Anais da Faculdade de Medicina de Olinda
Annals of Olinda Medical School

afmo.emnuvens.com.br ISSN: 2674-8487 Original article

Gamma-linolenic acid interacts with human voltage-gated calcium channel: aspects in the treatment of mastalgia

O ácido gama-linolênico interage com o canal de cálcio dependente de voltagem humano: aspectos no tratamento da mastalgia

Gidelson José da Silva Júnior[1] Gisele Evelin de Jesus Arruda[1]
Ana Carolina de Oliveira Aguiar[1] Lais Cristhinne Sabino Gondim[1]
Lise Reis Melo[2] Joelmir Lucena Veiga da Silva[1]

[1] Faculdade de Medicina de Olinda. Olinda, Pernambuco, Brazil.
[2] Hospital Universitário Lauro Wanderley. Universidade Federal da Paraíba. João Pessoa, Paraíba, Brazil.

Abstract

Objectives: To assess the correlation between gamma-linoleic acid (GLA) and the voltage-gated calcium channel CaV3.2 and compare it with pregabalin (PGB) to elucidate the potential analgesic mechanism of GLA in cases of mastalgia. **Methods:** This quantitative, experimental, *in silico* study employed GLA and PGB as ligand molecules and the CaV3.2 channel as the protein target. Molecular docking experiments were performed using the Dockthor platform and analyzed with the Chimera 1.14 software. The results of ligand simulations with CaV3.2 were organized based on binding affinity (BA) and compared using the t-test; p-values < 0.05 were considered statistically significant. **Results and discussion:** After one million simulations of GLA and PGB with CaV3.2, the three best docking poses were selected. No significant differences were observed between BA values of GLA and PGB (p = 0.15). Both ligands docked within the pore of the channel, forming hydrogen bonds with the same amino acid residues; PBG established one additional interaction. **Conclusion:** GLA binds to the CaV3.2 channel in a manner similar to the reference blocker PGB. The identified chemical interactions suggest a potential channel blockade,

Corresponding author: Joelmir Lucena Veiga da Silva
E-mail: joelmir.silva@fmo.education
Funding: Programa de Desenvolvimento Institucional de Iniciação Científica, PRODIIC / Faculdade de Medicina de Olinda, FMO.
Ethics approval: Not applicable
Received: 05/22/2024
Approved: 04/03/2025

How to cite: Silva Júnior **GJ**, Arruda **GEJ**, Aguiar **ACO**, Gondim **LCS**, Melo **LR**, Silva **JLV**. Gamma-linoleic acid interacts with the human voltage-gated calcium channel: implications for the treatment of mastalgia. An Fac Med Olinda 2025; 1(13):388 DOI: https://doi.org/10.56102/afmo.2025.388

which may explain the analgesic effect of gamma-linolenic acid in the management of mastalgia.

Keywords: Gamma-linoleic acid; Mastalgia; Analgesics; Drug modeling; Voltage-gated calcium channel

Resumo

Objetivos: Avaliar a correlação do ácido gama-linolênico (GLA) com o CaV3.2 e comparar com a pregabalina (PGB), como perspectiva de elucidar o mecanismo de ação analgésica do GLA em casos de mastalgia. **Métodos:** Refere-se a uma pesquisa quantitativa e experimental, do tipo *in silico*, que empregou como moléculas ligantes o GLA e a PGB, e, como alvo proteico, o canal CaV3.2. Os experimentos de docagem molecular foram realizados no portal Dockthor e analisados pelo programa Chimera 1.14. Os resultados das simulações dos ligantes com o CaV3.2 foram organizados pelas afinidades de ligação (AL). Para comparação das AL, foi utilizado o teste "t" e foram considerados significantes valores de $p < 0,05$. **Resultados** e **discussão**: Após 1 milhão de simulações entre GLA e PGB com o CaV3.2, selecionaram-se os três melhores posicionamentos. Não houve diferença significante entre os valores de AL do GLA e da PGB ($p = 0,15$). Os ligantes se posicionaram dentro do poro do canal e estabelecendo ligações de hidrogênios com os mesmos resíduos de aminoácido e PGB apresentou interação com um outro a mais. **Conclusão**: O GLA é capaz de se ligar ao CaV3.2 de maneira semelhante ao bloqueador controle pregabalina. As interações químicas mostradas sugerem um possível bloqueio do canal, o que justificaria o efeito no controle da mastalgia pelo ácido gama-linolênico.

Palavras-chave: Ácido gama-linolênico; Mastalgia; Analgésico; Modelagem de drogas; Canal de cálcio dependente de voltagem

INTRODUCTION

Mastalgia refers to episodes of breast pain and is frequent among women of reproductive age, typically between 15 and 40 years old. About 70% of women in this age group experience the condition and seek medical care. The severity of mastalgia varies from mild to severe and is often described as discomfort, heaviness, tightness, or a burning sensation. These symptoms may also interfere with daily tasks, social activities, and the quality of life of patients.[1,2]

According to normal clinical and radiological findings, approximately 85% of patients improve after guidance, reassurance, and lifestyle advice without the need for medication. However, about 15% continue to need treatment due to the impacts of the condition or the increased intensity and frequency of pain episodes after the first consultation. The most appropriate treatment includes conservative and pharmacological therapy.[1-3]

The first step in the pharmacological treatment includes the use of evening primrose oil, which is rich in linoleic acid. Once absorbed by the body, linoleic acid is converted into gamma-linoleic acid (GLA). GLA is considered a completely safe, non-toxic, and non-carcinogenic

compound.[4,5]

GLA has become the preferred therapeutic option for treating mastalgia. The standard recommended dose is one 1000 mg capsule per day (180 mg of GLA) administered over an initial period of 4 to 6 months.[6,7] After being absorbed and metabolized, GLA is converted into biologically active metabolites (eicosanoids, such as prostaglandins, leukotrienes, and related compounds) that regulate cellular activity.[8] The low levels of GLA and its metabolites result in reduced prostaglandin E1 (PGE1) levels and exaggerated response to prolactin, which would help explain why some women experience severe mastalgia even when prolactin levels are within the normal range. The peripheral effects of prolactin are inhibited by increasing PGE1 production, which likely occurs through the interaction of GLA with membrane receptors and the production of eicosanoids and prostaglandins.[3] As no other mechanisms explaining GLA-induced analgesia have been documented, it is relevant to investigate whether ion channels could serve as a possible target for therapeutic action.

CaV is a low-voltage channels responsible for regulating neuronal excitability that is essential in the peripheral nociceptive process.[8] Three subtypes of T-type CaV channels have been described: CaV3.1, CaV3.2, and CaV3.3.[9] Studies on their regional gene expression have shown that CaV3.2 is mostly found in essential areas involved in pain transmission (e.g., sensory neurons of the dorsal root ganglion and superficial lamina of the dorsal horn).[10-12] Due to its role in pain signaling, CaV3.2 is considered a promising target for the development of drugs to treat acute and chronic pain.[13-17]

Drug discovery is an extensive, time-consuming, and often unsuccessful process. However, technological advances have been contributing to minimizing the many steps of drug development. Molecular docking analysis, for example, plays a crucial role in research by providing valuable insights into the mechanisms of action of new molecules; thus, reducing the costs and time associated with the drug development process.[18,19] Therefore, this study evaluated the potential interaction of GLA with the CaV3.2 channel using molecular docking analysis and compared it with pregabalin (PGB).

METHODS

This was a quantitative, experimental, *in silico* study. Virtual screening via molecular docking is a computational method commonly used in drug discovery to predict binding interactions between a new drug and a therapeutic target of interest. This approach enables the identification of potential ligand hits by docking compounds from a database against one or more receptors of interest.[20-23]

The 3D structures of the GLA (CID: 5280933) and PGB (CID: 5486971) ligands, a CaV-blocking drug, were obtained from the PubChem database. The 3D structure of the protein

target (human CaV3.2 channel [PDB: 6KZO]) was obtained from the Protein Data Bank. Molecular docking simulations were performed using the Dockthor platform, and results were ranked in order of increasing binding affinity (BA).[24] The following parameters for the DMRTS algorithm were set: (i) 24 docking corrections, (ii) one million evaluations per docking correction, (iii) population size of 750 individuals, and (iv) 20 clusters generated for each correction. The scoring function used to predict the best binding poses was based on the sum of the following terms from the MMFF94S force field: (i) intermolecular interaction energy, calculated as the sum of electrostatic and Van der Waals potentials between receptor-ligand atom pairs; (ii) intramolecular interaction energy, calculated as the sum of electrostatic and Van der Waals potentials between 1 and 4 ligand atom pairs; and (iii) a ligand torsional energy term.

Further detailed analyses and molecular visualization were performed in the Chimera 1.14 and PyMOL 2.1 software.

The BA between the ligands and the CaV3.2 were compared using the Student's t-test (GraphPad Prism, La Jolla, USA). A p-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Table 1 presents the BA between GLA or PGB and CaV3.2. GLA exhibited a BA of -6.9 ± 0.3 kcal/mol, similar to PGB (BA = -6.4 ± 0.3 kcal/mol). No statistically significant difference was observed between these values (p = 0.15), suggesting an affinity between GLA and CaV3.2 similar to the PGB. This finding supports the ability of the GLA, an unsaturated fatty acid of low binding energy, to interact with biological targets.[25,26] The BA of GLA with CaV3.2 was lower than -5 kcal/mol, indicating spontaneous binding between this ligand and target.[27]

Table 1. Binding affinity values between GLA and PGB with the CaV3.2 channel.

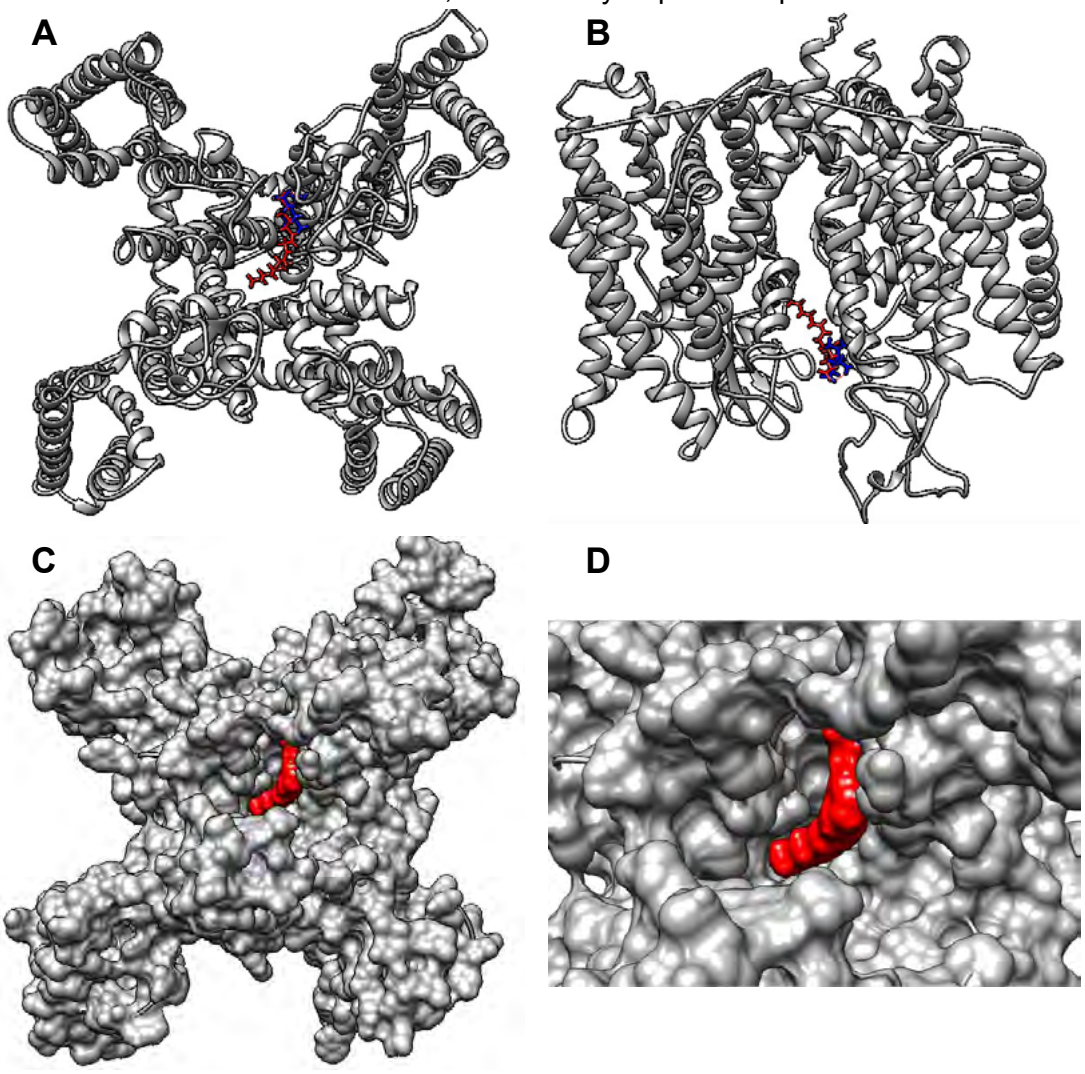
Ligand	Binding affinity (kcal/mol)
GLA	-6.9 ± 0.3
PGB	-6.4 ± 0.3

p = 0.15, Student's t-test (GLA vs. PGB).

Similar molecular docking simulations have been reported for cannabidiol and CaV3.2, suggesting that CaV3.2 may serve as a potential molecular target for cannabidiol in the treatment of chronic pain.[28] The CaV 3.1, CaV3.2, and CaV3.3, encoded by the CACNA1G, CACNA1H, and CACNA1I genes, respectively, have a very similar structure that suggests a possible affinity of GLA to CaV3.1 and CaV3.3.[29,30] Another molecular docking study revealed GLA interactions with the human voltage-dependent sodium channel 1.7 (NaV1.7), which is also targeted in pain therapies.[31,32] Thus, GLA may be a multitarget molecule that acts in other pain-related conditions beyond mastalgia.

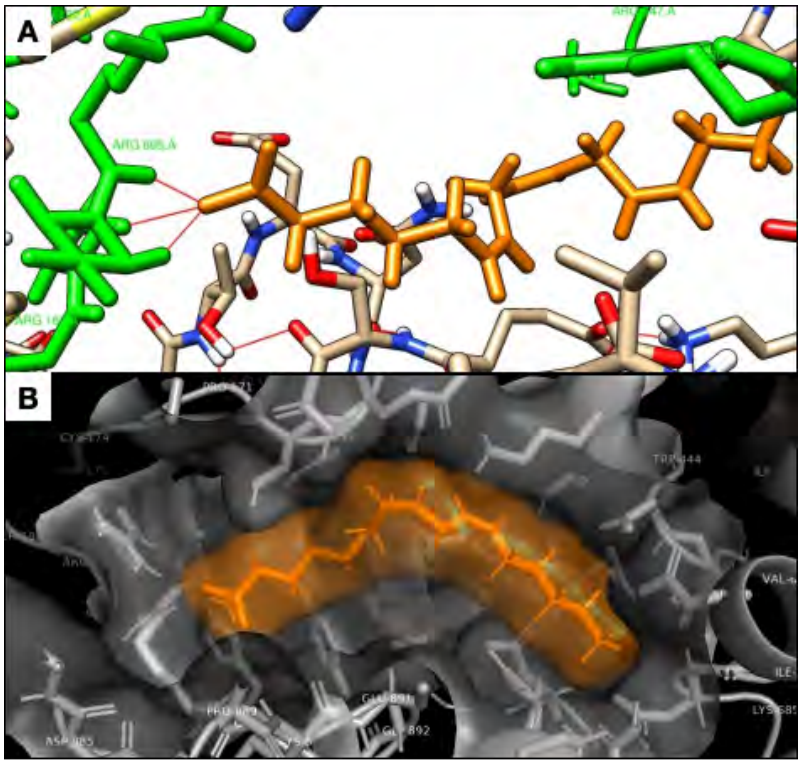
The 3D spatial analyses revealed that GLA and PGB occupied the same binding region on a loop of the CaV3.2 channel pore (Figures 1A and 1B). A long carbon chain of GLA also extended over the channel pore, possibly blocking it (Figures 1C and 1D). This blockade of CaV3.2 compromises the influx of calcium ions into the neurons and prevents the exocytosis of neurotransmitters in the synaptic cleft, triggering the pain impulse.[12]

Figure 1. Spatial arrangement of GLA and PGB over the CaV3.2. Loops (gray), GLA (red), and PGB (blue). A: top view of the channel; B: side view of the channel; C and D: hydrophobic representation.



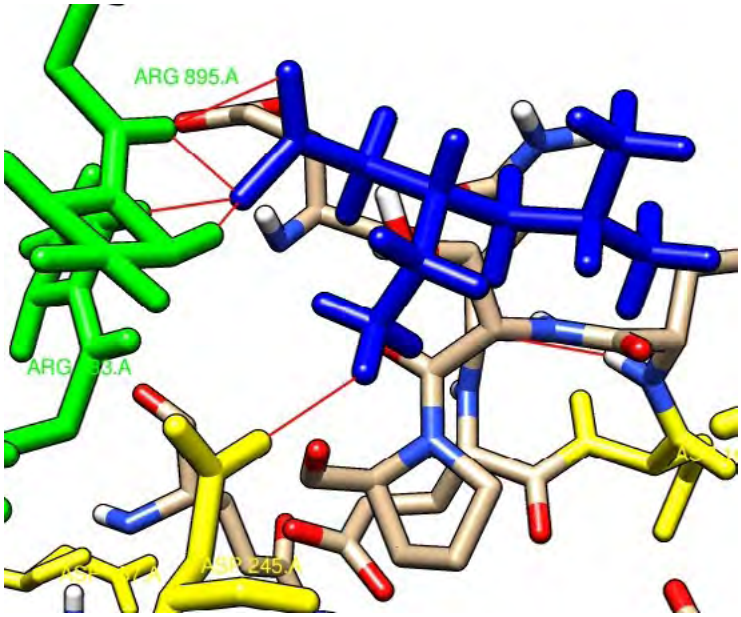
The binding pose between GLA and CaV3.2 was registered inside the channel pore (Figure 2). GLA established two hydrogen bonds with the amino acid residue Arg183 and one with Arg895 at distances of 2.2, 1.6, and 1.7 Å, respectively (Figure 2A). GLA was located in a pocket between CaV3.2 chains, where its long aliphatic chain formed hydrophobic interactions with the surrounding residues (Ser170, Glu169, Lys446, Asp443, Asn166, Asp690, Asn917, Lys923, Glu891, Pro889, Cys884, Gly894, Tyr241, and His896 [Figure 2B]).

Figure 2. Chemical interactions between GLA (in orange) and amino acid residues of the CaV3.2 channel. A: hydrogen bonds (red lines) and arginine amino acid (in green); B: electronic cloud (hydrophobic interactions).



Interestingly, PGB formed hydrogen bonds with the same amino acid residues besides the Asp245, establishing three hydrogen bonds with Arg895, one with Arg183, and one with Asp245, whose distances were 1.7, 2.3, 1.8, 1.4, and 2.3 Å, respectively (Figure 3). These chemical interactions between the same amino acid residues of GLA and the standard blocker (PGB) confirm their overlap within the channel pore and reinforce the probable blockade.

Figure 3. Hydrogen bonds between PGB (in blue) and amino acid residues of the CaV3.2 channel. Arginine (in green) and aspartate (yellow).



The ACT-709478 and ML218 have also been analyzed in the CaV3.2 structure as candidate molecules for potential analgesics. The ACT-709478 also made hydrogen bonds with the Ser1805, Asn412, and Gln1848 residues in regions distinct from GLA, while the ML218 interacted with the Phe1007.[33]

PGB is a CaV blocker and one of the therapeutic options in the management of chronic and neuropathic pain.[34,35] However, its use increases the risk of adverse events, leading to treatment discontinuation.[36] Therefore, the results obtained in this study support the use of GLA-based herbal medicines in the treatment of mastalgia over classic CaV blockers.

CONCLUSION

GLA binds to the CaV3.2 pore similarly to the control blocker PGB. This suggests a possible blockade of the channel pore, justifying its effect in controlling mastalgia. GLA is the primary chemical component of evening primrose oil, widely used as a therapeutic adjunct in the clinical treatment of this painful condition.

Future studies using *in vitro* models are required to confirm this mechanism of action of GLA on the voltage-gated calcium channel CaV3.2.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

FUNDING

Programa de Desenvolvimento Institucional de Iniciação Científica, PRODIIC / Faculdade de Medicina de Olinda, FMO.

AUTHOR CONTRIBUTIONS

GJSJ, GEJA, ACOA, and LCSG: Original Draft, Investigation, Visualization. **LRM**: Conceptualization, Writing - Revision and Editing. **JLVS**: Conceptualization, Supervision, Project Administration, Methodology, Writing - Revision and Editing. All authors read and agreed with the final version of the manuscript.

REFERENCES

1. Olawaiye A, Withiam-Leitch M, Danakas G, Kahn K. Mastalgia: a review of management. J Reprod Med. 2005;50(12):933-9. Disponível em: https://www.researchgate.net/publication/7328732_Mastalgia_A_review_of_management
2. Smith RL, Pruthi S, Fitzpatrick LA. Evaluation and management of breast pain. Mayo Clin Proc. 2004;79(3):353-72. https://doi.org/10.4065/79.3.353
3. Grullon S, Bechmann S. Mastodynia. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. 2022;32644675.
4. Dobryniewski J, Szajda SD, Waszkiewicz N, Zwierz K. Kwas gamma-linolenowy (GLA)—znaczenie terapeutyczne [The gamma-linolenic acid (GLA)—the therapeutic value]. Przegl Lek. 2007;64(2):100-2. Disponível em: https://www.researchgate.net/publication/5952444_The_gamma-linolenic_acid_GLA--the_therapeutic_value
5. Menke CH, et al. Rotinas em Mastologia. 1ª ed. Porto Alegre: Artes Médicas Sul; 2000;73.
6. Burbage J, Cameron L. An investigation into the prevalence and impact of breast pain, bra issues and breast size on female horse riders. J Sports Sci. 2017;35(11):1091-1097. https://doi.org/10.1080/02640414.2016.1210818
7. Functional nutrition and applicability of herbal medicines in the health of women with Premenstrual Syndrome: systematic review. Brazilian Journal of Health Review 2022;5(4):12766–12786. https://doi.org/10.34119/bjhrv5n4-067
8. Kapoor K, Huang Y-S. Gamma linolenic acid: an antiinflammatory omega-6 fatty acid. Current Pharmac Biotech 2006;7:531-534. https://doi.org/10.2174/138920106779116874
9. Zamponi GW, Striessnig J, Koschak A, Dolphin AC. The physiology, pathology, and pharmacology of voltage-gated calcium channels and their future therapeutic potential. Pharm. Rev. 2015;67(4):821–870. https://doi.org/10.1124/pr.114.009654
10. Choi S, Na HS, Kim J, Lee J, Lee S, Kim D, Park J, Chen CC, Campbell KP, Shin HS. Attenuated

pain responses in mice lacking Ca(V)3.2 T-type channels. Genes Brain Behav.2007;6(5):425–431. https://doi.org/10.1111/j.1601-183X.2006.00268.x

11. Talley EM, Cribbs LL, Lee JH, Daud A, Perez-Reyes E, Bayliss DA. Differential distribution of three members of a gene family encoding low voltage-activated (Ttype) calcium channels. J. Neurosci. 1999;19(6):1895–1911. https://doi.org/10.1523/jneurosci.19-06-01895.1999

12. Harding, E.K., Zamponi, G.W. Central and peripheral contributions of T-type calcium channels in pain. Mol Brain 15, 39 (2022). https://doi.org/10.1186/s13041-022-00923-w

13. Kamau PM, Li H, Yao Z, Han Y, Luo A, Zhang H, Boonyarat C, Yenjai C, Mwangi J, Zeng L, Yang S, Lai R, Luo L. Potent CaV3.2 channel inhibitors exert analgesic effects in acute and chronic pain models. Biomedicine & Pharmacotherapy 2022;153:113310. https://doi.org/10.1016/j.biopha.2022.113310

14. Jarvis MF, Scott VE, McGaraughty S, Chu KL, Xu J, Niforatos W, Milicic I, Joshi S, Zhang Q, Xia Z. A peripherally acting, selective T-type calcium channel blocker, ABT-639, effectively reduces nociceptive and neuropathic pain in rats, Biochem. Pharmacol. 2014;89(4):536–544. https://doi.org/10.1016/j.bcp.2014.03.015

15. Ziegler D, Duan WR, G. G, Thomas JW, Nothaft W. A randomized doubleblind, placebo-, and active-controlled study of T-type calcium channel blocker ABT-639 in patients with diabetic peripheral neuropathic pain. Pain 2015;156(10):2013–2020. https://doi.org/10.1097/j.pain.0000000000000263

16. Lee M. Z944: a first in class T-type calcium channel modulator for the treatment of pain. J. Peripher. Nerv. Syst. 2014;19(2):S11–S12. https://doi.org/10.1111/jns.12080_2

17. Snutch TP, Zamponi GW. Recent advances in the development of T-type calcium channel blockers for pain intervention. Br. J. Pharmacol. 2018;175(12):2375–2383. https://doi.org/10.1111/bph.13906

18. Asiamah I, Obiri SA, Tamekloe W, Armah FA, Borquaye LS. Applications of Molecular Docking in Natural Products-Based Drug Discovery. Scientific African 2023;S2468-2276. https://doi.org/10.1016/j.sciaf.2023.e01593

19. Teixeira LR, Silva Júnior JJ, Vieira PHS, Canto MVG, Figueirêdo AGM, Silva JLV. (2021). Tamoxifen inhibits the anion channel induced by Staphylococcus aureus α-hemolysin: electrophysiological and docking analysis. RSD [Internet]. 2021;10(2):e13010212326. https://doi.org/10.33448/rsd-v10i2.12326

20. Gentile F, Oprea TI, Tropsha A, Cherkasov A, (2023). Surely you are joking, Mr Docking! Chem. Soc. Rev. 2023;52:872–878. https://doi.org/10.1039/D2CS00948J

21. Sadybekov AV, Katritch V. Computational approaches streamlining drug discovery. Nature 2023;616:673–685. https://doi.org/10.1038/s41586-023-05905-z

22. Varela-Rial, A., Majewski, M., De Fabritiis, G., (2022). Structure based virtual screening: fast and slow. WIREs Comput. Mol. Sci. 2022;12:e1544. https://doi.org/10.1002/wcms.1544

23. Bender BJ, Gahbauer S, Luttens A, Lyu J, Webb CM, Stein RM, Fink EA, Balius TE, Carlsson J, Irwin JJ, Shoichet BK. A practical guide to large-scale docking. Nature Protoc. 2021;16:4799–4832. https://doi.org/10.1038/s41596-021-00597-z

24. Guedes IA, Silva MMPS, Galheigo M, Krempser E, Magalhães CS, Barbosa HJC, Dardenne LE. DockThor-VS: A Free Platform for Receptor-Ligand Virtual Screening. Journal of Molecular Biology 2024;436(17):168548. https://doi.org/10.1016/j.jmb.2024.168548

25. Kataria K, Dhar A, Srivastava A, Kumar S, Goyal A. A Systematic Review of Current Understanding and Management of Mastalgia. Indian J Surg. 2014;76(3):217–222. https://doi.org/10.1007/s12262-013-0813-8

26. Liu ZL, Li L, Ma HL, Zhong QS, Ke JY, Zhang H. Mechanism of action of Zhishi Daozhi decoction in the treatment of diarrhea based on network pharmacology and molecular docking. Drug Combination Therapy 2023;5(1):1-8. https://doi.org/10.53388/DCT20230003

27. Du G, Qu X, Hu J, Zhang Y, Cai Y. Identification of Taohong Siwu Decoction in Treating Chronic Glomerulonephritis Using Network Pharmacology and Molecular Docking. Natural Product Communications 2022;17(11):1-12. https://doi.org/10.1177/1934578X221139966

28. Silva Júnior GJ, Arruda GEJ, Lira NBD, Lira NBD, Costa AEA, Morioka CY, Silva JLV. Pharmacological prospection of cannabidiol analgesic action through molecular docking: interactions with voltage-gated sodium channel Nav1.7. Rsd [Internet]. 2023;12(3):E30340292. https://doi.org/10.33448/Rsd-V12i3.40292

29. Harding EK, Zamponi GW. Central and peripheral contributions of T-type calcium channels in pain. Mol Brain. 2022;15(1):39. https://doi.org/10.1186/s13041-022-00923-w

30. Weiss N, Zamponi GW. (2020). Genetic T-type calcium channelopathies. Journal of Medical Genetics 2020;57(1):1–10. https://doi.org/10.1136/jmedgenet-2019-106163

31. Silva JLV, Leite AI, Ferreira EN, Silva FM, Pavin JP, Oliveira LC, Marcela Eduarda Leite ME, Araújo MV, Ferreira RN, Lamares RJTC, Melo LR. Gamma-linolenic acid interactions with the human voltage-gated sodium channel 1.7 by molecular docking: its role in the action mechanism on mastalgia. IOSR Journal Of Pharmacy And Biological Sciences 2023;18,6(3):33-37. DOI: 10.9790/3008-1806033337. Disponível em: https://www.iosrjournals.org/iosr-jpbs/papers/Vol-18-issue6/Ser-3/G1806033337.pdf

32. Emery EE, Luiz AP, Wood JN. Nav1.7 and other voltage-gated sodium channels as drug targets for pain relief. Expert Opinion On Therapeutic Targets 2016;20(8): 975–983. http://dx.doi.org/10.1517/14728222.2016.1162295

33. Huang J, Fan X, Jin X, Lyu C, Guo Q, Liu T, Chen J, Davakan A, Lory P, Yan N. Structural basis for human Cav3.2 inhibition by selective antagonists. Cell Res 34, 440–450 (2024). https://doi.org/10.1038/s41422-024-00959-8

34. aylor CP. Mechanisms of analgesia by gabapentin and pregabalin--calcium channel alpha2-delta

[Cavalpha2-delta] ligands. Pain 2009;142(1-2):13-6. https://doi.org/10.1016/j.pain.2008.11.019

35. Calandre EP, Rico-Villademoros F, Slim M. Alpha2delta ligands, gabapentin, pregabalin and mirogabalin: a review of their clinical pharmacology and therapeutic use. Expert Rev Neurother. 2016;16(11):1263-1277. https://doi.org/10.1080/14737175.2016.1202764

36. Onakpoya IJ, Thomas ET, Lee JJ, Goldacre B, Heneghan CJ. Benefits and harms of pregabalin in the management of neuropathic pain: a rapid review and meta-analysis of randomised clinical trials. BMJ Open. 2019;9(1):e023600. https://doi.org/10.1136/bmjopen-2018-023600