Understanding the Molecular Mechanisms that Govern Opioid Potency Through a Course-embedded Computational Research Project

Keegan Gunderson, Faith Oldenburg, Sudeep Bhattacharyay, & Sanchita Hati**

Department of Chemistry and Biochemistry, University of Wisconsin - Eau Claire, Eau Claire, WI

https://doi.org/10.33697/ajur.2025.137

Students: gunderkr8370@uwec.edu, oldenbfa6097@uwec.edu*
*Mentors: bhattas@uwec.edu, hatis@uwec.edu**

ABSTRACT
In recent decades awareness surrounding the class of drugs known as opioids has risen due to what many have termed the "opioid epidemic." Rates in opioid-related drug overdoses have spiked due to increased opioid addiction and the illegal lacing of illicit drugs, such as marijuana, with opioid compounds like fentanyl, increasing their potency. Coexisting with the devastating realities of overdose and addiction, however, is the clinical demand for these drugs and their potency to manage extreme pain. Herein, we describe the results of a course-embedded computational research project to understand the factors responsible for opioid potency. In the present study, the chemical properties of commonly used opioids and their interactions with receptors are investigated using computational techniques. In particular, the molecular basis for the high potency of fentanyl is investigated. The drug molecules were constructed using a web-based tool, WebMO. The electronegativity and chemical hardness of nine opioids and one pain reliever were determined using a quantum chemistry software package named Q-Chem. The binding interactions between the same set of opioid molecules and their receptors were studied using AutoDock FR, interactions within the receptor's binding pocket were analyzed using Visual Molecular Dynamics (VMD), and SwissADME was used to investigate the pharmacokinetic properties of each drug molecule.

KEYWORDS
Opioid Addiction, Opioid-Receptor Interactions, Opioid-Receptor Binding Affinity, Electronegativity and Chemical Hardness, Course-embedded Research

INTRODUCTION

The pharmaceuticals known as opioids are a class of analgesic, or pain-relieving drugs that function as agonists of human opioid receptor (OR) proteins. Opioids are powerful pain relievers that are commonly used in medical settings, especially in acute care contexts. Drugs that fall beneath the opioid umbrella include codeine and morphine, which are naturally derived from opium, as well as semi-synthetically and synthetically derived drugs such as fentanyl, oxycodone, methadone, and illegal heroin.

While these drugs are necessary for pain management for patients who have undergone surgery, physical trauma, or certain chemotherapies, they have health risks. The mechanism of action of opioids triggers the same chemical response as the human body's dopamine reward system, causing them to be highly addictive for some individuals, especially when used to treat chronic pain.[1] Opioids function by binding to and stimulating G-protein coupled receptors (GPCRs) called ORs, found on the plasma membranes of cells in the human central nervous system and gastrointestinal tract.[2] They are attracted to and bound to their protein receptors via noncovalent interactions. There are four primary ORs, which belong to the family of cell membrane proteins GPCRs - mu (μ OR), delta (δ OR), kappa (k OR), and nociceptin/orphanin FQ (N/OFQ) receptors (**Figure 1**).[3,4] Once the surface proteins of the GPCR complexes are activated, they trigger a cascade of interactions within the cell, releasing endorphins, neurotransmitters involved with the mesolimbic system. Endorphins reduce feelings of pain by slowing down the release of proteins involved in pain signaling. These endorphins also catalyze the release of dopamine, which plays a vital role in the perception of pain and provides natural analgesia to various portions of the brain and body.[5] Because opioids activate the mesolimbic system, prolonged usage commonly results in the development of opioid drug tolerance and leads to a high probability that those who develop this tolerance will display signs of addiction and subsequent withdrawal when coming off the medication. In the last decade, opioid addiction has surged at an exponential rate. Recent data shows that 81,806 Americans died from opioid overdose in 2021.[6] Thus, there is rising concern within the medical and broader community surrounding their uses, their mechanism of action, and what precautions or treatment alternatives can be implemented to fight back against the destruction of opioid addiction.

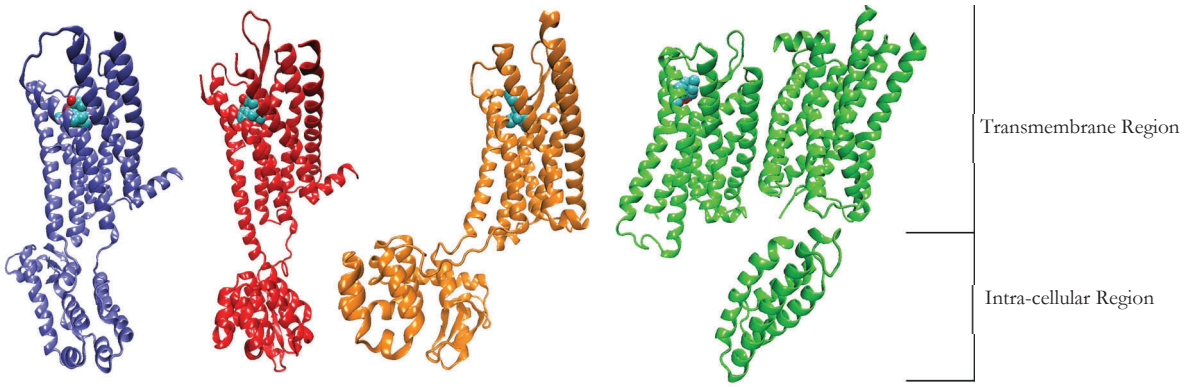

Figure 1. A visualization of the protein structure and binding pocket for each of the four opioid receptors.

The present study was conducted in a General Chemistry I Lab to provide students with hands-on experience with computation early in their college careers. It aimed to examine the molecular characteristics of nine opioids and the pain reliever imitrex and assess their potency based on their chemical and pharmacological properties. This involved computationally constructing the 3D molecular structures of the ten molecules and using these models to determine their electronegativity and chemical hardness values, which are key properties affecting chemical reactivity. It also aimed to identify basic chemical characteristics such as lipophilicity, gastrointestinal (GI) absorption, and blood-brain barrier permeability. Additionally, it determined the binding affinity values and analyzed the 3-dimensional structures of opioid-receptor complexes for all ten compounds (nine opioids and imitrex) and the four opioid receptors. Overall, the goal of this study was to identify molecular determinants of opioid potency, with a specific focus on fentanyl. This was achieved by analyzing the chemical properties of various opioids and their binding interactions with opioid receptors. These findings could be used in designing effective analgesics that pose fewer health risks, such as reduced potential for addiction or fatal overdose.

METHODS

The chemical and structural formulas of each opioid were obtained from PubChem.[7] Molecular structures were built using WebMO[8] and molecular geometry optimization was performed using QChem.[9] For geometry optimization, the Hartree-Fock method was used with the correlation-consistent polarized basis set with double zeta basis function.[10] This is abbreviated as cc-pVDZ. The convergence criteria for this optimization were 10^{-8} Hartree. Once the molecular optimization was completed, the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) energies for each opioid were collected. The HOMO and LUMO energies were used to determine the electronegativity (χ) and chemical hardness (η) of each molecule using equations 1 and 2 below:[11]

$$\chi = \frac{(IE + EA)}{2} = -\frac{1}{2}(\epsilon_{HOMO} + \epsilon_{LUMO}) \quad (1)$$

$$\eta = \frac{(IE - EA)}{2} = -\frac{1}{2}(\epsilon_{HOMO} - \epsilon_{LUMO}) \quad (2)$$

Electronegativity and chemical hardness are important chemical properties that characterize a molecule. Electronegativity (χ) is the ability of an atom or a group of atoms to attract the bonding pair of electrons towards itself.[12] The higher the electronegativity of an atom, the stronger its attraction for the bonding pair of electrons. On the other hand, chemical hardness (η) is defined as a measure of the tendency of a chemical species to localize charge density.[13] It is a qualitative indication of the polarizability of chemical species (atom, ion, or molecule). Chemically hard molecules are less polarizable, and chemically soft ones are more polarizable. The hardness η is an important chemical property that provides information about molecular reactivity and selectivity.[14-17]

To quantify the interaction between opioids and opioid receptors, the binding affinity was assessed for each opioid-receptor combination. To do this, the structural information of the opioid receptors was obtained from the Protein Data Bank.[18] The spatial data file (sdf) of the opioids was also obtained from PubChem.[7] These files contain information necessary to determine the size, 3-dimensional shape, and molecular characteristics of drug molecules and bound proteins. The molecular docking was performed using AutoDock for flexible receptors (ADFR) software.[19] ADFR uses a genetic algorithm-based approach and is validated using a protein ligand docking performance set called Astex Diverse Set.[20] This algorithm predicts the favored orientation of the ligand (an opioid) in the opioid receptor's binding pocket using the AutoDock 4 scoring function to estimate binding affinity.[21] The AutoDock 4 scoring function uses a semi-empirical force field to model non-bonding interactions between the opioid and receptor in various conformations. These models are quantified by finding the difference in energy before and

after ligand docking for Van der Waals forces, hydrogen bonding, electrostatic forces, and desolvation interactions. The binding affinity is calculated from the thermodynamic quantities of these interactions.[22] For each docking simulation, the genetic algorithm was run 50 times. In these simulations, the grid box size was calculated from the dimensions of the opioid receptor with a padding of 4 angstroms.

Additionally, interactions between opioids and receptors were investigated for fentanyl and meperidine bound to the μ and κ ORs. These two opioids were used because they displayed the highest and lowest binding affinities for the two opioid receptors. The μ OR was analyzed due to its contribution to opioid overdose deaths and the κ OR was used because it displayed the highest overall binding affinity when bound to fentanyl. This was done by visually analyzing and measuring the distance of significant polar and hydrophobic interactions between the opioid molecule and the receptor backbone using Visual Molecular Dynamics (VMD) software.[23] The ligand-bound binding pockets were manipulated to show their atom types using the "CPK," or ball-and-stick representation for the protein backbone, and the "licorice" representation for the opioid molecule. The alpha-carbon backbone surrounding the binding pocket was shown using the "NewCartoon" representation to help better visualize the location of the interactions relative to the protein. Layering several unique representations of the protein-ligand system allowed us to visualize noncovalent interactions between receptors and opioids within the binding pocket. These observations provided insight into the cause of variable binding affinity in each docking simulation.

Finally, the chemical characteristics, namely absorption, distribution, metabolism, elimination, and toxicity, or ADMET, of each opioid were obtained using SwissADME,[24] where the input files were in the simplified molecular-input line-entry system (SMILES) format.

RESULTS

Electronegativity and Chemical Hardness

First, the chemical properties of the nine opioids and Imitrex were compared. The χ and η values of these ten molecules range between 2.10 – 3.06 eV and 5.12 – 6.61 eV, respectively (**Figure 2, Table S1**). There is a notable spike in electronegativity and chemical hardness values for fentanyl, which is the most potent synthetic opioid, 50 to 100 times more potent than morphine.[25]

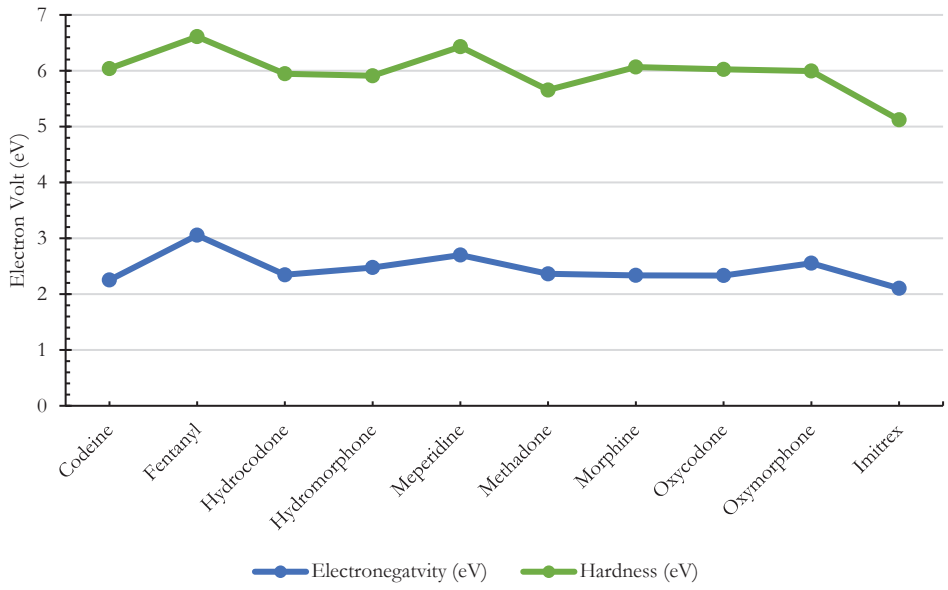

Figure 2. Electronegativity and chemical hardness, in electron volt (eV), of all nine opioids and Imitrex.

Docking and Binding Energy

The binding affinity for nine opioids and Imitrex, docked in the receptors binding pocket, were determined and shown in **Figure 3**. It is important to note that all binding affinity values are negative. Fentanyl showed the highest binding affinity for μ, κ, and δ ORs with values of -8.7 kcal/mol, -9.7 kcal/mol, and -9.2 kcal/mol, respectively. The N/OFQ OR had the best binding energy of -8.2 kcal/mol for methadone. The binding affinity was weakest (-6.4 kcal/mol, less tightly bound) for meperidine when bound to the μ OR. When comparing the binding affinities for different receptor proteins, the κ receptor exhibited tighter binding with a -

9.7 kcal/mol binding affinity for fentanyl. The κ OR exhibited an average binding affinity value of -8.12 kcal/mol while considering all 10 molecules. Also, fentanyl exhibited the highest binding affinity for three of the four receptors. However, the range for all the nine opioids for all the receptors was within 2.2 kcal/mol, suggesting all opioids are efficacious in stimulating the four primary opioid receptors in the body.

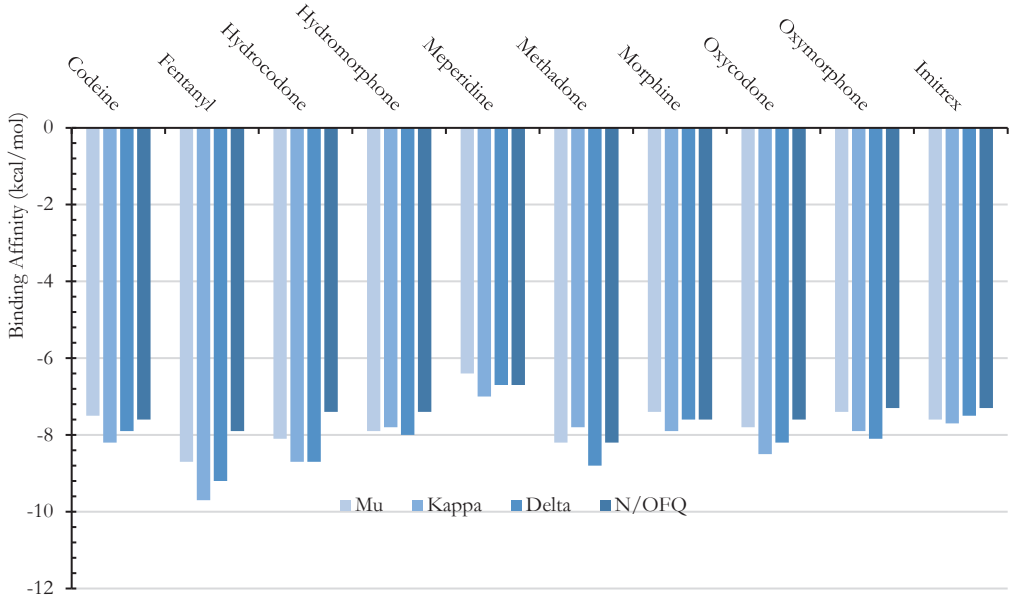

Figure 3. Computationally determined binding affinities for each of the four receptors, individually docked with all nine opioids and Imitrex.

Binding Pocket Analysis

To determine what kind of interactions were responsible for varying binding affinities among opioid-receptor pairs, the two extremes in binding affinity for κ and μ receptors, fentanyl and meperidine, were visualized using VMD. When these two molecules were bound to the κ OR (**Figure 4**), it was observed that their orientation in the pocket varied significantly, with fentanyl interacting more with the protein backbone (**Figure 4B, 4D**). Additionally, the two nonpolar phenyl groups of fentanyl (**Table S2**) appeared to have Van der Waals interactions with receptors that resulted in high binding affinity. Most notably, TRP287, ILE290, ILE294, and ILE316 in the binding pocket interact with one of fentanyl's two phenyl groups. Hydrogen bonding was also observed with residue ASP138 and fentanyl's carbonyl oxygen (**Figure 5A**). Hydrophobic interactions also could be observed for meperidine. The amino acid residues ILE294 and VAL230 interacted with the opioid's lone phenyl group. Weak interaction was also observed for residue HIS291 (**Figure 5B**). Hydrogen bonding was also observed between TYR139 and the oxygen group on meperidine. In the binding pocket of κ OR, hydrophobic interactions appear to be significant for both binding studies. However, polar interactions could also be observed.

When fentanyl and meperidine were bound to the μ OR (**Figure 6**), their orientation in the binding pocket appeared relatively similar (**Figure 6B, 6D**). However, fentanyl appeared to have more interactions. Again, fentanyl's two phenyl groups exhibited hydrophobic interactions with the protein backbone. This primarily occurred at amino acid residues VAL236, VAL300, TRP293, and TYR326. Also, a hydrogen bond was formed with fentanyl's carbonyl oxygen (**Table S2**) and ASP147 (**Figure 7A**). Similar interactions were observed to a lesser extent for meperidine. Namely, hydrophobic interactions occurred with amino acid residues VAL300 and TRP293. Also, a hydrogen bond interaction occurred between the amino acid residue ASP147 and the oxygen group in meperidine. A hydrogen bond also occurred between TYR326 and the nitrogen in meperidine (**Table S2**, **Figure 7B**).
For all four combinations, i.e. interactions of fentanyl and meperidine with κ and μ ORs, it is important to note that the noncovalent interactions between the receptors and opioids described here are those that were more significant to the opioid's binding; other amino acid residues also appeared to interact with the opioids (**Figures 6 and 7)**.

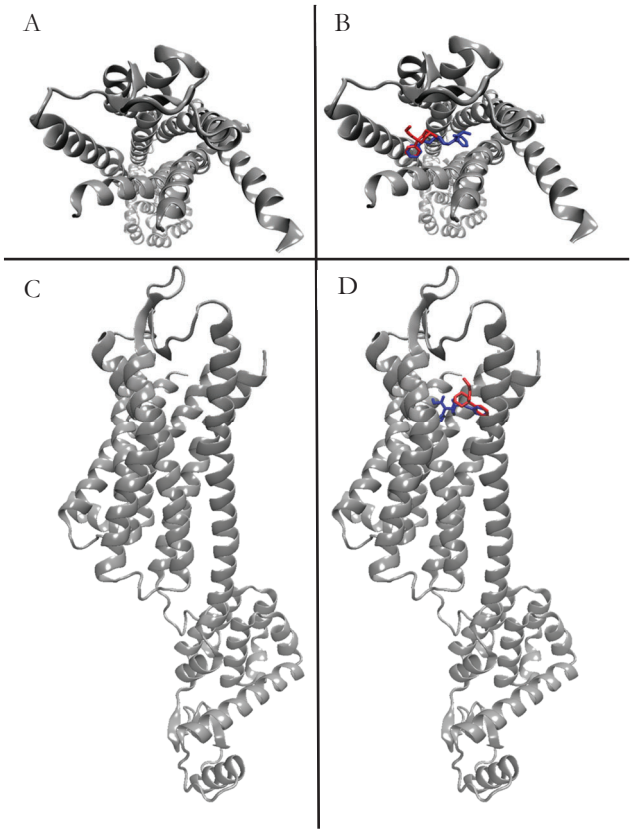

Figure 4. A) The top view of k-OR. B) k-OR displaying a bound meperidine (red) and fentanyl (blue). C) The front view of k-OR. D) k-OR front view displaying a bound meperidine (red) and fentanyl (blue).

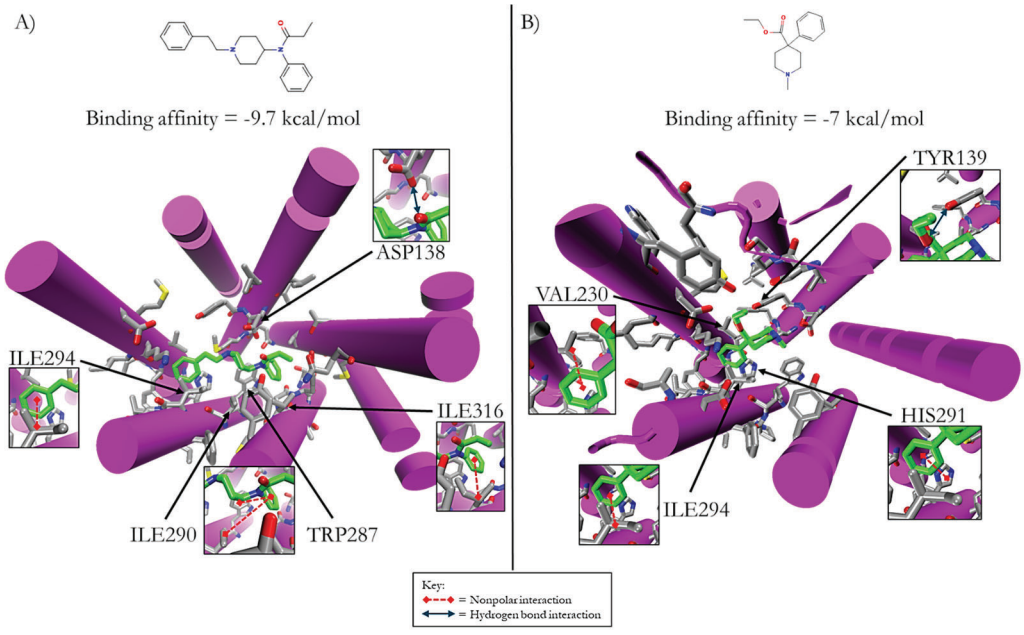

Figure 5. A) κ-OR bound to fentanyl. B) κ-OR bound to meperidine. Non-bonding interactions are depicted by the key and shown in the zoomed subpanels.

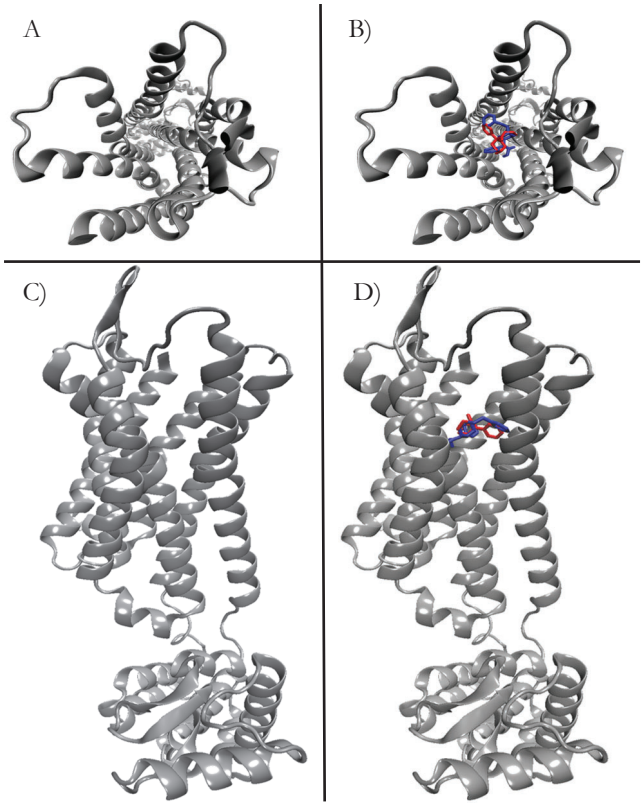

Figure 6. A) Top view of μ-OR. B) μ-OR displaying a bound meperidine (red) and fentanyl (blue). C) The front view of μ-OR. D) μ-OR front view displaying a bound meperidine (red) and fentanyl (blue) molecules.

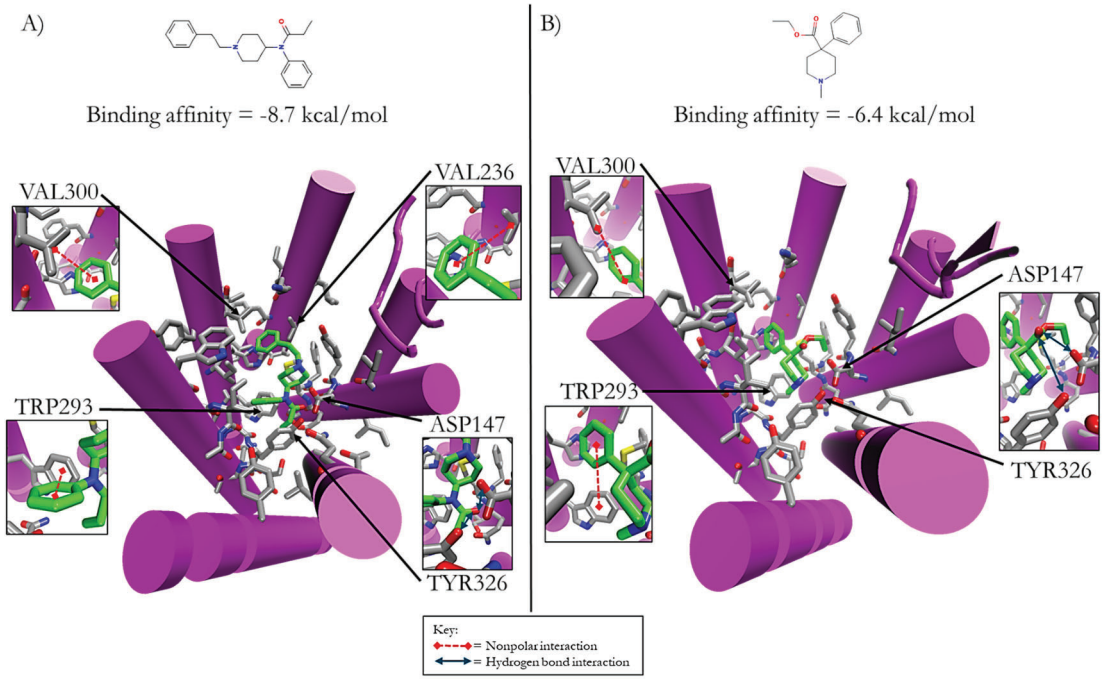

Figure 7. A) μ-OR bound to fentanyl. B) μ-OR bound to meperidine. Non-bonding interactions are depicted by the key and shown in the zoomed subpanels.

Absorption, Distribution, Metabolism, Elimination, and Toxicity (ADMET)

The ADMET values indicated that all tested drug molecules, apart from Imitrex, exhibited prominent levels of gastrointestinal absorption. It also demonstrated that all except three of the opioid molecules, Oxycodone, Oxymorphone, and the painkiller Imitrex, could permeate the body's blood-brain barrier complex, which is a vital function required for any pharmaceutical to be able to produce effects within the central nervous system tissues. These results are all shown in **Table 1.** One property of particular interest is lipophilicity which describes a chemical's ability to dissolve when placed into a hydrophobic solution. Highly lipophilic substances take longer to exit the body as they take longer to metabolize. They also more easily permeate membranes found in the body. Lipophilic opioids are effective because they can penetrate the blood-brain barrier faster and more effectively, leading to faster analgesic onset. They are also more readily absorbed into fatty tissues and are metabolized by the body at a lesser rate. This can lead to them having a more prolonged effect.[26] Notably, fentanyl and methadone exhibit heightened lipophilicity values relative to the other opioids tested. This may provide chemical insight into why fentanyl has such a dramatic effect on those who take it and why the level required for a dose of fentanyl to be lethal is so low, generally being estimated at 2 mg of the drug.[27] Methadone in contrast has an even higher lipophilicity, however, the other characteristics, such as electronegativity, chemical hardness, and 3-dimensional structure, are out of the ideal ranges, which may explain why it is not as potent.

Name	Lipophilicity	Water Solubility	GI absorption	BBB permeant	Drug-likeness	Lead-likeness	Synthetic accessibility
Codeine	2.88	Soluble	High	Yes	0.55	Yes	4.89
Fentanyl	**3.78**	Moderately Soluble	High	Yes	0.55	No; 1 violation	2.22
Hydrocodone	1.79	Very soluble	High	Yes	0.55	Yes	4.43
Hydromorphone	1.88	Soluble	High	Yes	0.55	Yes	4.33
Meperidine	2.53	Solubility	High	Yes	0.55	No; 1 violation	2.07
Methadone	**4.06**	Moderately Soluble	High	Yes	0.55	No; 1 violation	2.79
Morphine	1.47	Soluble	High	Yes	0.55	Yes	4.78
Oxycodone	1.12	Very soluble	High	No	0.55	No;1 violation	4.57
Oxymorphone	1.21	Very soluble	High	No	0.55	Yes	4.46
Imitrex	0.72	Very soluble	Low	No	0.55	No; 2 violations	3.07

Table 1. Absorption, distribution, metabolism, elimination, and toxicity values for each of the 10 opioids obtained using the SwissADME. GI and BBB stand for gastrointestinal and blood-brain barrier.

DISCUSSION

This study investigated the molecular characteristics of opioids and how they interact with opioid receptors. The goal was to understand opioid potency trends while also trying to find a chemical explanation for fentanyl's high potency.

The binding affinities determined computationally in this study aligned with published data for fentanyl and meperidine. Ellis et al. reported average docking scores for fentanyl and meperidine bound to the μ opioid receptor (μ OR) to be -9.43 and -7.77 kcal/mol, respectively.[28] These scores are comparable to the binding affinities of fentanyl (-8.7 kcal/mol) and meperidine (-6.4 kcal/mol) for μ OR found in this study. The small differences could be due to the use of different computational docking protocols. Additionally, the computed lipophilicity values obtained in this study were like other experimentally determined values, reinforcing conclusions about fentanyl and lipophilicity.[29,30] The computed electronegativity and chemical hardness values for fentanyl were also comparable to those reported by Sümeyya et al.[31] Overall, key findings for binding affinity, electronegativity, chemical hardness, lipophilicity, and binding pocket analysis were consistent with other studies. Future studies could validate these findings by performing multiple binding simulations to ensure consistency and by conducting bench-top experiments to determine if the results hold in a physical system. These steps would add more certainty to this study's conclusions.

Our findings show that fentanyl is an outlier in many ways. It exhibited the highest binding affinity for three of four opioid receptors, the highest electronegativity and chemical hardness, and the second-highest lipophilicity among opioids studied. When comparing binding pocket interactions between fentanyl, a highly potent opioid receptor agonist, to meperidine, a less potent opioid receptor agonist, it was observed that hydrophobic interactions may be important to fentanyl's high binding affinity. Specifically, fentanyl's long structure consisting of two phenyl groups on each end may allow it to interact more strongly with the μ OR. Specifically, these functional groups appeared to interact with the non-polar residues in the opioid receptor's binding pocket. This could help stabilize the compound and enhance opioid-receptor interactions. Similar studies have also highlighted

the importance of hydrophobic interactions in the μ OR-fentanyl complex. [32,33] This is also supported by fentanyl's high lipophilicity, which may promote the drug's interaction with the μ OR binding pocket. Additionally, the high fentanyl lipophilicity helps it to be absorbed more readily in the body and more easily cross the blood-brain barrier, which is key in opioids as they act on the central nervous system. Overall, these findings suggest that fentanyl's chemical properties are responsible for its high potency. This insight could aid in the development of effective and safer non-μ opioid receptor agonists.

The results of this study also explain how opioids, specifically fentanyl, can be linked to poor clinical outcomes. For example, it is known that stimulation of the μ OR triggers the body's reward system and, in overdose cases, the respiratory depression that leads to opioid overdose death. Fentanyl's high affinity for this receptor indicates that the risk of addiction and overdose is higher for fentanyl than that of other opioids. Furthermore, fentanyl's lipophilic nature allows it to cross the blood-brain barrier more easily, reaching opioid receptors efficiently. This characteristic means that fentanyl not only reaches opioid receptors effectively but also binds them strongly. Consequently, even trace amounts of fentanyl can provide analgesic effects, while a small overdose can be fatal. Ultimately, fentanyl's potency is highly beneficial in a clinical setting, but it also makes the drug extremely dangerous.

CONCLUSIONS

The present computational study demonstrated fentanyl to be the 'best fit' for three of the four opioid receptors in the body. Fentanyl's high affinity for the μ OR is significant because the binding of this receptor stimulates the release of endorphins and dopamine in the brain's limbic system and thus increases its addictiveness and potency relative to the other receptors studied. The μ OR is also associated with triggering respiratory depression, which causes death from an opioid overdose. In addition to its interactions with the μ OR, fentanyl was among the most lipophilic opioids, which means that the drug molecule takes a longer time to exit the body, granting it a longer period of action. The higher binding affinity for the μ OR combined with this elevated lipophilicity may provide chemical insight into the addictiveness and lethality of the drug.

As we currently stand amid a systemic opioid crisis, knowing why fentanyl is so lethal is essential information. This research highlights the lethal nature of fentanyl by identifying key chemical properties, such as lipophilicity and electronegativity, that contribute to its potency as a μ OR agonist. These insights could inspire the design of new opioids that avoid these properties, reducing the risks of addiction and overdose. The study also explores the chemical characteristics of strong agonists for other opioid receptors, potentially guiding targeted drug design for non-μ receptors. Minor variations in electronegativity, chemical hardness, and lipophilicity significantly impact opioid potency, suggesting that slight modifications in chemical structure could alter receptor selectivity and potency. Overall, this study provides valuable information on the structural and chemical factors influencing opioid-receptor binding, which could lead to the development of safer and more effective analgesics.

APPENDIX

The appendix contains two tables and is located on *https://ajuronline.org/*

ACKNOWLEDGMENTS

The authors thank all students enrolled in General Chemistry I (Sections 501 and 502) in Fall 2022 for performing the initial calculations, and the Blugold Center for High-Performance Computing at the University of Wisconsin-Eau Claire (NSF grant CNS 1920220) for the computational facilities.

REFERENCES

1. Chaves, C., Remiao, F., Cisternino, S., Decleves, X. (2017) Opioids and the Blood-Brain Barrier: A Dynamic Interaction with Consequences on Drug Disposition in Brain. *Curr Neuropharmacol* 15, 1156–1173. *https://doi.org/10.2174/1570159x15666170504095823*
2. Pathan, H., Williams, J. (2012) Basic opioid pharmacology: an update. *British Journal of Pain* 6, 11–16. *https://doi.org/10.1177/2049463712438493*
3. Faouzi, A., Varga, B. R., Majumdar, S. (2020) Biased Opioid Ligands. *Molecules* 25, 4257. *https://doi.org/10.3390/molecules25184257*
4. Dhaliwal A., Gupta, M. (2022) Physiology, Opioid Receptor, in *StatPearls [Internet]*. *https://www.ncbi.nlm.nih.gov/books/NBK546642/*
5. Ali, A. H., Ahmed, H. S., Jawad, A. S., Mustafa, M. A. (2021) Endorphin: function and mechanism of action. *Science Archives* 2, 09–13. *https://doi.org/10.47587/sa.2021.2102*
6. Drug Overdose Death Rates, National Institute on Drug Abuse (NIDA) *https://nida.nih.gov/research-topics/trends-statistics/overdose-death-rates* (accessed Mar 2023).
7. Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B., Thiessen, P., Yu, B., Zaslavsky, L., Zhang, J., Bolton, E. E. (2023) PubChem 2023 update. *Nucleic Acids Research* 51, D1373–D1380. *https://doi.org/10.1093/nar/gkac956*

8. Polik, W. F., Schmidt, J. R. (2021) WebMO: Web-based Computational Chemistry Calculations in Education and Research. *WIREs Computational Molecular Science* 12. *https://doi.org/10.1002/wcms.1554*

9. Shao, Y., Gan, Z., Epifanovsky, E., Gilbert, A. T. B., Wormit, M., Kussmann, J., Lange, A. W., Behn, A., Deng, J., Feng, X., Ghosh, D., Goldey, M., Horn, P. R., Jacobson, L. D., Kaliman, I., Khaliullin, R. Z., Kuś, T., Landau, A., Liu, J., Proynov, E. I., Rhee, Y. M., Richard, R. M., Rohrdanz, M. A., Steele, R. P., Sundstrom, E. J., Woodcock, H. L., Zimmerman, P. M., Zuev, D., Albrecht, B., Alguire, E., Austin, B., Beran, G. J. O., Bernard, Y. A., Berquist, E., Brandhorst, K., Bravaya, K. B., Brown, S. T., Casanova, D., Chang, C.-M., Chen, Y., Chien, S. H., Closser, K. D., Crittenden, D. L., Diedenhofen, M., DiStasio, R. A., Do, H., Dutoi, A. D., Edgar, R. G., Fatehi, S., Fusti-Molnar, L., Ghysels, A., Golubeva-Zadorozhnaya, A., Gomes, J., Hanson-Heine, M. W. D., Harbach, P. H. P., Hauser, A. W., Hohenstein, E. G., Holden, Z. C., Jagau, T.-C., Ji, H., Kaduk, B., Khistyaev, K., Kim, J., Kim, J., King, R. A., Klunzinger, P., Kosenkov, D., Kowalczyk, T., Krauter, C. M., Lao, K. U., Laurent, A. D., Lawler, K. V., Levchenko, S. V., Lin, C. Y., Liu, F., Livshits, E., Lochan, R. C., Luenser, A., Manohar, P., Manzer, S. F., Mao, S.-P., Mardirossian, N., Marenich, A. V., Maurer, S. A., Mayhall, N. J., Neuscamman, E., Oana, C. M., Olivares-Amaya, R., O'Neill, D. P., Parkhill, J. A., Perrine, T. M., Peverati, R., Prociuk, A., Rehn, D. R., Rosta, E., Russ, N. J., Sharada, S. M., Sharma, S., Small, D. W., Sodt, A., Stein, T., Stück, D., Su, Y.-C., Thom, A. J. W., Tsuchimochi, T., Vanovschi, V., Vogt, L., Vydrov, O., Wang, T., Watson, M. A., Wenzel, J., White, A., Williams, C. F., Yang, J., Yeganeh, S., Yost, S. R., You, Z.-Q., Zhang, I. Y., Zhang, X., Zhao, Y., Brooks, B. R., Chan, G. K. L., Chipman, D. M., Cramer, C. J., Goddard, W. A., Gordon, M. S., Hehre, W. J., Klamt, A., Schaefer, H. F., Schmidt, M. W., Sherrill, C. D., Truhlar, D. G., Warshel, A., Xu, X., Aspuru-Guzik, A., Baer, R., Bell, A. T., Besley, N. A., Chai, J.-D., Dreuw, A., Dunietz, B. D., Furlani, T. R., Gwaltney, S. R., Hsu, C.-P., Jung, Y., Kong, J., Lambrecht, D. S., Liang, W., Ochsenfeld, C., Rassolov, V. A., Slipchenko, L. V., Subotnik, J. E., Van Voorhis, T., Herbert, J. M., Krylov, A. I., Gill, P. M. W., Head-Gordon, M. (2014) Advances in Molecular Quantum Chemistry Contained in the Q-Chem 4 Program Package. *Molecular Physics* 113, 184–215. *https://doi.org/10.1080/00268976.2014.952696*

10. Hill, A. N., Meijer, A. J. H. M., Hill, J. G. (2022) Correlation Consistent Basis Sets and Core Polarization Potentials for Al–Ar with ccECP Pseudopotentials. *The Journal of Physical Chemistry A* 126, 5853–5863. *https://doi.org/10.1021/acs.jpca.2c04446*

11. Putz, M. V. (2021) Chemical Bonding by the Chemical Orthogonal Space of Reactivity. *Int J Mol Sci* 22, 223. *https://doi.org/10.3390/ijms22010223*

12. Parr, R. G., Donnelly, R. A., Levy, M., Palke, W. E. (1977) Electronegativity: The Density Functional Viewpoint. *J Chem Phys* 68, 3801–3807. *https://doi.org/10.1063/1.436185*

13. Pearson, R. G. (2005) Chemical Hardness and Density Functional Theory. *J Chem Sci* 117, 369–377. *https://doi.org/10.1007/BF02708340*

14. Parr, R. G., Pearson, R. G. (1983) Absolute Hardness: Companion Parameter to Absolute Electronegativity. *J Am Chem Soc* 105, 7512–7516. *https://doi.org/10.1021/ja00364a005*

15. Hati, S., Datta, D. (1996) Hardness: A Concept in Inorganic Chemistry. Some Aspects. *Proc Ind Acad Sci: Chem Sci* 108, 143–158. *https://doi.org/10.1007/BF02870020*

16. Hati, S., Datta, D. (1994) Hardness and Electric Dipole Polarizability. Atoms and Clusters. *J Phys Chem* 98, 10451–10454. *https://doi.org/10.1021/j100092a012*

17. Hati, S., Datta, D. (1994) Chemical Hardness of Metal Ions in the Gas Phase: A Thermochemical Approach. *J Chem Soc, Dalton Trans*, 2177–2180. *https://doi.org/10.1039/DT9940002177*

18. Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., Shindyalov, I. N., Bourne, P. E. (2000) The Protein Data Bank. *Nucl Acids Res* 28, 235–242. *https://doi.org/10.1093/nar/28.1.235*

19. Ravindranath, P. A., Forli, S., Goodsell, D. S., Olson, A. J., Sanner, M. F. (2015) AutoDockFR: Advances in Protein-Ligand Docking with Explicitly Specified Binding Site Flexibility. *PLoS Computational Biology* 11. *https://doi.org/10.1371/journal.pcbi.1004586*

20. Hartshorn, M. J., Verdonk, M. L., Chessari, G., Brewerton, S. C., Mooij, W. T. M., Mortenson, P. N., Murray, C. W. (2007) Diverse, High-Quality Test set for the validation of Protein−Ligand docking performance. *Journal of Medicinal Chemistry* 50, 726–741. *https://doi.org/10.1021/jm061277y*

21. Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S. and Olson, A. J. (2009) Autodock4 and AutoDockTools4: automated docking with selective receptor flexibility. *J Computational Chemistry* 16, 2785-2791. *https://doi.org/10.1002/jcc.21256*

22. Morris, G. M., Goodsell, D. S., Pique, M. E., Lindstrom, W., Huey, R., Forli, S., Hart, W. E., Halliday, S., Belew, R., Olson, A. J. (2014*)* AutoDock Version 4.2: Automated Docking of Flexible Ligands to Flexible Receptors, *AutoDock Tools.* https://autodock.scripps.edu/wp-content/uploads/sites/56/2021/10/AutoDock4.2.6_UserGuide.pdf (accessed Mar 2025).

23. Humphrey, W., Dalke, A., Schulten, K. (1996) VMD: Visual Molecular Dynamics. *J Mol Graph* 14, 33–38. *https://doi.org/10.1016/0263-7855(96)00018-5*

24. Daina, A., Michielin, O., Zoete, V. (2017) SwissADME: A Free Web Tool to Evaluate Pharmacokinetics, Drug-Likeness and Medicinal Chemistry Friendliness of Small Molecules. *Scientific Reports* 7, 42717. *https://doi.org/10.1038/srep42717*

25. Fentanyl Drug Facts, National Institute on Drug Abuse, *https://nida.nih.gov/publications/drugfacts/fentanyl* (accessed Mar 2025).

26. Morak-Młodawska, B., Jeleń, M., Martula, E., Korlacki R. (2023) Study of Lipophilicity and ADME Properties of 1,9-Diazaphenothiazines with Anticancer Action. *Int J Mol Sci* 24, 6970. *https://doi.org/10.3390/ijms24086970*

27. Facts about Fentanyl, United States Drug Enforcement Administration, *www.dea.gov/resources/facts-about-fentanyl* (accessed Mar 2025).

28. Ellis, C. R., Kruhlak, N. L., Kim, M. T., Hawkins, E. G., Stavitskaya, L. (2018) Predicting opioid receptor binding affinity of pharmacologically unclassified designer substances using molecular docking. *PLOS One* 13. *https://doi.org/10.1371/journal.pone.0197734*

29. Bird, H. E., Huhn, A. S., Dunn, K. E. (2023) Fentanyl absorption, distribution, metabolism, and excretion: narrative review and clinical significance related to illicitly manufactured fentanyl. *Journal of Addiction Medicine* 17, 503–508. *https://doi.org/10.1097/adm.0000000000001185*

30. Sutcliffe, K. J., Corey, R. A., Alhosan, N., Cavallo, D., Groom, S., Santiago, M., Bailey, C., Charlton, S. J., Sessions, R. B., Henderson, G., Kelly, E. (2022) Interaction with the lipid membrane influences fentanyl pharmacology. *Advances in Drug and Alcohol Research* 2. *https://doi.org/10.3389/adar.2022.10280*

31. Serîn, S., Utku, T., Kaya, G. (2021) Quantum chemical calculations on fentanyl used as potent analgesic. *MTU Journal of Engineering and Natural Sciences* 2. *https://doi.org/10.46572/naturengs.1022735*

32. Zhuang, Y., Wang, Y., He, B., He, X., Zhou, X. E., Guo, S., Rao, Q., Yang, J., Liu, J., Zhou, Q., Wang, X., Liu, M., Liu, W., Jiang, X., Yang, D., Jiang, H., Shen, J., Melcher, K., Chen, H., Jiang, Y., Cheng, X., Wang, M.-W., Xie, X., Xu, H. E. (2022) Molecular recognition of morphine and fentanyl by the human μ-opioid receptor. *Cell* 185, 4251–4253. *https://doi.org/10.1016/j.cell.2022.09.041*

33. Vo, Q., Mahinthichaichan, P., Shen, J., Ellis, C. (2021) How mu-Opioid Receptor Recognizes Fentanyl. *Nat Commun* 12, 984. *https://doi.org/10.1038/s41467-021-21262-9*

ABOUT THE STUDENT AUTHORS

Keegan Gunderson is a Biomedical Engineering student at the University of Wisconsin–Eau Claire. After graduation, he plans to go to medical school. He is passionate about using current clinical knowledge and research to provide exceptional patient care to individuals in rural communities.

Faith Oldenburg is a third-year student of Nursing and Spanish at the University of Wisconsin Eau Claire. After graduation, she hopes to pursue a doctoral degree to become a certified nurse midwife. She is passionate about advancing and promoting research-based practice in order to provide excellent healthcare to underserved communities, especially those experiencing elevated rates of maternal and infant mortality.

PRESS SUMMARY

Deaths from opioid overdoses are at an all-time high due to their use clinically and increasing prevalence in street drugs. This study intends to identify the mechanisms that make them such potent stimulants for opioid receptors, specifically the Mu opioid receptor, which causes respiratory depression and potentially death when overstimulated. Using computational chemistry techniques, it was found that multiple chemical properties, namely a high electronegativity and chemical hardness, high lipophilicity, and the presence of non-polar groups for hydrophobic interactions may be responsible for making some opioids potent ligands for opioid receptors. This knowledge is significant to the design of future analgesics that minimize health risks, including addiction and fatal overdose.