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14. ABSTRACT

The key positive findings of this grant are 7OH mitragynine (7OH) is a potent opioid analgesic devoid of respiratory depression separating its analgesic actions from classical mu opioid modulators like morphine, oxycodone and fentanyl. 7OH has distinct central analgesic mechanism of action which is dependent on the adrenergic α_{2A} receptors which in turn is dependent on 6TM-MOR-1 sites. Systemically, 7OH is less dependent on 6TM/MOR-1 receptors and primarily acts through 7TM-MOR-1 sites just like morphine. The negative findings are 7OH retains addictive and abuse liability similar to morphine irrespective of its route of administration. Both central as well as systemic receptor mechanisms lead to addiction liability. More conclusive studies where 6TM mechanisms can be separated from 7TM MOR-1 mechanisms may lead to better understanding of the target labeled by 7OH.

15. SUBJECT TERMS
6TM, 7TM, respiratory depression, analgesia, CPP, CYP3A

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INTRODUCTION

The purpose of this grant proposal was use the 7OH mitragynine template and identify the target labeled by the molecule integrating chemical synthesis, biochemistry and neuropharmacology. The goal was to synthesize analogs of 7OH as novel analgesics with a distinct mechanism of action devoid of the side-effects seen with opioid analgesics clinically used for treatment of pain.

1. KEYWORDS

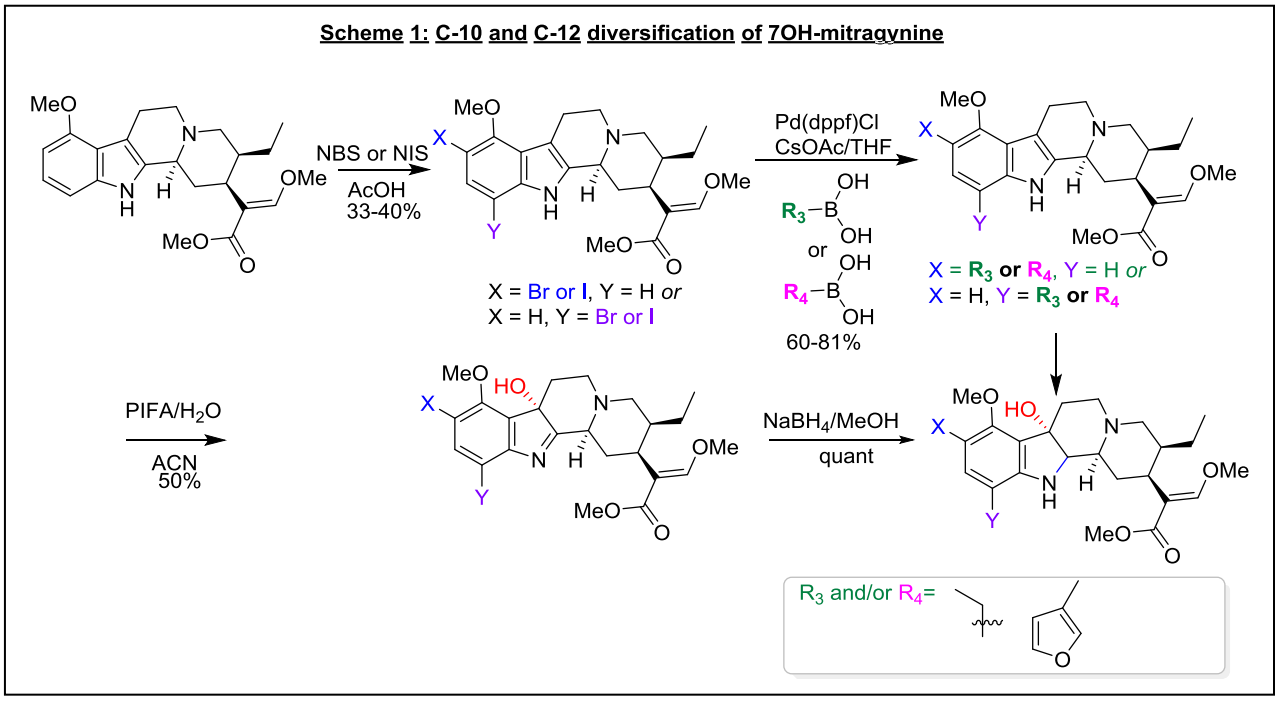
6TM, 7TM, respiratory depression, analgesia, CPP, CYP3A

2. ACCOMPLISHMENTS

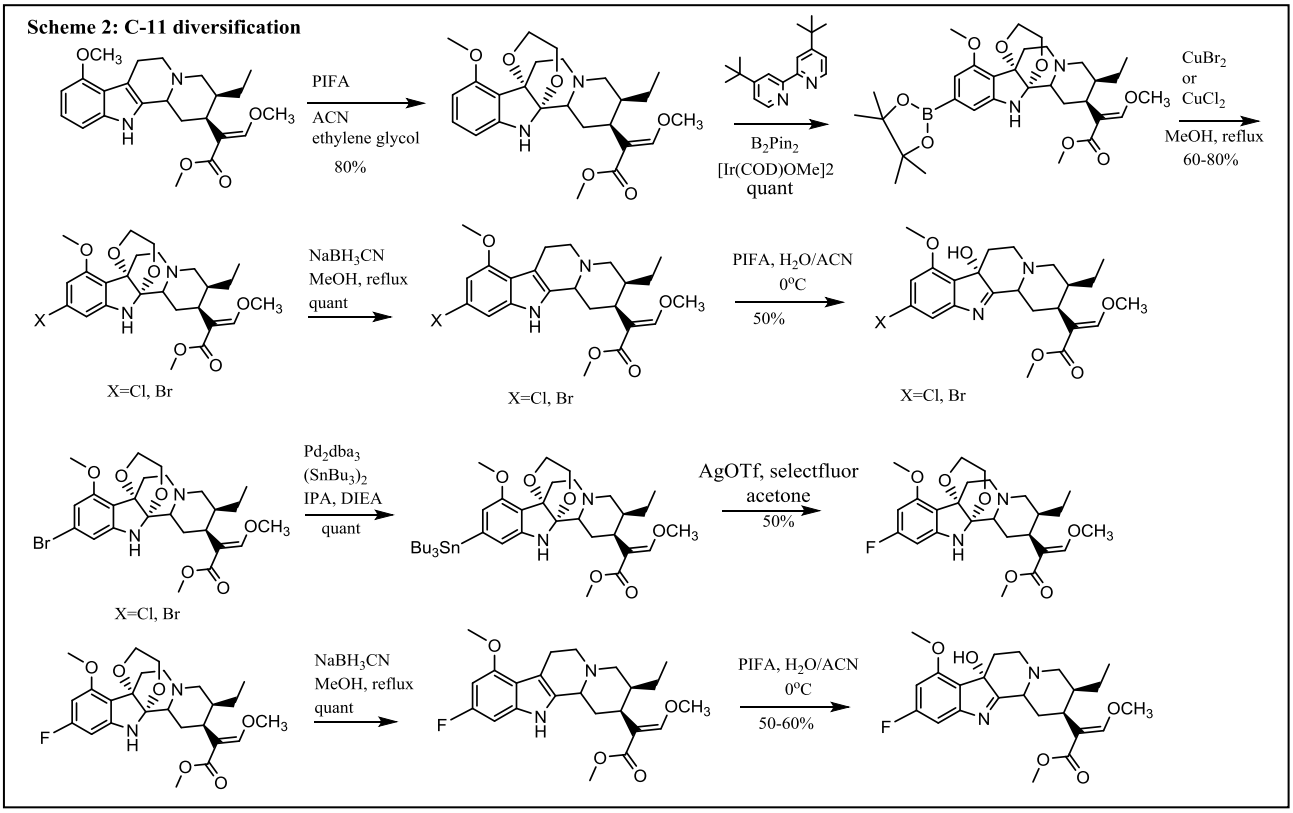
What were the major goals of the project?

The major goals of this project were to identify the receptor mechanisms responsible for the analgesic activity of 7OH mitragynine (7OH), a natural product related to mitragynine found in kratom leaves. We had proposed synthesizing a radiolabeled version of 7OH (^{3}H or ^{125}I) to identify the site labelled by 7OH in rodent brain. The synthesis would have enabled biochemical characterization of the7OH mitragynine site. The necessity for radiolabeling arose from the distinct pharmacology of 7OH in mice when this drug was administered supraspinally. The analgesia of this molecule was intact in mice lacking mu opioid receptors where the seven transmembrane mu opioid receptors (7TM-MOR-1) are lacking. Classical mu agonists/analgesics like morphine and DAMGO have activity in these knock out mice. 7OH was active in this KO mice. In a separate set of KO mice lacking six transmembrane domain mu opioid receptors (6TM MOR-1) where morphine is active as an analgesic, 7OH had vastly diminished analgesic activity. Analgesic potency was shifted by 40 fold in these KO mice lacking 6TM MOR-1 sites which had 7TM MOR-1 still present. Specific Aim 1 failed in our hands. Milestone under this aim was not achieved. A second goal was to synthesize 7OH analogs and probe there in vitro and in vivo pharmacology and map out 6TM/7TM structure activity relationships (SAR).
Specific aim 2: Major task 1(Subtask 1)-completed 1-3 months; Subtask 2-completed 4-5; Subtask 3-6-12 months. Milestone achieved -12 months. Major task 2: milestone achieved 8-12 months.

What was accomplished under these goals?



1) ***Major activities:*** Aim 1 in the proposal which involved synthesis of 7OH radiolabeled analog failed in our hands which set us back as a related sub aim was to biochemically characterize the binding site of 7OH using radioligand binding assays to determine a K_D for the site and competition assays using the radioligand to identify the partner protein and/or receptor involved in the actions of 7OH. We thereby took an alternate approach and used in vivo analgesia assays to identify which receptor system was involved in the actions of 7OH. We find that supraspinal actions



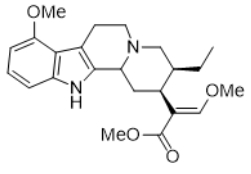
of 7OH are dependent on adrenergic α_{2A} receptor system which ultimately leads to its 6TM-MOR-1 actions. However we also find that systemic actions of this drug are more dependent on 7TM MOR-1 like morphine or DAMGO. 6TM-MOR-1 plays a less significant role in the pharmacology of this drug on systemic administration. We also find that irrespective of the route of administration the drug shows potent analgesia with no respiratory depression but retains its addictive potential. We also found that 7OH is a metabolite of kratom leaves which are used recreationally by number of Americans which we believe has high impact on the opioid epidemic in the country. Kratom is used anecdotally to treat opioid induced withdrawal. Thus, although we failed in using radiolabeling approaches to characterize the 7OH binding site we were still successful in identifying the mechanism of drug action of this natural product indirect using behavioral analgesia assays.

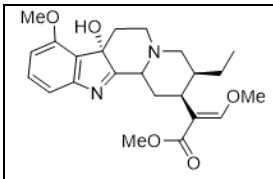
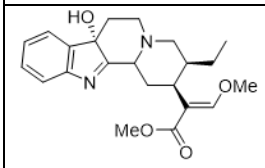
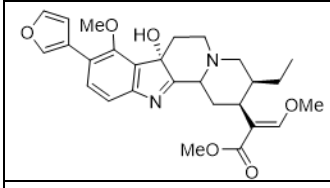
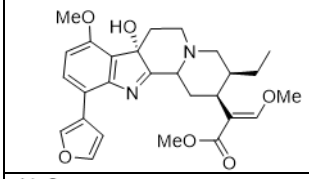
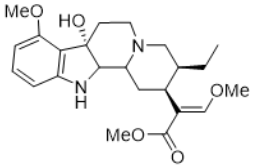
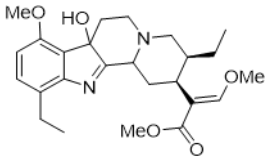
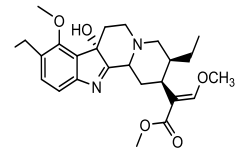
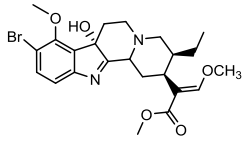
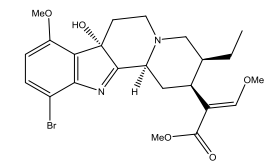
2) Specific activities:

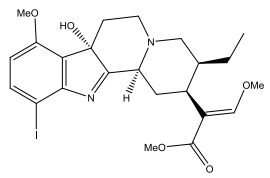
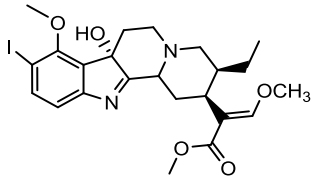
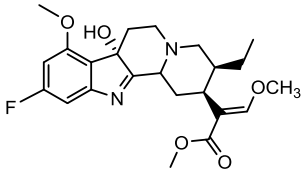
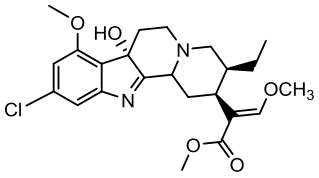
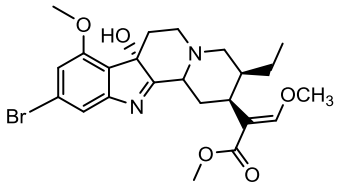
To map out the receptor mechanism mediating 7OH mitragynine pharmacology, analogs of 7OH mitragynine were synthesized.

Synthesis of 7OH analogs with diversification at C-10 and C-12 position were carried out as shown in Scheme 1. Briefly bromination or iodination was carried out using NBS or NIS. The mixture of 10 and/or 12 substituted products were purified by chromatography. Either these analogs were directly converted to corresponding 7OH analogs (not shown) or cross-coupling carried out on the 10 or 12 bromo substituted mitragynine derivative. Only two groups were looked at -ethyl and -3'-furanayl. We have previously published on synthetic methodology on these templates (Varadi et al J Med Chem 2016; 59(18):8381-97). Similarly C11-substituted analogs were synthesized as shown in Scheme 2. Only 3 compounds (with a F, Cl and Br) in this series were probed. Post synthesis these were characterized in radioligand binding assays (Table 1) and few for analgesia in mice by supraspinal administration. In general, -10 position substituted analogs showed higher binding affinity for kappa receptors (K_i<132 nM) over the parent (7OH) and were not further probed. 12-substituted analogs had poor opioid receptor affinity (K_i>37 nM) and were not found to be analgesic in mice. Among the C-11 analogs, C-11 iodo analog was not analgesic in mice while the C11-F and -Cl analog showed binding and analgesia with potency similar in both wild type and 6TM-E11 MOR-1 KO mice. Thus analog diversification did not lead to any analogs with selectivity for 6TM/MOR-1 sites.

Table 1: Radioligand binding assays and analgesia in mice

	Affinity (K_i nM)[a]			**Analgesia**	**Analgesia**
Compound Structure	**MOR-1**	**KOR-1**	**DOR-1**	**icv (µg)**	**E11 KO mice**
	230	231	1011	not tested	

	37	132	91	0.02	0.83
	23	781	147	0.1	not tested
	36	36	109		not tested
	117	338	>1000	>10	
	10.46	91.82	59.54	0.2	0.2
	108.2	783	88.13	>10	
	23	10		not tested	
	39	2.3		not tested	
	278	185	617	not tested	

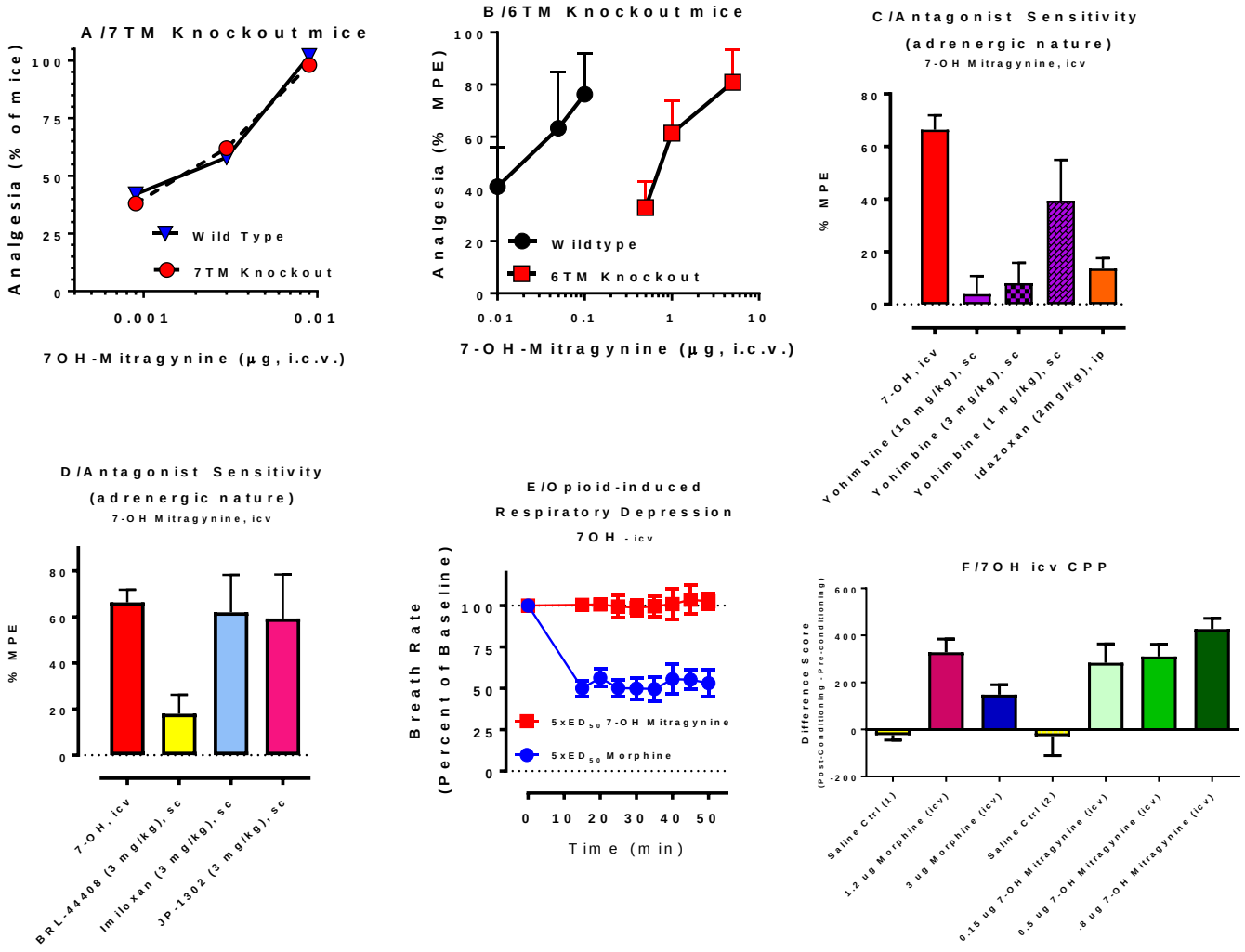
	364	204	>1000	not tested	
	28	1		not tested	
	13.65	20.96	35.81	0.1	0.1
	27.13	30.62	47.17	0.1	0.1
	32.37	81.12	57.74	>10	
DAMGO	3.3	-	-		
U50,488H	-	0.73	-		
DPDPE	-	-	1.39		
NTI	-	-	0.46		
norBNI	-	0.23	-		

[a]Competition studies were performed with the indicated compounds against ^{125}I-IBNtxA (0.1 nM) in membranes from CHO cells stably expressing the indicated cloned mouse opioid receptors. [b]Cumulative dose−response curves were carried out on groups of 129 mice (n = 10) using radiant heat tail-flick assays with indicated compound (icv), and antinociception was tested 15 min later at peak effect. Results from two independent experiments are shown as mean. "-" Denotes not determined or not applicable. Note: In vitro binding assays and analgesia in mice using tail flick assays have been described in our previous papers (Varadi et al *J Med Chem* **2016**; 59(18):8381-97 and Majumdar et

al., *PNAS* **2011**; 108 (49):19778-83).

Supraspinal pharmacology of 7OH

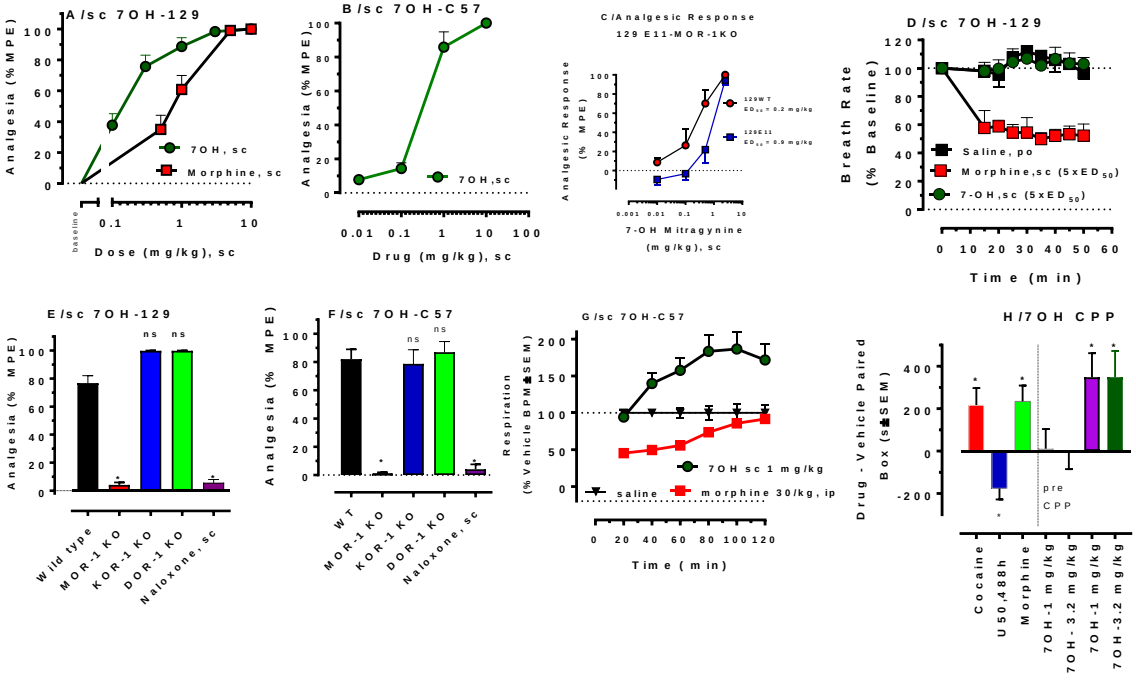
Figure 1. Supraspinal analgesic actions of 7OH are 6TM-MOR-1 dependent mediated by adrenergic α_{2A} receptors. 7OH shows no respiratory depression but shows abuse potential.



7OH mitragynine (7OH) is a unique analgesic. Structurally it is unrelated to morphinan class of opioid analgesics. It lacks a phenol characteristic of most mu opioid template and it has an indole based template. The supraspinal mechanism of 7OH is similar to IBNtxA, a small molecule modulator which labels 6TM-MOR-1 splice variants on which we have published before (Majumdar et al., PNAS 2011; 108 (49):19778-83). 7OH administered intracerebroventricularly (*icv*) like IBNtxA (administered subcutaneously, sc) retains activity in a MOR-1 KO lacking seven transmembrane domain (7TM) MOR-1 receptors (**Figure 1A**). Morphine and other classical mu

opioid agonists show no analgesic activity in KO mice. Again similar to IBNtxA, 7OH icv's analgesia was attenuated in a six transmembrane domain (6TM) MOR-1 KO animal. A 40 fold shift shift in analgesic potency was seen in mice lacking 6TM MOR-1 receptors (**Figure 1B**). We had previously utilized radiolabeled IBNtxA to tease out the 6TM site it labeled (Majumdar et al., J Med Chem 2012; 55(14):6352-62). However in spite of our best attempts we were niether able to make a ^{3}H or ^{125}I version of 7OH. We thus failed to make any progress in characterizing the biochemical site (aim 1 in the grant) characterization of 7OH in the absence of this radiolabel. We however used *in vivo* behavioral assays to probe what other receptor is labelled by 7OH. Non selective adrenergic antagonists like yohimbine and idazoxan were able to fully antagonize the supraspinal analgesia of 7OH in mice (**Figure 1C**). Similarly selective α_{2A} adrenergic antagonists like BRL44408 given systemically attenuated the7OH supraspinal analgesia while α_{2B} and α_{2C} antagonists did not antagonize the analgesia mediated by 7OH (**Figure 1D**). We have earlier reported that analgesia of clonidine and other adrenergics agents like dexmedetomidine is also dependent on 6TM MOR-1 variants (Marrone et al., PNAS 2016; 113(13):3663-8. These drugs like 7OH (K_i=360 nM) have no affinity for the 6TM site labeled by IBNtxA. Therefore, the 6TM mediated mechanism of action of 7OH may be mediated by an adrenergic mechanism. It is not clear if 7OH directly binds the α_{2A} site or complex of α_{2A} with another protein in the absence of any radiolabeling studies or if this α_{2A} mechanism is a downstream event. To summarize, the analgesic actions of 7OH administered supraspinally are mediated by a 6TM-MOR-1 receptors. α_{2A} receptors play a key role in mediating the analgesic actions of 7OH and possibly could account for the 6TM actions seen in vivo. The cross talk between α_{2A} and 6TM will be investigated in the near future. 7OH administered *icv*

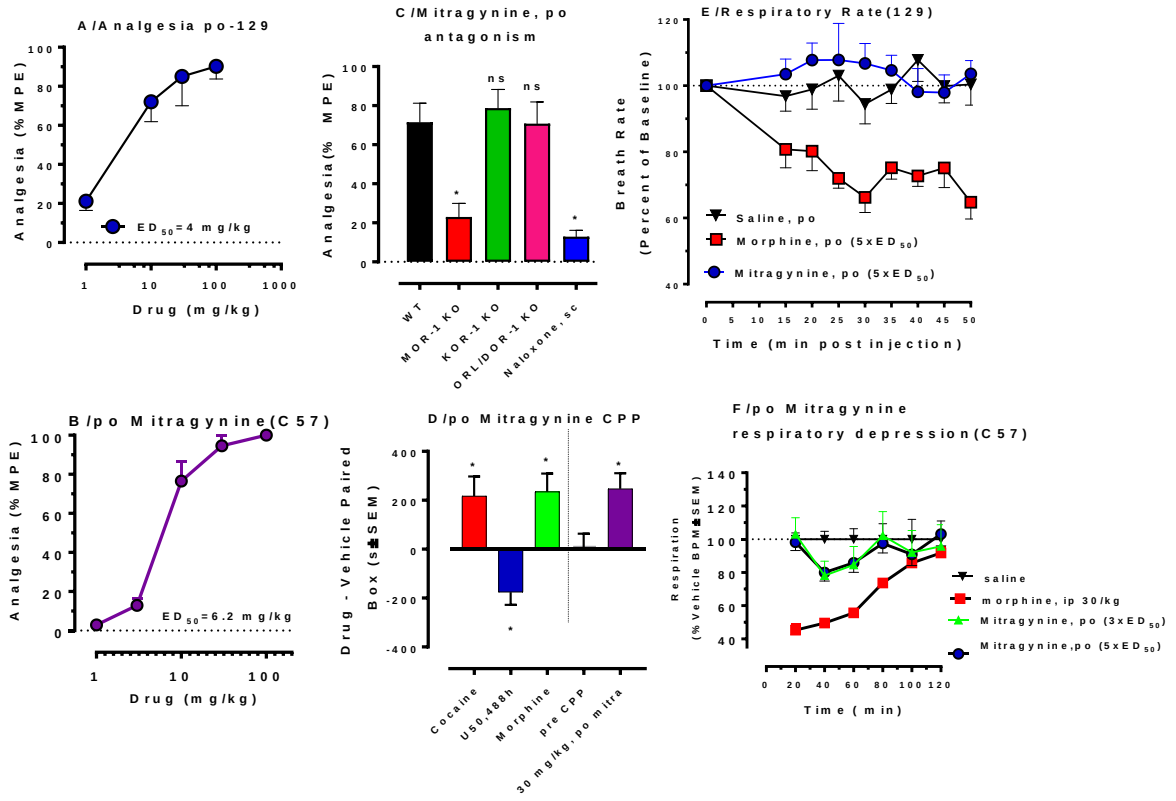
Figure 2. Pharmacology of 7OH



showed a lack of respiratory depressant effect characteristic of classical 7TM MOR-1 agonists like morphine (**Figure 1E**) while inspite of its novel supraspinal mechanism of action it retained the

abuse and addictive properties of opioid analgesics. It showed place preference in CPP assays mice when given *icv* at all tested doses (**Figure 1F**). To summarize, supraspinal analgesic actions of 7OH are 6TM-MOR-1 dependent mediated by adrenergic α_{2A} receptors. 7OH shows no respiratory depression but shows abuse potential. We consider the elucidation of addictive properties of 7OH as one of the negatives to come out from our studies.

Figure 4. *Per os* mitragynine pharmacology



Pharmacology of systemically administered 7OH

To further investigate the action of this drug, we studied it using systemic administration. The pharmacology of 7OH was studied in vivo in mice. 7OH was a potent analgesic (ED_{50}= 0.2 (0.07-0.6) mg/kg, sc in 129 (**Figure 2A**) and 0.33 (0.24-0.46) mg/kg in C57, **Figure 2B**) about 3-10 fold more potent than morphine sc (0.7(0.5-1) mg/kg in 129 and 4.1 (1.67-10.47) mg/kg in C57). To evaluate subtype selectivity analgesia was studied in a mu opioid knock out animals lacking 6-transmembrane domain splice variants of MOR-1 (**Figure 2C**). In contrast to supraspinal analgesia, the analgesia of 7OH systemically was less sensitive to 6TM MOR-1 variants. Only a 4 fold shift in analgesic potency was seen in 6TM MOR-1 KO mice compared to wild type animals in the 129 strain. A 40 fold shift in analgesia was seen when icv 7OH was administered in the same KO animals in the same strain (See **Figure 1B**). The reasons for this discrepancy remain unclear at this point and will be looked at as part of our future investigations. We did evaluate pK studies on sc

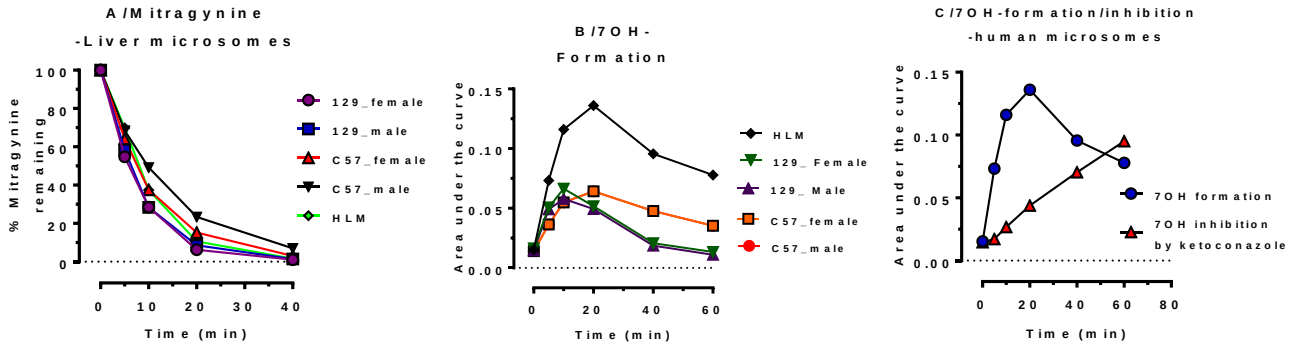
administered 7OH and penetration into the brain was not an issue. The brain:plasma ratio of 7OH was (1.5:1) (data not shown) suggestive of unique central versus peripheral mechanisms of action where peripheral systems overwhelm or is more efficacious. 7OH administered sc showed no respiratory depression in both 129 and C57 strain at doses 5 fold above its analgesic ED_{50} separating the pharmacology from typical mu opioid modulators like morphine (**Figure 2D and G**). The analgesia was dependent on 7TM MOR-1 receptors or classical mu opioid receptors just like morphine or DAMGO and independent of DOR or KOR (**Figure 2E-F**). The universal opioid antagonist naloxone attenuated the analgesia. Like morphine, 7OH showed reward like behavior in a place preference assay (**Figure 2H**). To summarize, 7OH was found to be a potent opioid analgesic whose molecular actions systemically where less dependent on 6TM MOR-1 splice variants and behaved similar to morphine. Like morphine or other 7TM-MOR-1 agonists, 7OH showed reward behavior but lacked the respiratory depression potential seen with typical MOR-1 modulators.

7OH mitragynine mediates the pharmacological actions of mitragynine (kratom)

Given the failure of aim 1 radiolabeling studies we wanted to investigate the origin of 7OH from kratom given it's an oxidized product of mitragynine. We hypothesized that it could be metabolically be made from mitragynine naturally found in kratom. Kratom is a tropical plant which has been in human use for centuries and native to South-East Asia. Kratom leaves are consumed recreationally across America and easily available commercially over the internet (https://www.drugabuse.gov/publications/drugfacts/kratom). Kratom leaves are consumed orally and have been reportedly used in humans both as an analgesic as well as an anti-withdrawal medication. The earliest reports on kratom dates back to1836 when Low observed that kratom could be useful opium substitute in local malayans. The major alkaloid present in kratom is mitragynine. About 66% of total alkaloid content of kratom leaves is mitragynine or 1-2% of kratom leaves dry weight is mitragynine. We have previously shown that mitragynine is weak mu opioid binder (K_i=230 nM) and agonist (EC_{50}=201 nM) (Varadi et al J Med Chem 2016; 59(18):8381-97). Given its weak potency it was rather surprising how it could act as an analgesic unless metabolism to a putative more active compound was involved. 7-OH mitragynine is an analog of mitragynine. Since the chemical synthesis of 7OH from mitragynine involves oxidation, we wondered if mitragynine metabolizes to 7OH in an endogenous setting.

a) Mitragynine metabolizes to 7OH in liver microsomes:

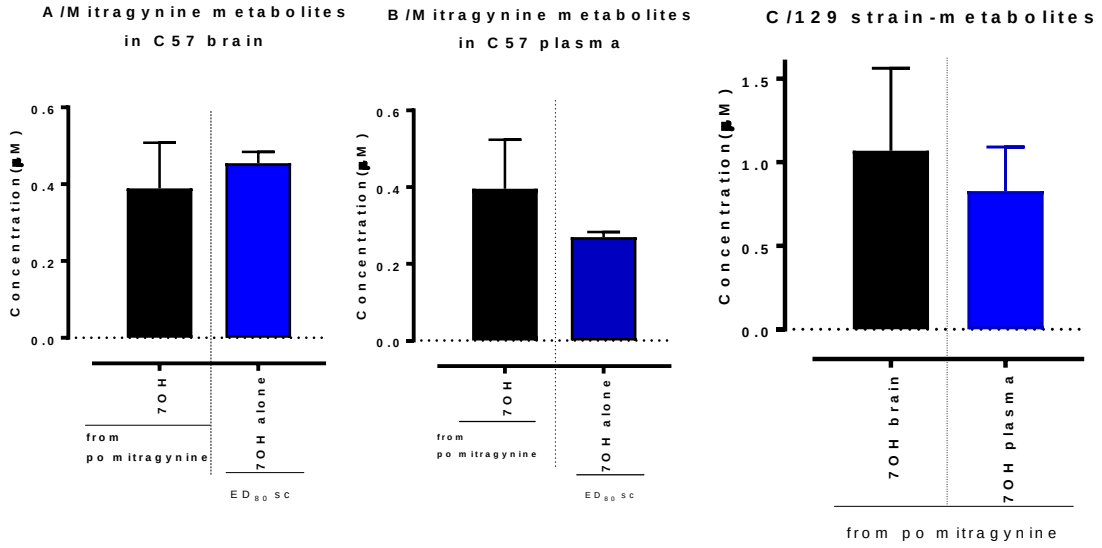
Figure 3. Microsomal metabolism of mitragynine to 7OH



We studied the metabolism of mitragynine in liver microsomes from mice (C57 and 129). Our studies conclusively show mitragynine is metabolically labile in liver microsomes of both male and female mice as well as human liver microsomes (**Figure 3A).** The formation of 7OH from mitragynine was also confirmed in these microsomal assays (**Figure 3B**). We also probed if CYP3A was involved in the conversion of mitragynine to 7OH. The formation of 7OH from mitragynine was blocked by CYP3A inhibitor ketoconazole (**Figure 3C**).

b) *Orally administered mitragynine metabolizes to 7OH in mice:*

Figure 5: Formation of 7OH from mitragynine in mice



We next wanted to probe the formation of 7OH from mitragynine in mice and evaluated pharmacokinetic studies on mitragynine administered orally (po). Before carrying out pK studies we wanted to see determine analgesia of mitragynine in mice. In our hands in C57 mice, po mitragynine was a potent analgesic in 129 (ED_{50}=4(2.21-7.16) mg/kg**), Figure 4A**) as well as C57

mice (ED_{50}=6.25 (4.49-8.68) mg/kg), **Figure 4B**) in radiant tail flick analgesia assay. Morphine administered subcutaneously (sc) in same strains was 0.7(0.5-1) mg/kg and 4.1 (1.67-10.47) mg/kg. Thereby mitragynine had analgesic potency similar to morphine. The peak effect was at 20 min and 15 min for po mitragynine in both C57 and 129 strain respectively (data not shown). To further probe the metabolism in mice, pK studies in mice were carried out at ED_{80} analgesic doses of mitragynine with drug administered po at 30 mg/kg, po in C57. Brain and plasma from mice were taken out at the peak analgesic effect of 20 min and concentrations of 7OH was measured in both brain as well as plasma samples. The pK of sc administered 7OH at ED_{80} dose of 1 mg/kg was also carried out to validate concentrations in brain as well as plasma. The results show that 400nM of 7OH is seen in both brain as well as plasma of C57 mice. The levels of 7OH seen match up well when 7OH is administered alone sc at the ED_{80} analgesic dose (See **Figure 5**). To confirm formation of 7OH from mitragynine, pK studies were also carried out in the 129 strain with po mitragynine, here again formation of 7OH was seen in both plasma and brain. The levels of 7OH seen were 8 fold more than the agonistic potency of 7OH at MOR-1 (EC_{50}=53 nM) and clarified why mitragynine is orally so much more potent as an analgesic by virtue of its first-pass metabolism to 7OH. The results thus validated the pharmacological actions of kratom administered orally involves CYP3A mediated conversion of mitragynine to 7OH. ***NOTE: The studies proposed in this section where not proposed in our original grant. But given the grant was on 7OH we felt obligated to understand the biosynthetic origin of 7OH from kratom leaves easily available across America. Kratom is banned by the US army and is under DEA radar because of its opioid like actions.***

c) Pharmacology of po mitragynine:

We next studied the pharmacology of po administered mitragynine in mice. Like 7OH, mitragynine analgesia is mediated by MOR-1 only and not DOR-1 and KOR-1 (**Figure 4C**). Analgesia was attenuated in MOR-1 KO mice only and reversed by the universal opioid antagonist naloxone. At doses 5 fold above the analgesic doses po mitragynine showed less respiratory depression in both C57 as well as 129 strains (**Figure 4E-F**). Po mitragynine showed place preference similar to 7OH sc (**Figure 4D**). Thus po mitragynine pharmacology was similar to 7OH sc again suggestive of mitragynine metabolism to 7OH and 7OH as the modulator responsible for mitragynine or kratom oral analgesic actions.

3) S*ignificant results or key outcomes*

The key positive findings of this grant are 7OH is potent analgesic devoid of respiratory depression separating its analgesic actions from classical mu opioid modulators like morphine, oxycodone and fentanyl. 7OH has distinct central analgesic mechanism which is dependent on the adrenergic α_{2A} receptors which in turn is dependent on 6TM-MOR-1 sites. Systemically, 7OH is less dependent on 6TM/MOR-1 receptors and primarily acts through 7TM-MOR-1 sites just like morphine. 7OH is the major metabolic product of mitragynine, the alkaloid responsible for all kratom's actions recreationally consumed by millions of Americans.

The negative findings are 7OH retains addictive and abuse liability similar to morphine irrespective of its route of administration. Both central as well as systemic receptor mechanisms lead to

addiction liability. More conclusive studies where 6TM mechanisms can be separated from 7TM MOR-1 mechanisms may lead to better understanding of the target labeled by 7OH.

What opportunities for training and professional development has the project provided?

Rajendra Uprety a postdoctoral fellow in the laboratory worked on this project. Rajendra was trained as a chemist but this project expanded his horizon beyond chemistry and he became an expert in drug metabolism and neuropharmacology of opioids in general. Rajendra did have one-on-one multiple meetings with his mentors including me. The work will be soon be presented and published in peer reviewed journals.

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?

Nothing to Report

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Mitragynine, among nearly 40 alkaloids found in kratom, is the major alkaloid (approximately 1-2% of dry leaves matter representing to 66% of total alkaloid content) thought to be responsible for its biological actions. The mechanism of molecular actions on kratom and its alkaloids remain largely unknown despite its use in humans over centuries and numerous related scientific studies. In 2016, the US Drug Enforcement Administration (DEA) put out a notice of intent to Mitragynine and its oxidized analog, 7OH mitragynine into Schedule I of the Controlled Substances Act (CSA). The notice was withdrawn two months later after public and national media outcry (https://www.scientificamerican.com/article/kratom-drug-ban-may-cripple-promising-painkiller-research/, see this for example where this author is mentioned). The US Food and Drug Administration (FDA) issued a statement on kratom on the 6th of February, 2018 describing its potential for abuse, addiction, and serious health consequences including death, ultimately concluding that kratom use has no health benefits.[28] The recent status of kratom based products use in the U.S. shall be estimated that there are about 10,000 retail outlets with an estimated market value of 207 million. We have also looked at the metabolites of mitragynine at peak effect in mice as a key step to understand the mechanism of its biological action. We evaluated the analgesic actions of kratom extract, its major alkaloid mitragynine and assess the key roles that these metabolites play in the biological actions. With these studies in mice, we report that analgesic action of kratom is mediated by mitragynine and its first pass metabolic products namely 7OH mitragynine (7OH) through MOR-1 while showing attenuated respiratory depression while still displaying reward behavior in mice. 7OH also has supraspinal adrenergic mechanisms which are dependent 6-transmembrane domain splice variants of the mu opioid receptor.

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Kratom leaves are consumed recreationally across America. Unlike marijuana, kratom is not regulated or controlled and easily available on the internet. There is very little 7-OH mitragynine present in kratom leaves on which this grant is based on. However we find that mitragynine is metabolized to 7OH mitragynine in mice and that leads to potent analgesic which is safe (as it leads to less respiratory depression) but is addictive. The grant mechanism thus has led to identification of the major chemical entity responsible for kratom's biological actions. The most recent report on kratom tea consumption was covered by CNN: https://www.cnn.com/2018/11/07/health/kratom-tea-neonatal-abstinence-syndrome-study/index.html. We believe consumption of kratom tea in this particular case led to withdrawal in the neonate because of conversion of mitragynine to the more potent 7OH mitragynine. Mitragynine is a weak opioid modulator while 7OH is potent analgesic about 10 fold more potent over morphine. So this study has significant impact on US society dealing with an opioid epidemic and looking at alternate ways to circumvent it.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to Report

Changes that had a significant impact on expenditures

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Nothing to Report

Books or other non-periodical, one-time publications.

Nothing to Report

Other publications, conference papers and presentations.

Chemistry and Pharmacology of mitragyna speciosa-invited talk at Drug Discovery Chemistry, April 8-12th, San Diego, CA.

- **Website(s) or other Internet site(s)**

Nothing to Report

- **Technologies or techniques**

Nothing to Report

- **Inventions, patent applications, and/or licenses**

Nothing to Report

- **Other Products**

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*Susruta Majumdar*
Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*6*
Contribution to Project:	*Dr. Majumdar supervised research associates, technicians and collaborated with Drs. Pasternak, Pan and Pintar.*
Funding Support:	*NIDA*

Name:	*Rajendra Uprety*
Project Role:	*Research Associate*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*2.91*
Contribution to Project:	*Dr. Uprety synthesized 7OH mitragynine and its analogs.*
Funding Support:	*NIDA*

Name:	*Ms. Amanda Hunkule*
Project Role:	*Research Technician*
Researcher Identifier (e.g. ORCID ID):	*NA*
Nearest person month worked:	*2.91*
Contribution to Project:	*Ms. Hunkule carried out in vitro/ in vivo characterization of 7OH and analogs.*
Funding Support:	*NIDA*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Yinxian Pan was awarded 2 grants:

1 R21 DA046714-01 (PI: Pan) 9/30/2018 - 8/31/2020 1.00 calendar
NIDA $ 161,199
Mapping mu agonist-induced receptor-protein interactions for OPRM1 7TM variants
Goals: Multiple full-length carboxyl terminal variants of mu opioid receptors generated through alternative pre-mRNA splicing of a single copy mu opioid receptor gene (OPRM1) play important roles in diverse actions of mu opioids in animals and humans. The proposed studies will explore molecular mechanisms and functions of the OPRM1 carboxyl terminal variants in mu opioid actions.
Specific Aims: Specific Aim 1: Mapping mu agonist-induced receptor-protein interactions for *Oprm1* C-terminal 7TM variants using APEX2-TMT proteomics
Specific Aim 2: Validate selected candidate receptor-interacting proteins
Agency Contact: Penny L Greene **Email**: penny.greene@nih.gov

5 R01 DA042888-02 (PI: Pan) 9/1/2017 - 5/31/2022 3.00 calendar
NIDA $ 240,300
Alternative pre-mRNA splicing of mu opioid receptor gene and mu opioid actions
Goals: The proposed studies aim to investigate mechanism and functions of OPRM1 alternative splicing, and provide potential targets for developing novel therapeutics for the treatment of pain and drug abuse.
Specific Aims: Specific Aim 1: Decoding molecular mechanisms underlying *OPRM1* 3' splicing
Specific Aim 2: Investigating the role of the *OPRM1* 3' splicing in mu opioid actions
Agency Contact: Cheryl A Nathaniel **Email**: nathanic@mail.nih.gov

What other organizations were involved as partners?

John E Pintar, Department of Neuroscience and Cell biology, Rutgers Robert Woodward Medical School
Collaboration: Dr. Pintar provided MOR, KOR and DOR KO mice. Analgesia of mitragynine, 7OH, 7OH analogs and kratom was tested in his laboratory.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *N/A*

QUAD CHARTS: *N/A*

9. APPENDICES: *None*