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Semi-synthesis in the exploration of opioid-targeting natural products

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Since the isolation of morphine from opium, chemists have sought to modify its chemical structure in hopes of developing a safer, less addictive pain killer. At the same time, these novel morphine derivatives have provided new chemical tools to study the opioid receptors. In this way, the field of semi-synthesis, that is, the synthetic modification of isolated natural products, has co-evolved alongside the field of opioid pharmacology. This review summarizes recent semi-synthetic studies of the opioid-targeting natural products mitragynine, akuammine, akuammicine, and salvinorin A. These studies have resulted in novel opioid ligands with improved affinity and potency, differing signaling profiles, and increased effects in animals. In addition to offering new tools to study the opioid receptors, these natural product analogues represent promising steps towards developing safer opioid analgesics.

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1. Introduction

When Friedrich Sertürner isolated morphine from opium in the beginning of the 19th century, it was a pivotal moment in the history of medicine and natural products.[1] Not only did this mark the discovery of an analgesic that remains the gold-standard in pain management, it also represented the first isolation of a plant natural product. However, even as morphine was isolated and its opium-like analgesic effects were confirmed, the less desirable effects of opium were also present. Morphine is, of course, a potent sedative (hence Sertürner named the molecule after Morpheus, the Greek deity of dreams) and highly addictive. In this way, Sertüner's discovery also inspired the birth of semi-synthesis – the use of synthetic chemistry to structurally modify a naturally occurring compound, typically with the goal of modifying its biological activity – as a tool in the quest for a better analgesic.[2,3]

Due to the synthetic chemistry available at the time, these early derivatives were generally limited to ethers and esters of morphine's phenol and secondary alcohol. Nevertheless, one of the first semi-synthetic morphine derivatives prepared would also become the most infamous: diacetylmorphine (heroin, Fig. 1). Diacetylmorphine was first synthesized by the chemist Charles Romley Alder Wright in 1874 and its pharmacological activity in animals was reported by physician F. M. Pierce.[4,5] And while several other reports of the activity of diacetylmorphine appeared in the late 19th century,[6,7] it was not made widely available until 1898 when Bayer marketed it under the name heroin as a cough suppressant and respiratory aid.[8] However, the addictive properties of heroin were recognized nearly immediately, and less than two decades after its introduction, the United States passed the Harrison Act that regulated its use.[8]

Despite, or perhaps in response to, the societal impact of heroin, the search for an improved morphine-like drug was continued.[2] One notable early accomplishment was the discovery by J. Pohl, who demonstrated that replacement of the *N*-methyl group with an allyl group, as in *N*-allylnorcodeine (Fig. 1), led to compounds that reversed the action of morphine (*i.e.*, antagonists).[9] This would lay the foundation for the development of compounds like naloxone (Narcan) that is now

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widely used a reversal agent for opioid overdose. As advancements in synthetic chemistry were made and the structure of morphine was elucidated by Sir Robert Robinson in 1925,[10] efforts were made to establish the structure–activity relationship (SAR) by modifying as many of the positions on the morphine scaffold as possible. Much of this effort was initiated in the US by the Committee on Drug Addiction of the National Research Council and later the National Institutes of Health. Bentley and Hardy later identified that adding to the morphinan scaffold, as exemplified in buprenorphine and etorphine (Fig. 1), dramatically improved affinity and potency for the opioid receptors.[11–14]

Beyond their use as medicines, several semi-synthetic morphine derivatives have served as fundamental tools in the field of opioid pharmacology. By the middle of the 20th century, it was generally agreed that morphine and its derivatives exerted their action by interacting with specific nervous system receptors, but the nature of these receptors and interactions was far from clear. Seminal work by Beckett and Casy, and later refined by Portoghese, had proposed a three-point receptor model that accounted for the observed stereospecificity of opioid ligands – that is that one enantiomer of a ligand generally was more active than the other – suggesting the opioid receptors were chiral.[15,16] Remarkably, in 1973 three separate laboratories independently demonstrated the binding of a small molecule to a specific protein receptor, each using a radiolabeled morphine derivative: [^{3}H]-naloxone, [^{3}H]-etorphine, and [^{3}H]-dihydromorphine.[17–20] We now know these studies were labeling the mu opioid receptor (μOR), which together with the kappa (κOR), delta (δOR), and nociceptor (NOP) receptors make up the family of opioid G-protein coupled receptors. In the proceeding years, highly selective semi-synthetic morphine derivatives like β-funaltrexamine (for μOR), naltrindole (for δOR), norbinaltrophimine (for κOR) would help define the role played by each of these opioid receptor subtypes in the analgesic action and adverse effects of opioid ligands. Later, through the incorporation of ^{11}C and ^{18}F, morphine derivatives like [^{11}C]DPN and [^{18}F]FE-DPN, were used as PET ligands to visualize the location of the opioid receptors both in living animals and humans.[21–23]

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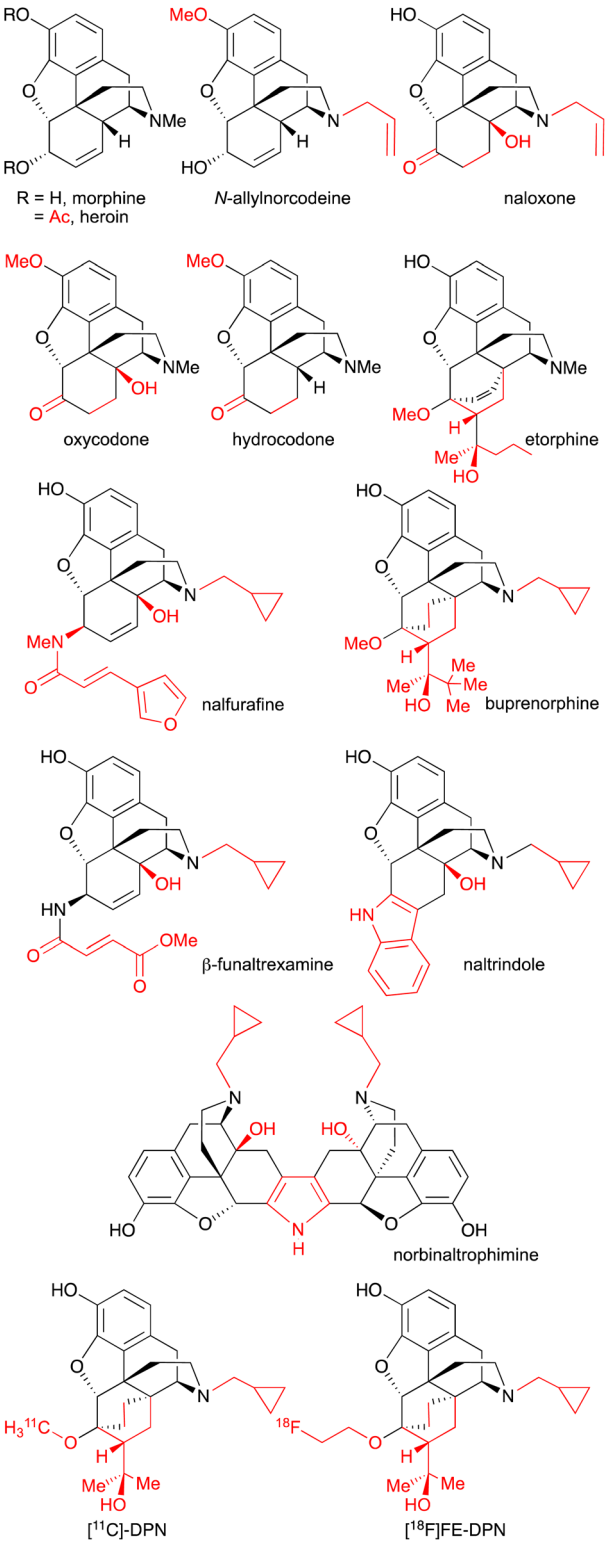


Fig. 1 Structures of morphine and representative semi-synthetic derivatives used as drugs and probes of the opioid receptors. Portions in red highlight structural modifications that were introduced *via* semi-synthesis from a naturally occurring opioid.

It is now well established that the analgesia and adverse effects of morphine and its derivatives are produced through the activation of the μOR. More specifically, activation of the μOR results in dissociation of the $G\alpha_{i/o}$ from the G-protein complex and subsequent inhibition of adenylate cyclase.[24] In addition to this canonical G-protein pathway, the opioid receptors can also signal through the β-arrestins, scaffolding proteins that are recruited to the receptor following its activation by most agonists. Early studies on the β-arrestins suggested that the G-protein pathway is responsible for the analgesic effects of morphine, whereas the adverse effects were more closely associated with β-arrestin recruitment, specifically β-arrestin 2.[25–27] These observations led to the development of so-called biased agonists (also known as functionally selective agonists) that could preferentially activate the G-protein pathway with reduced ability to induce β-arrestin recruitment. However, more recent studies have suggested at least some of the opioid side effects are β-arrestin-independent and G-protein biased agonists have resulted in only minimal improvements in side effect profiles.[27,28] This is a reminder that our understanding of the cellular mechanisms responsible for opioid analgesia and side effects is continually evolving. And in these mechanistic studies, semi-synthetic morphine derivatives, as well as those derived from other natural products discussed here, have been instrumental in shaping our understanding of the opioid receptors.

In all, hundreds of semi-synthetic morphine derivatives have synthesized and tested. Of these >20 have made their way to the clinic and include analgesics (oxycodone), antitussives (hydrocodone), antipruritic agents (nalfurafine), and treatments for opioid use disorder (buprenorphine). And yet, the quest for a safer, less addictive pain killer remains ongoing. In these efforts, chemists continue to draw inspiration from morphine. However, in recent years attention has also turned to natural products from kratom (*Mitragynine speciosa*), the akuamma plant (*Picralima nitida*), and salvia (*Salvia divinorum*). Chemists have continued to use semi-synthesis to explore these natural products, hoping that these novel structural scaffolds will provide avenues for developing enhanced medicines and new tools to study opioid receptors.

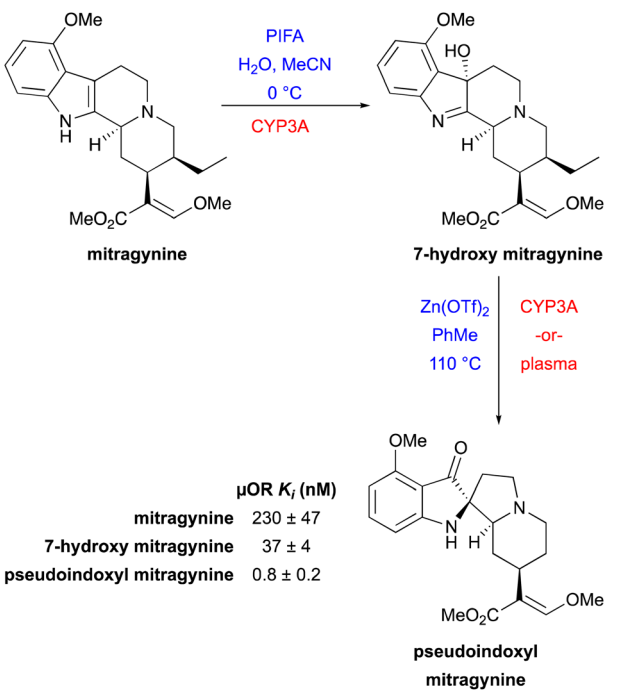
2. Mitragynine

In the search for safer analgesics, chemists have continued to turn to natural sources besides opium that have been used as traditional treatments for pain. Since at least 1836, the dried leaves of *Mitragynine speciosa*, commonly known as kratom, have been consumed for their psychoactive and pain killing effects. In its native Southeast Asia, the *M. speciosa* leaves are chewed, smoked, or consumed as a tea for its stimulant and analgesic effects and as an opium substitute.[29] The use of kratom has now grown well outside these geographical boundaries; some estimates suggest there are >2 million kratom users in the United States alone.[30] This widespread use of kratom is due in large part to the accessibility of kratom products, which are distributed through online and brick-and-mortar vendors in a largely unregulated fashion. Despite strong warnings from the US FDA concerning its adverse effects,[31] including the risk of developing a substance use disorder, the consumption of kratom remains legal in most of the world.

2.1. Opioid activity of mitragynine and its metabolites

The psychoactive and analgesics effects of kratom are largely attributed to mitragynine, which makes up 1–2% of leaf mass, and two-thirds of the alkaloid content.[32] Mitragynine was first isolated in 1921 by Ellen Field and in 1932 it was demonstrated to have anaesthetic action in the rabbit eye.[33,34] Remarkably, these early pharmacological studies occurred three decades before the chemical structure of mitragynine was reported (Scheme 1).[35–37]

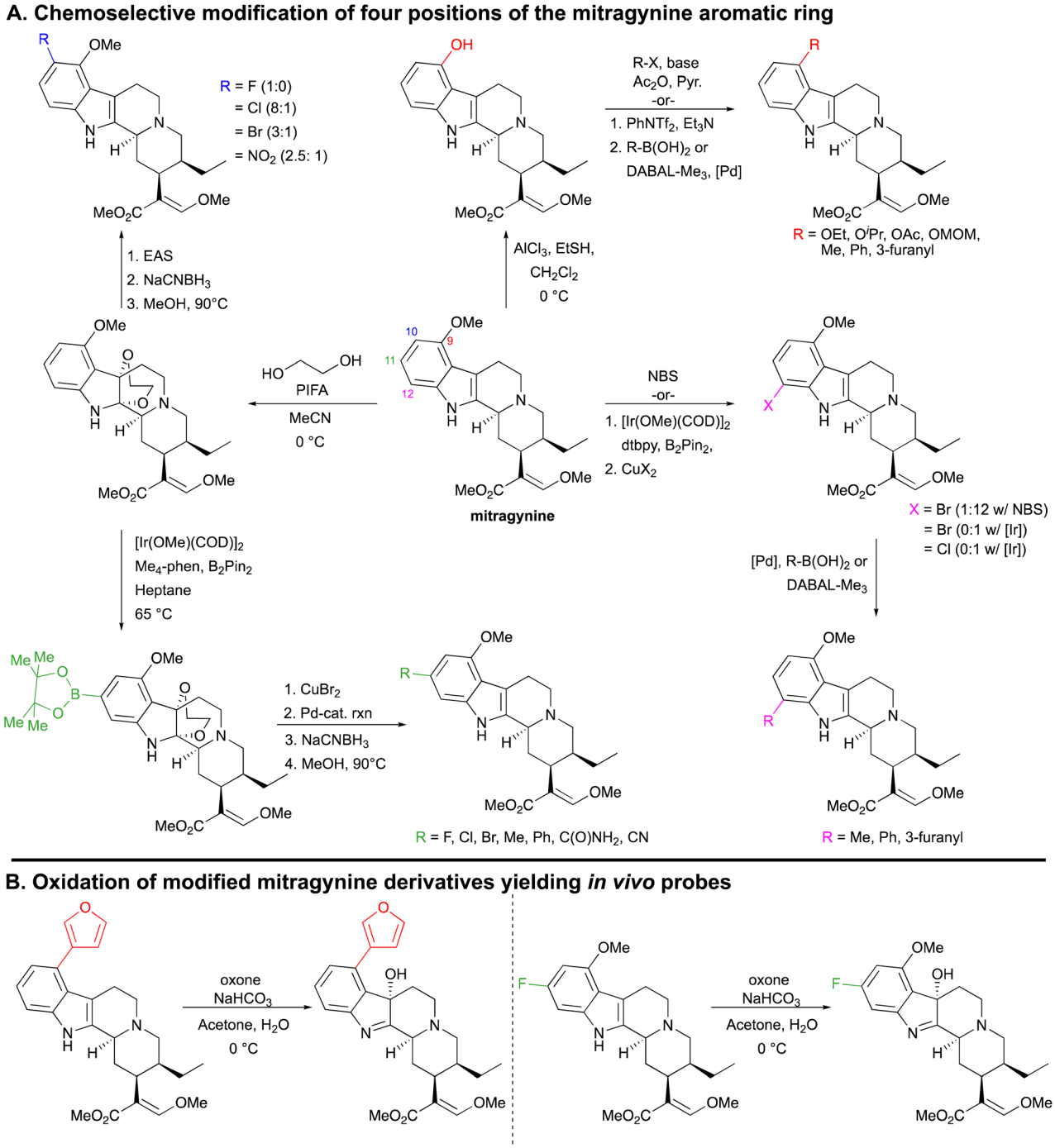
In 1972, Macko *et al.* demonstrated mitragynine's antinociceptive activity in mouse, rat, and dog.[38] This antinociceptive action was later shown to be reversed by the antagonist naloxone, suggesting these effects were μOR-dependent.[39] However, shortly after the initial demonstration of mitragynine's pain killing effects, it was postulated that the *in vivo* effects of mitragynine were actually elicited by one or more of its metabolites. This hypothesis was originally formulated after it was observed that biotransformation of mitragynine by the fungus *Helmin thosporum* sp. produced metabolites with increased antinociceptive activity.[40] Notably, 7-hydroxymitragynine and pseudoindoxyl mitragynine, the product of a 1,2-semipinacol rearrangement of 7-hydroxymitragynine, have 6- and 280-fold greater affinity to the μOR than mitragynine, respectively (Scheme 1).[41] While mitragynine, 7-



Scheme 1 Oxidation and semi-pinacol rearrangement of the mitragynine scaffold produces significant increases in μOR affinity. Conditions for the semisynthesis of mitragynine oxidative metabolites are shown in blue alongside the metabolic processes that produce the metabolites in red. Binding affinities and synthetic conditions from ref. 36.

hydroxymitragynine, and pseudoindoxyl mitragynine, activate the G-protein pathway of the μOR, they do not induce βArr2 recruitment to the receptor, making them highly G-protein biased agonists. This biased agonism profile may explain the reduced propensity of mitragynine and its oxidized derivatives to cause opioid-like side effects.[41] However, this improved side effect profile may also be due to the fact they are partial agonists of the μOR, as low intrinsic efficacy has also been proposed as a strategy to reduce opioid side effects.[42,43]

Further support to the bioactivation hypothesis has been provided by recent pharmacokinetic studies. Notably, mitragynine produces a more potent antinociceptive effect when given orally compared to subcutaneous delivery, suggesting that mitragynine is converted to an active metabolite *via* first-pass



Scheme 2 Optimization the reaction conditions and protecting groups strategy enables the chemoselective modification of the aromatic core of mitragynine. (A) Selective derivatization of four positions on the benzene ring mitragynine made possible through highly chemoselective transformations. Ratios in parenthesis indicate the C10 : C12 regioselectivity based on isolated products. (B) Oxidation of mitragynine analogues that produce lead compound with efficacy in animal models of pain.

metabolism.[38,44,45] Pharmacokinetic studies by several groups have now established that oxidative metabolism of mitragynine *via* CYP3A produces 7-hydroxymitragynine, which undergoes further rearrangement to pseudodindoxyl mitragynine, likely in the blood.[44–46] It is worth noting that the synthesis of these metabolites from mitragynine, made these pharmacokinetic studies possible by providing authentic samples to verify their identify and standards to quantify concentrations.

2.2. Substitution of mitragynine's benzene ring

The potent antinociceptive activity of mitragynine and its oxidized analogues has prompted the exploration of its SAR. Most of these semisynthetic studies have focused on modifying the benzene ring of mitragynine; indeed, through careful optimization of the reaction conditions each position (C9–C12) has been selectively targeted for modification (Scheme 2A). The earliest of these reports began by targeting the C9 methyl ether.[47] After selective demethylation of the aryl ether, a small collection of simple ethers and esters were synthesized.[47] This approach was later expanded to include C–C bonding forming reaction by first converting the phenol to a triflate thus allowing for palladium-catalysed Suzuki reactions to introduce substitutions at C9.[48] A similar approach has also been applied to the analogous triflate of pseudodindoxyl mitragynine.[41]

To access the C10–C12 positions, additional functional groups first needed to be introduced to the ring. Although early studies indicated that electrophilic aromatic substitutions (EAS) were generally unsuccessful,[49] bromination with NBS is possible and occurs with a strong preference for the C12 over C10 position.[48] Interestingly, Takayama *et al.* discovered that adding an equivalent of ethylene glycol across the 6,7-olefin of the indole scaffold drastically changed the reactivity of the mitragynine aromatic ring.[49] The resulting indoline was shown to undergo several EAS reactions with the major products having substitution at C10 rather than C12. The bridging ethylene glycol moiety can be removed with $NaCNBH_3$ to reveal the substituted mitragynine analogue. A similar trend has also been observed for iridium-catalysed borylation; in the unprotected mitragynine C–H activation occurs exclusively at C12, but once protected with ethylene glycol, reaction conditions could be modified to chemoselectively borylate and then further functionalize the C11 position.[50]

Beyond demonstrating an impressive ability to manipulate the reactivity of a complex natural product, the semi-synthetic studies on mitragynine have also provided new pharmacological tools to probe the opioid receptors. Two clear examples are represented by the two oxidized analogues SC13 and 11-fluoro-7-hydroxymitragynine (Scheme 2B).[48,50] During their respective SAR studies, both analogues were identified as μOR partial agonists. However, in both cases these compounds show considerable higher efficacy in assays that measure inhibition of cAMP, a downstream signalling event in the G-protein pathway cascade, compared to nanobody-based assays that measure intrinsic receptor activation. Indeed, in the cAMP assays, SC13 is a full agonist yet only produces an $E_{max} = 21\%$ in upstream receptor activation assays. This difference in efficacy measured is an illustrative example of the increased amplification produced when measuring downstream events such as cAMP inhibition. In mice, both SC13 and 11-fluoro-7-hydroxymitragynine are effective antinociceptive agents, albeit with 11-fluoro-7-hydroxymitragynine producing a significantly diminished maximal effect compared to 7-hydroxymitragynine and morphine. Perhaps more impressively, SC13, which is as effective as morphine in animal pain models, does not cause condition placed preference (a model of reward), respiratory depression, constipation, or hyperlocomotion. This ability of SC13 to effectively reduce pain, without causing classical morphine-like side effects suggests partial μOR agonists represent one promising strategy for developing safer opioid analgesics.

3. Akuamma alkaloids

In addition to the now wide-spread use of *M. speciosa*, the use of other botanicals to manage pain has also risen. The seeds of the akuamma plant (*Picralima nitida*) represent one such example. In its native West Africa, *P. nitida* has been used as a traditional treatment for a range of ailments including malaria, fever, and pain, and continues to be an important medicinal plant.[51–53] Recently, *P. nitida* seeds have been commercialized by online vendors and marketed as an alternative to kratom. The first chemical investigations into *P. nitida* seeds were reported by Henry and Sharp in 1927 and identified a family of related of alkaloids, known as the akuamma or akuammiline alkaloids, which include akuammine, pseudoakuammigine, picraline, akuammidine, akuammiline, and akuammicine (Fig. 2).[54,55] However, it took six decades for a potential target of the akuamma alkaloids to be discovered when Menzies *et al.* demonstrated they bind to and activate the opioid receptors using radioligand binding and tissue preparation assays.[56] More recently, Creed *et al.* through unbiased receptor screening *via* the PDSP and modern functional assays using cells lines that express the cloned human receptors confirmed this activity and determined the alkaloids generally had low affinity at other CNS receptors.[57] Moreover, this latter study suggested the akuamma

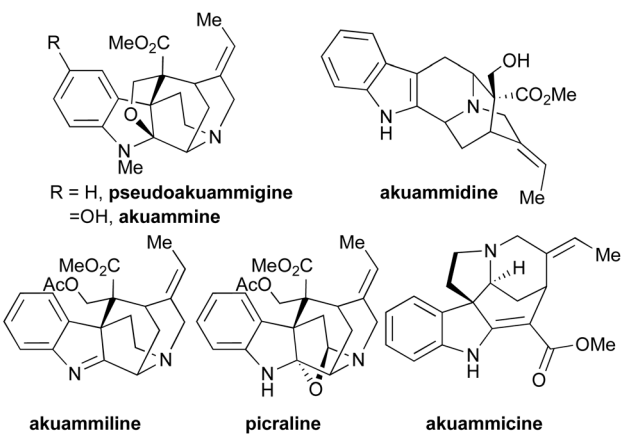


Fig. 2 Structures of the major alkaloids found in the seeds of the akuamma plant (*Picralima nitida*).

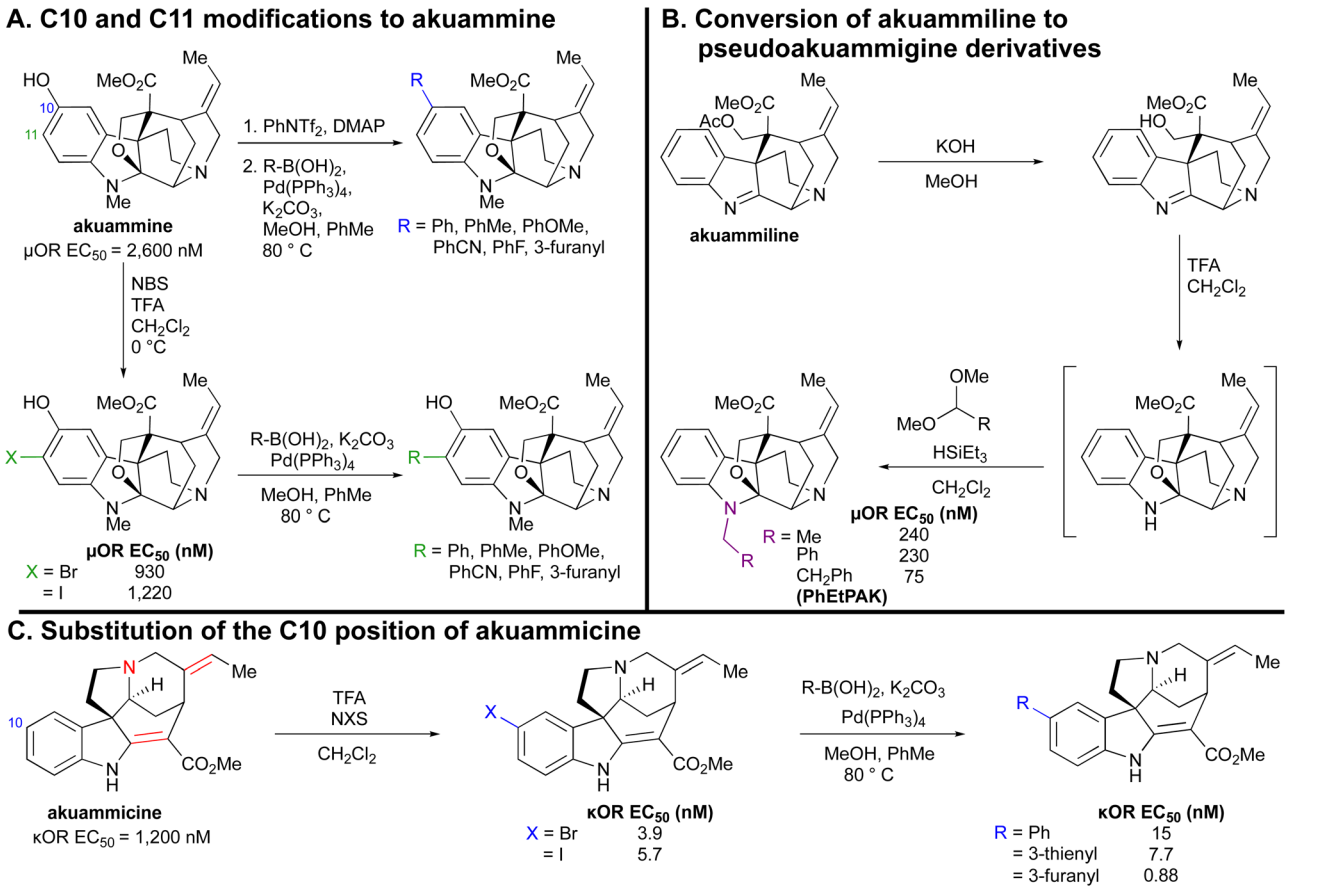
alkaloids were G-protein biased agonist with akuammine, pseudoakuammigine, and akuammidine acting preferentially at the μOR and akuammicine at the κOR.[57]

3.1. Akuammine and pseudoakuammigine

Given the traditional and contemporary use of *P. nitida* seeds in the treatment of pain, the μOR-active akuamma alkaloids were evaluated in animal models of pain.[57] However, in the hot-plate and tail-flick assays, akuammine, pseudoakuammigine, and akuammidine, showed limited anti-nociceptive activity in mice and were considerably less efficacious than morphine. This is in contrast to an earlier study that had reported pseudoakuammigine were anti-nociceptive and anti-inflammatory rats.[58] Although species differences could not be ruled out as a possible explanation, the low activity of the akuamma alkaloids in mice is likely due to their modest potency at the μOR; akuammine, pseudoakuammigine, and akuammidine are >1000-fold less potent than morphine at the μOR.

In response to the limited *in vivo* activity and limited potency, a campaign to elucidate SAR at the μOR was initiated.[59] The initial study focused on akuammine and pseudo-akuammigine because of their availability, which was facilitated by an isolation protocol developed using pH-zone refining countercurrent chromatography.[57] Due to its chemical reactivity and ability to form potential ligand–receptor interactions (*e.g.*, hydrogen bond donor/acceptor) the phenol of akuammine was a logical starting point. In addition to simple methylation and acetylation, the phenol was also converted to a triflate which allowed for the introduction of substitions at the C10 position *via* palladium-catalysed reactions (Scheme 3A). Similarly, halogenation of akuammine at the C11 position afforded similar function handles to substitute the adjacent position on the aryl ring. Unfortunately, despite the range of different substitutions explored, modifications at these positions generally led to considerable reductions in affinity at the μOR.

In contrast to substituting the aromatic ring, replacement of the *N*-methyl group of pseudoakuammigine led to more encouraging results. To access these *N*-modified pseudoakuammigine derivatives, a two-step route was developed using akuammiline, another akuamma alkaloid isolated from *P. nitida* (Scheme 3B). First akuammiline was deacetylated to reveal a primary alcohol intermediate. Under acidic conditions, this primary alcohol cyclizes to generate a hemiaminal ether that undergoes a reductive amination, ultimately yielding *N*-



Scheme 3 Modification of the akuamma alkaloids at select sites results in improved potency at the μOR or κOR. (A) Direct modification of the C10 and C11 positions on the akuammine scaffold generally results in reduced potency at the opioid receptors. (B) Conversion of akuammiline to pseudoakuammigine analogues bearing modifications at the N1 position results in significant increases in μOR potency. (C) substitution of the C10 position of akuammicine leads to dramatic increases in κOR potency. Modifications to the positions coloured red lead to reduction in κOR potency.

substituted pseudoakuammigine derivatives. Most notable among the analogues generated through this route was phenethyl-pseudoakuammigine (PhEtPAK), bearing a phenethyl (–CH_2–CH_2-Ph) substitution. PhEtPAK is 70-fold more potent at the μOR than pseudoakuammigine. Importantly this increased potency at the μOR translates into *in vivo* efficacy: using the same tail-flick and hot-plate pain models where the akuamma alkaloids failed to produce a meaningful response, PhEtPAK (80–100 mg kg^{-1}, s.c.) produces similar antinociceptive activity as morphine (10 mg kg^{-1}, s.c.). Although the doses required for PhEtPAK are roughly 10-fold higher than morphine, suggesting further improvements to the μOR potency may be necessary, the improvement over the naturally occurring akuamma alkaloids, both regarding *in vitro* potency and *in vivo* efficacy, is a clear testament to the power of semi-synthetic SAR studies.

3.2. Akuammicine

During the SAR studies that led to the discovery of PhEtPAK, concurrent investigations into the akuammicine scaffold were also underway.[60] In this case, a more comprehensive approach was taken and essentially every functional group on the akuammicine scaffold was modified including the aromatic core, olefin, vinylogous carbamate, and tertiary nitrogen. As is often the case when modifying natural products, most of these changes drastically reduce or completely eliminate their activity at the κOR. The exception to this trend were substitions to the C10 position in the aromatic core, which invariably produce increases in the κOR potency. The introduction of bromine or iodine atoms at this position stand out as particularly striking examples, as a single atom substitution (H → Br or H → I) results in 210-300-fold increases in κOR potency (Scheme 3C). Furthermore, these halogenated derivatives also serve as useful synthetic intermediates allowing for the exploration of addition substitutions using palladium-catalysed reactions, including the introduction of a 3-furan *via* a Suzuki–Miyaura coupling that resulted in the most potent akuammicine derivative identified to date. It is worth noting that while C10 substitutions to the akuammicine scaffold drastically improve κOR affinity and potency, similar changes are not observed at other receptors. As a result, in addition to being highly potent κOR ligands, these C10-modified akuammicine derivatives are also highly selective with >200-fold higher affinity at the κOR over all other CNS receptors investigated.

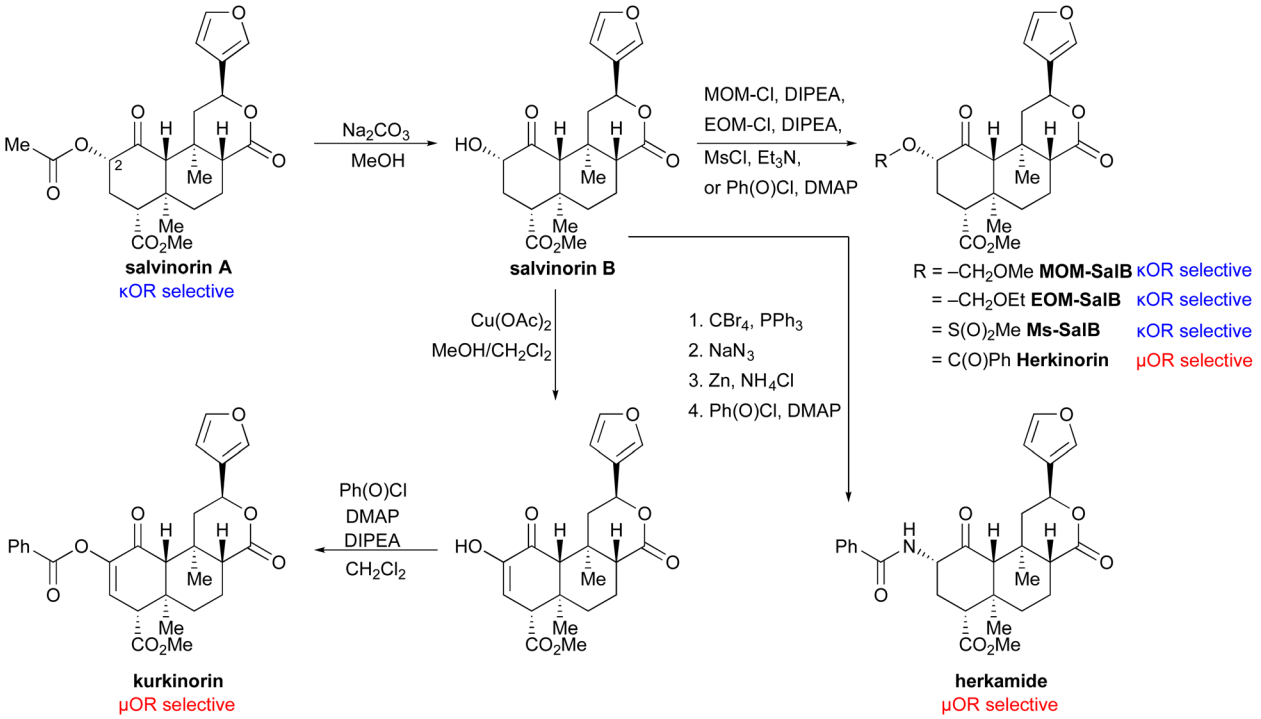
Given the dramatic increase in potency and affinity produced by halogenation of the C10 position, the *in vivo* effects of 10-bromo-akuammicine and 10-iodo-akuammicine have recently been investigated.[61] The κOR-specific morphine derivative nalfurafine was the first κOR agonist approved for use in humans and is used in the treatment of pruritus (*i.e.*, itch). The only FDA-approved κOR agonist, difelikefalin, is similarly used as an anti-pruritic agent. As such, 10-bromo-akuammicine and 10-iodo-akuammicine and were first shown to dose-dependently reduce scratching bouts induced by 48/80, a chemical that induces a histamine response. However, 10-bromo-akuammicine produces condition place avoidance (a model of aversion), reduces hyperlocomotion, and impairs performance on a rotarod test, all of which are adverse effects produced by most κOR agonists in mice. Interestingly, similar effects were also produced by 10-iodo-akuammicine, however the magnitude of these effects was reduced. This difference in behavioural effects may be due to the two compounds differing abilities to cause κOR internalization, which is a consequence of βArr2 recruitment. However, because the mechanisms responsible for the side effects produced by κOR remain largely undefined, these and future akuammicine derivatives represent important pharmacological tools to clarifying these pathways.

4. Salvinorin A

Much like the isolation of morphine from opium, the discovery of the kratom and akuamma alkaloids grew out of their plant sources being used as traditional medicines to treat pain. However, analgesia is not the only activity that has inspired searches for psychoactive natural products. Indeed, salvinorin A, one of the most potent naturally occurring opioid receptor ligands identified to date, was first investigated because it is the active component of the hallucinogenic plant *Salvia divinorum*. For centuries, Mazatec shamans in Oaxaca, Mexico have used *S. divinorum* in ritualistic divination rituals due to the intense, yet short-lived hallucinations produced from consumption of its leaves. Due to its legal status, recreational use of *S. divinorum* began in the late 1990s. The major metabolite found in the leaves of *S. divinorum* is salvinorin A, a labdane diterpene that was first isolated by Alfredo Ortega in 1982 (Scheme 4).[62] Later, Valdes *et al.* independently isolated and characterized salvinorin A and confirmed it was the psychoactive component of *S. divinorum* extracts.[63] However, it was not until 2002 that Bryan Roth and coworkers at the Psychoactive Drug Screening Program determined that salvinorin A derives its psychoactive effects through the potent and selective activation of the κOR.[64] This discovery was surprising for two reasons. First, unlike prototypical hallucinogens (*e.g.*, lysergic acid diethylamide, psilocybin, dimethyltryptamine) salvinorin A has no affinity for the serotonin receptors. Secondly, as salvinorin A is a terpene and not an alkaloid it contains no nitrogen, making it the first naturally occurring non-nitrogenous opioid receptor agonist identified. Moreover, the unique structure of salvinorin A, particularly this lack of a basic nitrogen, directly contradicted the long-established "message-address" concept that explains how endogenous opioid peptides and morphinans bind to the opioid receptors.[65,66] Due to its potent activity at the κOR and its structural dissimilarity to other opioid ligands, this initial report of its biological target spurred further investigation into salvinorin A.

4.1. Salvinorin A derivatives targeting the κOR

To date, nearly 600 analogues of salvinorin A have been reported, with the vast majority of these being produced *via* semi-synthesis.[67,68] These analogues explore the SAR by modifying many positions of the diterpene core but focus heavily on the C2 ester and furan ring. Because up until very recently there has been no structure of the κOR complexed to salvinorin A, SAR studies alongside mutation of receptor residues represented the



Scheme 4 Synthesis of salvinorin A derivatives modified at the C2 position. Substitution of the C2 acetyl ester witho acetate bioisosteres maintains salvinorin A's high selectivity for the κOR. Conversely, replacement of the C2 acetyl ester with aromatic esters and amides results in a unique change in receptor selectivity, converting salvinorin A to a potent and selective μOR agonist.

only tools to investigate how salvinorin A interacts with the κOR.[69] Although most modification of the salvinorin A structure perturb its activity at the opioid receptors, perhaps the most drastic changes in activity occur when altering the C2 acetate.

The C2 acetate moiety was originally viewed as a potential reason for salvinorin A's short duration of action, as the acetyl ester could be rapidly hydrolysed. The resulting alcohol, salvinorin B, which is also found in the leaves of *S. divinorum*, has no κOR agonist activity.[70] As a result, some of the first derivatives of salvinorin A focused on replacing the acetyl ester with bioisosteres, such as a methoxy methyl ether (MOM-SalB), ethoxy methyl ether (EOM-SalB), and methyl sulfonate (Ms-SalB).[71–73] To synthesize these derivatives, the acetyl ester of salvinorin A can be selectively cleaved with Na_2CO_3 in methanol yielding salvinorin B and the requisite sulfonate or ether appended (Scheme 4). These derivatives place an oxygen atom in a similar position as the carbonyl oxygen in salvinorin A and thus retain potent κOR agonist activity but are not prone to cleavage by esterases. As a result, MOM-SalB, EOM-SalB, and Ms-SalB have been employed in numerous *in vivo* studies that demonstrate their effects in animal models of pain and cocaine use.[74–77] Additionally, the cryoEM structure of MOM-SalB complexed with the κOR was recently reported, providing the first structure of the receptor bound to a salvinorin A derivative.[69]

4.2. Salvinorin A derivatives targeting the μOR

While salvinorin A derivatives containing simple alkoxy or alkyl sulfonate C2 substitions retain salvinorin A's high κOR selectivity, aromatic esters appended at this position lead to an interesting change in opioid receptor preference.[78] Most notably, replacement of the C2 acetate with a benzoate converts salvinorin A, a ligand with complete selectivity for the κOR, into a molecule, known as herkinorin, with 7.5-fold greater affinity at the μOR than the κOR (Scheme 4). Alternatively, a multi-step process involving the bromination of the C2 alcohol of salvinorin B, nucleophilic substitution with NaN_3, reduction, and benzoylation provides the C2 benzamide derivative, known as herkamide, which possesses >3000-fold greater affinity at μOR than the κOR (Scheme 4). Notably, herkinorin and herkamide are unique examples where the high selectivity of a natural product can be overridden in favour of a preference for a different receptor. Beyond this intriguing reversal in opioid receptor selectivity, the discovery of herkinorin was also a pivotal moment in understanding signalling bias for the opioid receptor. Herkinorin was the first μOR agonist that was shown not to recruit βArr2 or cause receptor internalization.[79,80] Intriguingly, despite the structurally similarities to herkinorin, herkamide lacks this signalling bias and does recruit βArr2 and promote receptor internalization.[80] Driven by these differences, further SAR studies on the A-ring of herkinorin identified a derivative, designated as kurkinorin, with an additional degree of unsaturation at the C2–C3 position that can be introduced using $Cu(OAc)_2$ as an oxidant (Scheme 4).[81] Like herkamide, kurkinorin is highly potent and selective for the μOR; however unlike herkamide, kurkinorin possesses the G-protein bias observed in herkinorin. By seemingly combining the positive attributes of herkinorin (G-protein bias) and herkamide (μOR potency and selectivity), kurkinorin and its

derivatives are as effective as morphine in animal models of pain but with reduced tolerance, condition place preference, and sedation.[81,82] The differences in selectivity, potency, signalling, and *in vivo* effects of herkinorin, herkamide, and kurkinorin highlights how even subtle changes in structure can result in dramatically different pharmacological effects. Additionally, the isolation of salvinorin A from a plant not traditionally used to treat pain, and the impressive number of opioid receptor ligands it has given rise to, underscores the importance of unbiased screening when looking for novel bioactivity.

5. Conclusions

For >150 years chemists have been drawn to idea that through careful manipulation of a natural product's structure, one can harness its opioid activity, remove its side effects, and arrive at a safer, more effective opioid medicine. Early on, these studies centred nearly exclusively on morphine and other alkaloids found in *P. somniferum* as they were the starting points chemists had at their disposal. However, the above examples demonstrate that the semi-synthesis of other opioid-targeting natural products remains an active area of research. By leveraging highly chemoselective transformations, semi-synthesis offers unique opportunities to quickly generate analogues and elucidate SAR trends, while bypassing the need for resource-intensive total syntheses. As a result, the field of opioid pharmacology and pain medicine has been shaped by the pharmacology tools provided by these natural product derivatives.

Semi-synthesis is clearly not without its drawbacks. Perhaps the most apparent challenge is the pre-requisite that the natural product be readily available, ideally in quantities that allow for multiple reactions to be performed at reasonable scales. Initially, milligram quantities may be sufficient for exploring SAR; however, for commercialized drugs like morphine and oxycodone, tons of raw material must be isolated. In many instances, the isolation of natural products from these natural sources is not trivial and requires specialized separation techniques to purify single compounds from the complex mixtures that make up natural product extracts. For instance, the isolation of the akuamma alkaloids relies heavily on the use of pH-zone refining countercurrent chromatography to separate the individual akuamma alkaloids.[57] Nevertheless, the isolation of these complex natural products is often the most direct route to accessing them and can provide an expedited means to begin studying their biological activity.

In instances where the supply from the natural source is limited, total synthesis can serve as a useful alternative to semi-synthesis. However, the total synthesis of a natural product is nearly always a time and resource intensive endeavour. Thus, the investment in pursuing a total synthesis campaign should be balanced by the prospect of identifying novel biological activity. Even when such evidence is present, total synthesis is not without its risk. The natural product collybolide provides one such cautionary tale; despite significant pharmacological data identifying collybolide isolated from *Collybia maculata* as a κOR agonist, further investigations with material provided from its total synthesis were unable to confirm these results.[83,84] However, it should be noted that in the long history of natural product total synthesis, the irreproducibility of a compound's biological activity is a relatively rare occurrence. Moreover, total synthesis has provided countless training opportunities for and synthetic challenges to inspire organic chemists.

In addition to the supply issue, another common criticism of semi-synthesis is that the sites of modifications are limited to the natural product's inherit reactivity. However, the functional groups commonly targeted through semi-synthesis (carbonyls, olefins, alcohols, amines, aromatic rings) are also capable of forming strong ligand–receptor interactions (hydrogen bonds, π–π stacking, cation−π, and electrostatic interactions). Thus, the most accessible sites are often the site a medicinal chemist would look to target to establish SAR trends. In recent years, this notion has been supported by structural biology data of morphine, mitragynine pseudoindoxyl, MOM-SalB, and GB18, bound to their respective opioid targets.[69,85–87] For instance, in the morphine-bound structure of the μOR, one observes a salt–bridge interaction between the amine of morphine and $Asp149^{3.32}$ (Ballesteros-Weinstein numbering), which has long been recognized as a highly conserved interaction for amine-containing opioid ligands. Additionally, $Tyr150^{3.33}$ forms a hydrogen bond to the aryl ether.[87] It should also be noted that hydrophobic interactions also heavily contribute to a ligand ability to bind a receptor. These recent cryoEM structures also demonstrate how despite being chemically quite divergent, each of the natural product's complex carbon skeletons are uniquely suited to fill the hydrophobic pockets found within the opioid receptors binding site.

As advancements in synthetic chemistry are made, particularly those suited for late-stage functionalization, previously unreactive sites on natural product scaffolds will be made accessible. The modifications of the aromatic core of mitragynine depicted in Scheme 2 provides an illustrative example. Whereas derivatives were initially limited to simple substitutions of the aryl ether, the advent of C–H functionalization, the application of palladium-catalysed cross-couplings, and the discovery a new process to protect the 2,3-π bond of indole rings enabled substitutions at C10–C12. In this way, the modification of natural products often inspires innovative chemistry and the structural complexity found in natural products should continue to be a fertile testing ground for new synthetic methods.

The medicinal chemistry of natural products is of course not limited to derivatives accessed through semi-synthesis. As noted, total synthesis can provide a useful solution to the supply problem and provides increased flexibility in the modifications that can be introduced. The recent total syntheses of salvinorin A, ibogaine, and GB18, highlight how total syntheses can complement semi-synthetic efforts to develop ligands for the opioid receptors.[88–90] The most successful of these approaches enable the rapid generation of compound collections by introducing diversification points late in the synthetic sequence as exemplified by the Shenvi lab's investigations into the furan and lactone of salvinorin A.[90] The additional flexibility of total synthesis also allows for the discovery of compounds like oxanoribogaine, a κOR-agonist analogue of ibogaine that replaces

the indole nitrogen with an oxygen, that would likely be impossible *via* semi-synthesis.[89] And finally, the recent synthesis of the *Galbulimima* alkaloid GB18 demonstrates how the total synthesis is necessary in instances where isolation from natural sources is not feasible.[88] In this case, the streamlined synthesis of GB18 provided sufficient material to identify its biological target as the μOR and κOR.

Moving forward, the semi-synthesis of natural product analogues will continue to play a pivotal role in the study of the opioid receptors. As assays to measure opioid receptor activity become more accessible, additional screening of natural products will lead to the discovery of new chemical scaffolds to explore *via* semi-synthesis. These semi-synthetic studies will be equally facilitated by the development of new, functional group-tolerant synthetic methods capable of modifying their complex structures. The resulting natural products derivatives are bound to advance our understanding of the opioid receptors and new drug leads.

6. Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

7. Conflicts of interest

There are no conflicts to declare.

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