Site Selective C-H Functionalization of *Mitragyna* Alkaloids Reveals a Molecular Switch for Tuning Opioid Receptor Signaling Efficacy

Srijita Bhowmik,[1#] Juraj Galeta,[1#,8] Václav Havel,[1] Melissa Nelson,[4,5] Abdelfattah Faouzi,[7] Benjamin Bechand,[1] Tomas Fiala,[1] Amanda Hunkele,[6,7] Andrew C. Kruegel,[1] Susruta Majumdar,[7] Jonathan A. Javitch,[4,5] Dalibor Sames[1,2,3]*

[1]Department of Chemistry, Columbia University, New York, NY, United States

[2]NeuroTechnology Center at Columbia University, New York, NY, United States

[3]The Zuckerman Mind Brain Behavior Institute at Columbia University, New York, NY,

[4]Department of Psychiatry, and Department of Pharmacology, Columbia University, New York, NY, United States

[5]Division of Molecular Therapeutics, New York State Psychiatric Institute, New York, NY, United States

[6]Department of Neurology and Molecular Pharmacology, Memorial Sloan Kettering Cancer Center, NY, NY 10021.

[7]Center for Clinical Pharmacology, St Louis College of Pharmacy and Washington University School of Medicine, St Louis, MO 63110.

[8]Current affiliation: The Institute of Organic Chemistry and Biochemistry of the Czech Academy of Science (IOCB Prague), Prague 6, Czech Republic

Abstract

Mitragynine is the most abundant alkaloid component of the psychoactive plant material "kratom", which according to numerous anecdotal reports shows efficacy in self-medication for pain syndromes, depression, anxiety, and substance use disorders. We developed a new synthetic method for selective functionalization of the unexplored C11 position of the mitragynine scaffold (C6 position in indole numbering) via the use of an indole-ethylene glycol adduct and subsequent iridium-catalyzed borylation. We discovered that C11 represents a key locant for fine-tuning opioid receptor signaling efficacy. In the 7-hydroxymitragynine (7OH) series, the high efficacy parent compound was transformed to an equipotent low efficacy agonist by introducing a

fluorine substituent in this position (11-F-7OH), as demonstrated in vitro at both mouse and human mu opioid receptors (mMOR/hMOR) and in vivo in mouse analgesia tests after systemic administration. Low efficacy opioid agonists are of high interest as candidates for generating safer opioid medications with mitigated adverse effects.

Introduction

In search of molecules with profound clinical effects in the area of CNS disorders and ability to repair synaptic function in the brain, we have been led to atypical modulators of endogenous opioid signaling.[1–4] In this context, we became interested in the psychoactive plant *Mitragyna speciosa* that has been used for centuries in Southeast Asia for treatment of pain, fatigue, opium dependence, and a number of other ailments. In the U.S., the use of the dry leaf material, known as "kratom", has been on the rise in the last decade, along with the number of anecdotal reports that point to the efficacy of kratom in a range of disorders with limited therapeutic options, including opioid dependence, treatment-resistant depression, anxiety, and pain syndromes.[5–10] A number of alkaloids in this plant, including mitragynine (MG) and its oxidation product 7-hydroxymitragynine (7OH, Fig. 1), have been found to bind to opioid receptors and represent novel molecular scaffolds for the development of opioid receptor modulators.[11,12] Mitragynine, as an indole alkaloid, shows no structural resemblance to traditional morphine-type compounds and represents an atypical opioid ligand with distinct signaling properties and physiological effects compared to clinically used opioid analgesics. For example, we have shown that mitragynine is a partial MOR agonist with a potential bias for G protein signaling (showing no β-arrestin-2 recruitment) in cell-based assays.[13,14] Further, we have found that 7OH is a more potent partial MOR agonist compared to mitragynine, and that it acts as a potent analgesic in mice.[13,14] The relative activities of mitragynine and 7OH are highly

relevant, as we recently reported that 7OH is formed *in vivo* from mitragynine and mediates mitragynine's analgesic effects in mice.[15] In additional preclinical studies, others have found that mitragynine exhibits antinociceptive effects in dogs comparable to those of codeine but with less respiratory depression,[16] and that the compound is not self-administered by rats, but instead, inhibits self-administration of morphine and heroin.[17,18]

The study and development of safer opioids is a long-standing scientific and societal goal, and a part of the scientific strategy proposed by the NIH initiative to address the opioid crisis.[19,20] Further, there is a growing interest in the use of opioid receptor modulators as medicaments for depression and anxiety disorders (and other psychiatric diseases).[1,21] Therefore, safer opioid modulators represent promising medications for disorders spanning a wide spectrum of physical and emotional pain. Accordingly, further understanding of kratom and its alkaloids has major implications for public health.

Our previous report provided a basic SAR map of mitragynine, in terms of *in vitro* opioid receptor pharmacology, with respect to the substituents on the saturated rings of mitragynine. This work was enabled by the total enantioselective synthesis of mitragynine developed in our laboratories.[13] Several other research teams have also reported total syntheses of mitragynine and related compounds.[22–26] These *de novo* synthetic approaches offer nearly unlimited exploration of complex mitragynine-related molecules, but they are labor intensive due to the high number of synthetic steps. Thus, the need to access many different derivatives at multiple positions demands more efficient synthetic strategies. Mitragynine can be extracted from kratom leaf matter in multigram quantities (~ 1% of dry kratom mass[12,13]), and therefore, there is a strong incentive to develop synthetic methods for direct functionalization of mitragynine, for example, via late-stage C-H functionalization, rather than laborious total synthesis.

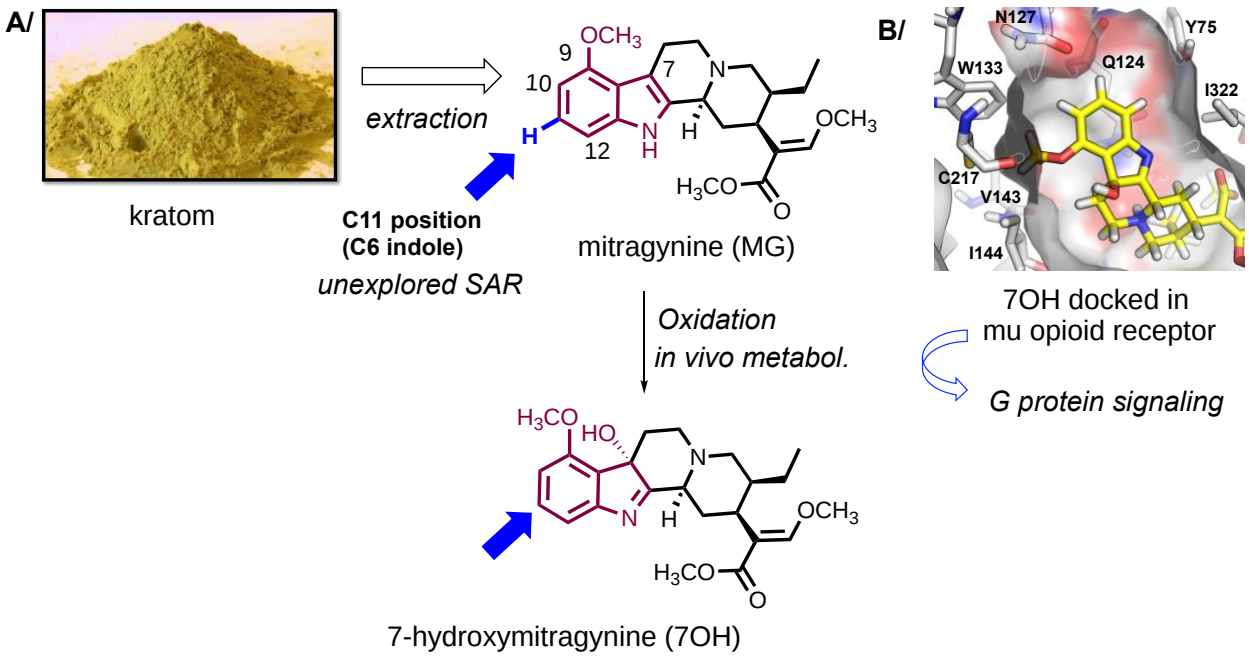

Fig 1. The rationale for selective C-H bond functionalization of mitragynine (MG) and 7-hydroxymitragynine (7OH). A/ MG is readily obtained in multigram quantities from kratom powder by extraction, hence there is an incentive to develop selective functionalization of mitragynine and related scaffolds via late-stage C-H bond functionalization. Specifically, the C11 position has not been explored in terms of mu-opioid receptor (MOR) signaling and other biological effects, due to the lack of chemistries for functionalization of this position. **B/** The top right panel shows a docking pose of 7OH in human MOR highlighting the aromatic ring and the proposed binding pocket in the receptor.

With respect to derivatization of the indole nucleus, the methoxy group at the C9 position is synthetically accessible by selective demethylation and subsequent functionalization of the free phenol.[14] For the adjacent C10 position, a small series of compounds has been prepared,[12,27] while the C11 position remained unexplored due to the lack of functionalization methods (see below). Our preliminary docking studies suggested that relatively small substituents (e.g., F, Cl, and Me) would be tolerated at C11 (Fig 1).[13] However, it is presently difficult to predict the effect of such substituents on opioid receptor activation potency, efficacy or signaling bias, demonstrating the need for new C-

H bond functionalization approaches compatible with the structural complexity presented by *Mitragyna* alkaloids.

More than a decade ago, we formulated the general concept of "C-H bonds as ubiquitous functionality" and demonstrated the strategic impact of C-H functionalization in both the construction of molecular frameworks and modification of existing complex cores.[28–31] For the latter, termed "complex core diversification" or "late-stage functionalization", the synthetic power of this concept is readily apparent as the positional (as well as stereoisomeric) analogs of complex starting materials are accessed with high efficiency when compared to lengthy *de novo* approaches.[28–34] Late-stage functionalization approaches have since been widely adopted and become a common part of chemists' armamentarium.[35–39] These concepts are being extended beyond C-H bond to include the possibilities of skeleton modification via C-C and other bond activation.[40–43]

In this paper, we describe application of these concepts to *Mitragyna* alkaloids in the context of mapping their neuropharmacology. We introduce a strategic temporary modification of the mitragynine alkaloid skeleton ("complex core restructuring"), to create a distinct chemotype disposed toward the desired C-H functionalization chemistry, namely C11 functionalization of mitragynine's indole nucleus. In this manner, we offer a solution that provides for rapid and selective functionalization of the aromatic ring of mitragynine at the C11 and C12 positions (C6 and C7 positions in indole numbering). We subsequently found that these novel analogs enable fine tuning of opioid receptor signaling efficacy, which represents one of the currently most promising strategies for creating safer opioid therapeutics.

Results

C-H Borylation of Mitragynine Yields C12-substituted Analogs. Mitragynine is a complex natural product of a corynanthe alkaloid type decorated with a number of functional groups, including enol ether, ester, tertiary amine, indole amine and aromatic methyl ether, arranged in a specific constitutional and geometrical configuration that underlies its opioid activity. It therefore poses an exciting challenge for late-stage functionalization. In this study, we focused on functionalization of the indole arene ring (the rationale is discussed in Introduction). Ir-catalyzed arene C-H borylation was selected due to its wide substrate scope, including basic heterocyclic compounds.[44,45]

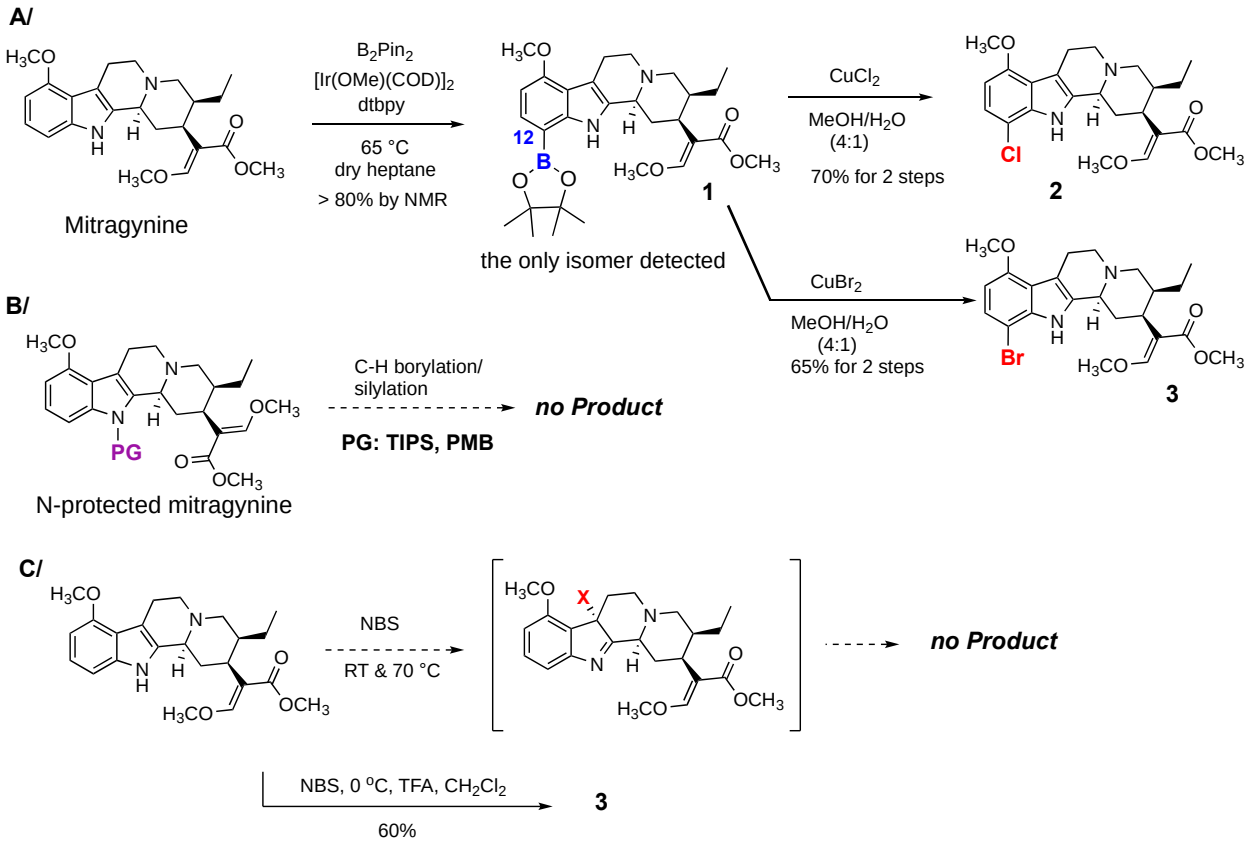

Fig 2. Examination of direct functionalization of mitragynine indole nucleus. **A/** C-H borylation of mitragynine provides exclusively C12-derivatives consistent with indole directing effects (C7 in indole numbering). The yields are a general range based on > 10 repetitions by two experimenters. **B/** The positional selectivity cannot be re-routed to C11-functionalization via previously reported TIPS protection of the indole nitrogen. **C/** The C3-indole halogenation-rearrangement process[46] is not applicable to mitragynine. Bromination under acidic conditions gave 12-bromo-mitragynine as the major product.

After exploration and optimization of reaction conditions (Supplementary Table 1), we found that borylation is compatible with the mitragynine chemotype. Using B_2pin_2 under catalytic $[Ir(COD)OMe]_2$ and dtbpy as the ligand in heptane at 65 ^{o}C for ≥17 h C12-boronate ester **1** was obtained as the single isomer (Fig 2A). This compound proved to be unstable and decomposed during silica gel column chromatography, and we thus confirmed its formation in the crude material via NMR, TLC and MS. The crude ester **1** was transformed to several derivatives without further purification in good yields. Namely, 12-chloro- (**2**) and 12-bromo-mitragynine (**3**) were prepared in two steps in 70% and 65% yield, respectively (Fig 2A).[47] The observed regioselectivity is consistent with the known directing effect of the indole amine and C7-borylation of 2,3-disubstituted indoles.[48,49]

Known C6 Indole Derivatization Methods Do Not Produce C11 Analogs of Mitragynine. After achieving C12 functionalization we turned our attention to the C11 position in mitragynine (C6 position in indole numbering), which remained unexplored due to the limited reaction repertoire available for C6-indole functionalization.[50] We considered several known approaches for C6 indole functionalization to gain access to these novel derivatives. Specifically, we examined the possibility of blocking the indole nitrogen's directing effect with a protecting group, along the path demonstrated in the context of tryptophan substrates, where protection with a bulky TIPS group led to C6-borylation under optimized C-H borylation conditions (Fig 2B).[51] However, this approach was not applicable to mitragynine as the TIPS-protected mitragynine decomposed under the catalytic conditions. On the other hand, PMB-mitragynine was either unreactive or formed undesired products under the reaction conditions. Boc-protected mitragynine provided the C11 boronate ester, but the subsequent functionalization reaction was

inefficient (Supplementary Fig. 3 and 4). The latter approach was not further optimized as an entirely different protection scheme was found to be successful (see below).

Another avenue of enquiry was inspired by an intriguing thermal rearrangement of 3-bromoindolenines to 6-bromoindoles,[46] which had been successfully applied in complex substrates *en route* to stephacidine A alkaloids.[40] However, this approach also failed in the mitragynine scaffold under similar reaction conditions (Fig 2C); namely, mitragynine was unreactive in the presence of NBS under neutral conditions, while under acidic conditions, 12-bromo-mitragynine **3** was the major product (Fig 2C). Apparently, the C9 methoxy group exerts an activating and directing effect in the benzene ring of the indole nucleus once the bromination reagent is sufficiently activated.[52]

Finally, the methods relying on remote directing effect of groups attached to the indole nitrogen, such as the copper catalyzed C6 functionalization of indoles using the phosphinimide directing group,[53] were not examined, as 1) the deprotection step involves harsh reduction conditions (e.g. $LiAlH_4$) incompatible with mitragynine's functionalities, or 2) the C6 functionalization is too restrictive in terms of the functionalization chemistry or substrate requirements.[54,55]

Complex Core Restructuring: C-H Borylation of Mitragynine-Ethylene Glycol Adduct (MG-EG). Direct functionalization of mitragynine gave C12-halo or -boronate ester analogs, while re-routing this regioselectivity was unsuccessful via either catalyst optimization or indole amine protection. The known methods for C6 indole functionalization – that possess the required generality and scope to pursue systematic SAR studies – failed to provide an efficient synthetic route to C11-substituted mitragynine analogs.

We therefore resorted to changing the reactivity of the mitragynine core by "removing" the indole double bond (2,3-π bond in indole numbering) by either reduction or oxidation, or oxidation followed by rearrangement (Fig 3). 7OH was examined as an indolenine substrate in the Ir-catalyzed borylation reaction, but there was no conversion to a borylated product. 7OH can be readily rearranged to mitragynine pseudoindoxyl with zinc triflate as reported by us previously,[14] however this pseudoindoxyl system afforded C12 borylation (Fig 3), while the corresponding 12-boronate ester (not shown) was unreactive in the subsequent halogenation reaction. Next, we investigated the possibility of using a reduced indole substrate, namely dihydromitragynine.[56] With this compound, we observed a partial conversion of starting material to the corresponding 11-boronate ester (50% conversion as determined by ^{1}H NMR). However, the following halogenation reaction gave complex mixtures of mitragynine and 2,3-dihydromitragynine as the major products, and 11-bromo-mitragynine and 11-bromo-2,3-dihydromitragynine as the minor products (Fig 3).

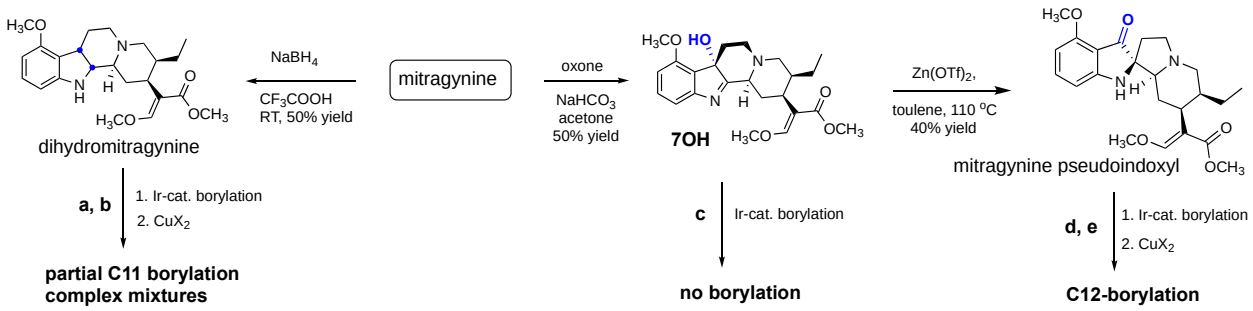

Fig 3. Catalytic borylation of mitragynine analogs with reduced, oxidized, or rearranged indole nucleus. a) B_2pin_2, dtbpy/Me_4-phen, $[Ir(COD)(OMe)]_2$, 65 °C, dry heptane; 50% conversion to 11-boronate ester by ^{1}H NMR. b) $CuBr_2$, MeOH/H_2O (4:1), 80 °C; using the crude borylation mixture, provided mixtures of mitragynine, 2,3-dihydromitragynine (major products, ~ 60% by ^{1}H NMR) and 11-bromo-dihydromitragynine and 11-bromo-mitragynine (minor products, ~ 30% by ^{1}H NMR). c) B_2Pin_2, dtbpy/Me_4-phen, $[Ir (COD)(OMe)]_2$, 65 °C, dry heptane; no borylation product was detected. d) B_2Pin_2, dtbpy/Me_4-phen, $[Ir (COD)(OMe)]_2$, 65 °C, dry heptane; complete conversion to C12-boronate by ^{1}H NMR). e) $CuBr_2$, MeOH/H_2O (4:1), 80 °C; no reaction.

Lastly, we focused on oxidative attachment of ethylene glycol to the indole nucleus, rendering a distinct chemotype: mitragynine-ethylene glycol (MG-EG, Fig 4A). This unusual

compound was first introduced by Takayama and co-authors to provide access to the 10-substituted analogs via electrophilic aromatic substitution, and this scaffold showed potent activity at MOR.[12,57] Aside from these studies, this indole-ethylene glycol adduct has not been explored in the context of new indole chemistry. We became interested in this compound as the ethylene glycol group not only masks the indole, which is particularly relevant in this study, but also dramatically alters the shape of the entire alkaloid scaffold. We crystallized MG-EG from methanol and confirmed its 3D-structure (Fig 4B, CCDC 1905559), which revealed the chair-like conformation of the dioxane ring and the propeller-like arrangement of the three rings converging on the C-C bond of the former indole ring.

When we applied the catalytic borylation protocol (with the dtbpy ligand) to MG-EG, a 1:1 ratio of C11 (**4a**) and C12 (**4b**) products was observed (Fig 4C), clearly confirming the different reactivity property of this chemotype, and unlocking the possibility of optimizing the reaction conditions to favor C11 functionalization.[51,58] Indeed, ligand screening (Supplementary Table 3) showed that Me_4-phen as the ligand, together with B_2pin_2 and $[Ir(COD)OMe]_2$ in heptane at 65 ^{o}C, gave the 11-borylated product (**4a**) as the major product with ratio > 16:1 of C11 (**4a**) : C12 (**4b**) products (Fig 4C, confirmed by the subsequent bromination).

We reasoned that by converting the indole nucleus to an unusual aniline derivative, the directing effects of the nitrogen would be diminished while its steric effects would gain importance, resulting in functionalization of the unhindered C11 position (after catalyst-ligand optimization). Boronates **4a** and **4b** were converted to the bromo derivatives **5** and **6** respectively[47] using copper(II) bromide. Thus, this sequence provided two 11-substituted MG-EG intermediates - boronate **4a** and bromide **5** - with versatile synthetic potential.

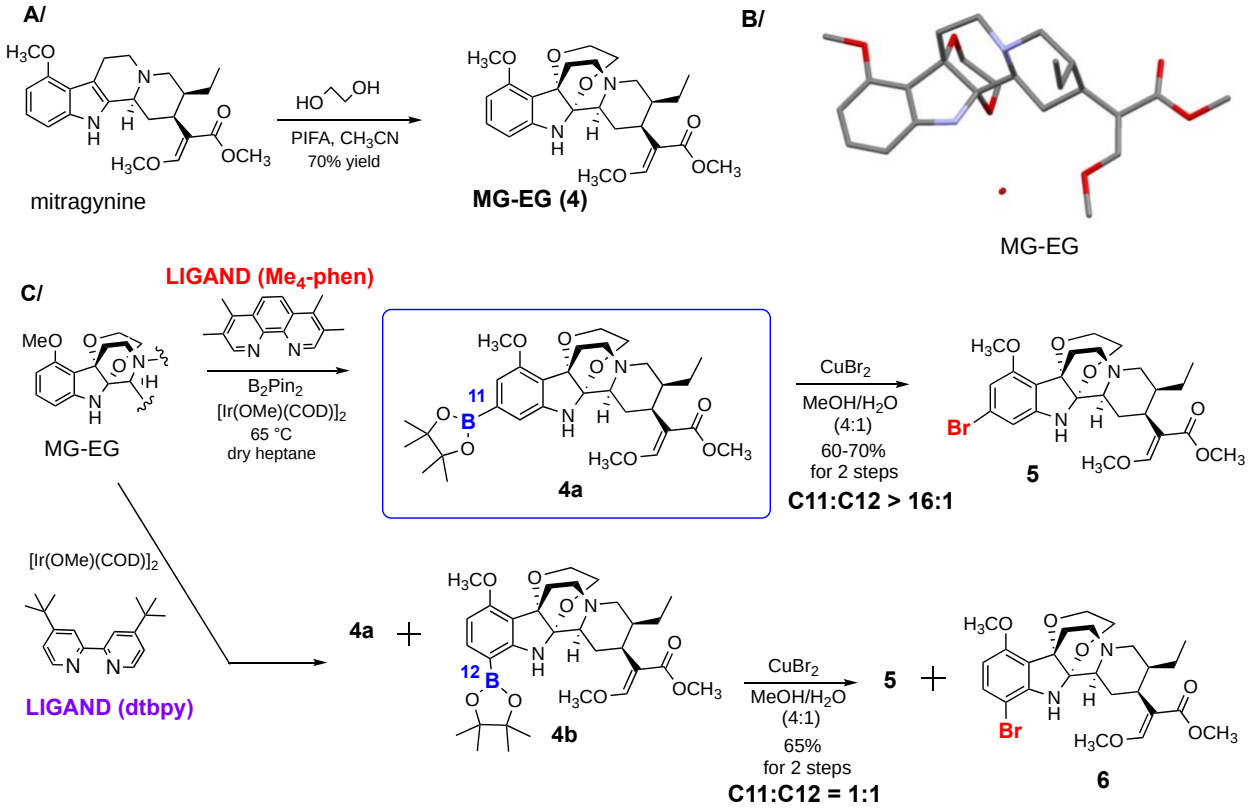

Fig 4. C11-selective C-H borylation via temporary conversion of mitragynine to mitragynine-ethylene glycol adduct (EG-MG). A/ One-step synthesis of MG-EG, a stable derivative of mitragynine. **B/** The 3D structure of MG-EG was confirmed by X-ray crystallography. ORTEP representation of MG-EG structure is shown without hydrogen atoms for clarity. **C/** C-H borylation of MG-EG gives a mixture of C11- and C12-boronates and the subsequent functionalization products. The high C11 selectivity was achieved by ligand optimization. The ratio was determined by ^{1}H NMR of crude reaction mixtures. The yields, given as a general range, are based on > 20 repetitions by 3 experimenters, on a scale up to 100 mg of substrate.

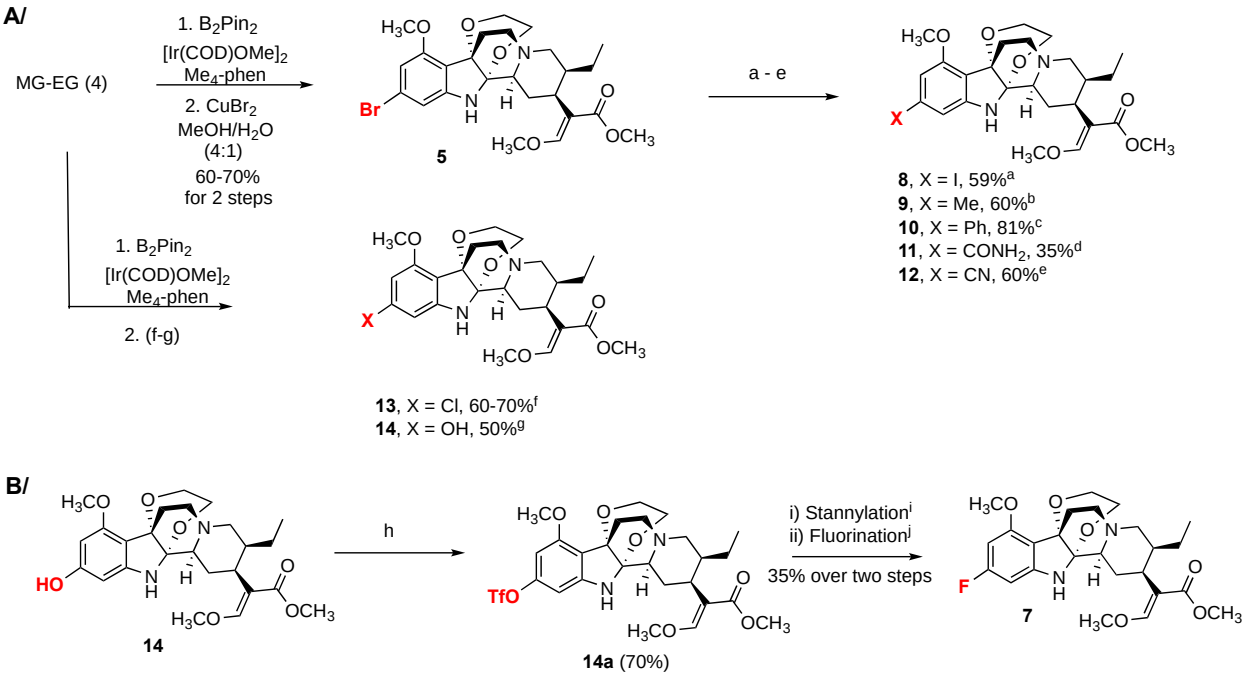

Fig 5. The MG-EG scaffold is compatible with a wide array of functionalization chemistries. Synthesis of C11-substituted analogs of MG-EG. a) NaI, CuI, N,N'-dimethylethylenediamine, 110 ^{o}C, 22 h. b) $Pd_2(dba)_3$, XPhos, DABAL-Me_3, THF, 60 ^{o}C, 2 h. c) $Pd(dppf)Cl_2 \cdot CH_2Cl_2$, $PhB(OH)_2$, CsOAc, THF, 70 ^{o}C, 7 h. d) $Pd(OAc)_2$, dppf, $Co_2(CO)_8$, DIPEA, NH_4Cl, imidazole, dioxane, 90 ^{o}C, 15 h. e) $Pd_2(dba)_3$, Zn dust, $Zn(CN)_2$, $[HPtBu_3]BF_4$, DMF, RT, 3 h. f) $CuCl_2 \cdot 2H_2O$, MeOH : H_2O (4:1), 80 ^{o}C, 12 h. g) H_2O_2, THF, 0 ^{o}C to RT, 30 min. h) $PhNTf_2$, DIPEA, DMF, 50 ^{o}C, overnight. i) ($^{n}Bu_3Sn)_2$, $Pd(PPh_3)_4$, LiCl, dioxane, 100 ^{o}C, overnight. j) AgOTf, F-TEDA-PF_6, RT, 20 min. The yields are based on > 2 repetitions.

Preparation of C11-Substituted Analogs of Mitragynine and Related Scaffolds. The 11-boronate ester (**4a**) was converted to the novel compounds 11-Cl-MG-EG (**13**) and 11-OH-MG-EG (**14**) using the appropriate substitution methods (Fig 5A).[47,60] For preparation of additional derivatives, the 11-Br-MG-EG (**5**) served as the key intermediate enabling preparation of compounds **7**-**12** in one synthetic step (Fig 5A), where X = I (**8**),[61] Me (**9**),[62] Ph (**10**),[63] $CONH_2$ (**11**),[64] and CN (**12**).[65] Considering the complexity of these compounds, the yields were satisfactory and more than sufficient to produce practical amounts of the compounds for preliminary pharmacological evaluation. However, the synthesis of 11-F-MG-EG (**7**) proved more difficult compared to its other halogen relatives. Relevant reported procedures were not effective in this context; for example, direct fluorination of 11-boronate ester **4a** using copper-

mediated fluorination with ($_t$BuCN)$_2$CuOTf or Cu(OTf)$_2$py$_4$ failed,[66,67] so did an indirect sequence of stannylation-fluorination.[68] Deoxyfluorination of the corresponding phenol **14** using PhenofluorMix was also unsuccessful.[69] Eventually, 11-F-MG-EG (**7**) was synthesized from bromide **5** via the sequence of stannylation and fluorination. However, this route yielded inseparable mixtures of MG-EG (**4**) along with the desired compound (not shown). Ultimately, compound **7** was obtained in pure form from phenol **14** through triflation, stannylation, and fluorination (Fig 5B).[70] The triflate **14a** represents an alternative intermediate (to the corresponding bromide **5**) for further functionalization; for example, carboxamide **11** was prepared via the latter method in 57% yield (versus 35% via the bromide, Supplementary Information).

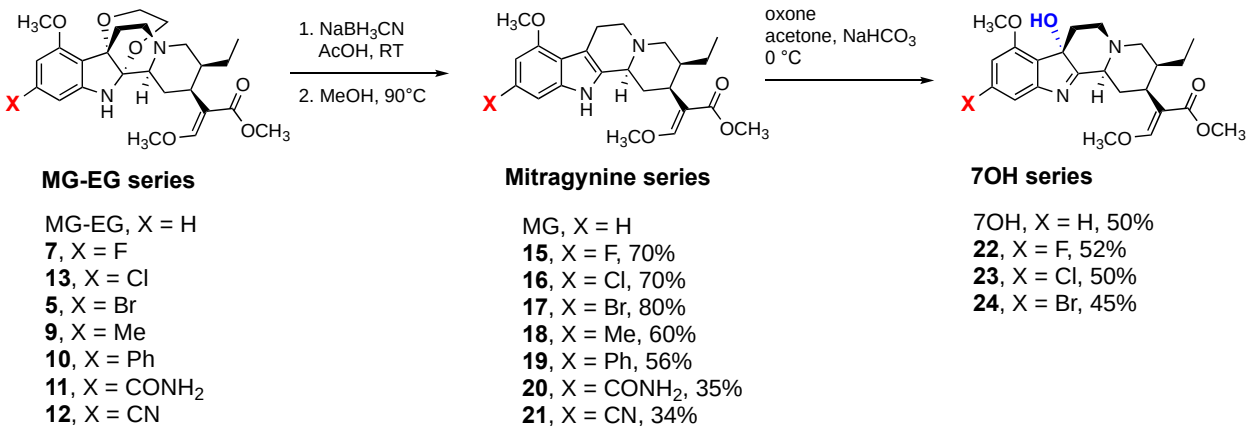

Fig 6. Reductive removal of ethylene glycol. This step renders 11-substituted mitragynine analogs, which in turn can be oxidized to the corresponding 7OH derivatives, all novel chemical entities. The yields are average based on at least 3 repetitions.

The ethylene glycol moiety can be readily removed under mild reductive condition in one step to yield 11-substituted mitragynine analogs (**15-21**, Fig 6) in moderate to good yields. Our strategy enables, for the first time, functionalization of the C11 position with a wide range of substituents

starting directly from the mitragynine natural product, thus permitting systematic exploration of SAR at this position.

To convert the mitragynine analogs to the corresponding 7OH series, we optimized a new set of conditions using oxone in acetone (Fig 6).[71,72] Mitragynine is prone to over-oxidation under these conditions and so we added 1.4 - 1.5 equivalents of oxone solution dropwise to the reaction mixture with continuous stirring at 0 ◦C. This procedure was successfully applied to the 11-substituted mitragynine analogs, as demonstrated by preparation of the F- (**22**), Cl- (**23**), and Br-7OH (**24**) analogs in yields around 50%. This oxidation protocol is superior to the previously reported approaches, namely PIFA gives complex mixtures of products and a lower yield of 7OH, while lead tetraacetate involves a toxic heavy metal and requires a second hydrolysis step.[73]

In summary, we developed access to 11-substituted analogs in three mitragynine-related molecular series, all novel compounds previously inaccessible via electrophilic substitution or other approaches, providing the means for a systematic SAR exploration of this position in multiple mitragynine-type scaffolds.

Neuropharmacology: *In vitro* Modulation of Opioid Receptors by the Novel 11-Substituted 7OH Analogs. With efficient access to the novel analogs, we examined the effect of C11 substitution on the opioid receptor pharmacology of mitragynine analogs. We focused the initial set of biological assays on the 7OH series as 7OH is the active metabolite of mitragynine and exhibits an order of magnitude greater potency compared to mitragynine as an MOR agonist.[15] First, we examined the 11-halo analogs of 7OH in radioligand binding studies to assess the affinity for the opioid receptors (Table 1). We found that the C11 halogen modulated affinity

across all three opioid receptors: the 11-fluoro compound **22** exhibited greater affinity compared to the parent 7OH, but this gain in binding was progressively lost as the halogen became larger (compounds **23** and **24**, Table 1). This effect was most pronounced at KOR, where the affinity of **22** was six times greater than that of the parent compound 7OH. The 11-fluoro compound **22** also had more than 2-fold greater affinity for DOR compared to 7OH. The effect of the C11 halogen on MOR binding was subtle across the halogen series. Since the parent compound 7OH acts as a potent agonist, we examined the functional modulation of mouse MOR (mMOR) activation in living cells. Specifically, we examined the signaling consequences of G protein activation via detection of the downstream signaling molecule cyclic adenosine monophosphate (cAMP). cAMP is formed from ATP by adenylyl cyclase (AC) and is inhibited by activation of the mu opioid receptor and its associated G proteins. Changes in the level of cAMP were measured using a bioluminescence resonance energy transfer (BRET) functional assay. The mMOR was co-expressed with $G\alpha_{oB}$, β_1, $\gamma 2$ and the BRET sensor CAMYEL (cAMP sensor using YFP-Epac-RLuc), to assay inhibition of cAMP formation. The BRET signal increases in response to decreasing amounts of cAMP as a result of conformational changes in the sensor. cAMP levels were raised prior to the assay by addition of forskolin as described previously (Fig 7A).[1,74]

Table 1. Binding affinities of C11 7OH *Mitragyna* analogs at the Mouse Opioid Receptors

Compound	K_i ± SEM (nM)[a]		
	mMOR	mKOR	mDOR
7OH	21.5 ± 0.8	119.0 ± 2.1	88.5 ± 9.9
C11-**F**-7OH (**22**)	13.7 ± 1.0	21.0 ± 3.2	35.8 ± 2.0
C11-**Cl**-7OH (**23**)	27.1 ± 1.1	30.7 ± 11.4	47.2 ± 2.4
C11-**Br**-7OH (**24**)	32.4 ± 1.4	81.1 ± 7.1	57.7 ± 6.7

[a]*All data points represent mean ± SEM (nM) of n = 3. [125I]BNtxA was used as the standard radiolabeled ligand.*[75]

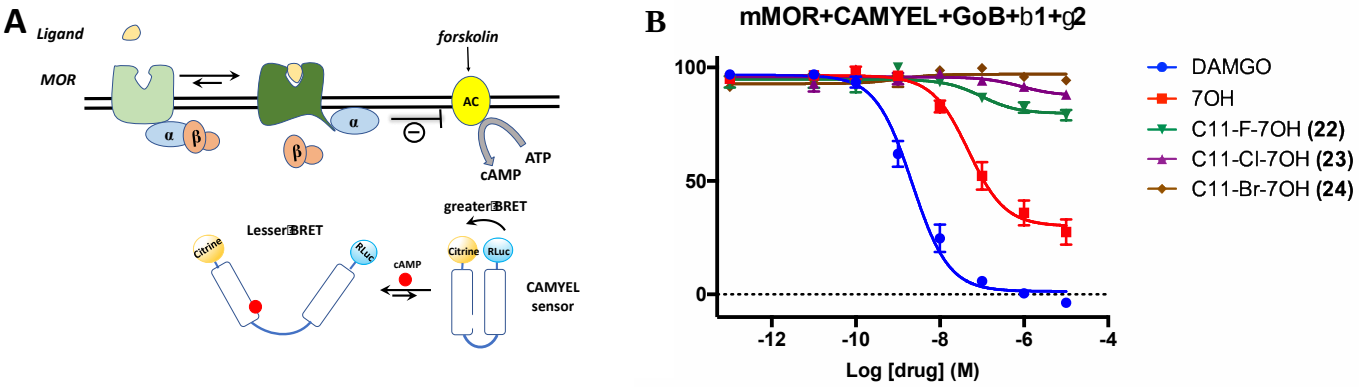

Fig 7. Activity of 7-hydroxymitragynine (7OH) analogs at the mouse mu-opioid receptor (mMOR). **A/** Conceptual representation of the MOR CAMYEL BRET assay. To measure G protein activation, MOR (light green) was co-expressed with G protein subunits $G\alpha_{oB}$, β_1, γ_2 and the BRET CAMYEL sensor. Forskolin activates adenylyl cyclase (AC), which converts ATP to cAMP. On activation of mMOR by ligand, the α subunit inhibits AC, resulting in a decrease of cAMP accumulation. The CAMYEL sensor comprises of citrine (mustard yellow) and *Renilla* luciferase (light blue) with human Epac1 (blue linker) interspaced between them. **B/** Agonist activity of 7OH at mMOR, presented as a percentage of cAMP inhibition produced by the positive control DAMGO ([D-Ala$_2$, N-Me-Phe$_4$, Gly$_5$-ol]-enkephalin); curves represent the average of n = 3, with error bars representing ± SEM.

Table 2. Functional Activity of 7OH analogs at mMOR as determined in CAMYEL BRET Assays

Compound	E_{max}[a]	pEC_{50}[b] ± SE (EC_{50} nM)
DAMGO	100	8.6 ± 0.1 (2.2)
7OH	70	7.3 ± 0.1 (48.0)
C11-**F**-7OH **(22)**	21	7.0 ± 0.3 (97.0)
C11-**Cl**-7OH **(23)**	<20	Not determined
C11-**Br**-7OH **(24)**	<20	Not determined

[a]*Agonist activity indicated by EC_{50} values, maximal efficacy is expressed as maximal inhibitory effect on cAMP levels relative to DAMGO (E_{max} = 100% – $cAMP_{(min)}$ %)*
[b]*All data points represent -log EC_{50} ± SE (EC_{50} nM) for n = 3 repetitions*

In the cAMP assay, 7OH was a potent agonist with relatively high signaling efficacy (E_{max} = 70%, compared to DAMGO ([D-Ala_2, N-Me-Phe_4, Gly_5-ol]-enkephalin), EC_{50} = 48 nM, Fig 7B, Table 2). Introduction of the 11-fluoro substitution in compound **22** dramatically reduced the efficacy, rendering a low efficacy agonist (E_{max} ~ 21%) (Fig 7B, Table 2). For the Cl- (**23**) and Br- (**24**) derivatives the agonist activity at mMOR was abolished. Accordingly, 11-halo substituents in the 7OH scaffold do not interfere with binding of these compounds to mMOR, but profoundly modulate the signaling efficacy at this receptor.

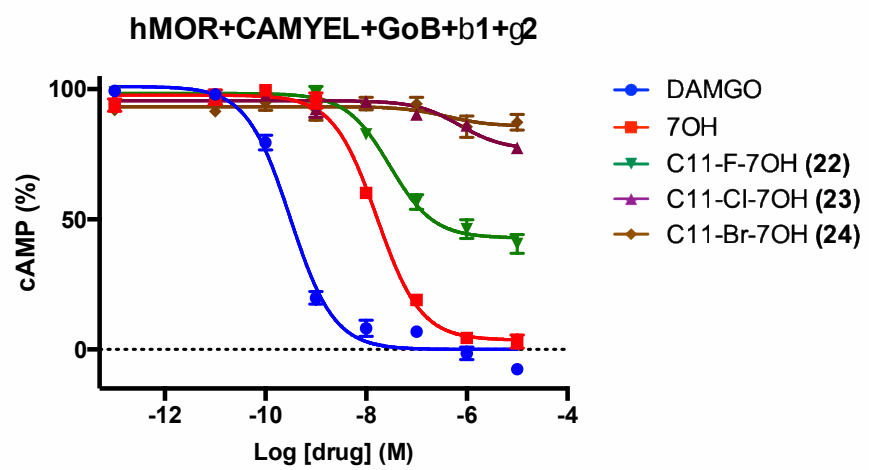

Fig 8. Agonist activity of 7OH series at human MOR (hMOR). Positive control = [D-Ala$_2$, N-Me-Phe$_4$, Gly$_5$-ol]-enkephalin (DAMGO); curves represent the average of *n* = 3, with error bars representing SEM as in Fig. 7.

Table 3. Functional Activity of 7OH analogs at hMOR as determined in CAMYEL BRET Assays

Compound	E_{max}[a]	pEC_{50}[b] ± SE (EC_{50} nM)
DAMGO	100	9.5 ± 0.10 (0.3)
7OH	96	7.8 ± 0.10 (16.4)
C11-**F**-7OH **(22)**	57	7.5 ± 0.10 (29.4)
C11-**Cl**-7OH **(23)**	<20	Not determined
C11-**Br**-7OH **(24)**	<20	Not determined

[a]*Agonist activity indicated by EC_{50} values, maximal efficacy is expressed as maximal inhibitory effect on cAMP levels relative to DAMGO (E_{max} = 100% – $cAMP_{(min)}$ %)*
[b]*All data points represent -logEC_{50} ± SE (EC_{50} nM) for n = 3 repetitions*

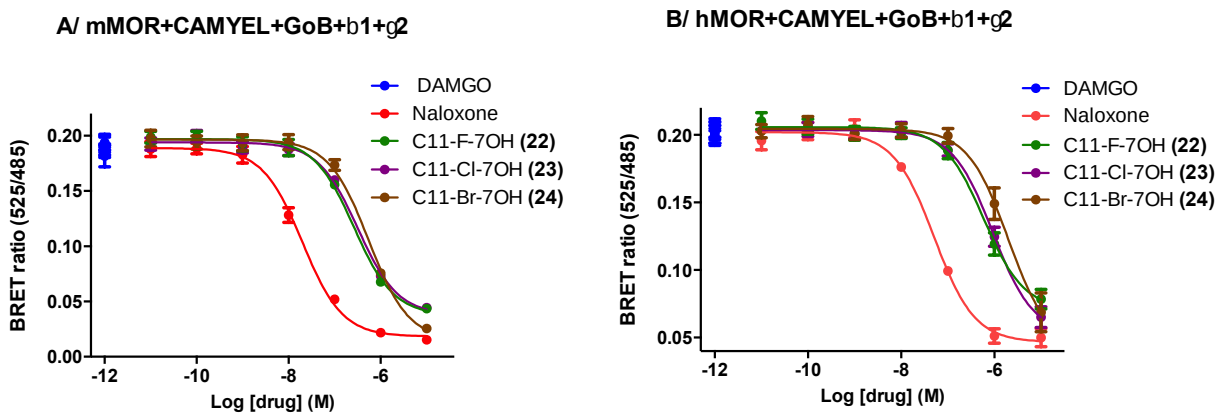

Fig 9. Antagonist activity of 7OH analogs at mMOR and hMOR. To assay G protein activation, mMOR and hMOR was coexpressed with G protein subunits $G\alpha_{oB}$, β_1, γ_2 and the BRET CAMYEL sensor. Antagonism was measured by the inhibition of DAMGO's effect on cAMP. **A/** Competitive antagonist activity of 7OH analogs at mMOR; positive control = naloxone. **B/** Competitive antagonist activity of 7OH analogs at hMOR; positive control = naloxone. Curves represent the average of *n* = 3, with error bars representing ± SEM.

Table 4: Functional Activity of 7OH analogs at mMOR as determined in CAMYEL BRET Assays

Compound	**pIC_{50c} ± SE (IC_{50} µM)**
Naloxone	7.7 ± 0.1 (0.02)
C11-**F**-7OH **(22)**	6.6 ± 0.1 (0.26)
C11-**Cl**-7OH **(23)**	6.5 ± 0.1 (0.32)
C11-**Br**-7OH **(24)**	6.3 ± 0.1 (0.55)

Table 5: Functional Activity of 7OH analogs at hMOR as determined in CAMYEL BRET Assays

Compound	**pIC_{50c} ± SE (IC_{50} µM)**
Naloxone	7.3± 0.1 (0.050)
C11-**F**-7OH **(22)**	6.2 ± 0.1 (0.59)
C11-**Cl**-7OH **(23)**	6.0 ± 0.1 (0.92)
C11-**Br**-7OH **(24)**	5.7 ± 0.1 (1.97)

[c]Antagonist activity indicated by IC_{50} values for inhibition of a reference agonist (DAMGO), all data points represent -logIC_{50} ± SE (IC_{50} µM) for n = 3 repetitions

To determine if the efficacy modulation trend holds at the human receptor, we examined the 7OH series in hMOR-CAMYEL assay (Fig. 8) using the same experimental setup as illustrated above (Fig. 7A). The parent compound 7OH was potent and highly efficacious (E_{max} = 96%, compared to the standard ligand DAMGO, EC_{50} = 16.4 nM, Fig. 8, Table 3), whereas 11-F-7OH (**22**) was a partial agonist with markedly reduced efficacy (E_{max} = 57%, EC_{50} = 29.4 nM, Fig 8, Table 3). The Cl- **(23)** and Br- **(24)** derivatives were essentially inactive and showed a very low efficacy (<20%). Hence, we can conclude that the trend in efficacy signaling

modulation in the halogen 7OH series is comparable between the two species and operative at hMOR.

To correlate the low/no agonist efficacy with antagonism, we next determined the antagonist activity of the 11-halogen-7OH series in a CAMYEL antagonism assay at mMOR (Fig. 9A, Table 4) and hMOR (Fig. 9B, Table 5) compared to the positive control naloxone. As expected, the compounds were able to inhibit the response elicited by the standard full agonist DAMGO at hMOR and mMOR down to the level predicted based on their agonist efficacy as determined above.

To complete the examination of this series at the opioid receptors, we performed the analogous cAMP BRET assays with mDOR and hDOR, and rat KOR (rKOR) and hKOR. The parent 7OH was found to act as a partial agonist at both mDOR (E_{max} = 72%, EC_{50} = 71.4 nM) and hDOR (E_{max} = 45%, EC_{50} = 99.9 nM, Supplementary Fig. 7).[76] However, 11-F-7OH showed considerable reduction in signaling efficacy at the receptors of both species, when compared to 7OH, without a major shift in the signaling potency (E_{max} = 41%, EC_{50} = 102.1 nM at mDOR; E_{max} = 26%, EC_{50} = 150.8 nM at hDOR,). The 11-Cl-7OH and 11-Br-7OH compounds were inactive (Supplementary Fig. 7). Remarkably, the C11 halogen trend described for MORs was also observed at DORs. In contrast, this compound series showed no agonist activity at the KOR receptors (Supplementary Fig. 8). Finally, we selected one representative compound of this series, 11-Br-7OH **(24),** and tested it for antagonism at the KOR and DOR, which showed antagonistic activity at these receptors (Supplementary Fig. 9 and 10) effecting full inhibition of reference agonists DPDPE ([D-$Pen_{2,5}$]-enkephalin) for DOR and U50488 for KOR.

In summary, these pharmacological studies indicate an important role for the C11 substitution in selective and profound modulation of the signaling efficacy at MORs and DORs

of the mitragynine-related scaffolds and provide a strong rationale for examining this effect *in vivo*.

11-Fluoro-7-hydroxymitragynine (11-F-7OH) is a Low-efficacy Partial Agonist *in vivo* in Mouse Analgesia Tests. We set out to examine if the gradual signaling efficacy modulation found *in vitro*, within the 7OH series, would translate to *in vivo* effects in living rodents. There is a strong rationale for pursuing such studies as partial MOR agonists may provide a path to safer opioid therapeutics (see Discussion below for more detail). We recently reported that 7OH was a potent and efficacious analgesic in mice using a tail-flick assay (thermal nociception assay),[14,15] consistent with a previous report by others.[77,78] On the basis of the previously determined ED_{50} value for 7OH in mouse tail-flick assay [ED_{50} = 0.57 mg/kg, (0.19-1.7, 95% CI)][15], and similar *in vitro* binding and signaling potencies of 7OH and 11-F-7OH at MOR, we selected a relatively high dose of 11-F-7OH (5 mg/kg, s.c. administration) for the initial time course profiling of the analgesic effect in CD1 mice (Fig 10A). The analgesic effect peaked at 15 minutes post injection, indicating rapid bioavailability *in vivo* comparable to the parent 7OH, followed by a decay to a residual analgesic effect that lasted for at least 120 minutes. Noteworthy is the partial analgesic efficacy of < 40%. To confirm that the maximum effect was reached, we examined even higher doses at a 15-minute time point, namely 10 and 25 mg/kg (s.c.), which showed that indeed the maximal analgesic effect was reached already at the 5 mg/kg dose (Fig 10B). The control compounds, 7OH and morphine, elicited a greater analgesic effect in the same mouse strain. In contrast, 11-Cl-7OH showed no analgesic effect at 5 or 25 mg/kg dose (Fig 10C).

Thus, the *in vitro* MOR pharmacological profile of the 7OH analogs translates well to the *in vivo* tail-flick test, an established physiological readout for MOR agonists that measures

antinociceptive effects of drugs to painful thermal stimuli. We next set out to rigorously confirm these results by 1) using a different and widely used mouse strain, C57BL/6J mice, 2) performing the experiments in a different location and research group, and 3) determining a full dose-curve for both the control (7OH) and the new compound (11-F-7OH, Fig 10D). 7OH showed a potent and high efficacy analgesic effect [ED_{50} = 0.25 (0.20 - 0.31) mg/kg, 95% CI, E_{max} is taken as 100%, comparable to morphine], consistent with previous results[15], while 11-F-7OH also elicited a analgesic potent effect [ED_{50} = 0.18 (0.04 - 0.78) mg/kg 95% CI], but with dramatically attenuated analgesic efficacy (E_{max} = 21%, Fig 10D). Thus, 11-F-7OH is equipotent to 7OH but exhibits low efficacy in antinociceptive effects. 7OH and 11-F-7OH provide an excellent probe pair for examining the effects of relative signaling efficacy on therapeutic (desired) and adverse effects, and the resulting preclinical measure of a therapeutic window.

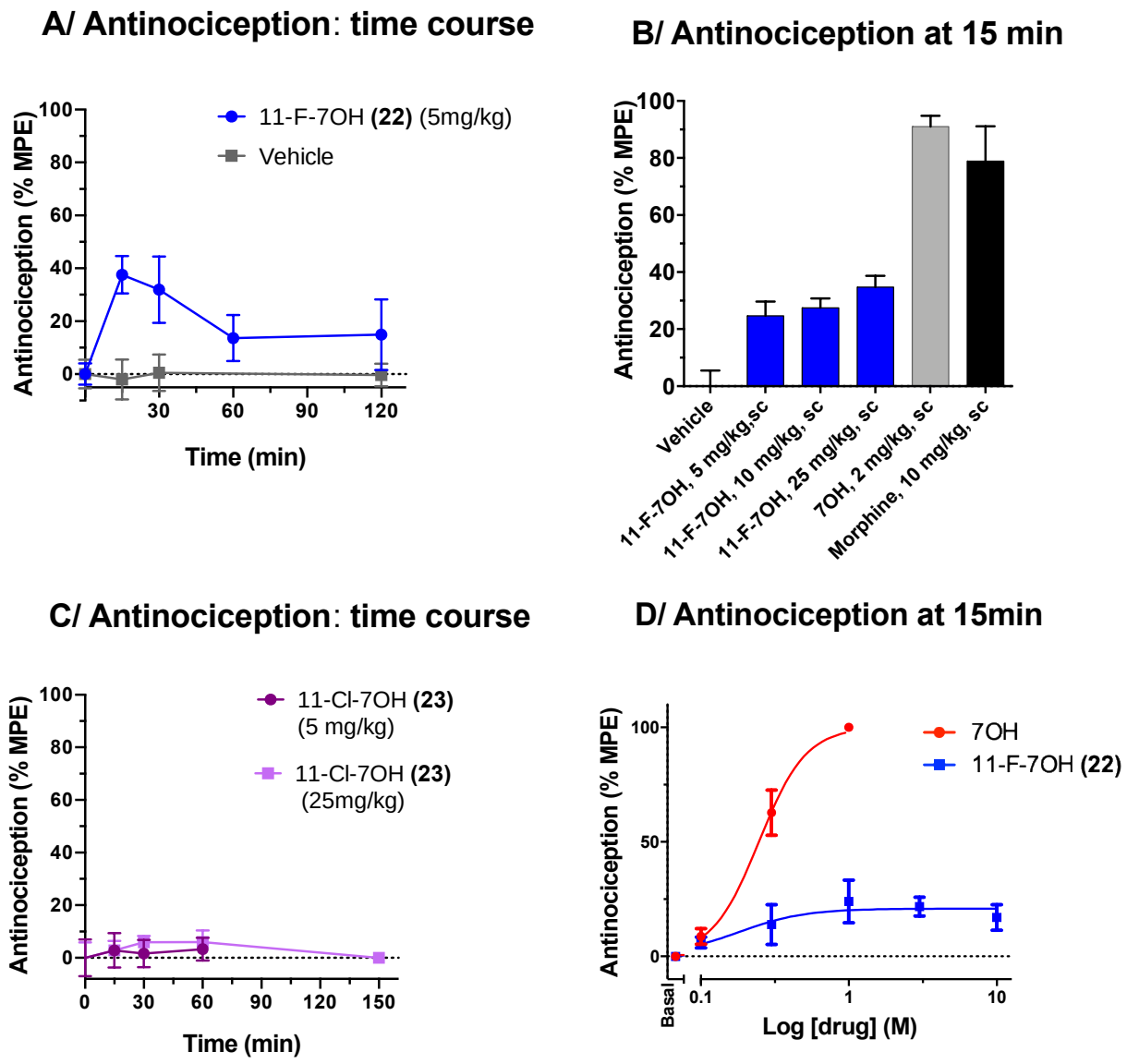

Fig 10. Antinociceptive effects of 7OH analogs in tail-flick assay in mice. **A/** Time course of the antinociceptive effect of 11-F-7OH (**22**, 5mg/kg. s.c.) in the tail flick assay, CD1 mice (*n* = 10). Each point represents mean ± SEM. %MPE, a percentage of the maximum possible effect. **B/** Tail-flick dose-response for the high dose range of 11-F-7OH (**22**), in direct comparison to controls 7OH and morphine. CD1 mice (*n* = 10), 15 minute time point. **C/** Time course of 11-Cl-7OH (**23**) at 2 doses (5 and 25 mg/kg, s.c.). **D/** Dose-response curves in mouse tail-flick assay for the parent 7OH (s.c.) and 11-F-7OH (**22**, s.c.), 15 minute time point, C57BL/6 mice (*n* = 10).

Discussion

Most recent estimates of kratom use, based on the tonnage of imported kratom and survey reports of dosing ranges, suggest 10 – 16 million users in the U.S. alone.[79] Numerous anecdotal

reports, including those collected by the United States Drug Enforcement Administration (>23,000 publicly available reports)[8], point to remarkable medicinal effects of kratom in self-medication for chronic pain, depression, anxiety, substance use disorders, and other ailments. Inspired by these clinical indicators, our laboratories have been studying the chemistry and biology of kratom alkaloids with the long-term goal of developing new drug leads based on these compounds. In this context, the aromatic ring SAR of mitragynine has not been mapped systematically, as only limited series of analogs have been synthesized and studied (e.g. C10 analogs)[12,14], and the C11 position has not been examined at all. To address this task, and to complement our total synthesis efforts,[13] we set out to examine the late stage functionalization of mitragynine, in this study focusing on the C11 position of the indole nucleus.

The known methods for C6 indole functionalization were not applicable to mitragynine owing to its structural and reactivity pattern complexity. For example, the lesser known 6-halogenation of indoles that proceeds via a 3-haloindolenine intermediate was not successful[46], due to the C9 methoxy group in mitragynine and its activating and directing effects on the benzene ring; bromination of mitragynine takes place preferentially in the C12 position. The iridium-catalyzed borylation gave the corresponding C12-boronate of mitragynine and subsequent C12 functionalization products, directed by the indole nitrogen. The structural complexity of mitragynine prevented the use of methods reliant on protecting or directing groups attached to the indole nitrogen. Further, the examination of analogs with reduced or oxidized indole nucleus (e.g. indolines and indolenines) was similarly unproductive. However, we found a solution by converting mitragynine's indole nucleus to the corresponding ethylene glycol adduct, a more stable oxidized intermediate, and showed that under standard conditions C-H borylation of the resulting indoline-like system was not selective, likely due to the diminished directing

power of the aniline (hemiaminal) group. This reduced directing effect ultimately allowed for optimization of the catalyst-ligand system to provide access to the C11-boronate of mitragynine with high positional selectivity. Protection of the pyrrole ring double bond with ethylene glycol represents an underutilized strategy in indole chemistry that offers several favorable features, including stability of the EG-adduct, attenuated basicity and reactivity of the resulting aniline nitrogen, and ease of deprotection and restoration of the indole nucleus. Further, in the milieu of complex substrates such as mitragynine, the installment of an EG unit is stereoselective and profoundly alters the electronics and reactivity of the aromatic ring, as well as the 3D structure of the entire polycyclic alkaloid system. Hence, MG-EG is a distinct chemotype and the strategy based on converting mitragynine to EG-MG serves as an example of polycyclic skeleton restructuring.

Years ago, we formulated the ideas of C-H bond functionalization and late-stage functionalization as general concepts with transformational potential for the thought and practice of chemical synthesis of carbon-based substances[28–34]. These concepts have since been widely adopted in both academia and industry and the C-H functionalization mindset, as well as corresponding methods, are indispensable components of today's synthetic repertoire[35–38]. Our team has been developing new methods for systematic and programmable functionalization of heteroarenes, which are essential building blocks in medicinal chemistry, including indoles, pyrroles, pyrazoles, imidazoles, pyridines and others[28–34]. In the present study, we focused on the benzene ring of highly complex indoles, in the context of specific neuropharmacological goals.

Our approach provided a practical route to C11-analogs of mitragynine, 7OH, and MG-EG, from the natural product, and systematic SAR exploration of the C11 position. The results indicate that substitution of this position plays an important role with respect to MOR signaling,

specifically in modulating the efficacy of receptor-triggered signaling events. Designing the receptor-signaling parameters of GPCR ligands *a priori* remains an unfulfilled potential of computational methods, and thus, a systematic examination of functional characteristics of receptor ligands and probes still relies largely on chemical synthesis. The new chemical approach described here provides a means to advance our examination and understanding of how molecular interactions of mitragynine-type scaffolds with the opioid receptors underlie functional outcomes such as G protein signaling and maximal signaling efficacy.

There has been considerable excitement and hope for generating MOR agonists that bias its signaling toward G protein-initiated pathways as a means for rational design of improved safety and therapeutic index of opioid analgesics ("G protein bias hypothesis" of opioids).[80] It was suggested that some of the typical adverse effects of opioids such as respiratory depression or reinforcing effects are linked to arrestin signaling. According to this approach, biasing the MOR-triggered signaling toward the G protein and away from arrestin would result in attenuation of the adverse effects, relative to the desired analgesic activity.[80] However, this hypothesis has been seriously challenged using both genetic and pharmacological tools.[81–86] An emerging alternative hypothesis is that partial agonism of MOR can lead to reduced side effects while preserving therapeutic effects ("signaling efficacy hypothesis").[86] An example is the superior safety profile of buprenorphine (a partial agonist *in vitro*) compared to the standard prescription opioids.[87] Buprenorphine is as efficacious as full agonists in treating pain but has substantially reduced ability to produce respiratory depression and thus reduced risk of opioid overdose death.[87] Such low potency compounds may have been incorrectly identified as G protein biased due both to receptor reserve and to the much greater amplification of the G protein signaling arm as compared to the much less sensitive arrestin pathway assays.[86] Lower efficacy opioids are also of

great interest for the treatment of opioid use disorder (OUD) where such compounds may provide efficacious opioid maintenance while having a reduced abuse potential. Therefore, close structural derivatives with a varying degree of G protein signaling efficacy are needed for direct and rigorous probing of this hypothesis. In this context the 11-halo series of 7OH provide valuable pharmacological tools.

The measured efficacy of G protein activation depends greatly on the receptor reserve pools, as with large reserve pools a partial agonist may appear as a full agonist or an extremely low efficacy compound may emerge as a moderate efficacy agonist. In our BRET assays the MORs are overexpressed and thus the measured signaling efficacy for partial agonists is exaggerated. In this respect, however, the correlation between the cAMP signaling efficacy obtained *in vitro* in cultured cells and the *in vivo* analgesic efficacy determined in living animals, for the 11-halo-7OH series, suggest that our *in vitro* system may mimic the receptor reserve and or receptor-effector situation in the neurons of relevant pain circuitry, and thus provides a useful model system. Considering the recent surveys that document much improved safety of kratom compared to the prescription or illicit opioids,[79,88,89] a further decrease of MOR's G protein signaling efficacy of the key mediator of kratom's opioid-like effects, 7OH[15], may lead to even safer compounds, and illustrates the potential importance of the present work. Further, a recent report showed that 7OH attenuated alcohol intake in drinking mice and that this effect was mediated by DOR activation, while 7OH exhibited rewarding effects on its own.[76] Thus, the attenuated MOR signaling efficacy of 11-F-7OH, while maintaining sufficient DOR signaling, may provide a therapeutic lead for OUD and other substance use disorders with no or much diminished abuse potential.

In summary, we describe a systematic examination of late stage functionalization of kratom alkaloids, which provided efficient access to novel mitragynine analogs and identified 11-F-7OH as an important lead compound for further investigations.

METHODS

General procedure for synthesis of C11-boronate ester MG EG

Starting material **MG EG** (50 mg, 0.11 mmol), $[Ir(COD)OMe]_2$ (3.6 mg , 5.5 µmol, 5 mol%), 3,4,7,8-tetramethyl-1,10-phenanthroline (3.9 mg, 16 µmol, 15 mol%) and B_2pin_2 (111 mg , 0.44 mmol, 4 eqv) were balanced into an oven dried vial. The vial was purged with argon, dry heptane (2.5 mL) was added under argon, and the vial was sealed with a Teflon-lined screw cap and heated to 65 °C. The RM became a dark red-brown solution after 5-15 minutes of heating. After 17-24 h, when LR-MS indicated complete consumption of SM, the RM was concentrated to give the crude boronate ester. This intermediate was immediately used to prepare the **13**, **15**, and **14** derivatives without further purification. Compound **7** was synthesized from **14** through a sequence of triflation, stannylation and fluorination.

General procedure for synthesis of C11-Halo-MG derivatives

Starting material (**5**, **7,** or **13,** 0.11 mmol) was dissolved in AcOH (2.0 mL) under argon and $NaCNBH_3$ (13.7 mg, 0.22 mmol, 2 equiv) was added to the solution. After stirring at RT for 15 min, another portion of $NaCNBH_3$ (13.7 mg, 0.22 mmol, 2 equiv) was added and stirring continued for 1 h. After this time, MeOH (81 µL) was added and the RM was heated to 90 °C for 1 h (for **15**) and 14 h (for **16** and **17**). The reaction mixture was added into a cold concentrated NH_4OH solution and extracted with DCM. After drying over Na_2SO_4, the DCM extract was

evaporated. Product was purified by preparative thin layer chromatography using an appropriate solvent mixture.

General procedure for synthesis of C11-Halo-7OH derivatives

Starting material (**15**, **16** or **17**, 73 µmol) was dissolved in acetone (2.2 mL), sat. aq. $NaHCO_3$ (1.5 mL) was added, and the stirred suspension was cooled in an ice bath (0 °C). Oxone (1.4 – 1.5 equiv) in H_2O (0.7 mL) was added dropwise over 20 min with vigorous stirring. (Care should be taken that the RM does not form lumps and should be stirred thoroughly). The reaction was monitored during the addition of oxone by TLC. After 25 min from the first addition, the reaction mixture was diluted with H_2O (10 mL) and extracted with EtOAc (3 × 10 mL). The combined extracts were washed with brine (10 mL), dried over Na_2SO_4, and concentrated. Product was purified by preparative thin layer chromatography using an appropriate solvent mixture to synthesize compounds **22**, **23** and **24**.

DATA AVAILABILITY

The authors declare that all the data supporting the findings of this study are available within the article and Supplementary Information files which contains synthetic procedures and NMR spectra for the featured compounds, additional functional data at rodent and human receptors, biological protocols for receptor binding, activity and antinociception assays. The X-ray crystallographic coordinates for structure **4** reported in this article has been deposited at the Cambridge Crystallographic Data Center (CCDC) under deposition numbers 1905559

REFERENCES

1. Gassaway, M. M., Rives, M.-L., Kruegel, A. C., Javitch, J. A. & Sames, D. The atypical antidepressant and neurorestorative agent tianeptine is a μ-opioid receptor agonist. *Translational Psychiatry* **4**, e411–e411 (2014).
2. Kruegel, A. C., Rakshit, S., Li, X. & Sames, D. Constructing *Iboga* Alkaloids via C–H Bond Functionalization: Examination of the Direct and Catalytic Union of Heteroarenes and Isoquinuclidine Alkenes. *The Journal of Organic Chemistry* **80**, 2062–2071 (2015).
3. Gassaway, M. M. *et al.* Deconstructing the *Iboga* Alkaloid Skeleton: Potentiation of FGF2-induced Glial Cell Line-Derived Neurotrophic Factor Release by a Novel Compound. *ACS Chemical Biology* **11**, 77–87 (2016).
4. Marton, S. *et al.* Ibogaine Administration Modifies GDNF and BDNF Expression in Brain Regions Involved in Mesocorticolimbic and Nigral Dopaminergic Circuits. *Front. Pharmacol.* **10**, 193 (2019).
5. E. Adkins, J., W. Boyer, E. & R. McCurdy, C. Mitragyna speciosa, A Psychoactive Tree from Southeast Asia with Opioid Activity. *Current Topics in Medicinal Chemistry* **11**, 1165–1175 (2011).
6. Kruegel, A. C. & Grundmann, O. The medicinal chemistry and neuropharmacology of kratom: A preliminary discussion of a promising medicinal plant and analysis of its potential for abuse. *Neuropharmacology* **134**, 108–120 (2018).
7. Swogger, M. T. *et al.* Experiences of Kratom Users: A Qualitative Analysis. *Journal of Psychoactive Drugs* **47**, 360–367 (2015).
8. DEA 3 Factor Analysis for mitragynine and 7-hydroxymitragynine https://www.regulations.gov/document?D=DEA-2016-0015-0004 August 2016.

9. Swogger, M. T. & Walsh, Z. Kratom use and mental health: A systematic review. *Drug and Alcohol Dependence* **183**, 134–140 (2018).
10. Fluyau, D. & Revadigar, N. Biochemical Benefits, Diagnosis, and Clinical Risks Evaluation of Kratom. *Frontiers in Psychiatry* **8**, (2017).
11. Takayama, H. Chemistry and pharmacology of analgesic indole alkaloids from the rubiaceous plant, Mitragyna speciosa. *Chem. Pharm. Bull.* **52**, 916–928 (2004).
12. Takayama, H. *et al.* New Procedure to Mask the 2,3-π Bond of the Indole Nucleus and Its Application to the Preparation of Potent Opioid Receptor Agonists with a Corynanthe Skeleton. *Organic Letters* **8**, 5705–5708 (2006).
13. Kruegel, A. C. *et al.* Synthetic and Receptor Signaling Explorations of the *Mitragyna* Alkaloids: Mitragynine as an Atypical Molecular Framework for Opioid Receptor Modulators. *Journal of the American Chemical Society* **138**, 6754–6764 (2016).
14. Váradi, A. *et al.* Mitragynine/Corynantheidine Pseudoindoxyls As Opioid Analgesics with Mu Agonism and Delta Antagonism, Which Do Not Recruit β-Arrestin-2. *Journal of Medicinal Chemistry* **59**, 8381–8397 (2016).
15. Kruegel, A. C. *et al.* 7-Hydroxymitragynine Is an Active Metabolite of Mitragynine and a Key Mediator of Its Analgesic Effects. *ACS Cent. Sci.* **5**, 992–1001 (2019).
16. Macko, E., Weisbach, J. A. & Douglas, B. Some observations on the pharmacology of mitragynine. *Arch Int Pharmacodyn Ther* **198**, 145–161 (1972).
17. Hemby, S. E., McIntosh, S., Leon, F., Cutler, S. J. & McCurdy, C. R. Abuse liability and therapeutic potential of the *Mitragyna speciosa* (kratom) alkaloids mitragynine and 7-hydroxymitragynine: kratom abuse liability. *Addiction Biology* (2018) doi:10.1111/adb.12639.

18. Yue, K., Kopajtic, T. A. & Katz, J. L. Abuse liability of mitragynine assessed with a self-administration procedure in rats. *Psychopharmacology* (2018) doi:10.1007/s00213-018-4974-9.

19. Volkow, N. D. & Collins, F. S. The Role of Science in Addressing the Opioid Crisis. *New England Journal of Medicine* **377**, 391–394 (2017).

20. Majumdar, S. & Devi, L. A. Strategy for making safer opioids bolstered. *Nature* **553**, 286–288 (2018).

21. Samuels, B. A. *et al.* The Behavioral Effects of the Antidepressant Tianeptine Require the Mu-Opioid Receptor. *Neuropsychopharmacology* **42**, 2052–2063 (2017).

22. Takayama, H. *et al.* The first total synthesis of (−)-mitragynine, an analgesic indole alkaloid in mitragyna speciosa. *Tetrahedron Letters* **36**, 9337–9340 (1995).

23. Ma, J., Yin, W., Zhou, H., Liao, X. & Cook, J. M. General Approach to the Total Synthesis of 9-Methoxy-Substituted Indole Alkaloids: Synthesis of Mitragynine, as well as 9-Methoxygeissoschizol and 9-Methoxy-N_b-methylgeissoschizol. *The Journal of Organic Chemistry* **74**, 264–273 (2009).

24. Kerschgens, I. P. *et al.* Total syntheses of mitragynine, paynantheine and speciogynine via an enantioselective thiourea-catalysed Pictet–Spengler reaction. *Chemical Communications* **48**, 12243 (2012).

25. Kim, J., Schneekloth, J. S. & Sorensen, E. J. A chemical synthesis of 11-methoxy mitragynine pseudoindoxyl featuring the interrupted Ugi reaction. *Chemical Science* **3**, 2849 (2012).

26. Sun, X. & Ma, D. Organocatalytic Approach for the Syntheses of Corynantheidol, Dihydrocorynantheol, Protoemetinol, Protoemetine, and Mitragynine. *Chemistry - An Asian Journal* **6**, 2158–2165 (2011).
27. Matsumoto, K. *et al.* Orally Active Opioid / Dual Agonist MGM-16, a Derivative of the Indole Alkaloid Mitragynine, Exhibits Potent Antiallodynic Effect on Neuropathic Pain in Mice. *Journal of Pharmacology and Experimental Therapeutics* **348**, 383–392 (2014).
28. Godula, K. C-H Bond Functionalization in Complex Organic Synthesis. *Science* **312**, 67–72 (2006).
29. Pastine, S. J., Gribkov, D. V. & Sames, D. sp 3 C−H Bond Arylation Directed by Amidine Protecting Group: α-Arylation of Pyrrolidines and Piperidines. *Journal of the American Chemical Society* **128**, 14220–14221 (2006).
30. Guo, P., Joo, J. M., Rakshit, S. & Sames, D. C–H Arylation of Pyridines: High Regioselectivity as a Consequence of the Electronic Character of C–H Bonds and Heteroarene Ring. *Journal of the American Chemical Society* **133**, 16338–16341 (2011).
31. Genovino, J., Sames, D., Hamann, L. G. & Touré, B. B. Accessing Drug Metabolites via Transition-Metal Catalyzed C−H Oxidation: The Liver as Synthetic Inspiration. *Angewandte Chemie International Edition* **55**, 14218–14238 (2016).
32. Lane, B. S., Brown, M. A. & Sames, D. Direct Palladium-Catalyzed C-2 and C-3 Arylation of Indoles: A Mechanistic Rationale for Regioselectivity. *Journal of the American Chemical Society* **127**, 8050–8057 (2005).
33. Goikhman, R., Jacques, T. L. & Sames, D. C−H Bonds as Ubiquitous Functionality: A General Approach to Complex Arylated Pyrazoles via Sequential Regioselective *C* -

Arylation and *N* -Alkylation Enabled by SEM-Group Transposition. *Journal of the American Chemical Society* **131**, 3042–3048 (2009).

34. Wang, X., Lane, B. S. & Sames, D. Direct C-Arylation of Free (NH)-Indoles and Pyrroles Catalyzed by Ar−Rh(III) Complexes Assembled In Situ. *Journal of the American Chemical Society* **127**, 4996–4997 (2005).
35. He, J., Wasa, M., Chan, K. S. L., Shao, Q. & Yu, J.-Q. Palladium-Catalyzed Transformations of Alkyl C–H Bonds. *Chemical Reviews* **117**, 8754–8786 (2017).
36. McMurray, L., O'Hara, F. & Gaunt, M. J. Recent developments in natural product synthesis using metal-catalysed C–H bond functionalisation. *Chemical Society Reviews* **40**, 1885 (2011).
37. Cernak, T., Dykstra, K. D., Tyagarajan, S., Vachal, P. & Krska, S. W. The medicinal chemist's toolbox for late stage functionalization of drug-like molecules. *Chemical Society Reviews* **45**, 546–576 (2016).
38. Yang, L. & Huang, H. Transition-Metal-Catalyzed Direct Addition of Unactivated C–H Bonds to Polar Unsaturated Bonds. *Chemical Reviews* **115**, 3468–3517 (2015).
39. Gensch, T., Hopkinson, M. N., Glorius, F. & Wencel-Delord, J. Mild metal-catalyzed C–H activation: examples and concepts. *Chemical Society Reviews* **45**, 2900–2936 (2016).
40. Mukai, K. *et al.* Bioinspired chemical synthesis of monomeric and dimeric stephacidin A congeners. *Nature Chemistry* **10**, 38–44 (2017).
41. Kerschgens, I., Rovira, A. R. & Sarpong, R. Total Synthesis of (−)-Xishacorene B from (*R*)-Carvone Using a C–C Activation Strategy. *Journal of the American Chemical Society* **140**, 9810–9813 (2018).

42. Roque, J. B., Kuroda, Y., Göttemann, L. T. & Sarpong, R. Deconstructive fluorination of cyclic amines by carbon-carbon cleavage. *Science* **361**, 171–174 (2018).

43. Liao, K. *et al.* Site-selective and stereoselective functionalization of non-activated tertiary C–H bonds. *Nature* (2017) doi:10.1038/nature24641.

44. Larsen, M. A. & Hartwig, J. F. Iridium-Catalyzed C–H Borylation of Heteroarenes: Scope, Regioselectivity, Application to Late-Stage Functionalization, and Mechanism. *Journal of the American Chemical Society* **136**, 4287–4299 (2014).

45. Preshlock, S. M. *et al.* A Traceless Directing Group for C□H Borylation. *Angewandte Chemie International Edition* **52**, 12915–12919 (2013).

46. Ikeda, M. & Tamura, Y. 3-Haloindolenines — Versatile Intermediates in the Indole Chemistry. *HETEROCYCLES* **14**, 867 (1980).

47. Murphy, J. M., Liao, X. & Hartwig, J. F. Meta Halogenation of 1,3-Disubstituted Arenes via Iridium-Catalyzed Arene Borylation. *Journal of the American Chemical Society* **129**, 15434–15435 (2007).

48. Paul, S. *et al.* Ir-Catalyzed Functionalization of 2-Substituted Indoles at the 7-Position: Nitrogen-Directed Aromatic Borylation. *Journal of the American Chemical Society* **128**, 15552–15553 (2006).

49. Homer, J. A. & Sperry, J. A short synthesis of the endogenous plant metabolite 7-hydroxyoxindole-3-acetic acid (7-OH-OxIAA) using simultaneous C–H borylations. *Tetrahedron Letters* **55**, 5798–5800 (2014).

50. Leitch, J. A., Bhonoah, Y. & Frost, C. G. Beyond C2 and C3: Transition-Metal-Catalyzed C–H Functionalization of Indole. *ACS Catalysis* **7**, 5618–5627 (2017).

51. Feng, Y. *et al.* Total Synthesis of Verruculogen and Fumitremorgin A Enabled by Ligand-Controlled C–H Borylation. *Journal of the American Chemical Society* **137**, 10160–10163 (2015).
52. Vadola, P. A. & Sames, D. Catalytic Coupling of Arene C–H Bonds and Alkynes for the Synthesis of Coumarins: Substrate Scope and Application to the Development of Neuroimaging Agents. *The Journal of Organic Chemistry* **77**, 7804–7814 (2012).
53. Yang, Y., Li, R., Zhao, Y., Zhao, D. & Shi, Z. Cu-Catalyzed Direct C6-Arylation of Indoles. *Journal of the American Chemical Society* **138**, 8734–8737 (2016).
54. Yang, G. *et al.* Pd(II)-Catalyzed *meta* -C–H Olefination, Arylation, and Acetoxylation of Indolines Using a U-Shaped Template. *Journal of the American Chemical Society* **136**, 10807–10813 (2014).
55. Leitch, J. A., McMullin, C. L., Mahon, M. F., Bhonoah, Y. & Frost, C. G. Remote C6-Selective Ruthenium-Catalyzed C–H Alkylation of Indole Derivatives via σ-Activation. *ACS Catalysis* **7**, 2616–2623 (2017).
56. W. Gribble, G., L. Johnson, J. & G. Saulnier, M. Stereoselective Reduction of 1,2,3,4,6,7,12,12b-Octahydroindolo[2,3-a]quinolizine with Sodium Borohydride in Trifluoeoacetic Acid. *HETEROCYCLES* **16**, 2109 (1981).
57. Okada, N., Misawa, K. & Kitajima, M. Preparation of Ethylene Glycol Adducts at 2,3-Positions of Indoles with Hypervalent Iodine. *HETEROCYCLES* **74**, 461 (2007).
58. Saito, Y., Segawa, Y. & Itami, K. *para* -C–H Borylation of Benzene Derivatives by a Bulky Iridium Catalyst. *Journal of the American Chemical Society* **137**, 5193–5198 (2015).

59. Litvinas, N. D., Fier, P. S. & Hartwig, J. F. A General Strategy for the Perfluoroalkylation of Arenes and Arylbromides by Using Arylboronate Esters and [(phen)CuRF]. *Angewandte Chemie International Edition* **51**, 536–539 (2012).

60. Eastabrook, A. S., Wang, C., Davison, E. K. & Sperry, J. A Procedure for Transforming Indoles into Indolequinones. *The Journal of Organic Chemistry* **80**, 1006–1017 (2015).

61. Meyer-Eppler, G. *et al.* Cheap and Easy Synthesis of Highly Functionalized (Het)aryl Iodides via the Aromatic Finkelstein Reaction. *Synthesis* **46**, 1085–1090 (2014).

62. Cooper, T., Novak, A., Humphreys, L. D., Walker, M. D. & Woodward, S. User-Friendly Methylation of Aryl and Vinyl Halides and Pseudohalides with DABAL-Me3. *Advanced Synthesis & Catalysis* **348**, 686–690 (2006).

63. Wang, B., Sun, H.-X. & Sun, Z.-H. A General and Efficient Suzuki-Miyaura Cross-Coupling Protocol Using Weak Base and No Water: The Essential Role of Acetate. *European Journal of Organic Chemistry* **2009**, 3688–3692 (2009).

64. Suresh, A. S., Baburajan, P. & Ahmed, M. Synthesis of primary amides by aminocarbonylation of aryl/hetero halides using non-gaseous NH3 and CO sources. *Tetrahedron Letters* **56**, 4864–4867 (2015).

65. Ramnauth, J., Bhardwaj, N., Renton, P., Rakhit, S. & Maddaford, S. P. The Room-Temperature Palladium-Catalyzed Cyanation of Aryl Bromides and Iodides with Tri- *t* -butylphosphine as Ligand. *Synlett* 2237–2239 (2003) doi:10.1055/s-2003-42069.

66. Fier, P. S., Luo, J. & Hartwig, J. F. Copper-Mediated Fluorination of Arylboronate Esters. Identification of a Copper(III) Fluoride Complex. *Journal of the American Chemical Society* **135**, 2552–2559 (2013).

67. Taylor, N. J. *et al.* Derisking the Cu-Mediated ^{18}F-Fluorination of Heterocyclic Positron Emission Tomography Radioligands. *Journal of the American Chemical Society* **139**, 8267–8276 (2017).

68. Furuya, T. & Ritter, T. Fluorination of Boronic Acids Mediated by Silver(I) Triflate. *Organic Letters* **11**, 2860–2863 (2009).

69. Tang, P., Wang, W. & Ritter, T. Deoxyfluorination of Phenols. *Journal of the American Chemical Society* **133**, 11482–11484 (2011).

70. Furuya, T., Strom, A. E. & Ritter, T. Silver-Mediated Fluorination of Functionalized Aryl Stannanes. *Journal of the American Chemical Society* **131**, 1662–1663 (2009).

71. Liu, S., Scotti, J. S. & Kozmin, S. A. Emulating the Logic of Monoterpenoid Alkaloid Biogenesis to Access a Skeletally Diverse Chemical Library. *The Journal of Organic Chemistry* **78**, 8645–8654 (2013).

72. Movassaghi, M., Schmidt, M. A. & Ashenhurst, J. A. Stereoselective Oxidative Rearrangement of 2-Aryl Tryptamine Derivatives. *Organic Letters* **10**, 4009–4012 (2008).

73. Ishikawa, H., Takayama, H. & Aimi, N. Dimerization of indole derivatives with hypervalent iodines(III): a new entry for the concise total synthesis of rac- and meso-chimonanthines. *Tetrahedron Letters* **43**, 5637–5639 (2002).

74. Jiang, L. I. *et al.* Use of a cAMP BRET Sensor to Characterize a Novel Regulation of cAMP by the Sphingosine 1-Phosphate/G ^{13} Pathway. *Journal of Biological Chemistry* **282**, 10576–10584 (2007).

75. Majumdar, S. *et al.* Generation of novel radiolabeled opiates through site-selective iodination. *Bioorganic & Medicinal Chemistry Letters* **21**, 4001–4004 (2011).

76. Gutridge, A. M. *et al.* G protein-biased kratom-alkaloids and synthetic carfentanil-amide opioids as potential treatments for alcohol use disorder. *Br J Pharmacol* **177**, 1497–1513 (2020).

77. Matsumoto, K. *et al.* Antinociceptive effect of 7-hydroxymitragynine in mice: Discovery of an orally active opioid analgesic from the Thai medicinal herb Mitragyna speciosa. *Life Sciences* **74**, 2143–2155 (2004).

78. Matsumoto, K. *et al.* Involvement of μ-opioid receptors in antinociception and inhibition of gastrointestinal transit induced by 7-hydroxymitragynine, isolated from Thai herbal medicine Mitragyna speciosa. *European Journal of Pharmacology* **549**, 63–70 (2006).

79. Henningfield, J. E. *et al.* Risk of death associated with kratom use compared to opioids. *Preventive Medicine* **128**, 105851 (2019).

80. Schmid, C. L. *et al.* Bias Factor and Therapeutic Window Correlate to Predict Safer Opioid Analgesics. *Cell* **171**, 1165-1175.e13 (2017).

81. Johnson, T. A. *et al.* Identification of the First Marine-Derived Opioid Receptor "Balanced" Agonist with a Signaling Profile That Resembles the Endorphins. *ACS Chem. Neurosci.* **8**, 473–485 (2017).

82. Hill, R. *et al.* The novel μ-opioid receptor agonist PZM21 depresses respiration and induces tolerance to antinociception: PZM21 depresses respiration. *British Journal of Pharmacology* **175**, 2653–2661 (2018).

83. Kliewer, A. *et al.* Phosphorylation-deficient G-protein-biased μ-opioid receptors improve analgesia and diminish tolerance but worsen opioid side effects. *Nat Commun* **10**, 367 (2019).

84. Kliewer, A. *et al.* Morphine-induced respiratory depression is independent of β-arrestin2 signalling. *Br J Pharmacol* bph.15004 (2020) doi:10.1111/bph.15004.

85. Conibear, A. E. & Kelly, E. A Biased View of μ -Opioid Receptors? *Mol Pharmacol* **96**, 542–549 (2019).

86. Gillis, A. *et al.* Low intrinsic efficacy for G protein activation can explain the improved side effect profiles of new opioid agonists. *Sci. Signal.* **13**, eaaz3140 (2020).

87. Pergolizzi, J. *et al.* Current Knowledge of Buprenorphine and Its Unique Pharmacological Profile: Current Knowledge of Buprenorphine. *Pain Practice* **10**, 428–450 (2010).

88. Saref, A. *et al.* Self-reported prevalence and severity of opioid and kratom (Mitragyna speciosa korth.) side effects. *Journal of Ethnopharmacology* **238**, 111876 (2019).

89. Garcia-Romeu, A., Cox, D. J., Smith, K. E., Dunn, K. E. & Griffiths, R. R. Kratom (Mitragyna speciosa): User demographics, use patterns, and implications for the opioid epidemic. *Drug and Alcohol Dependence* **208**, 107849 (2020).

ACKNOWLEDGEMENTS

This work was supported by the National Institute on Drug Addiction (NIDA) of the National Institute of Health (NIH), grants R01DA046487 (D. Sames, S. Majumdar, and J. A. Javitch) and R21DA045884 (S. Majumdar and D. Sames), and the Hope for Depression Research Foundation (J. A. Javitch). S.M. also acknowledges Faculty Research Incentive Funding (FRIF) from STLCOP and AA026949 from NIAAA. We thank Prof. Gerard Parkin and Matthew James Hammond for solving the X-ray crystal structure of MG-EG and helpful discussions. We also thank Dr. Mu Yang, Co-Director of the Mouse NeuroBehavior Core (MNBC) facility for

oversight of the *in vivo* experiments at Columbia University. This research was funded in part through the NIH/NCI Cancer Center Support Grant P30 CA008748 to MSKCC.

AUTHOR CONTRIBUTION

D.S. conceptualized and supervised the work. S.B. and J.G. carried out majority of the synthetic work including reaction development and optimization, and compound characterization. V.H. contributed to the synthesis and characterization of compounds, T.F. contributed to reaction optimization and compound characterization. M.N. performed the BRET functional assays, A.C.K. contributed to the design of compounds and supervised the interpretation of the pharmacological data, S.M. supervised the binding assays, tail flick mice assay and contributed to global planning of the project, A.H. performed the binding assays, A.F. and B.B. performed the tail flick mice assay, JAJ supervised the BRET functional assays and interpretation of the corresponding results. S.B. and D.S. wrote the manuscript with significant help from J.G., V.H., J.A.J. and A.C.K. All authors contributed to editing of the manuscript.

# S.B. and J.G. contributed equally to this work.

CONFLICTS OF INTEREST

S. B., J. G., V. H., A.C.K., J.A.J., S.M., and D.S., are named as inventors on patent applications related to mitragynine analogs. A.C.K., J.A.J. and D.S. are co-founders of Kures, Inc. SM is a co-founder of Sparian biosciences.

AUTHOR INFORMATION

Corresponding author

Prof. Dalibor Sames, Ph.D., email ds584@columbia.edu

ORCID iD:

S.B 0000-0001-5951-4557
J.G. 0000-0002-7729-1825
A.F. 0000-0002-9059-4791
V.H 0000-0002-6911-4669
T.F. 0000-0001-6949-4561
S.M. 0000-0002-2931-3823
J.A.J. 0000-0001-7395-2967
D.S. 0000-0001-6911-2260