A Chemical Overview of Opioid Receptors and Their Agonists

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**Introduction**

Opioids are not a new concept in medicine as they have been studied in use for more than 5000 years, dating all the way back to ancient Greece and Egypt. The first symptoms that were observed including pain alleviation, anti-diarrheal, and mood changes; making opium a promising medication and dangerous recreational drug. In 2009, the World Drug Report sited that opioid related drugs generated a revenue of about 70 billion dollars worldwide. In 1805, German chemist Friedrich Sertuner isolated the active agent in opium, morphine, for the first time. Likewise, this led to the use of morphine in therapy and for a search for other analogues with reduced abuse potential and side effects. The next compound that was synthesized was diacetylmorphine (heroin) which was the first analogue boasting to be more potent than morphine and free from potential of abuse. As we know today, no such drug exists but remains to be the end goal of opioid research.  

The synthesis and isolation of more opioid related compounds led to two classifications of similar acting drugs. Opiates refer to products that are direct products of the opium poppy and morphine derivatives. Opioids are compounds that still target the opioid receptor but are not directly made from the opium poppy. Opioid research focused on new drug synthesis until 2012, when four opioid receptors were imaged: Mu, delta, kappa, and nociception (labeled μ, δ, κ, and nociception respectively). The nociception ligand has a high sequence identity which is why it is classified as an opioid receptor, however, few opioids have strong binding affinity for this receptor and will not be elaborated on in this paper. The loss in binding affinity is thought to have come from mutations that enlarge the binding pocket of the nociception receptor, which makes the hydrophobic interaction with morphinan rings ineffective.

Most opioids can be structurally categorized into three different groups: Morphinans, ketomorphinans, and benzomorphans. Morphinans have the basic structure of morphine and are highlighted
by a phenol group, two six-membered rings, and a nitrogen group that sits on top. Keto-morphinans are the same compound as morphine except also have a ketone on one of the six-membered rings. The most common keto-morphinan would be 10-keto-morphine. Finally, benzomorphans lack a third ring and contain only a benzene ring attached to a nitrogen containing eight-membered ring that is bridged in the middle. Opioids can then be classified even further as natural, semi-synthetic, and synthetic based on how they were synthesized or extracted.\(^5\)

\[
\begin{align*}
\text{morphinans} & \quad \text{10-Keto-morphinans} & \quad \text{Benzomorphans}
\end{align*}
\]

**Figure 1. Groups of Opioids.** The three different groups of opioids are represented here.\(^5\)

With the crystal structure of each receptor, researchers can study how compounds bind to each receptor and how opioids are metabolized in much greater detail. The crystal structure of all four receptors were captured in ligand-bound conformations. A ligand-bound conformation means that the receptor is in the inactive state because the ligands attached to each blocks the receptor; the ligand is called an antagonist because of its blocking ability. In order for the receptor to be activated, the activating chemical must be able to sterically fit around the antagonist.\(^6\)

An important theory in understanding the binding of opioids to their receptor is the Message-Address Model. This has been the supposed method of receptor binding since the 1980’s and has not been proven wrong. This theory states that every receptor has a specific binding site that acts as an
address while every antagonist has a specific shape that fits this address. The antagonist acts as the message, and instructs the receptor on what to do. Researchers can then use this concept by looking at known opioid antagonists to determine the address or shape of the binding site in the opioid receptor. Once the binding site has been determined, we can predict which other antagonists will activate the receptor.

The opioid receptor is often studied in mice because of its similarity to the human receptor and they are much more dispensable. The mouse δ-opioid receptor (mMOR) is 84% equal to the human δ-OR and differs mainly in DNA structure that builds the proteins. Some antagonists are taken up by the mouse μ-OR at different efficiencies than the human μ-OR. This is not a result from the structure of the binding site, but from the structure of the surrounding areas of the receptor. This makes the mouse μ-OR an optimal test subject before moving into opioid related human trials.

**Activation of Opioid Receptors**

The direct activation of opioid receptors is well understood. Activation of the opioid receptors is controlled by several non-covalent interactions between agonists and receptors. The primary interactions include hydrogen bonding, hydrophobic, hydrophilic, non-polar, and polar. Each receptor’s binding pocket is unique due to the different interactions of the specific residues that are in each receptor. Other aspects that contribute to binding pocket specificity are internalized molecules such as water which gives rise to hydrogen bonding to opioid agonists within the binding pocket.
Figure 2 Binding Site Interactions. Pictured is the μ-OR and the polar interactions that occur between beta-Funaltrexamine and the μ-OR. Water molecules, represented as red circles, are embedded in the binding pocket and add to polar interactions.²

When an agonist enters the binding pocket, specific interactions must be achieved to activate the receptor. If the interactions are not met, the molecule will often not enter the binding pocket because the interactions will repel each other. However, if these interactions are successful, then the agonist will activate the receptor and induce a conformational change within the receptor. This conformational change is then what we identify as the activated state of the receptor which have not been imaged yet.⁹

GCPRs are separated into three subunits: α, β, and γ. When inactive, all three subunits are bound together into one body. When a GCPR is activated, the β and γ subunits dissociate from the α unit; These two separate units are denoted as the Gβγ and Gα units respectively. Once dissociated, the GCPR is considered active until the subunits are reunited into its whole protein.¹¹
Only some effects that are caused by the activation of opioid receptors are understood; some are involved with neuron and brain activity, rather than the direct stimulation of the receptor. For example, the direct activation of the opioid receptors and the reduction of pain is well understood due to our knowledge of GCPRs and neuron stimulation. All four opioid receptors can inhibit neuron activity by inhibiting the repolarization of the cell. When activated, the $G_{\beta\gamma}$ binds directly to calcium ion channels. Once bound, the ion channels of the neuron remain open which prevents calcium removal from the cell. There is no charge buildup between the interior and exterior of the neuron when the flow of calcium is stopped. The ultimate result is no action potential, rendering the neuron unable to send any signal.

**Crystal Structure of the Mu-Opioid Receptor**

The first thing that can be learned from the crystal structure of the $\mu$-opioid receptor ($\mu$-OR) is how it is bound to the membrane. The $\mu$-OR has alternating aqueous and lipid layers that pair into dimers and tightly associate through the transmembrane $\alpha$-helices. In all, there are seven transmembrane $\alpha$-helices that form three extracellular and intracellular loops apiece. With the addition of several disulfide bridges, this creates an extremely stable base that anchors to a membrane through $\alpha$-helices. The transmembrane structure of the $\mu$-OR is going to be referred to as the bottom of the receptor for the remainder of this section.

One important site that was confirmed by the crystallization of the $\mu$-OR was the presence of Ala and Met residues that serve as a covalent anchor to the membrane of the cell. The presence of these residues were predicted in 1996 using fluorescent-tagged residues on Chinese hamster receptors. This is an important factor of the opioid receptors because without it, the proteins would not stay anchored in the membrane of the cell and would be free floating in the cytoplasm. This would render the receptor
dysfunctional because signaling molecules would not be able to escape the cell and arrive at their destination.\textsuperscript{12}

The top of the receptor in our orientation would be the exposed ligand-binding pocket, which is highlighted by what looks like a column formed by $\alpha$-helices. This is the location where chemicals bind to the receptor and activate it, which makes it a very promising and interesting area to study. This area varies in structure in each GCPR. One example is how exposed this binding pocket is. The further this binding pocket is buried into the column of helices the longer it will take for antagonists to dissociate from the GCPR. In the $\mu$-OR, the binding pocket is mostly exposed which creates relatively short half-lives of potent opioids. Some examples are 44 minutes, 36 minutes, 30 minutes, and less than one minute from duprenorphine, diprenorphine, alvimopan, and etorphine respectively. These are extremely short compared to other drugs like tiotropium (which uses a different receptor) that have a half-life of 34.7 hours. While the binding affinity is relatively unaffected by the exposed binding pocket, the dissociation is changed and gives the $\mu$-OR traits.\textsuperscript{2}

\textbf{Figure 3. Exposed $\mu$-OR Binding Pocket.} The binding pocket of the $\mu$-OR is particularly shallow and open compared to other GCPRs.

Two of the traits that the shallow binding pocket of $\mu$-OR has the ability to reverse heroin overdoses and the ability to use opioids as fast and effective anesthesia in veterinary applications.\textsuperscript{2}
Naloxone is a narcotic used during heroin overdose because it competes for the same binding site in the μ-OR and prevents heroin from activating the μ-OR until it is metabolized. The reason that this works is because naloxone has a slightly higher binding affinity to the μ-OR, and the shallow binding pocket allows for quick dissociation of the agonist. Etorphine is a preferred anesthetic in racehorses because it quickly knocks out the animal and when it is safe for the animal to wake up, diprenorphine is injected and takes the place of etorphine on the μ-OR much like naloxone in humans with heroin overdose symptoms.

The μ-OR has 14 residues that directly impact the specificity of the binding pocket. Nine of the residues are also in the κ receptor and 11 are found in the δ receptor. Some of the most important residues within the μ-OR include H297, E229, K303, and W318. The H297 residue directly interacts with the aromatic ring of morphine. Something unique that was found is that the ring does not directly interact to the residue through hydrogen bonding, but through two water molecules positioned inside of the residue. The other three residues mentioned are unique to the μ-OR and seem to be results of point mutations. The point mutation in W318 dramatically increases the binding affinity of some opioid agonists like naltrindole because it is less sterically hindered.

**Crystal Structure of the Delta-Opioid Receptor**

The δ-OR plays a similar physiological role as the μ-OR as it serves as a target for therapeutically treating pain. In general, the δ-OR can be split up into three distinct regions: The upper and lower portions of the binding pocket and the portion embedded in the cell membrane that anchors the cell. The binding pocket has two unique regions, the lower part is unique to each receptor while the upper portion of the pocket is very similar throughout all types of opioid receptors. This common binding pocket contains a region where β-sheets layer on top of each other using hydrogen bonding to create a “hairpin” loop. This hairpin loop creates a wide space in the primary binding pocket that allows many
agonists to enter. This may explain why there are a wide variety of ligands that activate these receptors while still being rapidly reversible.\textsuperscript{6} The second, more variable aspect of the binding pocket is the lower half, where the main interactions between the receptor and agonist take place.\textsuperscript{4}

The accuracy of the \(\delta\)-OR is ensured in its crystallization because its antagonist ligand is covalently bound to the receptor. This is unique to the \(\delta\)-OR because the \(\mu\)-OR and \(\kappa\)-OR have different residues at the 300 position. The \(\delta\)-OR is extremely similar to the \(\mu\)-OR, only differing in three residues. However, despite the similar sequence identity, these three residues create a binding site that is the most unique of the three main opioid receptors. The residues create a binding pocket that is slightly longer than the \(\mu\) and \(\kappa\) binding pockets. This creates a binding pocket that favors long agonists.\textsuperscript{4}

The expanded binding pocket also explains why many \(\mu\)-OR agonists will not activate the \(\delta\)-OR. Since agonists induce a conformational change through non-covalent interactions like hydrogen bonding, the agonist must be the proper length in order to activate the receptor. If the agonist is too short, all the binding site locations will not be reached, and the agonist will simply diffuse in and out of the receptor without doing much of anything. If the agonist is too long, then it won’t fit into the binding pocket and the receptor will not activate. This is a testament to how changes on the molecular level, no matter how small, can have a large impact.\textsuperscript{4}

Another unique aspect of the \(\delta\)-OR is a sodium ion positioned within the receptor that aids in allosteric effects. While water molecules have been found to aid in hydrogen bonding in all three opioid receptors, the \(\delta\)-OR contains a site surrounded by five oxygen atoms formed by 16 residues that harbors an allosteric sodium. 15 of these residues are consistent in all three receptors. The unique residue in the \(\delta\)-OR that helps contain the allosteric sodium is an ASP residue. While the direct impact of this allosteric sodium is currently unknown, it is hypothesized that this ion impacts binding properties of many agonists.\textsuperscript{14}
Figure 4. Opioid Receptor’s Open Binding Pocket. The opioid binding pocket is left open in part by the beta hairpin loop that is labeled as ECL2. All three opioid receptors are pictured and laid on top of each other.  

Crystal Structure of the Kappa-Opioid Receptor

The overall structure of the κ-OR deviates very little from the previously two mentioned receptors. It follows the same seven transmembrane bundle of α-helices along with the β-hairpin turn near the active site of the receptor. Another interesting discovery is the presence of a salt bridge near the end of the α-helices bundle that serves as a locking mechanism. This is predicted to stabilize the inactive form of the receptor. Receptors that are in the same class as κ-OR and lack this salt bridge are thought to be much more easily activated and stay activated for longer periods of time. One aspect that was discovered in the κ-OR crystal structure is an extra disulfide bond that gives extra stability to the receptor. While this extra disulfide bond can be found in all three receptors, it was originally found in the κ-OR structure.
The κ-OR binding pocket is unique in its class of receptors for several reasons. Like the μ-OR and δ-OR, the κ-OR has a binding pocket that is partially covered and larger than most other GCPRs. The κ-OR has a narrower and deeper binding pocket than the other opioid receptors that results from an inward shift of the sixth α-helix. This shift has a key role in controlling the binding properties and shows a preference to small, bulky ligands.\(^{15}\)

After revealing the crystal structure of the κ-OR, an in-depth analysis of how two κ-OR selective opioids bind to the receptor was done. Norbinaltorphimine (nor-BNI) and 5’-guanidinonaltrindole (GNTI) were used in this experiment because they are known to be κ-OR selective. Both opioid ligands consistently bind to the receptor with low energies because they are small and bulky. When bound to the κ-OR, both ligands form an amino group salt bridge and hydrogen bond on opposite sides of the binding pockets. These two locations (Asp 138 & Tyr 139) have been shown to be important anchoring points for opioid agonists within the κ-OR. Three other residues that were found to have major impacts in binding affinities of ligands were deeper in the binding pocket. Ile 294 creates hydrophobic attraction, Glu 209 is attracted to bulky polar groups, and His 291 adds attraction to aromatic groups.\(^{15}\)

A mutation worth mentioning resides in the His 291 group. Two common mutations that occur in this group is the replacement of histidine to phenylalanine or lysine. If mutated to phenylalanine, the binding affinity of nor-BNI and GNTI were either unaffected or minutely affected. However, if the residue is mutated to a lysine then all binding to the tested ligands stopped. This is a result of the lysine side chain disrupting the aromatic group of the ligand.

**Dimerization of Opioid Receptors**

A recent development in opioid research is the formation of dimers and oligomers between opioid receptors. It has been observed that opioids form oligomers of the same receptors (homodimers), of different receptors (heterodimers) and with other GCPR classes such as the
cannabinoid receptor.\textsuperscript{11} While the oligomerization of receptors have been confirmed using fluorescent and bioluminescent tagging, the application and frequency are debated. Despite this, it remains a subject of research due to the potential of targets for new drugs. Advances in mouse genetics and imaging will help resolve some of the questions that surround the dimerization of opioid receptors.\textsuperscript{11}

Current research is being done on the dimerization of receptors that happen more naturally. While it is hypothesized that opioid receptors dimerize on their own, none have been isolated. Some work has been done on the dimerization of the δ-OR and somatostatin receptors (sst), a class of receptors that control hormone release such as insulin and glucagon. In order to confirm the possibility of these two receptors form dimers naturally and to ensure proper application of the research, the central nervous system of a rat was analyzed to see how many cells expressed both receptors. The sst and δ-OR were heavily expressed in regions of the brain stem and spinal cord. After confirmation of simultaneous expression in cells, the two receptors can be used to learn about their effects and hypothesize the effects of other kinds of dimers.\textsuperscript{16}

\textbf{Figure 5. Heterodimer Electrophoresis.} The sst and μ-OR have similar molecular weights of 70-90 kDa. This gel was done with unaltered proteins and the heterodimer can be found at the correct weight of about 160kDa.
Some hypothesized effects of dimerization include the alteration of the ligand binding and signal transduction of the opioid receptors. However, this effect is not consistent with every heterodimer and depends on the pair of receptors. For example, the heterodimer sst$_{2A}$ and δ-OR contained two separate binding pockets with little to no change in ligand binding properties. Another effect is the alterations of how an agonist is internalized by the receptor and how the receptor is desensitized to a specific agonist. Looking again to the sst$_{2A}$-δ-OR heterodimer, when exposed to an agonist that binds for either receptor both are desensitized to their respective agonists. This outcome is a result of an attachment that is localized where both receptors are subject to a conformational change. Therefore, when one receptor undergoes conformational change from an agonist, the other receptor is forced to change to a different conformation. This also suggests that the dimer remains intact throughout binding despite the constant shift in shape from both receptors.

Another outcome of the sst-opioid receptor research is the fact that sst and δ-OR share only 38% of sequence identity. This shows that receptors need to be much less related than originally thought to produce heterodimers both artificially and naturally. Precipitation of tagged sst receptors prior to sample preparation were found to already have formed dimers with the δ-OR proving the natural dimerization of these two receptors. This opens the opportunity to future research on many more receptor combinations than previously thought.

**Desensitization of Opioid Receptors**

The desensitization of opioid receptors is an important area of study because of the dependence and tolerance that people build up by taking opioids. Dependence and tolerance then make a deadly combination that often result in overdoses and sometimes death. After taking opioids for an extended period, the receptor becomes desensitized and require more of the agonist in order to have the same outcome of pain relief and euphoria. By studying the mechanism of desensitization, we can begin
combat overdoses by knowing how to prevent tolerance of opioids or by being able to prescribe proper medication more effectively.  

Desensitization occurs in two forms. In the first, the receptor is still functional but cannot be activated, much like an action potential in a neuron that needs to repolarize. This phase only takes a few hours after removal of the agonist to recover and become fully functional again. The second form is associated with tolerance of an agonist and can last in a range of many hours to many days. If exposed to an agonist long enough a loss of total number of functional receptors may result. There are several reasons that lead to loss of functional receptors. One study has found that receptors can relocate to different areas of the cell that are more difficult for agonists to reach. So while still being fully functional, they are activated less often due to needing a higher concentration of the agonist in order to reach a binding site.  

Both phases of desensitization of opioid receptors can attributed to the phosphorylation of the opioid GPCR by G Protein Receptor Kinase (GRK). In phase one, phosphorylation requires an agonist to be effective because GRK is sterically hindered by the entire receptor. When the receptor is activated and the $G_{\beta\gamma}$ subunit dissociates from the protein, phosphorylation can take place. There are about 20 suggested sites that GRK can phosphorylate $\mu$-OR and are all near the C-terminal end of the receptor. When mutational studies of different amino acid residues of the $\mu$-OR where conducted, the residue that had the most profound effect was Ser375. Once phosphorylated, the receptor undergoes a conformational state that prevents it from being activated again for two reasons. The first being that agonists cannot reach the binding site and the second is that $G_{\beta\gamma}$ cannot reattach to $G_{\alpha}$.  

There are two outcomes of the opioid receptor once it has been phosphorylated. The first is that phosphate dissociates, and the receptor returns to normal and is ready to be activated again. The second fate of the receptor is the phosphorylation is permanent and the receptor is no longer functional.
until it is endocytosed, recycled, and replaced. The mechanism for how the receptor reacts to phosphorylation is unknown. One suggested mechanism involves the phosphorylation of several residues and the overexpression of GRK. The more times the receptor is phosphorylated, the harder it is for the receptor to rebind all its subunits and become active again making it more likely to be needed to be endocytosed and recycled. This is linked to tolerance because the more often a receptor is activated, the longer the Gα subunit of the μ-OR is exposed to GRK and phosphorylation. Therefore, more receptors are becoming dysfunctional from being overexposed to GRK and the cell cannot recycle and replace the dysfunctional receptors fast enough.  

Figure 6. Phosphorylation of the μ-OR. This two-dimensional representation of the μ-OR shows the amino acid residues of the βγ subunits which begin at residue 337. The magenta colored residues are the three most common sites for phosphorylation.  

Aminothiazole-Derived Opioid Agonists

The other area of study involving opioids and how they affect the body is dealing with the agonists themselves. Researching agonists are just as important as studying the receptors because different agonists target different receptors; One subject of study is supplemented by information with
the other. By knowing what agonist and functional groups they contain, we open the door to several treatment plans based on level of pain as well as potentially eliminating unwanted side effects.

All opioid agonists have two specific functional group in common. Both nitrogen and a phenol (more specifically the hydroxyl group on the phenol) are necessary for site binding due to the hydrogen bonding involved with the receptor. This poses the first problem to designing an agonist because the free hydroxyl group on the phenol is potential location for metabolism, conjugation with other molecules, or simply excreted by the body before it reaches the opioid receptor. Therefore, the first group that is subjected to alteration when designing different opioid drugs is the phenolic hydroxyl group. By changing this group, researchers are hoping to create an agonist that have a longer duration of action and are more available in the body. ¹⁸

One recent study attempted to add a 2-aminothiazole group to the phenolic group of several opioid compounds. The 2-aminothiazole group does not replace the phenolic group but removes the hydroxyl group and extends aromatic function outward. As a result, the aromaticity is kept with a six-membered aromatic ring with the extended polar aminothiazole group capable of binding to the opioid receptor while also being less likely to be metabolized by the body, increasing bioavailability of the agonist. This substitution is already being used as a dopamine agonist in anti-Parkinson agents. ¹⁸

Zhang used three opioid derivatives (levorphanol, cyclorphan, and morphine) to make several different isomers to test binding affinity. The synthesis procedure is as follows: First, a triflate group replaced the hydrogen on the hydroxyl group of the opioid substrate. A Buchwald-Hartwig amination was then performed to add amines. This reaction utilizes palladium to catalyze coupling reactions of amines with aryl halides, resulting in an amine where the triflate group was in our reaction. Once the hydroxyl group is replaced by the amine, a simple replacement reaction is used to swap the amine with
the 2-aminothiazole group. This procedure gave relatively good yields of 62% in aminothiazole-derived morphine.\textsuperscript{18}

$\text{\begin{align*}
\text{Morphine} & \xrightarrow{\text{i}} \text{10} \\
\text{10} & \xrightarrow{\text{ii}} \text{11 (X = TBDPS)} \\
\text{14} & \xrightarrow{\text{vi}} \text{13 (X = TBDPS)} \\
\text{13 (X = TBDPS)} & \xrightarrow{\text{v}} \text{12 (X = TBDPS)} \\
\end{align*}}$

\textsuperscript{a} Reagents and conditions: (i) PhNTf$_2$, Et$_3$N; (ii) TBDSCI, imidazole, THF; (iii) Pd(OAc)$_2$, BINAP, Ph$_3$C=NH, Cs$_2$CO$_3$; (iv) NaOAc, NH$_2$OH.HCl; (v) KSCN, Br$_2$, AcOH; (vi) TBAF, THF.

\textbf{Figure 7. 2-Aminothiazole Synthesis Mechanism.} The reaction mechanism for morphine derived aminothiazole including reagents and catalysts.\textsuperscript{18}

The following procedure yielded one morphine derivative, two levorphanol derivatives, and five cyclorphan derivatives. Cyclorphan had the most derivatives because it included three different R groups off the original nitrogen group as well as two derivatives that did not include the third six-membered ring. Every product was tested for binding affinities for all three common opioid receptors. Every product had negligible results to the δ-OR and had a higher selectivity for the κ-OR than the μ-OR. In all but one product, the binding affinity for the aminothiazole-derivative was lower than that of the phenolic originals ranging from a two and a half-fold to thirty-fold decrease in binding affinity. The cyclobutylmethyl analogue (one of the cyclorphan compounds) was the one product that had a higher binding affinity in all three opioid receptors.\textsuperscript{18}

The compounds synthesized by Zhang have not been tested for their effects and possible use for treatment. However, the study is useful in providing a procedure that can successfully replace the
hydroxyl group on the phenol with an aminothiazole group. The future of this specific procedure can be used if the κ-OR is wanting to be targeted over the other two receptors as the aminothiazole products were full κ-agonists and partial μ-agonists. The intended next steps in aminothiazole-derived opioid research is to analyze the pharmaceutical properties these derivatives such as half-life in vivo, pain treatment, and potential side effects.\textsuperscript{18}

**Dimeric Opioid Ligands**

Another area of study that has emerged is the synthesis and administration of opioid ligands in the form of dimers. This is because targeting both the μ-OR and the κ-OR at the same time have reduced self-administered opioids in non-human primates and include less unwanted side effects.\textsuperscript{19} In an effort to target both the μ-OR and the κ-OR, Knapp has synthesized and tested different opioid ligands that have formed dimers with each other.\textsuperscript{20} The hypothesized effect of a dimer agonist is that it will target both receptors to prevent a dependence and tolerance to one kind of ligand.\textsuperscript{19}

Five opioid ligands were chosen to create the opioid dimers: Cyclorphan, MCL-101, Levorphanol, Norlevorphanol, and Ethylketocyclazocine. These compounds were chosen due to being partial agonists of the κ-OR and the μ-OR unlike morphine which usually targets only the μ-OR. All but one of the products were connected by the hydroxyl groups connected to the phenol. Norlevorphanol was unique and connected by a hydrocarbon carbon chain attached to both nitrogen groups. Norlevorphanol was attached this way in attempt to retain the hydroxyl group that is required for binding to the receptor. The result for this compound was a dramatic decrease in binding affinity at all three receptors (20-fold at κ-OR, 140-fold at δ-OR, and 228-fold at μ-OR) which is less than ideal.

In attempt to raise the binding affinities of the other dimer compounds, the compounds were bridged at the hydroxyl group. In order to keep the necessary polar group at that location, varying lengths of ester and ether groups were tested. This allowed the connection of two agonists together
while at the same time keeping an area of high electron density around the oxygen and retaining the polar group that allows for binding at the receptor. While both yielded higher binding affinities than the norlevorphanol product, the ester bridge displayed the highest binding affinity.

![Diagram of a molecule with an ester bridge](image)

**Figure 8. MCL-135.** This is the general structure of many of the products in this study. The product with the highest binding affinity was MCL-135 which included two methylene groups.\(^ {19} \)

The most interesting part of the ester bridge was the different binding affinities that resulted from different lengths of the spacer between the two opioid groups. The most effective agonists had two and eight methylene groups between each oxygen. Both compounds had a higher binding affinity to both receptors than the original monomeric compound. Any other number of methylene groups gave worse binding affinity than their monomeric derivative. Another result were three compounds that had a change in receptor selectivity. Compounds with one, seven, and nine methylene groups had slightly decreased binding affinity but increased selectivity towards the μ-OR and κ-OR while decreasing selectivity towards δ-OR from 45 to 120-fold. This suggests that one of the most important parts of designing a dimerized agonist is the length of spacer between each group.
<table>
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<th>κ-OR Binding Affinity (nM)</th>
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Table 1. Binding Affinities of Compounds with Varying Bridge Lengths. The length of the ester bridge had a dramatic impact on binding affinity. The lower quantitative number for binding affinity is better because nM is a ration of amount of substrate off the ligand over the amount of substrate on the ligand.¹⁹

**Nitrogen-Substituted Agonists**

The last group of agonists that was studied were those that had a variety of functional groups that substituted the methyl group on the nitrogen. The study by Zhang synthesized and observed the binding properties of 43 different compounds. The overall goal of this study was like that of the dimeric agonists in they were searching for one that targets both the μ and κ receptors due to the less addictive properties. While binding affinity was usually lower than the original compound selectivity trended towards the μ and κ receptors. Zhang also hypothesizes that the selectivity towards the two receptors is a result of a large hydrophobic pocket in the μ and κ receptors that causes the area around the nitrogen to accept several different functional groups.⁵

The synthesis of the products followed 7 different reaction schemes with several different opioid substrates represented for reactants. Ethylketocyclazocine, three keto-morphinans, and three morphinans were used for synthesis. The first reaction scheme was used to synthesize the keto-
morpheins from levorphanol. After an addition reaction to add the ketone group, 3 different keto-
morphine reactants were created: 10-keto-morphine, and then the same compound with an ether
group replacing the hydroxyl on the phenol, and one with a methyl group on the nitrogen. Reaction
scheme 2 then took 10-keto-morphine and added three different carbon chains in an addition reaction
utilizing bromine. The carbon groups that were added included ethylcyclopropane, ethylcyclobutane,
and methane. DMF was used to add the carbon group to both the nitrogen and phenolic group while a
reflux reaction utilizing ethanol yielded the carbon group only on the nitrogen. The other five reaction
schemes build off each other to create a large number of final products. When one reaction is done,
some product would be set aside to test binding properties while the rest was used to synthesize
another product.\textsuperscript{5}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{structure.png}
\caption{10-Ketomorphinan Series. The general structure of all the compounds produced.\textsuperscript{5}}
\end{figure}

The first major contribution found was the study of the 10-keto-morphine compounds. In most
of the keto-morphine products, binding affinity went down which is thought to be a result of the polar
ketone group disrupting some important binding motifs in the receptors. However, some nitrogen
substituted groups seemed to compensate for the ketone group by increasing binding affinity of the
original compound. These compounds contained ethyl-cyclo-2-oxypentane (product 11), ethyl-
cyclobutane (13), 2-methyl-2pentene (14), and propylphenol (18) substituted onto the nitrogen group.
Products 11 and 18 are thought to have a higher binding affinity due to hydrogen bonding that can take
place at the polar oxygen groups on each product. It is not really known why the other carbon chains
increase binding affinity in some of the products.\textsuperscript{5} Since both chains are about the same length, I would
suspect that the nonpolar carbon chains shift the agonist to fit into the binding pocket slightly better. Since the polar ketone group shifts the agonist slightly out of the binding pocket, the carbon chain pushes the opposite way to correct some of the force from the ketone group and increase binding affinity.

All the base morphinan compounds had impressive binding affinities, with the ethyl-cyclopropane nitrogen substituted group having the highest. To complete this extensive study, binding properties were also taken from hydroxyl substitution on the phenol group of the morphinan compounds. Every substitution for the hydroxyl group resulted in negative binding effects except for the addition of an amide group. The cause of the increase of binding affinity from the amide group remains unknown.5

This work by Zhang remains to be one of the most successful nitrogen substituted opioid compound studies yet. The first and most important result of the study were elevated binding properties by substituting functional groups on the nitrogen of morphinan groups, a procedure that has troubled attempts like in the dimeric opioid study. Finally, the compounds that were synthesized also had exclusive selectivity to the μ and κ receptors, a property that has shown lower addictiveness and less side effects. Future research of this topic includes pharmaceutical properties of the most successful agonists and increased yields from reactions to make the synthesis more economical for drug companies.

**Conclusion**

The imaging of the three major opioid receptors has opened the door for new research of opioid ligands and imaging. The procedure used to image the receptors can be used and adapted to image the receptor at different stages of activation. The current images have been used to find which interactions
are necessary for binding site activation. In turn, this tells us what groups on opioid agonists must remain consistent and which groups can be changed in attempt to alter agonist side effects.\textsuperscript{21}

Further research on opioids will continue to go in two directions. One that trends towards the receptor and reaction mechanisms and one that trends towards making new opioid agonists. Despite the split in research, there is a common goal to continue to effectively reduce pain while also limiting side effects and addictiveness. The most pressing matter on the receptor and mechanics side is to completely image every receptor in every possible phase. The crystal structure highlighted in this paper was during the inactive state. Having the active state image would help us understand what happens with the receptor after activation, what kind of conformational changes occur and how that effects dissociation and binding properties, and finally give us clues on the pathway of the G\textsubscript{βγ} subunits and the respective pathways of signaling molecules.\textsuperscript{3}

Agonist research is well underway especially after boosted research funding by the United States government following a highly elevated opioid overdose rate. Some of the most promising agonists utilize the research and pathways described in this paper. Despite the increase in research, new drugs hit the market slow due to a long testing and approval process. Agonist research is supplemented by receptor research. As we continue to learn more about the pathway of the opioid receptor and what signaling molecules are being utilized in the body, we can develop new agonists that work more effectively and have less negative impacts on our bodies.\textsuperscript{3}

After extensive research on the opioid receptor and the agonists that have the most potential to change how we view opioids, I think that another category of drugs needs to be researched; whether this is an entirely new class of drugs to treat pain or an already discovered one. No matter how much we change the agonist, the same receptor and same pathway is always being activated. The nature of addiction and how it develops is a highly debated and poorly understood process. But the way I see it, if
the same receptor and pathway is being activated, addiction will always be a problem. I think that pain should be treated on a case by case basis, especially for chronic pain. Rather than automatically prescribing opioids as pain killers, we should be looking at what is causing that pain, and if it can be treated with something else. For example, the μ-OR has been found to dimerize with the cannabinoid receptor which may give rise to potential treatments that target the cannabinoid receptor rather than opioid receptors. 11 So while research on opioids is important and should continue due to promising procedures, such as targeting both the μ and κ receptors simultaneously, I think that we should be researching other methods of pain treatment just as urgently.
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