G-SPC, cut 4 mm below pedestal), one into each hemisphere, were inserted into the rostral part of the dorsal striatum (at a 10° angle from midline relative to bregma: AP+1.7mm, ML+3.5mm; DV-2.0mm; FIGs. 2A and 2B).

[00194] After establishing a post-surgery baseline (average of two days), 2 μl of 20 μmol/l JQ1 was injected in awake animals 5 minutes before heroin self-administration testing on two consecutive days. To test for potential protracted effects of JQ1, animals underwent 3 additional heroin self-administration sessions 24 hours, 48 hours, and 96 hours after the last JQ1 delivery. To test JQ1's effect on drug-seeking behavior, the last heroin self-administration was followed by a 96 hours abstinent period. Then animals were injected with JQ1 and underwent a non-reinforced cue-induced drug-seeking reinstatement session. The reinstatement session was 1 hour long. All testing was carried out using a counter-balanced experimental design and conducted at a similar time of the day.

Quantitative Real-Time Polymerase Chain Reaction (qPCR).

[00195] Messenger RNA (mRNA) was extracted from approximately 10 mg of pulverized putamen tissue using the RNAqueous Micro Kit (Ambion®, Foster City, CA, USA) and following the manufacturer's protocol. First-strand cDNA was synthesized using qScript

cDNA SuperMix (Quanta Biosciences, Gaithersburg, MD, USA) and subjected to qPCR analysis using Taqman-based probes. Primers and probes against GRIA1 (Hs00181348_m1), GRM5 (Hs00168275_m1), HOMER1 (Hs00188676_m1), DLG1 (Hs00938204_m1), DLG4 (Hs00176354_m1), NCOA1 (Hs00186661_m1), HDAC5 (Hs00608366_m1), SUV39H1 (Hs00957892_m1) and JMJD1C (Hs00405469_m1) were obtained from Applied Biosystems (Life Technologies, Carlsbad, CA, USA). Eukaryotic 18S rRNA (Applied Biosystems, product # 4319413E) was included in each multiplex PCR as an internal control. Real-time PCR and subsequent analysis were performed with a Roche LightCycler 480 Real-Time PCR system (LightCycler 480 software; Roche, Basel, Switzerland).

[00196] Quantification of target gene expression in all samples, performed in triplicate, was normalized to 18S rRNA by the equation $C_{T(target)} - C_{T\,(18S)} = \Delta C_T$, where C_T is the threshold cycle number. Differences between control and heroin subjects, including individual variation, were calculated by the equation $\Delta C_{T\,(individual\ subject)} - \Delta C_{T\,(mean\ control)} = \Delta\Delta C_T$. Changes in target gene expression (n-fold) in each sample were calculated by $2^{-(\Delta\Delta C_T)}$ from which the means and standard errors of the mean (SEM) were derived.

Fluorescence Assisted Cell Sorting (FACS) of Neuronal and Non-Neuronal Nuclei.

[00197] Fifty (50) mg of frozen post-mortem brain tissue was homogenized in cold lysis buffer (0.32M Sucrose, 5 mM CaCl2, 3 mM Mg(Ace)2, 0.1 mM, EDTA, 10mM Tris-HCl, pH 8, 1 mM DTT, 0.1% Triton X-100) and filtered through a 40µm cell strainer. The flow-through was underlaid with sucrose solution (1.8 M Sucrose, 3 mM Mg(Ace)2, 1 mM DTT, 10 mM Tris-HCl, pH 8) and subjected to ultracentrifugation at 24,000 rpm for 1 hour at 4°C. Pellets were thoroughly resuspended in 500µl DPBS and incubated in BSA (final concentration 0.1%) and anti-NeuN antibody (1:1000, Alexa488 conjugated; Millipore, Burlington MA, USA) under rotation for 1 hour, at 4 °C, in the dark. Prior to FACS sorting, DAPI (Thermo Scientific, Waltham, MA, USA) was added to a final concentration of 1µg/ml. DAPI positive neuronal (NeuN+) and non-neuronal (NeuN-) nuclei were sorted using a FACSAria flow cytometer (BD Biosciences, Franklin, NJ, USA).

Generation of ATAC-seq Libraries.

[00198] ATAC-seq (assay for transposase accessible chromatin) reactions were performed using an established protocol (Buenrostro *et al.*, 2015a) (Illumina Cat #FC-121-1030; Illumina, San Diego, CA, USA) and the resulting libraries amplified using the Nextera index kit (Illumina Cat #FC-121-1011; Illumina, San Diego, CA, USA). Optimal library amplification was determined by visualization with Bioanalyzer High Sensitivity DNA Chips (Agilent Technologies Cat#5067-4626; Agilent Technologies, Santa Clara, CA, USA).

Libraries were amplified for a total of 12–17 cycles and were quantified by Qubit HS DNA kit (Life Technologies, Carlsbad, CA, USA) and by quantitative PCR (KAPA Biosystems Cat#KK4873; Wilmington, MA, USA) prior to sequencing. Finally, ATACseq libraries were sequenced on Hi-Seq2500 (Illumina, San Diego, CA, USA) obtaining 50 bp paired-end reads.

Statistical Analysis.

[00199] Statistical evaluation was carried out using JMP (SAS Institute, Cary, NC, USA). Data were tested for normality and normalized by natural log transformation, if not normally distributed. Univariate statistical analyses were used to study the effect of each independent demographic variable (e.g. age, gender, postmortem interval, brain pH, RIN, years of heroin use, former overdose) and toxicology data (e.g. blood and urine alcohol, morphine, 6-mono-acetyl-morphine, codeine levels) on mRNA expression or protein levels. Variables with a p value of less than 0.1 were included in the final multiple regression model. If there were no such variables, group differences were determined by one-way analysis of variance. Correlational analyses were carried out according to Pearson.

Results.

Genome-wide Analysis of Chromatin Accessibility of the Post-mortem Human Brain Identified Heroin-related Changes in the Dorsal Striatum.

[00200] Heroin-related alterations in chromatin accessibility across the genome were profiled by performing ATAC-seq in the post-mortem putamen of long-term human heroin users and matched controls. Considering the high cellular heterogeneity of the striatum, ATAC-seq on FACS-sorted neuronal and glial cell populations based on the presence or absence of nuclear marker NeuN (feminizing locus on X-3), a nuclear protein specific to neurons in vertebrates (Mullen *et al.*, 1992) were carried out. Using MACS2 peak calling, 106247 accessible regions were observed in neurons and 67750 accessible regions in glia. At FDR 1% and using group (heroin or control), post-mortem interval (PMI) and gender as covariates, 45509 neuron-specific peaks and 47377 glia-specific peaks were identified, suggesting marked differences in chromatin accessibility and epigenetic regulation of gene expression between the two cell populations.

[00201] Interestingly, heroin users had an overall decrease in chromatin accessibility in both cell types. At $p<0.01$ significance level, 712 regions were downregulated and 17 regions were upregulated in neurons, while 1040 regions were downregulated and 72 regions were upregulated in glia. After FDR correction, gene ontology analysis of downregulated (more closed chromatin) neuronal regions (FIG. 3A) showed enrichment for plasma membrane components (p=4.03x10-6), transmembrane receptor activity (p=5.6x10-3),

transcription factor activity (p=5.6x10-3), RNApolII activity (p=1.59x10-3) as well as regulation of nervous system development (p=2.14x10-6), neurogenesis (p=4.61x10-6) and morphogenesis of branching (p=7.59x10-3). More open neuronal chromatin regions (FIG. 3B) were enriched for genes related to synaptic membrane (p=5.74x10-7), neuronal projections (p=3.24x10-4), post-synaptic density (p=1.3x10-3), dendrite (p=2.89x10-2), glutamate receptor activity (p=4.5x10-3), as well as regulation of nervous system development (p=8.81x10-4), modulation of synaptic transmission (p=3.7x10-3) and regulation of synaptic plasticity (p=6.48x10-3). Taken together, the genome wide ATAC-seq data suggest strong neuronal chromatin impairments at genes related to synaptic activity in the striatum, which might contribute to the transcriptional regulation of long term adaptations underlying heroin use disorder.

[00202] To further demonstrate the effects of heroin use on chromatin accessibility, the major factors explaining variability at consensus ATAC peaks in our post-mortem population were identified. As shown in FIG. 4, most of the variance (38.9%) was explained by cell-type. The second-most important factor was disease (heroin or control), explaining 7.5% of the variance in neuronal peaks and 4.9% of the variance in glial peaks. Strikingly, the contribution of disease thus exceeded additional factors such as individual variability (7.4%), gender (3.2%) or post-mortem interval (2.7%). Taken together, these data indicate that long-term heroin use has profound effects on striatal chromatin accessibility in a cell-type specific manner. These changes appear to be due to heroin-induced impairments of epigenetic regulation and contribute to drug-related gene expression alterations in this brain region that plays a crucial role in reward and goal-directed behavior, thus underlying neuronal maladaptations in substance use disorders.

Chronic Heroin Exposure Leads to Increased Accessibility and Expression of SRC Family Tyrosine Kinase FYN.

[00203] Consensus neuronal ATAC peaks were ranked based on the contribution of disease state to variance in peak amplitude (FIG. 4; third plot from left). The analysis identified a peak in the promoter region of FYN, member of the SRC tyrosine kinase family, as the locus most significantly affected by heroin in the genome. This is especially intriguing considering that FYN is a member of the glutamatergic post-synaptic density that has been shown to play a central role in the regulation of drug-seeking behavior and relapse (LaLumiere and Kalivas, 2008; Knackstedt and Kalivas, 2009; Okvist *et al.*, 2011; Bossert *et al.*, 2012; Egervari *et al.*, 2016a). FYN has also been strongly implicated in Alzheimer's disease pathology (Yang *et al.*, 2011; Nygaard *et al.*, 2014; Kaufman *et al.*, 2015) and

inhibitors of this kinase are currently being tested in clinical trials of this neurodegenerative disorder (Nygaard *et al.*, 2015). As such, FYN might contribute to neuronal maladaptations underlying substance use disorders and thus, represents a promising candidate for targeted interventions.

[00204] In order to characterize FYN more directly, chromatin accessibility throughout the FYN gene was investigated. Interestingly, the promoter region of FYN was more accessible in heroin users compared to controls specifically in neurons (FIG. 5A), while no such difference was observed in the glial cell population (FIG. 5B).

[00205] Increased chromatin accessibility in this region suggests enhanced transcriptional activity of FYN, and a trend for increased FYN expression in the RNA-seq data was observed based on homogenate tissue samples. The RNA-seq findings was verified using quantitative RT-PCR, which detected a trend for elevation of FYN mRNA levels in the putamen of human heroin users compared to matched controls (fold change=1.2, p=0.0697, FIG. 6A).

[00206] In addition, FYN mRNA levels tended to positively correlate with years of previous drug use (r=0.37, p=0.1036, FIG. 6C) and showed a significant negative correlation with urine morphine levels at the time of autopsy (r=0.40, p=0.0472, FIG. 6D), demonstrating antagonistic effects of chronic heroin use and acute morphine toxicity on FYN expression. These findings emphasize the dynamic nature of epigenetic and transcriptional dysregulation in relation to the acute and long-term effects of heroin use.

[00207] Since FYN is an important component of the glutamatergic post-synaptic density (PSD), heroin-related alterations of this transcript are in line with impairments of glutamatergic neurotransmission in the striatum, which has long been implicated in the regulation of drug-seeking behavior and relapse (LaLumiere and Kalivas, 2008; Knackstedt and Kalivas, 2009; Bossert et al., 2012; Egervari et al., 2016a). Interestingly, FYN mRNA levels showed positive correlations with several PSD members (GRIA1, GRM5, HOMER1 and DLG4) in heroin users but not in controls (FIGs. 7A, 7B, 7C, and 7C) further emphasizing the important role of FYN in heroin use-related PSD pathology.

[00208] In order to assess the causal relationship between heroin use and FYN pathology, drug-related changes in Fyn expression in animal models of heroin use disorder was determined. Adult male Long-Evans rats were trained to self-administer heroin and were sacrificed 24 hours following the last self-administration session. Consistent with the human findings, *Fyn* mRNA was significantly increased in the dorsal striatum (CPu) of heroin self-administering rats (fold change=1.15, p=0.0269, FIG. 8A) and showed a dose-dependent increase in rat primary striatal neurons in vitro (p=0.0115, FIG. 8B). Intriguingly, *Fyn*

expression was significantly decreased by intra-CPu delivery of the small molecule bromodomain inhibitor JQ1 (FIG. 8C), which inhibits the functional read-out of acetylated histones, suggesting that elevated *Fyn* levels are driven by heroin-induced histone hyperacetylation. In fact, *Fyn* mRNA showed a significant positive correlation with histone H3 acetylation in human heroin users (r=0.63, p=0.0008, Fig. 6B).

Pharmacological inhibition of Fyn reverses addiction behavior in heroin self-administering rats.

[00209] Based on convergent evidence for heroin-related epigenetic and transcriptional alterations in relation to FYN obtained from our human studies and translational animal models, it was hypothesized that this tyrosine kinase could be a promising candidate for targeted therapeutic interventions in heroin users. Further support for this hypothesis comes from the fact that FYN inhibitors are already used and well tolerated in clinical studies of Alzheimer's disease. As such, whether pharmacological inhibition of FYN affects addiction behavior in animal models was examined next.

[00210] In order to test FYN's role in heroin taking and seeking behaviors, adult male Long-Evans rats were trained to self-administer heroin. Upon reaching the maintenance phase, rats were injected either with vehicle (2% DMSO, 30% PEG-300 in ddH2O, i.p.) or FYN inhibitor saracatinib (AZD0530, 5 mg/kg in vehicle, i.p.) 30 minutes prior to self-administration sessions.

[00211] Strikingly, saracatinib treatment resulted in a strong, immediate attenuation of heroin self-administration behavior on all 3 test days (group effect in two-way ANOVA, F1, 29= 6.852, p=0.0139, FIG. 9A). Importantly, no differences were observed in inactive lever pressing and general locomotor activity and there were no group differences in active lever pressing prior to testing (p=0.834) or 72 hours after the last saracatinib injection (p=0.970), emphasizing the specificity of saracatinib on heroin-taking behavior.

[00212] Following the last heroin self-administration session, animals underwent 7 days of abstinence and were then re-exposed to the drug taking environment in a 1 hour long, cue-induced, non-reinforced drug seeking session. Thirty (30) minutes prior to testing, animals received i.p. injections of 5 mg/kg saracatinib or vehicle. Similarly to the active drug self-administration session, saracatinib strongly decreased heroin seeking behavior (group effect in two-way ANOVA, F1,132=7.376, p=0.0075, FIG. 9B). Once again, no difference in inactive lever pressing or general locomotor activity was observed. Taken together, the data indicate that pharmacological inhibition of the FYN tyrosine kinase strongly decreases heroin

taking and seeking behaviors and thus represents a new, targeted therapy of heroin use disorder.

Discussion.

[00213] Using a genome-wide, direct assessment of chromatin state, the tyrosine kinase FYN was identified as the most differentially accessible locus in striatal neurons of human heroin users. The initial ATAC-seq screening was followed up by transcriptional profiling of this specific gene that showed increased mRNA levels in human heroin users, as well as heroin self-administering rats and primary neurons undergoing chronic morphine treatment *in vitro*. In addition, it was demonstrated that the FYN kinase inhibitor saracatinib potently decreased heroin-taking behavior in self-administering rats and inhibited drug-seeking behavior following abstinence. Thus, the findings herein suggest that FYN inhibitors are promising therapeutic tools for heroin use disorder.

[00214] FYN belongs to the SRC family of protein tyrosine kinases (Ingley, 2008), is highly expressed in the human brain (Yagi *et al.*, 1993) and plays an important role in the central nervous system (Ohnishi *et al.*, 2011). Intriguingly, FYN was shown to be localized in the post-synaptic density of glutamatergic synapses and to associate with the NR2B subunit of the NMDA receptor (Trepanier *et al.*, 2012). This interaction is mediated by the scaffolding protein RACK1 (receptor of activated protein C kinase 1) (Yaka *et al.*, 2002; Thornton *et al.*, 2004) and leads to phosphorylation of NR2B (Tezuka *et al.*, 1999; Yaka *et al.*, 2003; Sato *et al.*, 2008). FYN-induced phosphorylation of NR2B in turn enhances NMDA receptor function (Yaka *et al.*, 2002; Yaka *et al.*, 2003; Trepanier *et al.*, 2012) by increased surface expression in post-synaptic membranes (Nakazawa *et al.*, 2001; Dunah *et al.*, 2004; Prybylowski *et al.*, 2005). FYN has been strongly implicated in Alzheimer's disease (AD). It has been shown that binding of A-beta-oligomers to cellular prion protein triggers mGluR5-dependent signaling, leading to the activation of FYN (Nygaard *et al.*, 2014). Importantly, Tau, the hyperphosphorylation of which plays a central role in AD neuropathology, is a direct substrate of FYN and can be phosphorylated by this kinase on its tyrosine-18 residue (Lee *et al.*, 2004). These events lead to synaptic malfunction and loss (Nygaard *et al.*, 2014), which strongly contribute to AD-related neurodegeneration and dementia.

[00215] Intriguingly, the inventors have previously demonstrated that heroin induces hyperphosphorylation of Tau in specific areas of the human brain (Kovacs *et al.*, 2015). Using immunohistochemistry, phosphorylated Tau (p-Tau) was observed in more anatomical regions and to a larger extent in the central nervous system of heroin users compared to

matched controls. While p-Tau levels correlated with age in both groups in certain predilection areas (*e.g.* entorhinal cortex, frontal cortex), this relationship was strongly exacerbated by heroin, suggesting accelerated AD-related changes in drug users. The fusiform cortex also exhibited marked p-Tau pathology, which is intriguing considering this area has recently been identified as part of a brain network related to impulsivity in heroin users (Xie *et al.*, 2011). Considering FYN's role in regulating p-Tau pathology in AD (Nygaard *et al.*, 2014), it is feasible that heroin-induced changes of FYN expression and activity underlie the observed Tau hyperphosphorylation, which might contribute to several addiction-related behaviors ranging from impulsivity to impaired inhibitory control.

[00216] Currently, it is unknown whether FYN activity contributes to heroin-related Tau phosphorylation. In future experiments, we plan to assess the effects of heroin exposure on p-Tau levels in the presence or absence of saracatinib in our *in vitro* and *in vivo* models. Of note, while our current studies focused on the striatum, Tau phosphorylation was mostly localized to the cortex in human drug users. As such, brain region specificity of FYN and Tau impairments as well as the involvement of additional FYN targets will be important to address.

[00217] FYN is composed of a regulatory and a catalytic domain and enhancement of its activity is mediated by phosphorylation of tyrosine residue 420 (Engen *et al.*, 2008). Conversely, dephosphorylation of tyrosine-420 inhibits FYN activity (Engen *et al.*, 2008). In the striatum, this dephosphorylation is maintained by striatal-enriched protein tyrosine phosphatase 61 (STEP-61), a brain specific tyrosine phosphatase highly expressed in this region (Lombroso *et al.*, 1991; Nguyen *et al.*, 2002). Prior to drug exposure, STEP-61 is localized to the post-synaptic density and dephosphorylates FYN as well as NR2B, reducing the spontaneous activity of NMDA receptors (Pelkey *et al.*, 2002). Intriguingly, Darcq and colleagues showed that ethanol exposure leads to protein kinase A-mediated phosphorylation of STEP-61 in rodent striatum (Darcq *et al.*, 2014). This resulted in increased FYN and NR2B phosphorylation and activity and was maintained even after prolonged withdrawal. Moreover, specific knock-down of STEP-61 in the striatum enhanced ethanol-induced FYN activation and increased ethanol intake (Darcq *et al.*, 2014). It is currently unknown how heroin leads to enhanced FYN expression and activity.

[00218] The present data suggest that histone hyperacetylation plays a role, as *Fyn* mRNA levels were correlated to pan-acetylated histone H3 in post-mortem human subjects and were decreased by JQ1 bromodomain inhibitor in our rat model.

[00219] In future studies, histone acetylation directly at the FYN promoter will be assessed. In addition, complex formation with STEP-61, RACK1 and NR2B and potential heroin-induced disruption thereof will be assessed using immunoprecipitation experiments.

[00220] Of note, in the above-mentioned ethanol studies, FYN-dependent glutamatergic facilitation was restricted to the dorsomedial part of the striatum (Wang *et al.*, 2010a; Wang *et al.*, 2011), suggesting that important regional differences might exist within this brain structure with respect to FYN pathology. This distribution relates to the idea of a gradual transition from limbic to motor functions along the ventromedial to dorsolateral axis of the ventral and dorsal striatum, in line with the lack of clear anatomical and functional demarcation between striatal sub-regions (Haber *et al.*, 2000). Nonetheless, prominent alterations of chromatin accessibility and gene expression of FYN were observed in our post-mortem human studies that focused on a more dorsal part of the putamen (FIG. 1).

[00221] Potential differences in the exact nature and localization of impairments that might be due to differences in species, experimental paradigm or drug need to be further explored. Nonetheless, the transcriptional changes observed in the homogenate samples were in line with enhanced neuronal chromatin accessibility as found increased *Fyn* mRNA levels in human, rat and *in vitro* models. The significant functional contribution of FYN to heroin abuse was causally demonstrated in the translational rodent model. Systemic administration of the FYN inhibitor saracatinib was found to potently decreased heroin self-administration as well as drug-seeking behavior following one week of forced abstinence.

[00222] Specific FYN inhibitors are available and currently being tested in the clinic for Alzheimer's disease. While their efficacy remains to be determined, most drugs targeting FYN appear to be well tolerated and are therefore excellent candidates for clinical studies focusing on SUDs.

INCORPORATION BY REFERENCE

[00223] The contents of all references, patents, pending patent applications and published patents, cited throughout this application are hereby expressly incorporated by reference.

EQUIVALENTS

[00224] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the disclosure described herein. Such equivalents are intended to be encompassed by the following claims. It is understood that the detailed examples and embodiments described herein are given by way of example for illustrative purposes only, and are in no way considered to be limiting to the disclosure. Various modifications or changes in light thereof will be suggested to persons

skilled in the art and are included within the spirit and purview of this application and are considered within the scope of the appended claims. For example, the relative quantities of the ingredients may be varied to optimize the desired effects, additional ingredients may be added, and/or similar ingredients may be substituted for one or more of the ingredients described. Additional advantageous features and functionalities associated with the systems, methods, and processes of the present disclosure will be apparent from the appended claims. Moreover, those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the disclosure described herein. Such equivalents are intended to be encompassed by the following claims.

REFERENCES (Each of which are incorporated by reference)

[00225] A, Fagergren P, Whittard J, Garcia-Osta A, Drakenberg K, Horvath MC, Schmidt CJ, Keller E, Bannon MJ, Hurd YL (2011) Dysregulated postsynaptic density and endocytic zone in the amygdala of human heroin and cocaine abusers. Biological psychiatry 69:245-252.

[00226] Oncken C, Arias AJ, Feinn R, Litt M, Covault J, Sofuoglu M, Kranzler HR (2014) Topiramate for smoking cessation: a randomized, placebo-controlled pilot study. Nicotine Tob Res 16:288-296.

[00227] Orphanides G, LeRoy G, Chang CH, Luse DS, Reinberg D (1998) FACT, a factor that facilitates transcript elongation through nucleosomes. Cell 92:105-116. Ortin A, Lake AM, Kleinman M, Gould MS (2012) Sensation seeking as risk factor for suicidal ideation and suicide attempts in adolescence. J Affect Disord 143:214-222.

[00228] Padmanabhan B, Mathur S, Manjula R, Tripathi S (2016) Bromodomain and extra-terminal (BET) family proteins: New therapeutic targets in major diseases. J Biosci 41:295-311.

[00229] Pankevich DE, Altevogt BM, Dunlop J, Gage FH, Hyman SE (2014) Improving and accelerating drug development for nervous system disorders. Neuron 84:546-553.

[00230] Parker CC, Chen H, Flagel SB, Geurts AM, Richards JB, Robinson TE, Solberg Woods LC, Palmer AA (2014) Rats are the smart choice: Rationale for a renewed focus on rats in behavioral genetics. Neuropharmacology 76 Pt B:250-258.

[00231] Pascual M, Do Couto BR, Alfonso-Loeches S, Aguilar MA, Rodriguez-Arias M, Guerri C (2012) Changes in histone acetylation in the prefrontal cortex of ethanol-exposed adolescent rats are associated with ethanol-induced place conditioning. Neuropharmacology 62:2309-2319.

[00232] Pasini D, Malatesta M, Jung HR, Walfridsson J, Willer A, Olsson L, Skotte J, Wutz A, Porse B, Jensen ON, Helin K (2010) Characterization of an antagonistic switch between histone H3 lysine 27 methylation and acetylation in the transcriptional regulation of Polycomb group target genes. Nucleic Acids Res 38:4958-4969.

[00233] Pawlak CR, Ho YJ, Schwarting RK (2008) Animal models of human psychopathology based on individual differences in novelty-seeking and anxiety. Neurosci Biobehav Rev 32:1544-1568.

[00234] Peavy KM, Banta-Green CJ, Kingston S, Hanrahan M, Merrill JO, Coffin PO (2012) "Hooked on" prescription-type opiates prior to using heroin: results from a survey of syringe exchange clients. J Psychoactive Drugs 44:259-265.

[00235] Pelkey KA, Askalan R, Paul S, Kalia LV, Nguyen TH, Pitcher GM, Salter MW, Lombroso PJ (2002) Tyrosine phosphatase STEP is a tonic brake on induction of long-term potentiation. Neuron 34:127-138.

[00236] Pfeiffer A, Brantl V, Herz A, Emrich HM (1986) Psychotomimesis mediated by kappa opiate receptors. Science 233:774-776.

[00237] Piazza PV, Deminiere JM, Le Moal M, Simon H (1989) Factors that predict individual vulnerability to amphetamine self-administration. Science 245:1511-1513.

[00238] Pierce RC, Meil WM, Kalivas PW (1997) The NMDA antagonist, dizocilpine, enhances cocaine reinforcement without influencing mesoaccumbens dopamine transmission. Psychopharmacology 133:188-195.

[00239] Pietrzykowski AZ, Friesen RM, Martin GE, Puig SI, Nowak CL, Wynne PM, Siegelmann HT, Treistman SN (2008) Posttranscriptional regulation of BK channel splice variant stability by miR-9 underlies neuroadaptation to alcohol. Neuron 59:274-287.

[00240] Pistis M, Muntoni AL, Pillolla G, Gessa GL (2002) Cannabinoids inhibit excitatory inputs to neurons in the shell of the nucleus accumbens: an in vivo electrophysiological study. The European journal of neuroscience 15:1795-1802.

[00241] Pollini RA, Banta-Green CJ, Cuevas-Mota J, Metzner M, Teshale E, Garfein RS (2011) Problematic use of prescription-type opioids prior to heroin use among young heroin injectors. Subst Abuse Rehabil 2:173-180.

[00242] Pontieri FE, Tanda G, Di Chiara G (1995) Intravenous cocaine, morphine, and amphetamine preferentially increase extracellular dopamine in the "shell" as compared with the "core" of the rat nucleus accumbens. Proceedings of the National Academy of Sciences of the United States of America 92:12304-12308.

[00243] Prybylowski K, Chang K, Sans N, Kan L, Vicini S, Wenthold RJ (2005) The synaptic localization of NR2B-containing NMDA receptors is controlled by interactions with PDZ proteins and AP-2. Neuron 47:845-857.

[00244] Pulvirenti L, Balducci C, Koob GF (1997) Dextromethorphan reduces intravenous cocaine self-administration in the rat. Eur J Pharmacol 321:279-283.

[00245] Ray R, Doyle GA, Crowley JJ, Buono RJ, Oslin DW, Patkar AA, Mannelli P, DeMaria PA, Jr., O'Brien CP, Berrettini WH (2005) A functional prodynorphin promoter polymorphism and opioid dependence. Psychiatr Genet 15:295-298.

[00246] Reid MS, Palamar J, Raghavan S, Flammino F (2007) Effects of topiramate on cue-induced cigarette craving and the response to a smoked cigarette in briefly abstinent smokers. Psychopharmacology 192:147-158.

[00247] Ren Y, Whittard J, Higuera-Matas A, Morris CV, Hurd YL (2009) Cannabidiol, a nonpsychotropic component of cannabis, inhibits cue-induced heroin seeking and normalizes discrete mesolimbic neuronal disturbances. The Journal of neuroscience: the official journal of the Society for Neuroscience 29:14764-14769.

[00248] Renthal W, Nestler EJ (2008) Epigenetic mechanisms in drug addiction. Trends Mol Med 14:341-350.

[00249] Renthal W, Nestler EJ (2009) Chromatin regulation in drug addiction and depression. Dialogues Clin Neurosci 11:257-268.

[00250] Renthal W, Carle TL, Maze I, Covington HE, 3rd, Truong HT, Alibhai I, Kumar A, Montgomery RL, Olson EN, Nestler EJ (2008) Delta FosB mediates epigenetic desensitization of the c-fos gene after chronic amphetamine exposure. The Journal of neuroscience : the official journal of the Society for Neuroscience 28:7344-7349.

[00251] Renthal W, Maze I, Krishnan V, Covington HE, 3rd, Xiao G, Kumar A, Russo SJ, Graham A, Tsankova N, Kippin TE, Kerstetter KA, Neve RL, Haggarty SJ, McKinsey TA, Bassel-Duby R, Olson EN, Nestler EJ (2007) Histone deacetylase 5 epigenetically controls behavioral adaptations to chronic emotional stimuli. Neuron 56:517-529.

[00252] Renthal W, Kumar A, Xiao G, Wilkinson M, Covington HE, 3rd, Maze I, Sikder D, Robison AJ, LaPlant Q, Dietz DM, Russo SJ, Vialou V, Chakravarty S, Kodadek TJ, Stack A, Kabbaj M, Nestler EJ (2009) Genome-wide analysis of chromatin regulation by cocaine reveals a role for sirtuins. Neuron 62:335-348.

[00253] Ritchie T, Noble EP (2003) Association of seven polymorphisms of the D2 dopamine receptor gene with brain receptor-binding characteristics. Neurochem Res 28:73-82.

[00254] Robison AJ, Nestler EJ (2011) Transcriptional and epigenetic mechanisms of addiction. Nat Rev Neurosci 12:623-637.

[00255] Robison AJ, Vialou V, Mazei-Robison M, Feng J, Kourrich S, Collins M, Wee S, Koob G, Turecki G, Neve R, Thomas M, Nestler EJ (2013) Behavioral and structural responses to chronic cocaine require a feedforward loop involving DeltaFosB and calcium/calmodulin-dependent protein kinase II in the nucleus accumbens shell. The Journal of neuroscience: the official journal of the Society for Neuroscience 33:4295-4307.

[00256] Rodriguez-Munoz M, Sanchez-Blazquez P, Vicente-Sanchez A, Berrocoso E, Garzon J (2012) The mu-opioid receptor and the NMDA receptor associate in PAG neurons: implications in pain control. Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology 37:338-349.

[00257] Rogge GA, Wood MA (2013) The role of histone acetylation in cocaine-induced neural plasticity and behavior. Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology 38:94-110.

[00258] Rosenfeld WE (1997) Topiramate: a review of preclinical, pharmacokinetic, and clinical data. Clin Ther 19:1294-1308.

[00259] Russell SE, Puttick DJ, Sawyer AM, Potter DN, Mague S, Carlezon WA, Jr., Chartoff EH (2016) Nucleus Accumbens AMPA Receptors Are Necessary for Morphine-Withdrawal-Induced Negative-Affective States in Rats. The Journal of neuroscience : the official journal of the Society for Neuroscience 36:5748-5762.

[00260] Russo SJ, Dietz DM, Dumitriu D, Morrison JH, Malenka RC, Nestler EJ (2010) The addicted synapse: mechanisms of synaptic and structural plasticity in nucleus accumbens. Trends Neurosci 33:267-276.

[00261] Sadakierska-Chudy A, Filip M (2015) A comprehensive view of the epigenetic landscape. Part II: Histone post-translational modification, nucleosome level, and chromatin regulation by ncRNAs. Neurotox Res 27:172-197.

[00262] Saify K, Saadat I, Saadat M (2014) Association between VNTR polymorphism in promoter region of prodynorphin (PDYN) gene and heroin dependence. Psychiatry Res 219:690-692.

[00263] Sakharkar AJ, Zhang H, Tang L, Shi G, Pandey SC (2012) Histone deacetylases (HDAC)-induced histone modifications in the amygdala: a role in rapid tolerance to the anxiolytic effects of ethanol. Alcohol Clin Exp Res 36:61-71.

[00264] Sanchis-Segura C, Borchardt T, Vengeliene V, Zghoul T, Bachteler D, Gass P, Sprengel R, Spanagel R (2006) Involvement of the AMPA receptor GluR-C subunit in alcoholseeking behavior and relapse. The Journal of neuroscience : the official journal of the Society for Neuroscience 26:1231-1238.

[00265] Sartor GC, St Laurent G, 3rd, Wahlestedt C (2012) The Emerging Role of Non-Coding RNAs in Drug Addiction. Front Genet 3:106.

[00266] Sartor GC, Powell SK, Brothers SP, Wahlestedt C (2015) Epigenetic Readers of Lysine Acetylation Regulate Cocaine-Induced Plasticity. The Journal of Neuroscience: the official journal of the Society for Neuroscience 35:15062-15072.

[00267] Sato Y, Tao YX, Su Q, Johns RA (2008) Post-synaptic density-93 mediates tyrosinephosphorylation of the N-methyl-D-aspartate receptors. Neuroscience 153:700-708.

[00268] Schaefer A, Im HI, Veno MT, Fowler CD, Min A, Intrator A, Kjems J, Kenny PJ, O'Carroll D, Greengard P (2010) Argonaute 2 in dopamine 2 receptor-expressing neurons regulates cocaine addiction. J Exp Med 207:1843-1851.

[00269] Schneider MF, Bailey JE, Cicero TJ, Dart RC, Inciardi JA, Parrino M, Munoz A (2009) Integrating nine prescription opioid analgesics and/or four signal detection systems to summarize statewide prescription drug abuse in the United States in 2007. Pharmacoepidemiol Drug Saf 18:778-790.

[00270] Schroeder FA, Penta KL, Matevossian A, Jones SR, Konradi C, Tapper AR, Akbarian S (2008) Drug-induced activation of dopamine D(1) receptor signaling and inhibition of class I/II histone deacetylase induce chromatin remodeling in reward circuitry and modulate cocaine-related behaviors. Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology 33:2981-2992.

[00271] Schuckit MA (1980) Self-rating of alcohol intoxication by young men with and without family histories of alcoholism. J Stud Alcohol 41:242-249.

[00272] Scofield MD, Heinsbroek JA, Gipson CD, Kupchik YM, Spencer S, Smith AC, Roberts-Wolfe D, Kalivas PW (2016) The Nucleus Accumbens: Mechanisms of Addiction across Drug Classes Reflect the Importance of Glutamate Homeostasis. Pharmacol Rev 68:816-871.

[00273] See RE (2005) Neural substrates of cocaine-cue associations that trigger relapse. Eur J Pharmacol 526:140-146.

[00274] Shan H, Zhang Y, Lu Y, Zhang Y, Pan Z, Cai B, Wang N, Li X, Feng T, Hong Y, Yang B (2009) Downregulation of miR-133 and miR-590 contributes to nicotine-induced atrial remodelling in canines. Cardiovasc Res 83:465-472.

[00275] Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, Hergueta T, Baker R, Dunbar GC (1998) The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry 59 Suppl 20:22-33;quiz 34-57.

[00276] Shelton KL, Balster RL (1997) Effects of gamma-aminobutyric acid agonists and N-methyl-D-aspartate antagonists on a multiple schedule of ethanol and saccharin selfadministration in rats. The Journal of pharmacology and experimental therapeutics 280:1250-1260.

[00277] Shen H, Moussawi K, Zhou W, Toda S, Kalivas PW (2011) Heroin relapse requires long-term potentiation-like plasticity mediated by NMDA2b-containing receptors.

Proceedings of the National Academy of Sciences of the United States of America 108:19407-19412.

[00278] Shen HW, Scofield MD, Boger H, Hensley M, Kalivas PW (2014) Synaptic glutamate spillover due to impaired glutamate uptake mediates heroin relapse. The Journal of neuroscience : the official journal of the Society for Neuroscience 34:5649-5657.

[00279] Shen HY, Kalda A, Yu L, Ferrara J, Zhu J, Chen JF (2008) Additive effects of histone deacetylase inhibitors and amphetamine on histone H4 acetylation, cAMP responsive element binding protein phosphorylation and DeltaFosB expression in the striatum and locomotor sensitization in mice. Neuroscience 157:644-655.

[00280] Sheng J, Lv Z, Wang L, Zhou Y, Hui B (2011) Histone H3 phosphoacetylation is critical for heroin-induced place preference. Neuroreport 22:575-580.

[00281] Shi Y, Ethell IM (2006) Integrins control dendritic spine plasticity in hippocampal neurons through NMDA receptor and Ca2+/calmodulin-dependent protein kinase IImediated actin reorganization. The Journal of neuroscience: the official journal of the Society for Neuroscience 26:1813-1822.

[00282] Shin VY, Jin H, Ng EK, Cheng AS, Chong WW, Wong CY, Leung WK, Sung JJ, Chu KM (2011) NF-kappaB targets miR-16 and miR-21 in gastric cancer: involvement of prostaglandin E receptors. Carcinogenesis 32:240-245.

[00283] Shippenberg TS, Bals-Kubik R, Herz A (1987) Motivational properties of opioids: evidence that an activation of delta-receptors mediates reinforcement processes. Brain Res 436:234-239.

[00284] Sillivan SE, Whittard JD, Jacobs MM, Ren Y, Mazloom AR, Caputi FF, Horvath M, Keller E, Ma'ayan A, Pan YX, Chiang LW, Hurd YL (2013) ELK1 transcription factor linked to dysregulated striatal mu opioid receptor signaling network and OPRM1 polymorphism in human heroin abusers. Biological psychiatry 74:511-519.

[00285] Skoubis PD, Lam HA, Shoblock J, Narayanan S, Maidment NT (2005) Endogenous enkephalins, not endorphins, modulate basal hedonic state in mice. The European journal of neuroscience 21:1379-1384.

[00286] Slingerland M, Guchelaar HJ, Gelderblom H (2014) Histone deacetylase inhibitors: an overview of the clinical studies in solid tumors. Anticancer Drugs 25:140-149.

[00287] Smith AC, Kupchik YM, Scofield MD, Gipson CD, Wiggins A, Thomas CA, Kalivas PW (2014) Synaptic plasticity mediating cocaine relapse requires matrix metalloproteinases. Nature neuroscience 17:1655-1657.

[00288] Solinas M, Ferre S, You ZB, Karcz-Kubicha M, Popoli P, Goldberg SR (2002) Caffeine induces dopamine and glutamate release in the shell of the nucleus accumbens. The Journal of neuroscience : the official journal of the Society for Neuroscience 22:6321-6324.

[00289] Soule J, Messaoudi E, Bramham CR (2006) Brain-derived neurotrophic factor and control of synaptic consolidation in the adult brain. Biochem Soc Trans 34:600-604.

[00290] Stathis A, Zucca E, Bekradda M, Gomez-Roca C, Delord JP, de La Motte Rouge T, Uro-Coste E, de Braud F, Pelosi G, French CA (2016) Clinical Response of Carcinomas Harboring the BRD4-NUT Oncoprotein to the Targeted Bromodomain Inhibitor OTX015/MK-8628. Cancer Discov 6:492-500.

[00291] Steiner H, Gerfen CR (1998) Role of dynorphin and enkephalin in the regulation of striatal output pathways and behavior. Exp Brain Res 123:60-76.

[00292] Stephens DN, Brown G (1999) Disruption of operant oral self-administration of ethanol, sucrose, and saccharin by the AMPA/kainate antagonist, NBQX, but not the AMPA antagonist, GYKI 52466. Alcohol Clin Exp Res 23:1914-1920.

[00293] Stice E, Yokum S, Bohon C, Marti N, Smolen A (2010) Reward circuitry responsivity to food predicts future increases in body mass: moderating effects of DRD2 and DRD4. Neuroimage 50:1618-1625.

[00294] Stoltenberg SF, Mudd SA, Blow FC, Hill EM (1998) Evaluating measures of family history of alcoholism: density versus dichotomy. Addiction 93:1511-1520.

[00295] Strahl BD, Allis CD (2000) The language of covalent histone modifications. Nature 403:41-45.

[00296] Surmeier DJ, Ding J, Day M, Wang Z, Shen W (2007) D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. Trends Neurosci 30:228-235.

[00297] Svenningsson P, Nairn AC, Greengard P (2005) DARPP-32 mediates the actions of multiple drugs of abuse. AAPS J 7:E353-360.

[00298] Swanson CJ, Baker DA, Carson D, Worley PF, Kalivas PW (2001) Repeated cocaine administration attenuates group I metabotropic glutamate receptor-mediated glutamate release and behavioral activation: a potential role for Homer. The Journal of neuroscience : the official journal of the Society for Neuroscience 21:9043-9052.

[00299] Taniguchi M, Carreira MB, Smith LN, Zirlin BC, Neve RL, Cowan CW (2012) Histone deacetylase 5 limits cocaine reward through cAMP-induced nuclear import. Neuron 73:108-120.

[00300] Tapper AR, McKinney SL, Nashmi R, Schwarz J, Deshpande P, Labarca C, Whiteaker P, Marks MJ, Collins AC, Lester HA (2004) Nicotine activation of alpha4* receptors: sufficient for reward, tolerance, and sensitization. Science 306:1029-1032.

[00301] Taqi MM, Bazov I, Watanabe H, Sheedy D, Harper C, Alkass K, Druid H, Wentzel P, Nyberg F, Yakovleva T, Bakalkin G (2011) Prodynorphin CpG-SNPs associated with alcohol dependence: elevated methylation in the brain of human alcoholics. Addiction biology 16:499-509.

[00302] Tarter RE, Kirisci L, Mezzich A, Cornelius JR, Pajer K, Vanyukov M, Gardner W, Blackson T, Clark D (2003) Neurobehavioral disinhibition in childhood predicts early age at onset of substance use disorder. The American journal of psychiatry 160:1078-1085.

[00303] Taunton J, Hassig CA, Schreiber SL (1996) A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. Science 272:408-411.

[00304] Tezuka T, Umemori H, Akiyama T, Nakanishi S, Yamamoto T (1999) PSD-95 promotes Fynmediated tyrosine phosphorylation of the N-methyl-D-aspartate receptor subunit NR2A. Proceedings of the National Academy of Sciences of the United States of America 96:435-440.

[00305] Thornton C, Tang KC, Phamluong K, Luong K, Vagts A, Nikanjam D, Yaka R, Ron D (2004) Spatial and temporal regulation of RACK1 function and N-methyl-D-aspartate receptor activity through WD40 motif-mediated dimerization. J Biol Chem 279:31357-31364.

[00306] Tie F, Banerjee R, Saiakhova AR, Howard B, Monteith KE, Scacheri PC, Cosgrove MS, Harte PJ (2014) Trithorax monomethylates histone H3K4 and interacts directly with CBP to promote H3K27 acetylation and antagonize Polycomb silencing. Development 141:1129-1139.

[00307] Tomasiewicz HC, Jacobs MM, Wilkinson MB, Wilson SP, Nestler EJ, Hurd YL (2012) Proenkephalin mediates the enduring effects of adolescent cannabis exposure associated with adult opiate vulnerability. Biological psychiatry 72:803-810.

[00308] Treadway MT, Buckholtz JW, Cowan RL, Woodward ND, Li R, Ansari MS, Baldwin RM, Schwartzman AN, Kessler RM, Zald DH (2012) Dopaminergic mechanisms of individual differences in human effort-based decision-making. The Journal of neuroscience: the official journal of the Society for Neuroscience 32:6170-6176.

[00309] Trepanier CH, Jackson MF, MacDonald JF (2012) Regulation of NMDA receptors by the tyrosine kinase Fyn. FEBS J 279:12-19.

[00310] Tronick E, Hunter RG (2016) Waddington, Dynamic Systems, and Epigenetics. Frontiers in behavioral neuroscience 10:107.

[00311] Tsai WW, Wang Z, Yiu TT, Akdemir KC, Xia W, Winter S, Tsai CY, Shi X, Schwarzer D, Plunkett W, Aronow B, Gozani O, Fischle W, Hung MC, Patel DJ, Barton MC (2010) TRIM24 links a non-canonical histone signature to breast cancer. Nature 468:927-932.

[00312] Van Etten ML, Anthony JC (1999) Comparative epidemiology of initial drug opportunities and transitions to first use: marijuana, cocaine, hallucinogens and heroin. Drug Alcohol Depend 54:117-125.

[00313] Vavouri T, Lehner B (2012) Human genes with CpG island promoters have a distinct transcription-associated chromatin organization. Genome Biol 13:R110.

[00314] Vo N, Klein ME, Varlamova O, Keller DM, Yamamoto T, Goodman RH, Impey S (2005) A cAMP-response element binding protein-induced microRNA regulates neuronal morphogenesis. Proceedings of the National Academy of Sciences of the United States of America 102:16426-16431.

[00315] Vogelauer M, Wu J, Suka N, Grunstein M (2000) Global histone acetylation and deacetylation in yeast. Nature 408:495-498.

[00316] Vsevolozhskaya OA, Anthony JC (2016) Transitioning from First Drug Use to Dependence Onset: Illustration of a Multiparametric Approach for Comparative Epidemiology. Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology 41:869-876.

[00317] Waldhoer M, Bartlett SE, Whistler JL (2004) Opioid receptors. Annu Rev Biochem 73:953-990.

[00318] Wang HL, Qi J, Zhang S, Wang H, Morales M (2015a) Rewarding Effects of Optical Stimulation of Ventral Tegmental Area Glutamatergic Neurons. The Journal of neuroscience: the official journal of the Society for Neuroscience 35:15948-15954.

[00319] Wang J, Lanfranco MF, Gibb SL, Ron D (2011) Ethanol-mediated long-lasting adaptations of the NR2B-containing NMDA receptors in the dorsomedial striatum. Channels (Austin) 5:205-209.

[00320] Wang J, Lanfranco MF, Gibb SL, Yowell QV, Carnicella S, Ron D (2010a) Long-lasting adaptations of the NR2B-containing NMDA receptors in the dorsomedial striatum play a crucial role in alcohol consumption and relapse. The Journal of neuroscience: the official journal of the Society for Neuroscience 30:10187-10198.

[00321] Wang J, Carnicella S, Phamluong K, Jeanblanc J, Ronesi JA, Chaudhri N, Janak PH, Lovinger DM, Ron D (2007) Ethanol induces long-term facilitation of NR2B-NMDA receptor activity in the dorsal striatum: implications for alcohol drinking behavior. The Journal of neuroscience: the official journal of the Society for Neuroscience 27:3593-3602.

[00322] Wang L, Lv Z, Hu Z, Sheng J, Hui B, Sun J, Ma L (2010b) Chronic cocaine-induced H3 acetylation and transcriptional activation of CaMKIIalpha in the nucleus accumbens is critical for motivation for drug reinforcement. Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology 35:913-928.

[00323] Wang WS, Kang S, Liu WT, Li M, Liu Y, Yu C, Chen J, Chi ZQ, He L, Liu JG (2012) Extinction of aversive memories associated with morphine withdrawal requires ERK-mediated epigenetic regulation of brain-derived neurotrophic factor transcription in the rat ventromedial prefrontal cortex. The Journal of neuroscience: the official journal of the Society for Neuroscience 32:13763-13775.

[00324] Wang Y, Lai J, Cui H, Zhu Y, Zhao B, Wang W, Wei S (2015b) Inhibition of histone deacetylase in the basolateral amygdala facilitates morphine context-associated memory formation in rats. J Mol Neurosci 55:269-278.

[00325] Wang Z, Zang C, Cui K, Schones DE, Barski A, Peng W, Zhao K (2009) Genome-wide mapping of HATs and HDACs reveals distinct functions in active and inactive genes. Cell 138:1019-1031.

[00326] Watanabe S, Radman-Livaja M, Rando OJ, Peterson CL (2013) A histone acetylation switch regulates H2A.Z deposition by the SWR-C remodeling enzyme. Science 340:195-199.

[00327] Wayman GA, Davare M, Ando H, Fortin D, Varlamova O, Cheng HY, Marks D, Obrietan K, Soderling TR, Goodman RH, Impey S (2008) An activity-regulated microRNA controls dendritic plasticity by down-regulating p250GAP. Proceedings of the National Academy of Sciences of the United States of America 105:9093-9098.

[00328] Weber T, Vogt MA, Gartside SE, Berger SM, Lujan R, Lau T, Herrmann E, Sprengel R, Bartsch D, Gass P (2015) Adult AMPA GLUA1 receptor subunit loss in 5-HT neurons results in a specific anxiety-phenotype with evidence for dysregulation of 5-HT neuronal activity. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 40:1471-1484.

[00329] Wei SG, Zhu YS, Lai JH, Xue HX, Chai ZQ, Li SB (2011) Association between heroin dependence and prodynorphin gene polymorphisms. Brain Res Bull 85:238-242.

[00330] Whyte WA, Orlando DA, Hnisz D, Abraham BJ, Lin CY, Kagey MH, Rahl PB, Lee TI, Young RA (2013) Master transcription factors and mediator establish super-enhancers at key cell identity genes. Cell 153:307-319.

[00331] Wolf ME (2010) The Bermuda Triangle of cocaine-induced neuroadaptations. Trends Neurosci 33:391-398.

[00332] Wu Q, Law PY, Wei LN, Loh HH (2008) Post-transcriptional regulation of mouse mu opioid receptor (MOR1) via its 3' untranslated region: a role for microRNA23b. FASEB J 22:4085-4095.

[00333] Wu Q, Zhang L, Law PY, Wei LN, Loh HH (2009) Long-term morphine treatment decreases the association of mu-opioid receptor (MOR1) mRNA with polysomes through miRNA23b. Mol Pharmacol 75:744-750.

[00334] Xi ZX, Stein EA (2002) Blockade of ionotropic glutamatergic transmission in the ventral tegmental area reduces heroin reinforcement in rat. Psychopharmacology 164:144-150.

[00335] Xi ZX, Ramamoorthy S, Baker DA, Shen H, Samuvel DJ, Kalivas PW (2002) Modulation of group II metabotropic glutamate receptor signaling by chronic cocaine. The Journal of pharmacology and experimental therapeutics 303:608-615.

[00336] Xie C, Li SJ, Shao Y, Fu L, Goveas J, Ye E, Li W, Cohen AD, Chen G, Zhang Z, Yang Z (2011) Identification of hyperactive intrinsic amygdala network connectivity associated with impulsivity in abstinent heroin addicts. Behav Brain Res 216:639-646.

[00337] Xie CW, Lewis DV (1991) Opioid-mediated facilitation of long-term potentiation at the lateral perforant path-dentate granule cell synapse. The Journal of pharmacology and experimental therapeutics 256:289-296.

[00338] Xu P, Li M, Bai Y, Lu W, Ling X, Li W (2015) The effects of piracetam on heroin-induced CPP and neuronal apoptosis in rats. Drug Alcohol Depend 150:141-146. Xuei X, Dick D, Flury-Wetherill L, Tian HJ, Agrawal A, Bierut L, Goate A, Bucholz K, Schuckit M, Nurnberger J, Jr., Tischfield J, Kuperman S, Porjesz B, Begleiter H, Foroud T, Edenberg HJ (2006) Association of the kappa-opioid system with alcohol dependence. Mol Psychiatry 11:1016-1024.

[00339] Yager LM, Garcia AF, Wunsch AM, Ferguson SM (2015) The ins and outs of the striatum: role in drug addiction. Neuroscience 301:529-541.

[00340] Yagi T, Shigetani Y, Okado N, Tokunaga T, Ikawa Y, Aizawa S (1993) Regional localization of Fyn in adult brain; studies with mice in which fyn gene was replaced by lacZ. Oncogene 8:3343-3351.

[00341] Yaka R, He DY, Phamluong K, Ron D (2003) Pituitary adenylate cyclase-activating polypeptide (PACAP(1-38)) enhances N-methyl-D-aspartate receptor function and brain-derived neurotrophic factor expression via RACK1. J Biol Chem 278:9630-9638.

[00342] Yaka R, Thornton C, Vagts AJ, Phamluong K, Bonci A, Ron D (2002) NMDA receptor function is regulated by the inhibitory scaffolding protein, RACK1. Proceedings of the National Academy of Sciences of the United States of America 99:5710-5715.

[00343] Yang K, Belrose J, Trepanier CH, Lei G, Jackson MF, MacDonald JF (2011) Fyn, a potential target for Alzheimer's disease. J Alzheimers Dis 27:243-252.

[00344] Yao L, McFarland K, Fan P, Jiang Z, Inoue Y, Diamond I (2005) Activator of G protein signaling 3 regulates opiate activation of protein kinase A signaling and relapse of heroin-seeking behavior. Proceedings of the National Academy of Sciences of the United States of America 102:8746-8751.

[00345] Yizhar O, Fenno LE, Davidson TJ, Mogri M, Deisseroth K (2011) Optogenetics in neural systems. Neuron 71:9-34.

[00346] Young JW, Winstanley CA, Brady AM, Hall FS (2016) Research Domain Criteria versus DSM V: How does this debate affect attempts to model corticostriatal dysfunction in animals? Neurosci Biobehav Rev.

[00347] Yuferov V, Ji F, Nielsen DA, Levran O, Ho A, Morgello S, Shi R, Ott J, Kreek MJ (2009) A functional haplotype implicated in vulnerability to develop cocaine dependence is associated with reduced PDYN expression in human brain. Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology 34:1185-1197.

[00348] Zhang B, Day DS, Ho JW, Song L, Cao J, Christodoulou D, Seidman JG, Crawford GE, Park PJ,

[00349] Pu WT (2013) A dynamic H3K27ac signature identifies VEGFA-stimulated endothelial enhancers and requires EP300 activity. Genome Res 23:917-927.

[00350] Zhang W, Bone JR, Edmondson DG, Turner BM, Roth SY (1998) Essential and redundant functions of histone acetylation revealed by mutation of target lysines and loss of the Gcn5p acetyltransferase. EMBO J 17:3155-3167.

[00351] Zhang Y, Venkitaramani DV, Gladding CM, Zhang Y, Kurup P, Molnar E, Collingridge GL, Lombroso PJ (2008) The tyrosine phosphatase STEP mediates AMPA receptor endocytosis after metabotropic glutamate receptor stimulation. The Journal of neuroscience: the official journal of the Society for Neuroscience 28:10561-10566.

[00352] Zhang Z, Zhang MQ (2011) Histone modification profiles are predictive for tissue/cell-type specific expression of both protein-coding and microRNA genes. BMC Bioinformatics 12:155.

[00353] Zheng H, Chu J, Zeng Y, Loh HH, Law PY (2010a) Yin Yang 1 phosphorylation contributes to the differential effects of mu-opioid receptor agonists on microRNA-190 expression. J Biol Chem 285:21994-22002.

[00354] Zheng H, Zeng Y, Chu J, Kam AY, Loh HH, Law PY (2010b) Modulations of NeuroD activity contribute to the differential effects of morphine and fentanyl on dendritic spine stability. The Journal of neuroscience: the official journal of the Society for Neuroscience 30:8102-8110.

[00355] Zheng H, Zeng Y, Zhang X, Chu J, Loh HH, Law PY (2010c) mu-Opioid receptor agonists differentially regulate the expression of miR-190 and NeuroD. Mol Pharmacol 77:102-109.

[00356] Zhou L, Furuta T, Kaneko T (2003) Chemical organization of projection neurons in the rat accumbens nucleus and olfactory tubercle. Neuroscience 120:783-798.

[00357] Zimprich A, Kraus J, Woltje M, Mayer P, Rauch E, Hollt V (2000) An allelic variation in the human prodynorphin gene promoter alters stimulus-induced expression. J Neurochem 74:472-477.

[00358] Zinkernagel C, Naef MR, Bucher HC, Ladewig D, Gyr N, Battegay M (2001) Onset and pattern of substance use in intravenous drug users of an opiate maintenance program. Drug Alcohol Depend 64:105-109.

[00359] Zuckerman M (1993) P-impulsive sensation seeking and its behavioral, psychophysiological and biochemical correlates. Neuropsychobiology 28:30-36.

[00360] Zuckerman M (2002) Zuckerman-Kuhlman Personality Questionnaire. In: Big Five Assessment (ZKPQ), an alternative five-factorial model (Raad BD, Perugini M, eds), pp 377-396. Seattle, WA: Hogrefe & Huber.

What is claimed is:

CLAIMS

1. A method of treating an opioid and/or opiate addiction in a subject comprising administering a therapeutically effective amount of saracatinib or a derivative of saracatinib, or a pharmaceutically acceptable salt thereof.

2. The method of claim 1, wherein the subject has a tolerance, dependence, or an addiction to at least one opioid or opiate drug.

3. The method of claim 1, wherein the opioid and/or opiate is heroin.

4. The method of claim 1, wherein the opioid and/or opiate is oxycodone, hydrocodone, fentanyl, or naloxone.

5. The method of claim 1, further comprising co-administering a therapeutically effective amount of anopioid replacement therapy.

6. The method of claim 4, wherein the opioid replacement therapy is methodone therapy.

7. The method of claim 4, wherein the opioid replacement therapy is buprenorphine/naloxone and/or naltrexone therapy.

8. The method of claim 1, further comprising co-administering a therapeutically effective amount of a histone acetylation inhibitor and/or a bromodomain inhibitor.

9. The method of claim 1, wherein the saracatinib is administered orally, sublingually, intramuscularly, subcutaneously, intravenously, or by inhalation.

10. A method of treating an opioid and/or opiate addiction in a subject comprising administering a pharmaceutical composition comprising a therapeutically effective amount of saracatinib or a derivative of saracatinib, or a pharmaceutically acceptable salt thereof.

11. The method of claim 10, wherein the subject has a tolerance, dependence, or an addiction to at least one opioid or opiate drug.

12. The method of claim 10, wherein the opioid and/or opiate is heroin.

13. The method of claim 10, wherein the opioid and/or opiate is oxycodone, hydrocodone, fentanyl, or naloxone.

14. The method of claim 10, further comprising co-administering a therapeutically effective amount of an opioid replacement therapy.

15. The method of claim 14, wherein the opioid replacement therapy is methodone therapy.

16. The method of claim 14, wherein the opioid replacement therapy is buprenorphine/naloxone and/or naltrexone therapy.

17. The method of claim 10, further comprising co-administering a therapeutically effective amount of a histone acetylation inhibitor and/or a bromodomain inhibitor.

18. The method of claim 10, wherein the pharmaceutical composition is administered orally, sublingually, intramuscularly, subcutaneously, intravenously, or by inhalation.

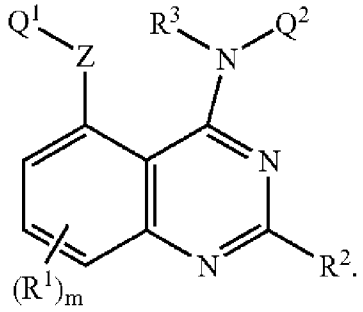
19. A method of treating an opioid and/or opiate addiction in a subject comprising administering a therapeutically effective amount of an inhibitor of FYN activity or expression, or a pharmaceutically composition comprising same.

20. The method of claim 19, wherein the inhibitor of FYN activity or expression is a small molecule inhibitor.

21. The method of claim 20, wherein the small molecule inhibitor is saracatinib or derivative thereof, dasatinib or derivative thereof, bosutinib or derivative thereof, A 419259 trihydrochloride or derivative thereof, herbimycin A or derivative thereof, 1-naphthyl PP1 or

derivative thereof, PP 1 or derivative thereof, PP 2 or derivative thereof, SU6656 or derivative thereof, or combinations thereof.

22. The method of claim 20, wherein the small molecule inhibitor is a compound of Formula IV



(IV),

wherein each of Q^1, Z, m, R^1, R^2, R^3, and Q^2 have any of the meanings as defined or claimed in PCT/GB2001/002424 (published as WO/2001/094341).

23. The method of claim 19, wherein the inhibitor of FYN activity or expression is a polypeptide inhibitor.

24. The method of claim 23, wherein the polypeptide inhibitor is an anti-FYN antibody.

25. The method of claim 19, wherein the inhibitor of FYN activity or expression is a nucleic acid inhibitor.

26. The method of claim 25, wherein the polypeptide inhibitor is an siRNA that targets FYN mRNA.

27. The method of claim 19, wherein the subject has a tolerance, dependence, or an addiction to at least one opioid or opiate drug.

28. The method of claim 19, wherein the opioid and/or opiate is heroin.

29. The method of claim 19, wherein the opioid and/or opiate is oxycodone, hydrocodone, fentanyl, or naloxone.

30. The method of claim 19, further comprising co-administering a therapeutically effective amount of an opioid replacement therapy.

31. The method of claim 30, wherein the opioid replacement therapy is methodone therapy.

32. The method of claim 30, wherein the opioid replacement therapy is buprenorphine/naloxone and/or naltrexone therapy.

33. The method of claim 19, further comprising co-administering a therapeutically effective amount of a histone acetylation inhibitor and/or a bromodomain inhibitor.

34. The method of claim 19, wherein the FYN inhibitor is administered orally, sublingually, intramuscularly, subcutaneously, intravenously, or by inhalation.

35. The method of claim 33, wherein the histone acetylation inhibitor is at least one of anacardic acid or a derivative thereof, C 646 or a derivative thereof, EML 425 or a derivative thereof, PU139 or a derivative thereof, PU141 or a derivative thereof, MB-3 or a derivative thereof, CPTH2 or a derivative thereof, ISOX DUAL or a derivative thereof, L002 or a derivative thereof, Lys-CoA or a derivative thereof, NU 9056 or a derivative thereof, curcumin or a derivative thereof, garcinol or a derivative thereof, or a combination thereof.

36. The method of claim 33, wherein the bromodomain inhibitor is at least one of JQ1 or a derivative thereof, ISOX DUAL or a derivative thereof, I-CBP 112 or a derivative thereof, SCG-CBP30 or a derivative thereof, I-BET151 (GSK1210151A) or a derivative thereof, I-BET762 (GSK525762) or a derivative thereof, OXF BD 02 or a derivative thereof, MS 436 or a derivative thereof, XMD 8-92 or a derivative thereof, PFI 1 or a derivative thereof, PF1 4 or a derivative thereof, PF CBP1 or a derivative thereof, XD 14 or a derivative thereof, bromosporine or a derivative thereof, RVX-208 or a derivative thereof, BMS-986158 or a derivative thereof, OTX-015 or a derivative thereof, ischemin sodium salt or a derivative thereof, TEN-010 or a derivative thereof, CPI-0610 or a derivative thereof, PLX-51107 or a derivative thereof, INCB054329 or a derivative thereof, GSK2820151 or a derivative thereof, BAY 1238097 or a derivative thereof, or a combination thereof.

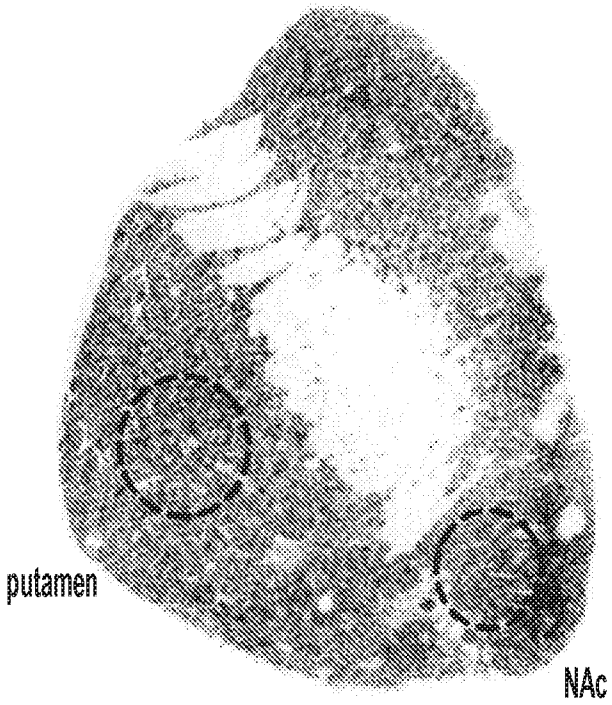


FIG. 1

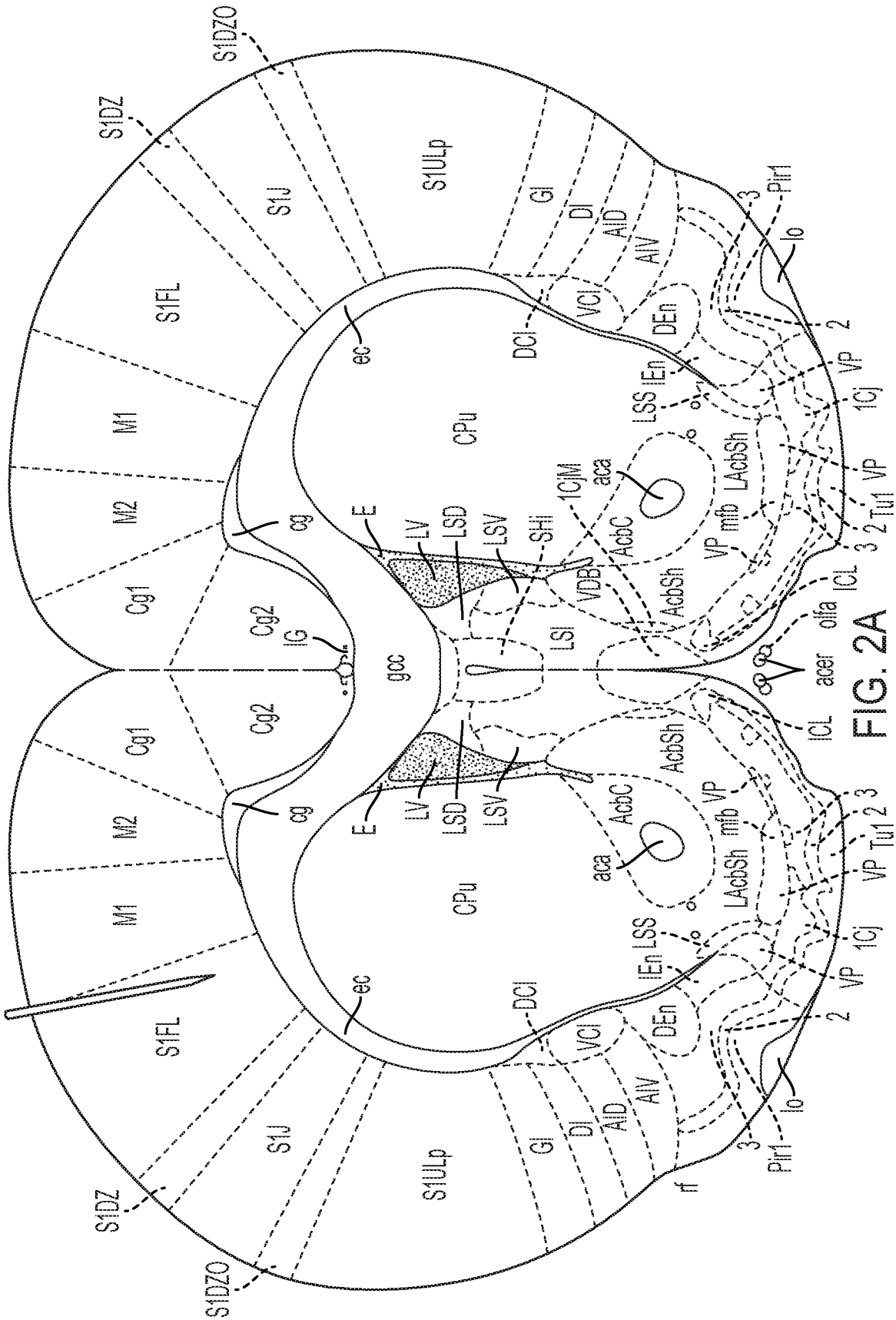

S1DZO
S1DZ
S1J
S1ULp
S1FL
M1
M2
Cg1
Cg2
cg
IG
gcc
ec
CPu
GI
DI
AID
AIV
Pir1
Io
DCl
VCl
DEn
IEn
LSS
2
1Cj
VP
E
LV
LSD
LSV
SHi
1CjM
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FIG. 2A

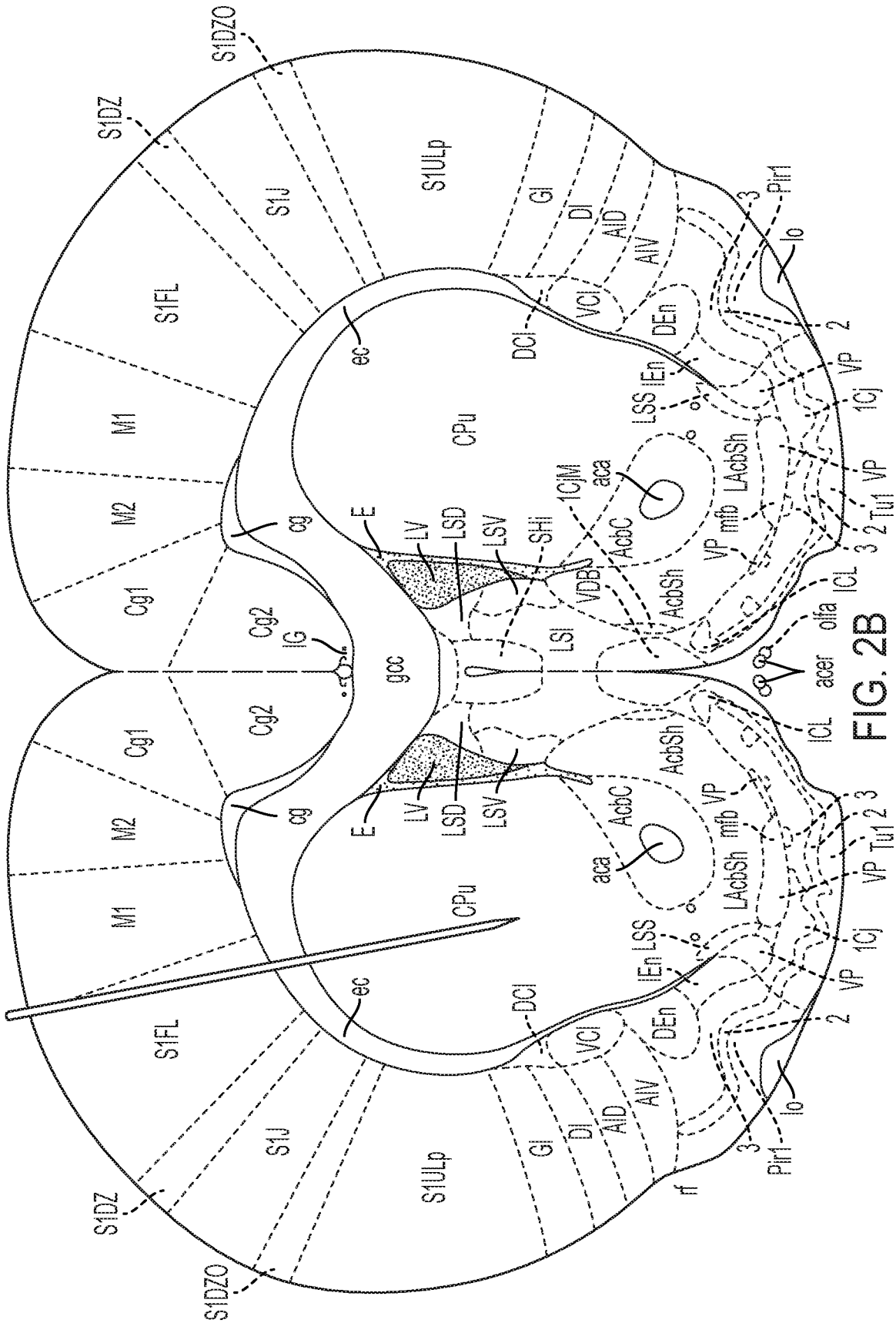

S1DZO
S1DZ
S1J
S1ULp
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M1
M2
Cg1
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AID
AIV
DCI
VCI
DEn
IEn
LSS
LAcbSh
mfb
VP
1CjM
aca
AcbC
AcbSh
VDB
SHi
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FIG. 2B

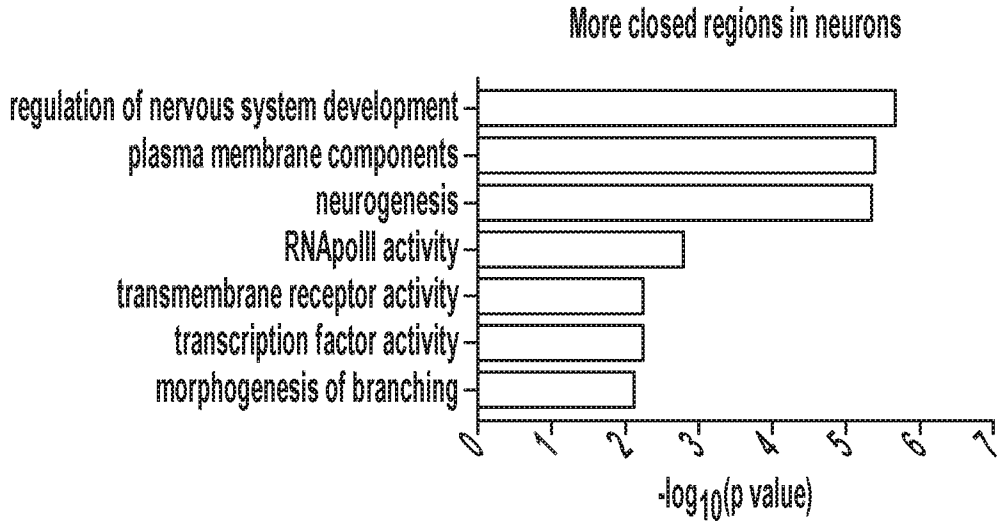

More closed regions in neurons
regulation of nervous system development
plasma membrane components
neurogenesis
RNApolII activity
transmembrane receptor activity
transcription factor activity
morphogenesis of branching
0
1
2
3
4
5
6
7
-log10(p value)

FIG. 3A

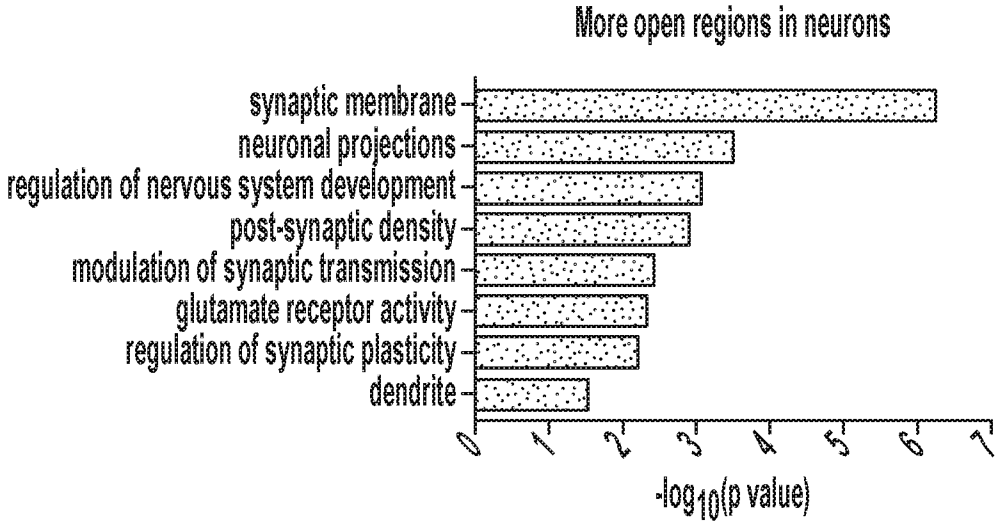

More open regions in neurons
synaptic membrane
neuronal projections
regulation of nervous system development
post-synaptic density
modulation of synaptic transmission
glutamate receptor activity
regulation of synaptic plasticity
dendrite
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6
7
-log10(p value)

FIG. 3B

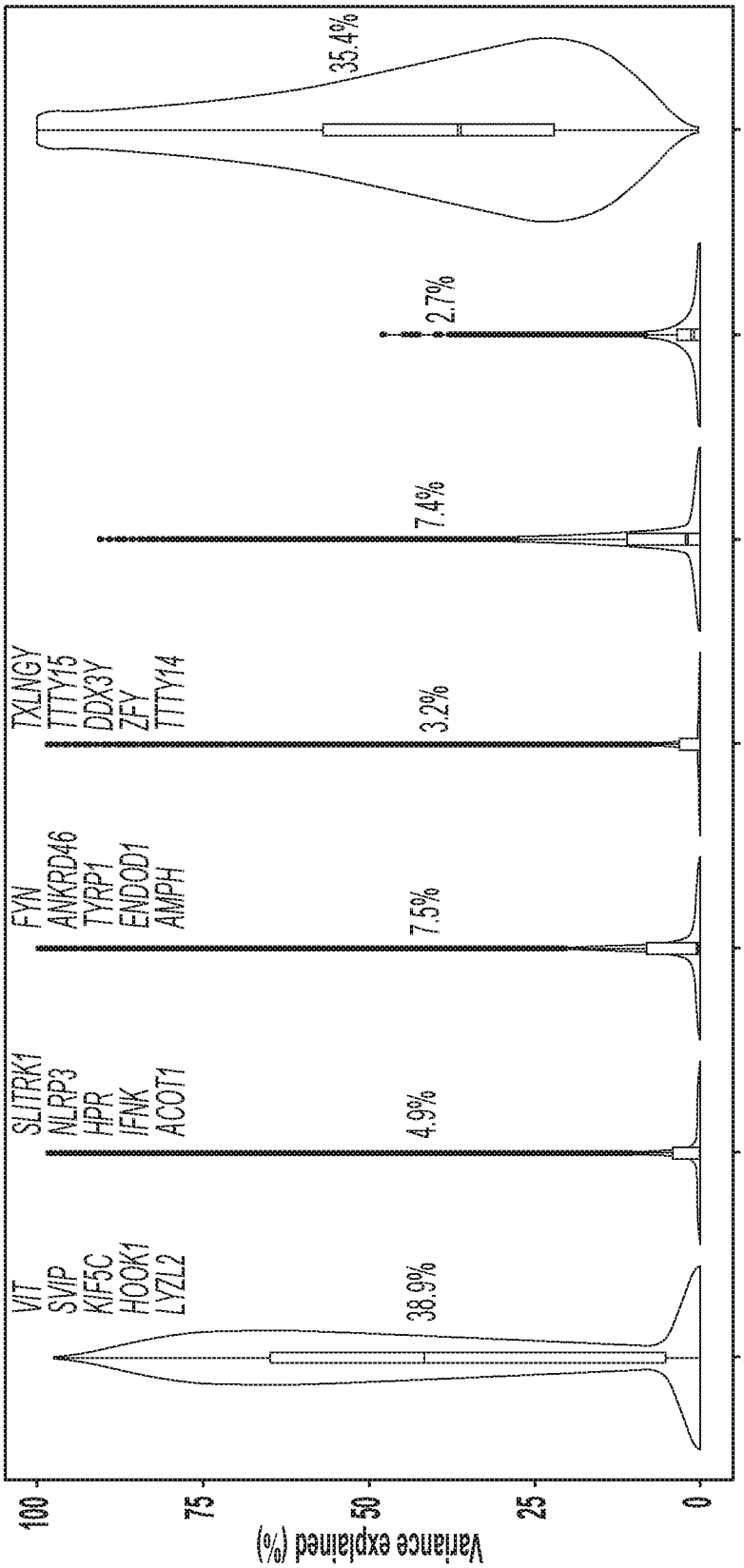

Variance explained (%)
100
75
50
25
0
VIT
SVIP
KIF5C
HOOK1
LYZL2
38.9%
SLITRK1
NLRP3
HPR
IFNK
ACOT1
4.9%
FYN
ANKRD46
TYRP1
ENDOD1
AMPH
7.5%
TXLNGY
TTTY15
DDX3Y
ZFY
TTTY14
3.2%
7.4%
2.7%
35.4%
Cell
CellIGlialDx
CellNeuronDx
Gender
Individual
PMI
Residuals

FIG. 4

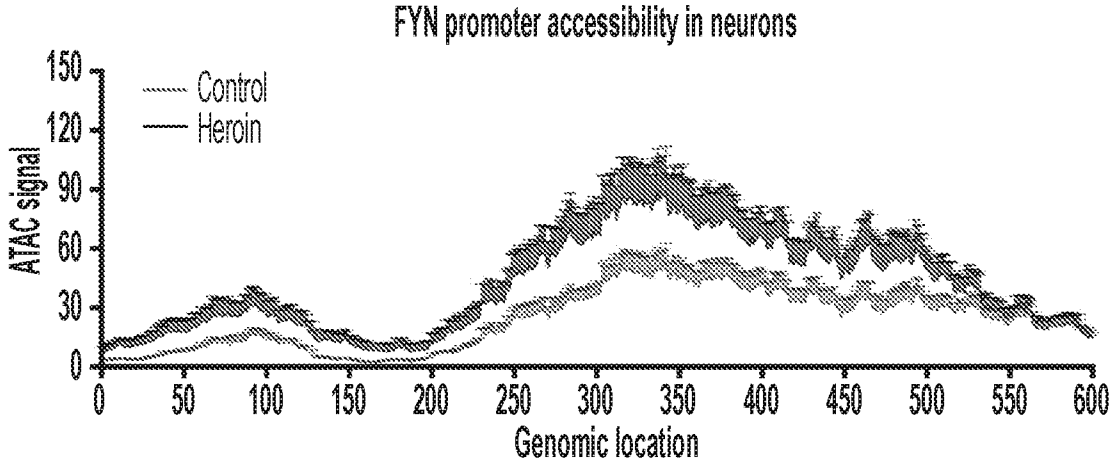


FIG. 5A

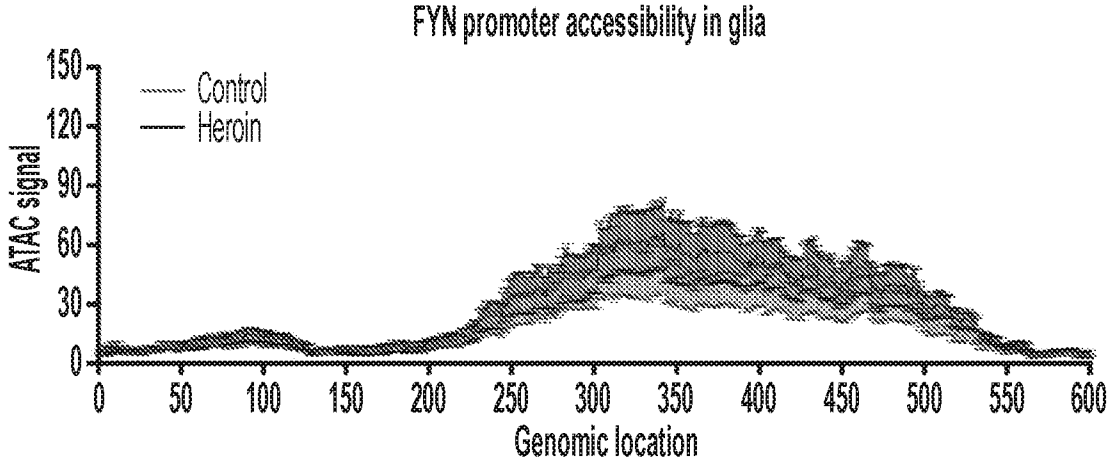


FIG. 5B

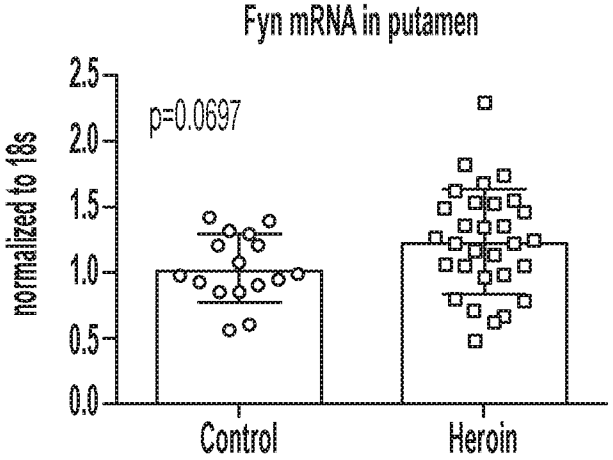


FIG. 6A

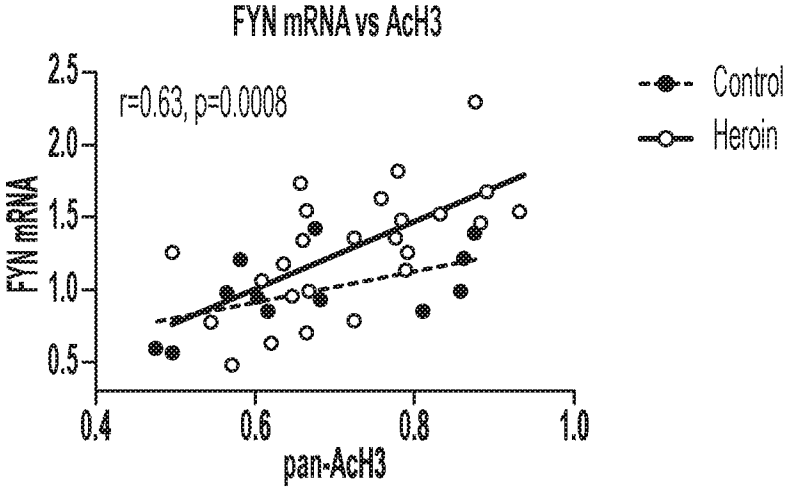


FIG. 6B

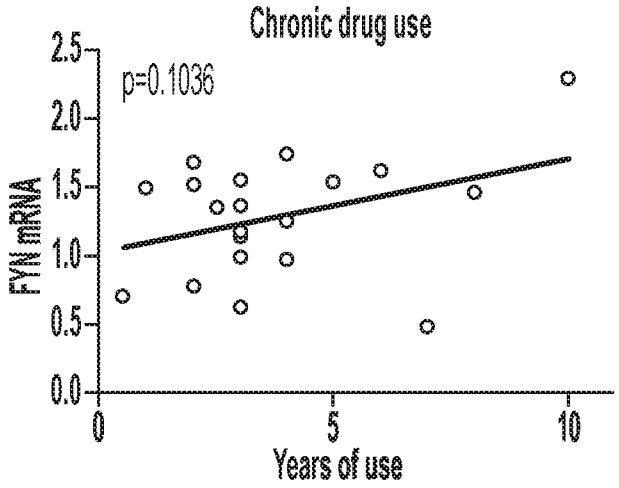


FIG. 6C

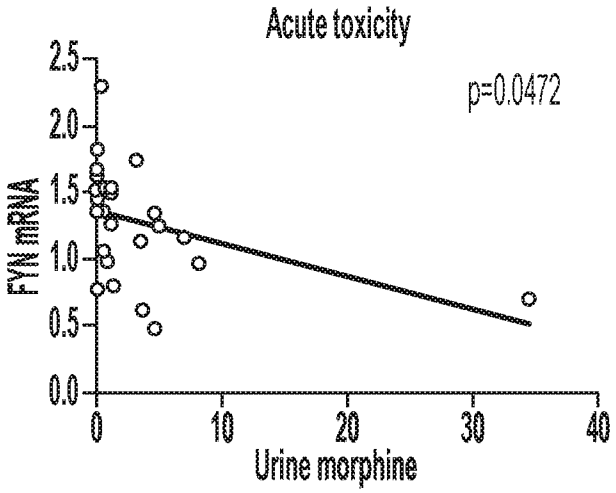


FIG. 6D

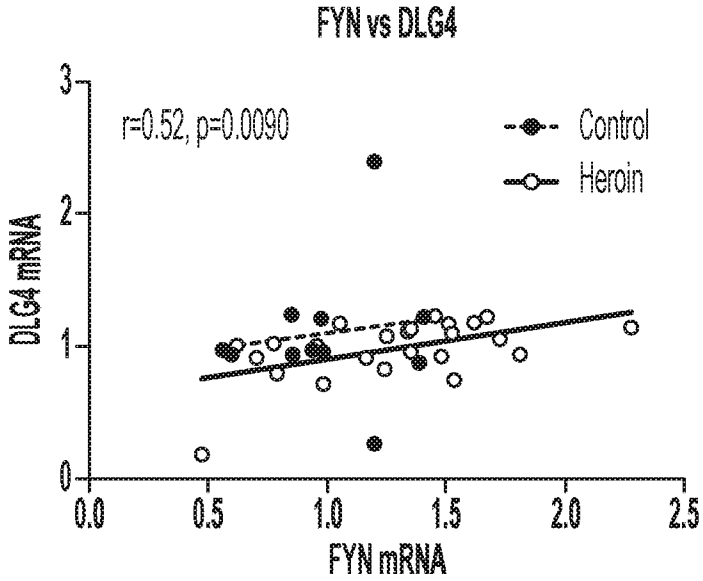

FYN vs DLG4
r=0.52, p=0.0090
Control
Heroin
DLG4 mRNA
FYN mRNA
0
1
2
3
0.0
0.5
1.0
1.5
2.0
2.5

FIG. 7A

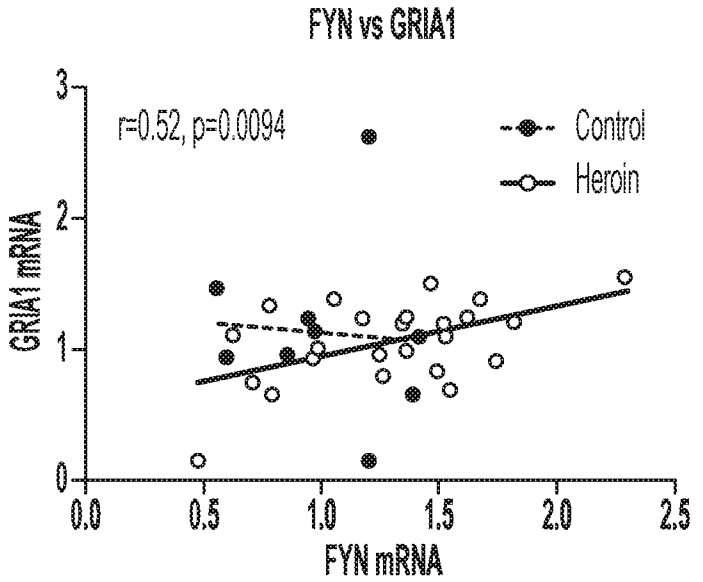

FYN vs GRIA1
r=0.52, p=0.0094
Control
Heroin
GRIA1 mRNA
FYN mRNA
0
1
2
3
0.0
0.5
1.0
1.5
2.0
2.5

FIG. 7B

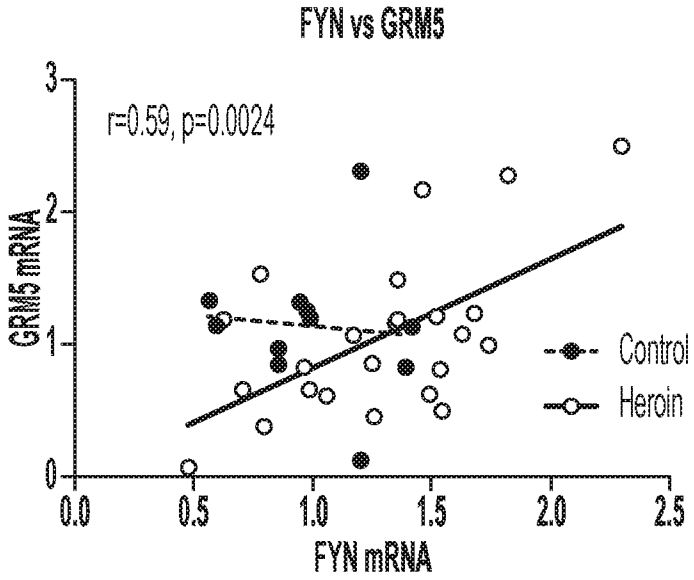

FYN vs GRM5
r=0.59, p=0.0024
GRM5 mRNA
FYN mRNA
Control
Heroin
0
1
2
3
0.0
0.5
1.0
1.5
2.0
2.5

FIG. 7C

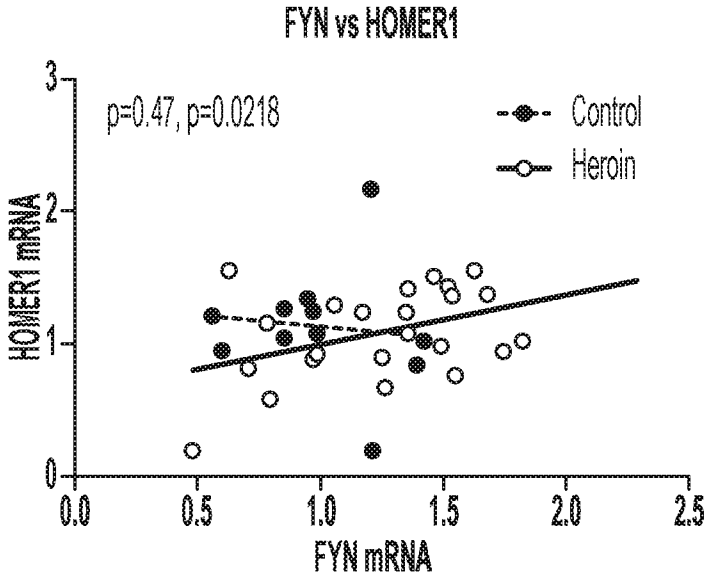

FYN vs HOMER1
p=0.47, p=0.0218
HOMER1 mRNA
FYN mRNA
Control
Heroin
0
1
2
3
0.0
0.5
1.0
1.5
2.0
2.5

FIG. 7D

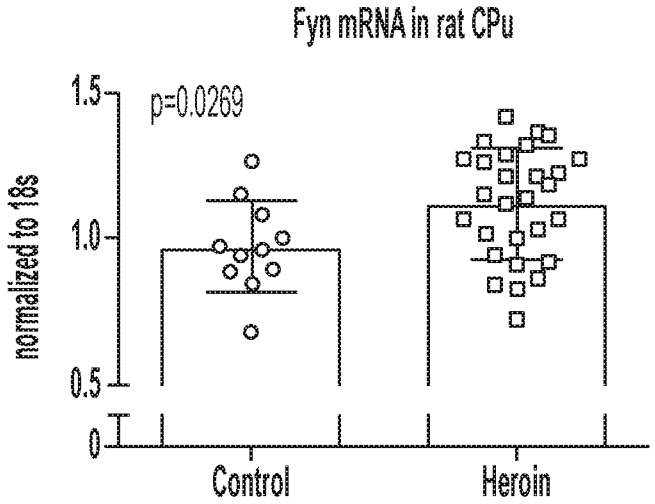

Fyn mRNA in rat CPu
p=0.0269
normalized to 18s
1.5
1.0
0.5
0
Control
Heroin

FIG. 8A

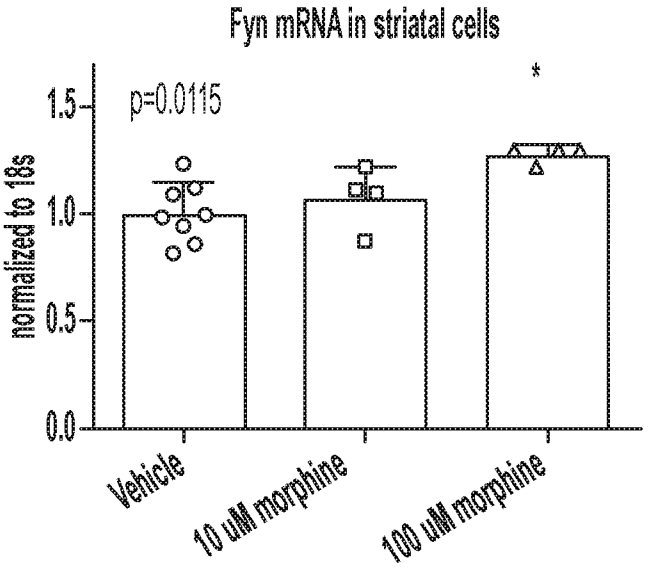

Fyn mRNA in striatal cells
p=0.0115
*
normalized to 18s
1.5
1.0
0.5
0.0
Vehicle
10 uM morphine
100 uM morphine

FIG. 8B

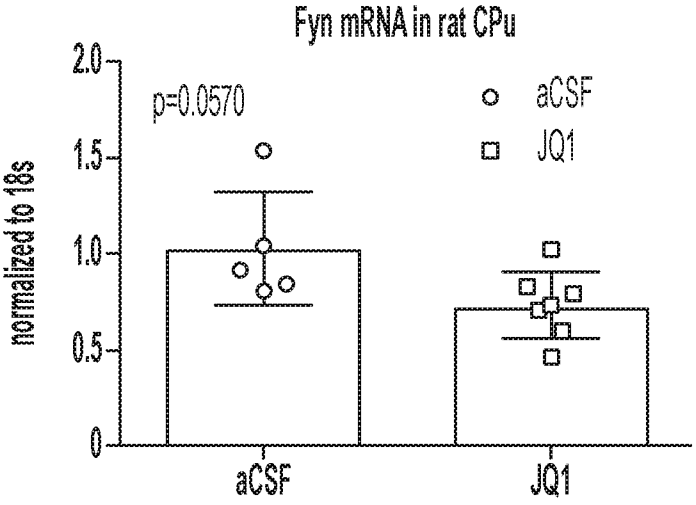

Fyn mRNA in rat CPu
p=0.0570
aCSF
JQ1
normalized to 18s
2.0
1.5
1.0
0.5
0
aCSF
JQ1

FIG. 8C

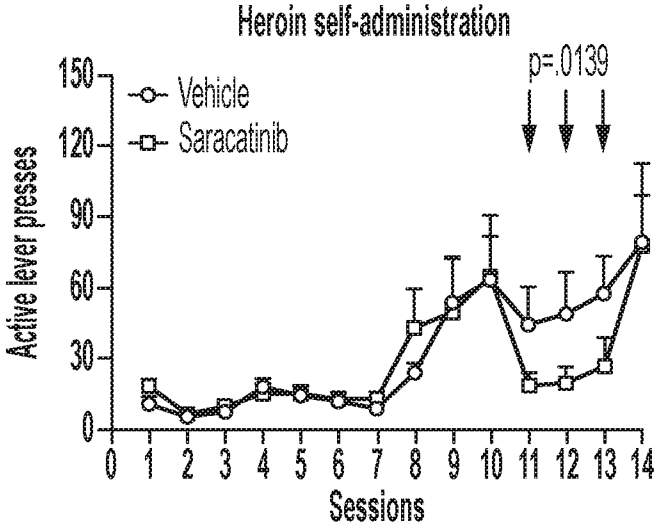

Heroin self-administration
p=.0139
Vehicle
Saracatinib
Active lever presses
150
120
90
60
30
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Sessions

FIG. 9A

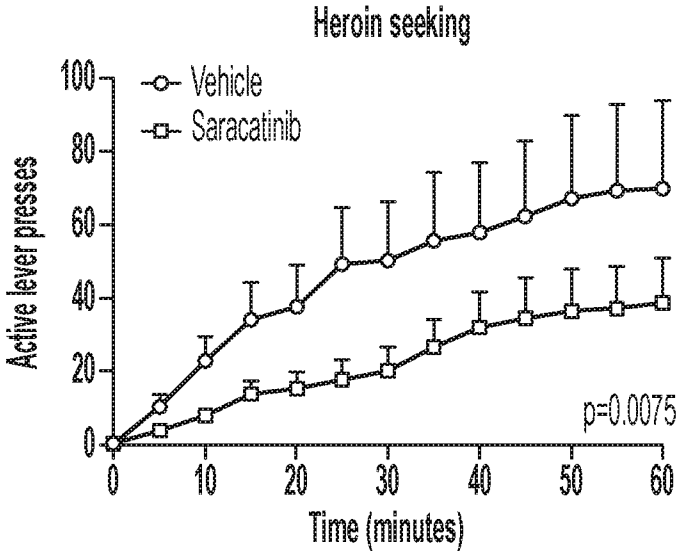

Heroin seeking
Vehicle
Saracatinib
Active lever presses
100
80
60
40
20
0
p=0.0075
0 10 20 30 40 50 60
Time (minutes)

FIG. 9B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/17020

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.: 22
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:

Claim No. 22 is an unsearchable omnibus claim.

3. ☐ Claims Nos.:
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.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/17020

A. CLASSIFICATION OF SUBJECT MATTER
IPC - A61K 31/485; A61P 25/04, 25/36; C07D 405/14 (2019.01)
CPC - A61K 31/485; A61P 25/04, 25/36; C07D 405/14

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.
X -- Y	(ZHANG, L et al.) Src-dependent phosphorylation of Mu-opioid receptor at Tyr336 modulates opiate withdrawal. EMBO Molecular Medicine. November 2017, Epub 17 August 2017, Vol. 9, No. 11; pages 1521-1538; abstract, page 1526, 1st column, 1st paragraph; page 1533, 1st column, 1st paragraph and sidebar, 1st and 3rd paragraphs; figure 5B; DOI: 10.15252/emmm.201607324	1-4, 10-13, 19-21, 23-29 ---------- 5-9, 14-18, 30-36
Y	(EGERVARI, G et al.) Striatal H3K27 acetylation linked to glutamatergic gene dysregulation in human heroin abusers holds promise as therapeutic target. Biological Psychiatry. 1 April 2017, Epub 28 September 2016, Vol. 81, No. 7; page 585-594; abstract; DOI: 10.1016/j.biopsych.2016.09.015	8, 17, 33, 36
Y	(FURY, MG et al.) Phase II Study of Saracatinib (AZD0530) for Patients with Recurrent or Metastatic Head and Neck Squamous Cell Carcinoma (HNSCC). Anticancer Research. January 2011, Vol. 31, No. 1; pages 249-253; page 252, 1st column, 4th paragraph	9, 18, 34
Y	(LIANG, DY et al.) Epigenetic Regulation of Opioid-Induced Hyperalgesia, Dependence and Tolerance in Mice. The Journal of pain. January 2013, Vol. 14, No. 1; pages 36-47; page 9, 4th paragraph; DOI: 10.1016/j.jpain.2012.10.005	33, 35
A	(STOTTS, AL et al.) Opioid Dependence Treatment: Options In Pharmacotherapy. Expert Opinion on Pharmacotherapy. August 2009, Vol. 10, No. 11; pages 1727-1740; page 2, 2nd paragraph, page 3, 4th paragraph; page 7, 2nd paragraph; DOI: 10.1517/14656560903037168	5-7, 14-16, 30-32

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
30 April 2019 (30.04.2019)	13 MAY 2019
Name and mailing address of the ISA/ 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 PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)