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Computational Evaluation of Curcumenol as a Potential Inhibitor against Calcineurin Protein

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Abstract

Calcineurin protein is a multifunctional serine-threonine protein phosphatase vital to several cellular functions, such as immune response control, signal transduction, and cardiac function. Its relevance in preserving cellular homeostasis is highlighted by its involvement in T-cell activation, calcium signalling pathways, etc. The dysregulation of the calcineurin protein has been reported to be linked with neurodegenerative diseases, and hence calcineurin inhibitors may be beneficial as a treatment option. Existing FDA-approved calcineurin inhibitors are used under stringent guidelines and require careful monitoring because of possible side effects and toxicity. Consequently, as a therapeutic option, the discovery and development of novel calcineurin inhibitors is imperative. For this reason, the current study aims to characterize the calcineurin inhibitory action of curcumenol, a naturally occurring chemical by employing diverse *in-silico* techniques. Curcumenol was checked for its ADME-T and molecular interactional stability and was predicted to have impressive inhibitory potential with a docking score of -9.1kcal/mol and a mean RMSD of the backbone atoms of the Calcineurin-curcumenol complex as 4.55Å when simulated in a molecular dynamics system for 100ns. The*in-silico*findings were further justified using *in vitro* Calcineurin Phosphatase activity assayto quantitively analyse the inhibitory efficacy of curcumenol, defined in terms of IC_{50} value,which was found to be 0.113 µM. The overall study suggested that curcumenol holds the characteristic molecular and interactional properties and hence can be developed as a prospectiveand safe calcineurin inhibitor which can be ultimately employed as a promising therapeutic candidate against neurodegenerative diseases.

Keywords: Natural inhibitor, Molecular Docking, Molecular dynamics simulation, Inhibitory concentration 50

Introduction

Calcineurin is a heterodimeric, eukaryotic protein and functions as a direct mediator between calcium signaling and other phosphorylating states in several physiological pathways. Its structural components include a catalytic subunit known as Calcineurin A with an active site that is blocked by an autoinhibitory peptide, and a regulatory subunit with a calmodulin-binding site known as calcineurin B (1). The binding of calmodulin to the regulatory subunit is facilitated by a rise in cellular calcium concentration, which then displaces the autoinhibitory peptide from the active site and hence activates the calcineurin protein.It plays vital roles in many physiological processes, including memory and cognition, apoptosis, muscle and cardiac function, cell cycle regulation, T-cell activation, and transcription regulation. More significantly, it is the only calcium-dependent phosphatase found in the brain and is abundantly expressed within the brain, particularly in the forebrain (2). Hence, the regulation of calcineurin expression and its activation plays an essential part in a variety of developmental and differentiating processes (3). Consequently, its dysregulation can play a major role in the pathogenesis of diseases affecting the diverse organ systems; some of these diseases have already been

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documented, and they include skeletal muscle hypertrophy, autoimmune diseases, neurodegenerative diseases, spermatogenesis, inflammatory bowel disease, allergies and asthma, diabetes, liver fibrosis, and more (4).

Given calcineurin's well-studied ability to activate immune system T cells, typically through the NFAT pathway, its inhibitors are designed to prevent unintended immune activation. At the moment, calcineurin inhibitors are used as a class of immunosuppressants in clinical applications. The FDA-approved calcineurin inhibitors like cyclosporin, tacrolimus, voclosporin, and pimecrolimus are used to treat autoimmune diseases including connective tissue disorders, lupus nephritis, idiopathic inflammatory myositis, interstitial lung disease, atopic dermatitis, as well as during solid organ transplantation to manage the unwanted immune reaction (5).

Recently these inhibitors, especially cyclosporin and tacrolimus have demonstrated their ability of neuroprotection in different neurodegenerative cell line models as well as animal models such as forebrain ischemia in rat models, mouse models of Alzheimer's disease, rotenone-treated rat models, etc (6,7,8). However, the concern lies in the direct application of these inhibitors against a neurodegenerative disease as they possess significant toxicity as well as innate side effects (9). Therefore, there is a strong need to find and develop new calcineurin inhibitors to provide treatment for numerous distinct neurodegenerative conditions.

Repurposing of drugs is becoming a revolutionary trend in drug discovery which in turn facilitates the use of natural products with substantial potential involving a wide range of pharmacological activity, and considerable structural diversity, as well as less toxicity (10, 11). Curcumenol which is a sesquiterpene primarily isolated from the rhizomes of *Curcuma zedoaria*is one such natural active molecule that has the potential to be developed as a promising medication (12). It is known to possess innumerable medicinal properties like antimicrobial properties, applicability as an anti-inflammatory agent for intervertebral disc degeneration, and anti-cancerous activity in lung cancer disease models. Its employment in the future clinical setting will be highly favored due to reduced cytotoxicity and adverse effects (13).

The protective effect of different bioactive components extracted from *Curcuma zedoaria* on oxidative stress in neuroblastoma glioma hybrid cell lines, induced by hydrogen peroxide has previously been studied. In the same study, it was found that increasing the concentration of curcumenol enhanced this protective effect (14). The mechanism underlying this, nonetheless, remains unknown. Accordingly, exploring curcumenol's inhibitory effects on the calcineurin protein may reveal information on the underlying neuroprotective processes. The current study hereby aims at the evaluation of curcumenol as a potential calcineurin inhibitor using different *in silico* techniques mainly including molecular docking studies and molecular dynamics simulation studies for predicting the molecular interactional behaviour in the simulated virtual systems mimicking the physiological environment and to further confirm its inhibitory efficiency via *in vitro* assays. The study consequently proposes the hypothesis that if curcumenol can be proved to be a potential calcineurin inhibitor then it can be developed further for its employment as a prospective neuroprotective remedy.The current therapeutic strategies against diverse neurodegenerative conditions like Alzheimer's disease, Parkinson's disease, Huntington's disease, etc, demand serious attention and the application of sophisticated research and development approaches is imperative.In this context, this work can open new avenues for the development of novel therapeutic options.

Materials and Methods

Retrieval of the 3D structure of Curcumenol and Prediction of ADME-Toxicity

The present work commenced by retrieving 3D structural data of curcumenol

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from PubChem (15). The structural data in both SMILES, as well as ".sdf" formats, were downloaded. The 3D structure in ".sdf" format was further converted to ". pdbqt" via the OpenBabel tool (16). The structural data in SMILES format were used as the input for predicting the ADME properties and toxicity details via the open web servers SWISS-ADMEand ProToxII accordingly (17, 18). The predicted properties and toxicity profiles were studied thoroughly and understood the possibility of employing curcumenol as a possible drug candidate.

Retrieval of the 3D structure of Calcineurin protein and evaluation of molecular interactional stability

Further, the 3D structure of the Calcineurin protein was retrieved from RCSB-PDB with PDB id 1MF8 (19). The initial preparation of the protein structure was done and Binding Site coordinates were predicted using Biovia Discovery Studio (20). The protein structure in ".pdbqt" was used for docking against the 3D structure of curcumenol in ".pdbqt" using POAP, which is a software pipeline based on GNU Parallel that is configured to operate Open Babel and the AutoDock suite in highly ideal parallelization (21). The docking score obtained was studied and determined whether the calcineurin-curcumin interaction was spontaneous or not. The 2-D interaction diagram was then used to study the key residues of protein and curcumenol involved in the interaction along with the type of interactions. Further, the interactional behavior was scrutinized in a virtual dynamic system mimicking a physiological system using Desmond Maestro, Molecular Dynamics Simulation platform, procured from Schrodinger.Inc (22). The simulation event analysis panel of the software facilitated the analysis of structural stability and behavioral pattern of calcineurin alone and when interacted with curcumenol for a while of 100ns by converting the trajectory data into performance metrics like RMSD (Root mean square deviation) as well as Radius of Gyration plots.

***In vitro* confirmation of Calcineurin inhibition by curcumenol**

The computational analysis of the molecular inhibitory ability of curcumenol was then validated via *in vitro* assay using the calcineurin phosphatase activity kit provided by Abcam (ab139461) (23). Calcineurin protein is a phosphatase that removes the phosphate group from its substrates. Hence when curcumenol is added to the reaction mixture if it has an inhibitory effect then there will be a decrease in the released phosphate concentration. The phosphate release was detected by adding the malachite green solution and measuring the absorbance at a wavelength of 620nm after 30 mins of incubation at dark. A standard phosphate graph was further plotted to derive equations to calculate the Phosphate release and Percentage inhibition. Varying concentrations of curcumenol ranging from 1 mM, 100 µM, 10 µM, 1 µM and 0.1 µM were assayed and the regression plot was obtained by plotting concentration against percentage inhibition which was then employed to get the minimum inhibitory concentration (IC_{50}) of curcumenol against calcineurin protein.

Results and Discussion

Owing to the implicit side effects and toxicity demonstrated by the currently available FDA approved calcineurin inhibitors, the present study intended to evaluate the potential of curcumenol as a possible candidate via diverse computational evaluations.

Retrieval of the 3D structure of Curcumenol and Prediction of ADME-Toxicity

For that objective, the structural data of curcumenol was initially retrieved from the PubChem Database (Table 1).

Besides having the intended biological activity, a prospective chemical must also have appropriate or ideal pharmacokinetics and safety features to be taken into consideration as a possible medicine. Therefore, we used the Swiss-ADME *in silico* toolset to examine the curcumenol, to find appropriate

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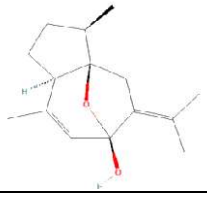
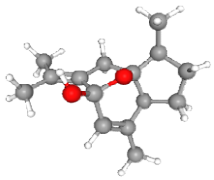
Table1: Chemical structure of Curcumenol (https://pubchem.ncbi.nlm.nih.gov/compound/167812)	
2D structure	
3D structure	
SMILES format	CC1CCC2C13CC(=C(C)C)C(O3)(C=C2C)O

pharmacokinetics, drug-likeness, and ideal medicinal chemistry properties (24). Hence in the preliminary screening, curcumenol was found to be soluble, blood-brain barrier permeable, highly gastrointestinal absorbable, and possess no cytochrome inhibition, as predicted by the SWISS-ADME website. With a bioavailability score of 0.55, which is discussed to be ideally admissible and readily absorbed by the human body as stated by other authors, curcumenol is predicted to be successfully developed as an oral drug (25). Curcumenol is further predicted to be Lipinski's rule-following compound in addition to Mugge's, Egan's, and Verber's Rules which shows that curcumenol can be developed as an oral drug candidate with good pharmacokinetic druggability characteristics (26).The ProTox II webserver estimated that curcumenol has an LD_{50} value of 6000 mg/kg for acute oral toxicity, placing it in class 6 toxicity. According to the globally harmonized system of categorization and labeling of substances (GHS), class 6 toxicity is classified as non-toxic. Additionally, it was determined to be inert in terms of immunotoxicity, cytotoxicity, mutagenicity, hepatotoxicity, and carcinogenicity (27). Overall, curcumenol's toxicity profile was favorable. Hence it was concluded that

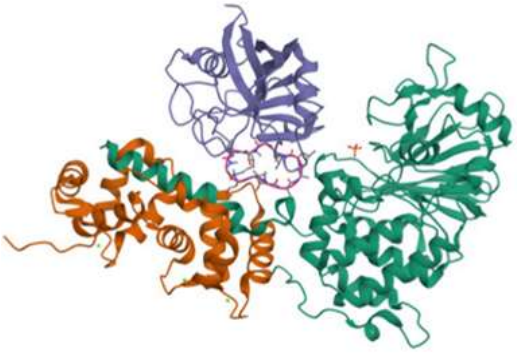


Figure 1: 3D Structure of the human calcineurin-cyclosporin-cyclophilin complex with PDBid: 1MF8. (https://www.rcsb.org/structure/1MF8)

curcumenol is a promising candidate for drug development under clinical conditions, according to initial screening based on ADME and toxicity predictions.

Retrieval of the 3D structure of Calcineurin protein and evaluation of molecular interactional stability

Consequently, the PDB file containing the 3.10 Å resolution protein structure of the human calcineurin-cyclosporin-cyclophilin complex (PDB id: 1MF8) has been retrieved from the Protein Data Bank (Figure 1). The retrieved .pdb file was prepared and binding site coordinates were found using Biovia Discovery studio as x: -36.754, y: 15.366, and z: 23.538.

The docking score obtained via POAP software for curcumenol against calcineurinprotein in terms of binding energy was -9.1 kcal/mol, which indicated the better inhibitory capability of curcumenol in a static environment against the calcineurin protein. The inhibition constant (Ki) was then found using equation (1), where ΔG is the Binding energy, R (universal gas constant) has the value of 1.985×10^{-3} kcal/molK, and T (temperature) is 298.15 K and it was found to be 2.1 (Figure 2).

$$Ki = \exp^{(\Delta G/RT)} \quad (1)$$

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A chemical is generally considered to be more active if its docking score is more negative. Consequently, curcumenol can be contemplated as an active inhibitor of curcumenol reviewing the discussions made in similar studies (28).

The 2D interaction diagram of calcineurin-curcumenol complex was then analyzed using Biovia Discovery studio and key interactions including van der Waals, Conventional Hydrogen bond, Alkyl, and Pi-alkyl bonds, were found to be the common interactional forces seen in most stable target protein-drug complexes (29). The major underlying interactions were conventional hydrogen bonds with residue Val: D: 9, van der Waals interaction with residues Asn: B: 122, Thr: C: 73 and Ser: A: 353, and alkyl bonds with residues Met: B:118, Trp: A: 352, Phe: A: 356, Pro: A: 355 and Val: B: 119 of calcineurin protein (Figure 3).

Ultimately, molecular dynamics simulation was used to verify the effectiveness of curcumenol as a molecular

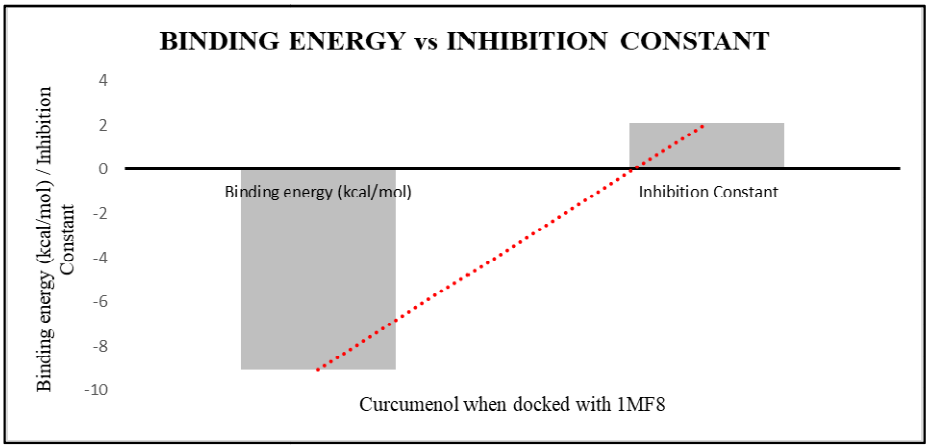

Figure 2: Binding energy vs Inhibition constant

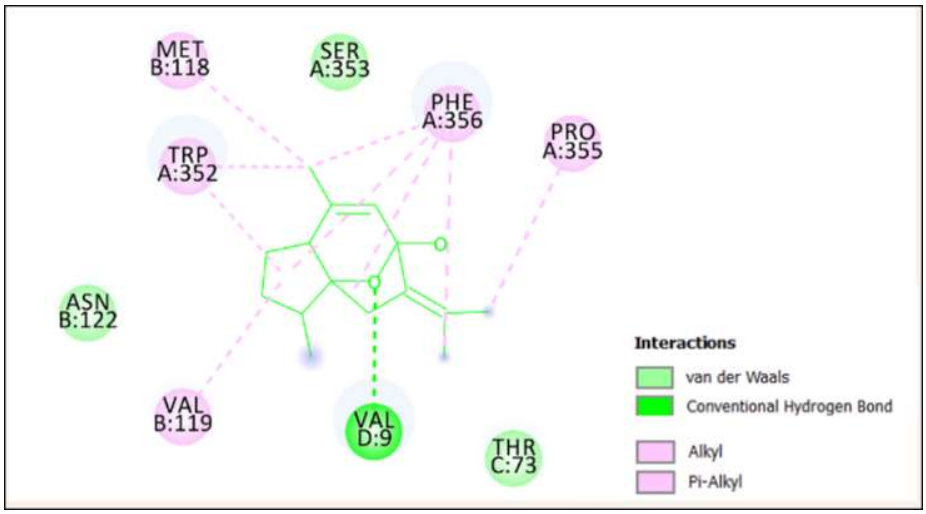

Figure 3: 2D interactional Diagram

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inhibitor against calcineurin in a simulated SPC-solvated system. Molecular biology and drug development have benefited greatly from the application of molecular dynamics (MD) simulations in recent years. These simulations provide a fine temporal resolution and complete atomic information on the behavior of proteins and other biomolecules (30).This information can hence be deducted for evaluating the applicability of our candidate molecule as a potential inhibitor. Consequently, the interactional stability of curcumenol with calcineurin in a dynamic virtualcondition mimicking the physiological environment was studied for 100ns, which revealed the behavior of trajectories in terms of performance metrices like Root mean square deviation and Radius of Gyration. The mean RMSD (Root mean square deviation) for trajectories of backbone atoms was obtained as;5.032 Å for calcineurin protein alone and 4.55 Å, when complexed with curcumenol, correspondingly. The small and relatively comparable values of RMSD of apoprotein and holoprotein implied that the backbone atoms had relatively constant movement in the dynamic physiological condition. Reports state that RMSD should ideally be zero, but deviance results from statistical errors. Therefore, the more spatially comparable the two compared structures are, the smaller the variance. The more configurations deviate from the reference structure, the worse the RMSD value becomes (31). In the current study, as shown in Figure 4, the blue line indicates the plot of reference structure or structure of the protein (1MF8) alone and the red line indicates the structure to be evaluated or 1MF8-curcumenol complex structure. The plot showed that both structures were following the same trajectories for most time in simulation with very negligible deviations. Further, the radius of gyration (ROG) of atom trajectories within the simulated period was used to assess the compactness of the structures of calcineurin alone and the calcineurin curcumenol complex. Plotting the RMSD and ROG provided additional evidence of the interactional stability. Moreover, all plots indicated the overall structural stability in a significant manner as scrutinized by other authors (Figure 5) (31).

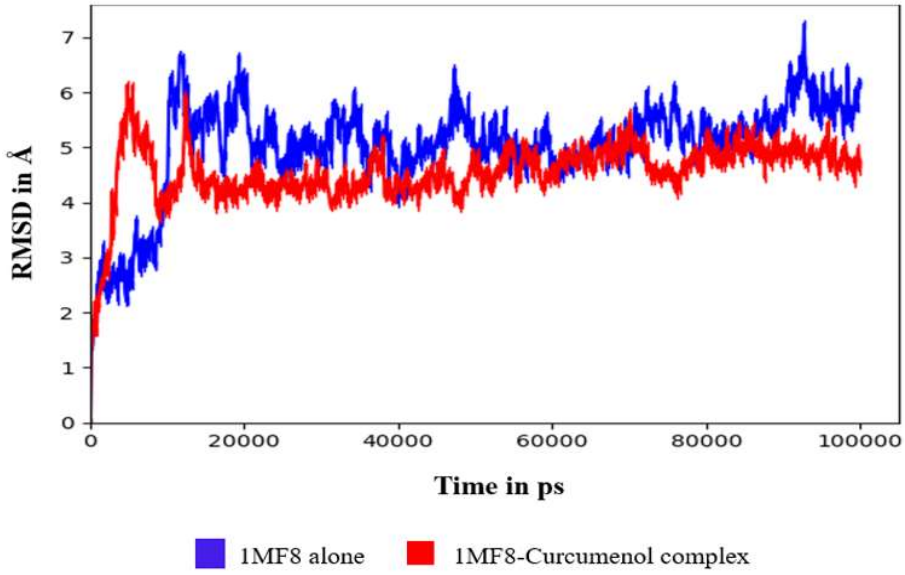

Figure 4: Root mean square deviation of backbone atoms of calcineurin protein alone and curcumenol-calcineurin complex

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***In vitro* confirmation of Calcineurin inhibition by curcumenol**

The computational evaluations carried out hence predicted that curcumenol is a possible calcineurin inhibitor by having fairly acceptable molecular inhibitory and interactional characteristics. Accordingly, the inhibitory efficacy of curcumenol was lastly assayed using real-time *in vitro* assay employing a calcineurin phosphatase activity kit. The inhibitory effect was calculated based on the decrease in released phosphate concentration, which was obtained using equation (2) derived from the standard phosphate graph (Figure 6).

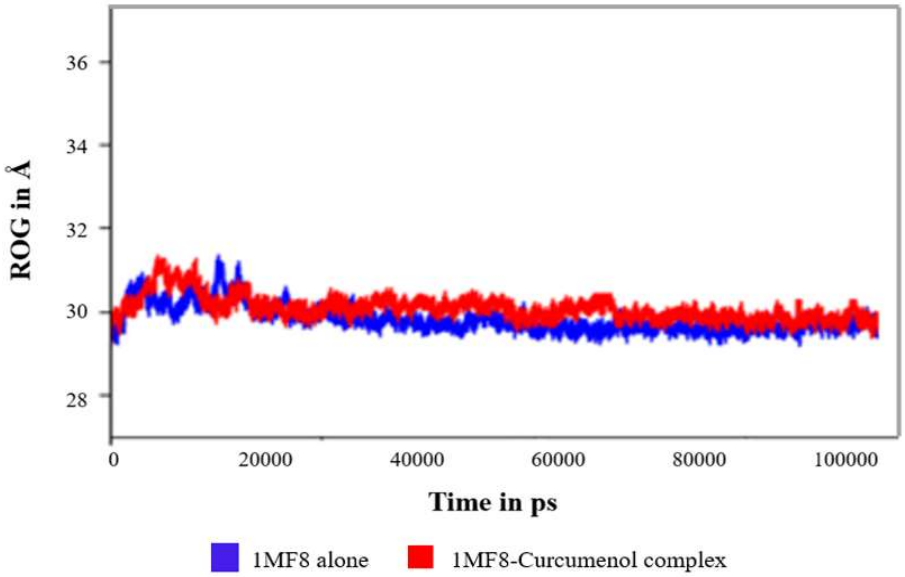

Figure 5: Radius of Gyration Plot of backbone and sidechain atoms of calcineurin protein alone and curcumenol-calcineurin complex

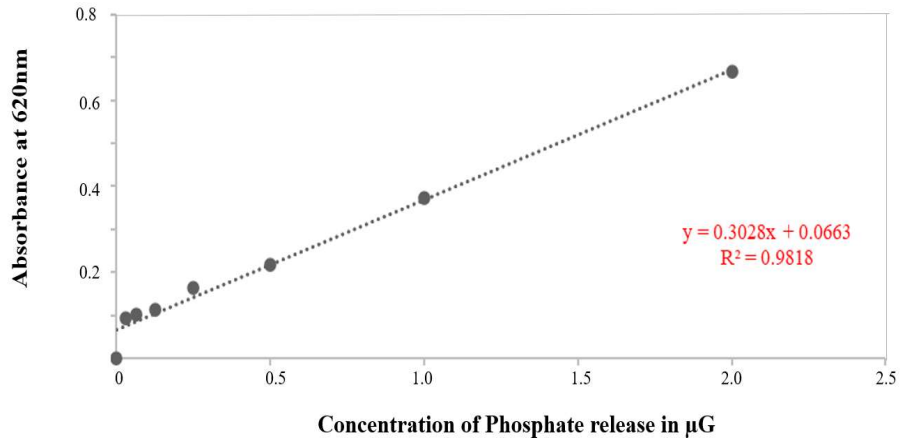

Figure 6: Phosphate Standard Curve

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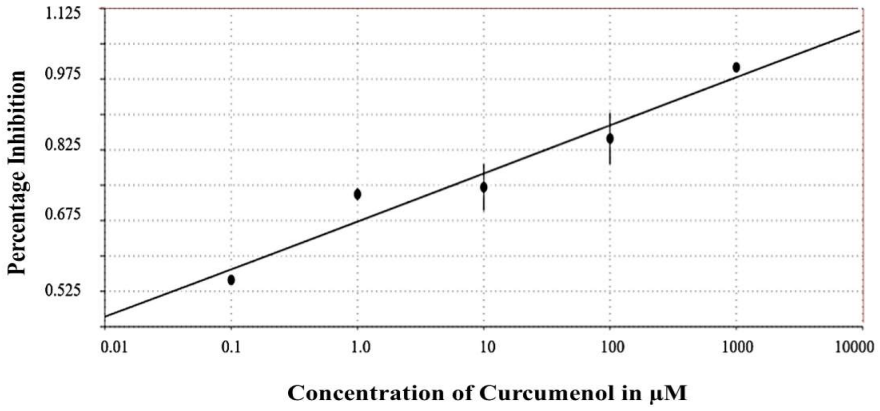

Figure 7: Percentage Inhibition Regression Curve

$$\text{Phosphate Released} = \frac{(\text{OD at } 620\text{nm} - \text{Y intercept})}{\text{Slope}} \quad (2)$$

Curcumenol in 5 different concentrations was used to assay its inhibitory effect. The percentage inhibition was consequently calculated using equation (3).

$$\text{Percentage Inhibition} = \frac{(\text{Positive control} - \text{Test well})}{\text{Positive control}} * 10 \quad (3)$$

The percentage inhibition was plotted against the concentration of curcumenol to obtain the regression plot and the minimum inhibitory concentration value of curcumenol as 0.113 µM using the online software AAT Bio Quest IC_{50} Calculator (Figure 7) (32).

The low IC_{50} value of curcumenol that was found is similar to the investigative calcineurin inhibitors reported by other authors (33). After analyzing all of these data, it was determined that curcumenol possessed adequate inhibitory activity. These characteristics were predicted by *in silico* studies and replicated through *in vitro* assays, in the current study. Thus, more thorough research on curcumenol may be necessary to assess and develop it as a potential treatment for diverse neurodegenerative diseases.

Conclusion

The application of calcineurin inhibitors is suggested as the best course of treatment for a variety of ailments, including neurodegenerative conditions like Parkinson's Disease, Alzheimer's disease, Amyotrophic Lateral Sclerosis, Huntington's disease, Transmissible spongiform encephalopathies, and so on. Current FDA-approved calcineurin inhibitors must be administered by rigorous guidelines and need to be closely monitored due to potential toxicity and side effects. Therefore, finding new calcineurin inhibitors is essential. According to the results of the current study, curcumenol possesses the necessary molecular and interactional characteristics as a potential calcineurin inhibitor. Its effectiveness as a potent calcineurin inhibitor was ultimately confirmed using *in vitro* assay. In light of these findings, we conclude by proposing further research and investigations on advancing curcumenol's potential as a treatment option against neurodegenerative diseases.

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References

1. Rusnak, F., and Mertz, P. (2000). Calcineurin: form and function, Physiological reviews, 80(4): 1483–1521.
2. Creamer, T. P. (2020). Calcineurin. Cell Communication and Signaling, 18(1).
3. Schulz, R. A., andYutzey, K. E. (2004). Calcineurin signaling and NFAT activation in cardiovascular and skeletal muscle development. Developmental biology, 266(1): 1-16.
4. Chen, L., Song, M., and Yao, C. (2022). Calcineurin in development and disease. Genes and Diseases, 9(4): 915-927.
5. Lee, J. U., Kim, L. K., and Choi, J. M. (2018). Revisiting the Concept of Targeting NFAT to Control T Cell Immunity and Autoimmune Diseases. Frontiers in immunology, 9: 2747.
6. Uchino, H., Minamikawa-Tachino, R., Kristián, T., Perkins, G., Narazaki, M., Siesjö, B.K. and Shibasaki, F., (2002). Differential neuroprotection by cyclosporin A and FK506 following ischemia corresponds with differing abilities to inhibit calcineurin and the mitochondrial permeability transition. Neurobiology of disease, 10(3): 219-233.
7. Sullivan, P.G., Sebastian, A.H. and Hall, E.D. (2011) “Therapeutic window analysis of the neuroprotective effects of cyclosporine-A after traumatic brain injury,” Journal of Neurotrauma, 28(2): 311–318.
8. Singh, S., Ganguly, U., Pal, S., Chandan, G., Thakur, R., Saini, R. V., Chakrabarti, S. S., Agrawal, B. K., and Chakrabarti, S. (2022). Protective effects of cyclosporine A on neurodegeneration and motor impairment in rotenone-induced experimental models of Parkinson's disease. European journal of pharmacology, 929: 175129.
9. Naesens, M., Kuypers, D. R., and Sarwal, M. (2009). Calcineurin inhibitor nephrotoxicity. Clinical Journal of the American Society of Nephrology, 4(2): 481-508.
10. Pushpakom, S., Iorio, F., Eyers, P.A., Escott, K.J., Hopper, S., Wells, A., Doig, A., Guilliams, T., Latimer, J., McNamee, C. and Norris, A. (2019). Drug repurposing: progress, challenges and recommendations. Nature reviews Drug discovery, 18(1): 41-58.
11. Newman, D. J., and Cragg, G. M. (2016). Natural products as sources of new drugs from 1981 to 2014. Journal of natural products, 79(3): 629-661.
12. U. C. Rahayu, D., Hartono, and Sugita, P. (2018). Antibacterial activity of curcumenol from rhizomes of Indonesian Curcuma aeruginosa (Zingiberaceae). Rasayan Journal of Chemistry, 11(2): 762–765.
13. Zhang R, Pan T, Xiang Y, Zhang M, Xie H, Liang Z, Chen B, Xu C, Wang J, Huang X, Zhu Q, Zhao Z, Gao Q, Wen C, Liu W, Ma W, Feng J, Sun X, Duan T, Lai-Han Leung E, Xie T, Wu Q, Sui X. (2021). Curcumenol triggered ferroptosis in lung cancer cells via lncRNA H19/miR-19b-3p/FTH1 axis. Bioactive materials, 13: 23–36.
14. Hamdi, O. A. A., Ye, L. J., Kamarudin, M. N. A., Hazni, H., Paydar, M., Looi, C. Y., and Awang, K. (2015). Neuroprotective and Antioxidant Constituents from Curcuma zedoaria Rhizomes. Records of Natural Products, 9(3): 349-355
15. Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B. A., Thiessen, P. A., Yu, B., Zaslavsky, L., Zhang, J., and Bolton, E. E. (2023). PubChem 2023 update. Nucleic Acids Res., 51(D1): D1373–D1380.
16. O'Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., & Hutchison, G. R. (2011). Open Babel: An open chemical toolbox. Journal of cheminformatics, 3: 33.
17. Daina, A., Michielin, O., and Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific reports, 7: 42717.
18. Banerjee, P., Eckert, A. O., Schrey, A. K., and Preissner, R. (2018). ProTox-II: a webserver for the prediction of toxicity of chemicals. Nucleic acids research, 46(W1), W257–W263.
19. Jin, L., and Harrison, S. C. (2002). Crystal structure of human calcineurin complexed with cyclosporin A and

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10.5530/ctbp.2024.4s.6

human cyclophilin. Proceedings of the National Academy of Sciences, 99(21): 13522–13526.
20. SYSTÈMES, D. (2016). BIOVIA Discovery Studio Dassault Systems BIOVIA, Discovery Studio Modeling Environment, Release 2017. Dassault Systemes
21. Samdani, A., and Vetrivel, U. (2018). POAP: A GNU parallel based multithreaded pipeline of open babel and AutoDock suite for boosted high throughput virtual screening. Computational biology and chemistry, 74: 39–48.
22. Ivanova, L., Tammiku-Taul, J., García-Sosa, A. T., Sidorova, Y., Saarma, M., and Karelson, M. (2018). Molecular dynamics simulations of the interactions between glial cell line-derived neurotrophic factor family receptor GFRα1 and small-molecule ligands. ACS Omega, 3(9): 11407–11414.
23. Zhang, H. L., Zhao, B., Han, W., Sun, Y. B., Yang, P., Chen, Y., Ni, D., Zhang, J., and Yin, D. M. (2021). Acetylation of calmodulin regulates synaptic plasticity and fear learning. The Journal of Biological Chemistry, 297(3): 101034.
24. Ononamadu, C. J., and Ibrahim, A. (2021). Molecular docking and prediction" of ADME/drug-likeness properties of potentially active antidiabetic compounds isolated from aqueous-methanol extracts of Gymnemasylvestre and Combretum micranthum. Biotechnologia, 102(1): 85–99.
25. Martin, Y.C. (2005). A bioavailability score, Journal of Medicinal Chemistry, 48(9): 3164–3170.
26. Benet, L. Z., Hosey, C. M., Ursu, O., and Oprea, T. I. (2016). BDDCS, the Rule of 5 and drugability. Advanced drug delivery reviews, 101: 89–98.
27. Bhat, V., and Chatterjee, J. (2021). The use of in silico tools for the toxicity prediction of potential inhibitors of SARS-COV-2. Alternatives to Laboratory Animals, 49(1–2): 22–32.
28. Macip, G., Garcia-Segura, P., Mestres-Truyol, J., Saldivar-Espinoza, B., Ojeda-Montes, M. J., Gimeno, A., Cereto-Massagué, A., Garcia-Vallvé, S., and Pujadas, G. (2022). Haste makes waste: A critical review of docking-based virtual screening in drug repurposing for SARS-CoV-2 main protease (M-pro) inhibition. Medicinal research reviews, 42(2): 744–769.
29. Dhorajiwala, T., Halder, S., and Samant, L. (2019). Comparative in silico molecular docking analysis of L-threonine-3-dehydrogenase, a protein target against African trypanosomiasis using selected phytochemicals. Journal of Applied Biotechnology Reports, 6(3): 101–108.
30. Hollingsworth, S. A., and Dror, R. O. (2018). Molecular Dynamics Simulation for All. Neuron, 99(6): 1129–1143.
31. Arnittali, M., Rissanou, A.N. and Harmandaris, V. (2019) ‘Structure of biomolecules through molecular dynamics simulations’, Procedia Computer Science, 156: 69–78.
32. AAT Bioquest, Inc Quest Graph™ IC50 Calculator. Accessed on 26 February 2024, (Available online: www.aatbio.com/tools/ic50-calculator).
33. Mukherjee, A., Cuanalo-Contreras, K., Sood, A., and Soto, C. (2022). Development of a novel pharmacophore model to screen specific inhibitors for the serine-threonine protein phosphatase calcineurin. Biochemistry and Biophysics Reports, 31: 101311.