

MOLECULAR MODELING OF PARACETAMOL AND CODEINE INTERACTIONS WITH CYP3A4: COMPUTATIONAL INSIGHTS

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Abstract. The combination of paracetamol and codeine is widely used for the treatment of moderate acute pain, with their complementary mechanisms of action improving analgesic effects and overall pain control. Paracetamol is primarily metabolized in the liver through glucuronidation and sulfation, while a minor fraction is oxidized by cytochrome P450 enzymes, particularly CYP2E1 and, to a lesser extent, CYP1A2 and CYP3A4, generating N-acetyl-p-benzoquinone imine (NAPQI), a reactive metabolite that may cause severe hepatotoxicity when glutathione reserves are depleted. Codeine is metabolized through several pathways, including UGT2B7-mediated glucuronidation, CYP3A4-dependent N-demethylation to norcodeine, and CYP2D6-dependent O-demethylation to morphine. Therefore, modulation of CYP3A4 activity may influence paracetamol and codeine metabolism and consequently affect their pharmacological and safety profile. Combination analgesic therapy may enhance pain management; however, conventional molecular docking generally evaluates individual ligands and provides limited information regarding potential intermolecular interactions between co-administered drugs. In this study, molecular docking was employed to investigate the interaction of paracetamol, codeine, and their proposed 1:1 and 2:1 non-covalent assemblies with CYP3A4. The assemblies exhibited distinct docking scores and binding orientations compared with the individual compounds, suggesting that molecular stoichiometry and orientation may influence their predicted interactions with the enzyme. These findings indicate that supramolecular organization may contribute to differences in the theoretical interaction profiles of paracetamol and codeine. However, the docking scores represent relative computational estimates rather than experimentally validated binding affinities, while the short-timescale molecular dynamics simulations provide only preliminary evidence of assembly stability.

Keywords: paracetamol; codeine; CYP3A4; molecular docking; drug interaction.

1. INTRODUCTION

The combination of paracetamol (acetaminophen) and codeine is a widely used analgesic therapy for the management of moderate pain when treatment with paracetamol or

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other non-opioid analgesics alone is insufficient. The rationale for combining these two drugs is based on their complementary mechanisms of action and pharmacological properties. Paracetamol provides predominantly central analgesic and antipyretic effects, whereas codeine is an opioid prodrug whose analgesic activity is partly dependent on its conversion to morphine [1,2].

The combination may produce a greater analgesic efficacy than either component administered alone. Paracetamol acts mainly through central mechanisms involving modulation of prostaglandin synthesis and descending pain pathways, while codeine and its active metabolites interact with opioid receptors, particularly the μ -opioid receptor. Consequently, the combination can provide additive or synergistic analgesia and may allow adequate pain control with lower doses of the opioid component. This approach is particularly useful in acute postoperative pain, dental pain, musculoskeletal pain, and other conditions associated with moderate acute pain [3,4].

Cytochrome P450 (CYP) enzymes represent a large family of heme-containing monooxygenases that play a central role in the metabolism of numerous endogenous compounds and xenobiotics, including medicinal drugs. These enzymes catalyze mainly oxidation reactions and thereby influence the pharmacokinetics, therapeutic efficacy, drug-drug interactions, and toxicity of pharmacological agents. Among the approximately 50 CYP enzymes expressed in humans, CYP3A4 is particularly important, as it is responsible for the metabolism of a large proportion of clinically used drugs. Its contribution to the metabolism of paracetamol and codeine, however, is markedly different [5].

Paracetamol and codeine are extensively used analgesic drugs whose therapeutic effects and safety profiles depend substantially on their hepatic metabolism, with both compounds undergoing extensive biotransformation in the liver. Paracetamol is primarily metabolized in the liver through conjugation reactions, with approximately 90% of a therapeutic dose undergoing glucuronidation and sulfation, generating water-soluble metabolites that are subsequently eliminated in the urine [1]. A smaller proportion, approximately 5–10%, is oxidized predominantly by cytochrome P450 enzymes, particularly CYP2E1 and, to a lesser extent, CYP1A2 and CYP3A4, producing the highly reactive metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI) [6]. Under normal circumstances, NAPQI is rapidly detoxified through conjugation with hepatic glutathione, but in overdose situations or when glutathione reserves are depleted, NAPQI accumulates and binds to cellular proteins, potentially causing severe hepatocellular injury and acute liver failure [6-8]. However, the specific contribution of CYP3A4 remains comparatively controversial. Experimental studies using recombinant enzymes and human liver microsomes show that CYP3A4 can convert paracetamol to NAPQI, but CYP3A4 should not be considered the principal determinant of paracetamol-induced hepatotoxicity, although its involvement is relevant given the overall complexity of paracetamol metabolism and possible enzyme-mediated interactions [7,9].

Codeine, in contrast, is a prodrug whose analgesic activity depends partly on its conversion to morphine. The metabolic fate of codeine illustrates a more clinically significant role for CYP3A4. The majority of codeine is metabolized through glucuronidation by UGT2B7; approximately 5–10% undergoes *N*-demethylation by CYP3A4 to norcodeine, a metabolite considered to have little or no analgesic activity, and about 5–10% of codeine is *O*-demethylated by CYP2D6 to morphine, which has substantially greater analgesic activity than codeine itself [2,10]. CYP3A4 activity is clinically relevant because its inhibition or induction can alter the metabolic disposition of codeine and potentially modify the balance between codeine, norcodeine, and the CYP2D6-dependent pathway [2,5,10].

Molecular docking is a computational technique widely used in structural bioinformatics and computer-aided drug discovery to predict the preferred orientation of a

small molecule (ligand) when bound to a macromolecular target, usually a protein or nucleic acid. The primary objective of docking is to characterize potential ligand-target interactions and to estimate the relative binding affinity of different compounds [11,12]. According to Meng et al. [13], molecular docking has become an important approach for identifying and optimizing potential drug candidates, particularly during the early stages of drug development.

The docking process generally involves two fundamental components: searching for possible ligand conformations and orientations within the binding site, followed by scoring these poses according to their predicted binding affinity. Common docking programs employ computational algorithms to explore the conformational space of ligand-receptor complexes and use scoring functions to rank the resulting binding modes [14,15]. The predicted interactions may include hydrogen bonds, hydrophobic interactions, electrostatic forces, van der Waals interactions, and π - π interactions, all of which can contribute to ligand recognition and stabilization within the binding pocket [16].

Molecular docking is frequently used for virtual screening, lead identification, binding-mode prediction, and structure-based optimization of drug candidates. However, docking scores should not be interpreted as direct experimental measurements of binding affinity. The accuracy of the results depends on several factors, including the quality of the protein structure, treatment of receptor flexibility, ligand protonation and tautomeric states, and the limitations of the scoring function [16]. Consequently, docking predictions should ideally be complemented by experimental approaches or additional computational methods such as molecular dynamics simulations.

Therefore, molecular docking represents a valuable and relatively cost-effective tool for investigating molecular recognition and guiding drug-discovery research. When appropriately designed and critically interpreted, it can provide important insights into ligand-receptor interactions and help prioritize compounds for subsequent experimental investigation.

2. MATERIALS AND METHODS

2.1. MATERIALS

Chemical modeling of paracetamol and codeine was performed using HyperChem (version 8.0.8, HyperCube, Gainesville, FL, USA) [17]. The two-dimensional molecular structures were initially constructed in the software and subsequently converted into three-dimensional conformations. Geometry optimization was performed using both the MM+ molecular mechanics force field and the semi-empirical PM3 method, as implemented in HyperChem.

Energy minimization was conducted using the Polak-Ribiere conjugate gradient algorithm with an RMS gradient convergence criterion of 0.1 kcal/(Å·mol). For each compound, the lowest-energy conformation obtained from the optimized structures was selected as the most stable conformation and used in the subsequent docking analyses.

Molecular docking simulations were performed using HEX software (version 8.0.0) [18] to investigate the potential interactions between the studied compounds and CYP3A4. The "Shape+Electro" correlation mode was selected, allowing the docking procedure to consider both molecular shape complementarity and electrostatic interactions [18,19].

2.2. METHODS

As an initial step, supramolecular assemblies of paracetamol and codeine were generated by reciprocal docking of the two compounds. This analysis assessed whether the assignment of ligand and receptor, and consequently the relative orientation of the two molecules, affected the predicted stability and docking energy of the resulting complexes. Two reciprocal 1:1 configurations were investigated: *paracetamol_codeine*, in which paracetamol was defined as the ligand and codeine as the receptor, and *codeine_paracetamol*, in which the roles were reversed. For each configuration, the 100 highest-ranking docking poses were generated and ranked according to their calculated docking energies. The resulting non-covalent paracetamol–codeine assemblies were subsequently extracted and treated as dual-drug ligands for docking against CYP3A4. In addition to the 1:1 assemblies, 2:1 paracetamol–codeine configurations were generated by considering the different possible molecular arrangements. The resulting complexes were evaluated according to their docking energies and predicted interaction geometries.

The CYP3A4 receptor structure was obtained from the Protein Data Bank (PDB) [20]. During receptor preparation, crystallographic water molecules and heteroatoms were removed using the standard procedures implemented in HEX. The receptor was appropriately oriented relative to the coordinate system, and docking calculations were performed based on shape and electrostatic complementarity. The highest-ranking poses were retained for subsequent structural analysis, with particular attention given to docking energy, molecular orientation, and the predicted binding region.

This methodological approach, which first models drug-drug interactions and then examines their interactions with the target enzyme, ensures a comprehensive assessment of the molecular behavior of combination analgesics. This approach offers valuable insights into potential synergistic or competitive binding mechanisms that may influence their therapeutic efficacy [21,22].

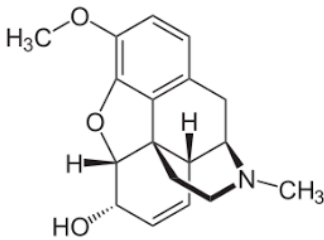
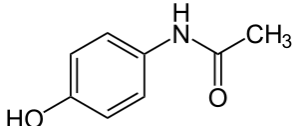
The docking results were interpreted as theoretical predictions of molecular recognition and interaction. The calculated docking energies were used for comparative assessment of the investigated configurations rather than as direct experimental measurements of binding affinity. Consequently, experimental validation is needed to confirm the predicted binding modes and supramolecular assemblies and establish their stability and biological relevance.

3. RESULTS AND DISCUSSION

3.1. RESULTS

To evaluate how paracetamol and codeine may interact at the molecular level, several molecular docking configurations were analyzed. Table 1 presents the structures and compounds included in the intermolecular-interaction analysis, providing the basis for the subsequent evaluation of the stability of the resulting complexes. Both binary interactions between paracetamol and codeine and higher-order molecular configurations were considered to highlight the influence of molecular orientation and spatial organization on the interaction energy and overall stability of the resulting supramolecular complexes.

Table 1. Chemical structure of the studied compounds.

Structure	Compound
Codeine	
Paracetamol	

The molecular docking analysis revealed that the interaction between paracetamol and codeine depends on the orientation and relative positioning of the two molecules within the supramolecular complex. Differences in docking energy between the analyzed configurations may reflect variations in the intermolecular contacts established by the ligands, including hydrogen bonding, electrostatic interactions, and other non-covalent forces. In particular, the amide NH and carbonyl oxygen groups of paracetamol can participate in hydrogen-bonding interactions, while the functional groups of codeine provide additional interaction sites that may contribute to complex stabilization.

The comparison of the two binary arrangements, paracetamol–codeine and codeine–paracetamol, indicates that reversing the ligand–receptor assignment can result in differences in the predicted interaction energy and complex stability. This finding suggests that the docking orientation is an important factor when characterizing supramolecular drug–drug interactions [23–25]. Even relatively small changes in the spatial arrangement of the molecules may modify the accessibility and geometry of key functional groups and, consequently, the strength of the predicted intermolecular interactions.

To further investigate the structural organization of these systems, ternary complexes containing two paracetamol molecules and one codeine molecule were also evaluated. The three configurations: paracetamol–paracetamol–codeine, paracetamol–codeine–paracetamol, and codeine–paracetamol–paracetamol were compared to determine whether the position of codeine within the assembly influences the energetic profile and intermolecular interactions. The differences observed among these arrangements indicate that molecular sequence and spatial organization may affect the overall stabilization of higher-order complexes.

Overall, the docking results suggest that the molecular association of paracetamol and codeine cannot be described solely by their individual chemical structures. Rather, the predicted stability of the complexes depends on the specific orientation, stoichiometry, and network of intermolecular interactions established between the molecules [14]. The evaluation of multiple binary and ternary configurations therefore provides a more comprehensive description of the possible supramolecular organization of the paracetamol–codeine system. These findings also emphasize the importance of considering alternative docking orientations when investigating potential drug–drug interactions using computational approaches.

Subsequently, the biological target selected for the molecular docking analysis was established. The receptor structure was retrieved from the Protein Data Bank (PDB), using entry 1W0E, corresponding to cytochrome P450 3A4 (CYP3A4) [20]. CYP3A4 was selected as a relevant biological target because it metabolizes both paracetamol and codeine, making it suitable for investigating potential changes in their molecular interactions after supramolecular complex formation.

To assess whether supramolecular complexation between paracetamol and codeine at a 1:1 molar ratio may influence their interaction with CYP3A4, molecular docking simulations were performed using this enzyme as the selected biological target. Two alternative docking configurations of the 1:1 supramolecular complex were evaluated, namely codeine–paracetamol and paracetamol–codeine. This approach tested whether the relative orientation of the two molecules within the supramolecular assembly affects their predicted binding affinity and interaction profile. The docking energies obtained for the two complexes were subsequently compared with those calculated for the individual, uncomplexed compounds, namely free codeine and free paracetamol. This comparative analysis assessed the potential effect of supramolecular complexation on receptor affinity and binding behavior, while also highlighting the importance of molecular orientation within the resulting drug-drug complex.

Table 2. The results of docking combinations of paracetamol and codeine (1:1) with CYP3A4 [18].

Ligand	Energy(kcal/mol)
paracetamol_codeine	-271.54
codeine_paracetamol	-254.53
codeine	-237
paracetamol	-179.32

The docking results presented in Table 2 indicate a clear difference in the predicted interaction energies of free compounds and the paracetamol–codeine supramolecular complexes with CYP3A4. The most favorable docking energy was obtained for the paracetamol–codeine complex (-271.54 kcal/mol), followed by the codeine–paracetamol configuration (-254.53 kcal/mol). In comparison, free codeine and paracetamol exhibited less favorable predicted energies of -237.00 kcal/mol and -179.32 kcal/mol, respectively. The more negative docking energies observed for the two supramolecular complexes suggest that complexation between paracetamol and codeine may enhance their predicted interaction with CYP3A4 compared with the individual compounds. Notably, the paracetamol–codeine configuration showed a more favorable energy than the codeine–paracetamol configuration, indicating that the relative orientation of the two molecules within the complex may influence its interaction with the enzyme. This difference may arise from changes in the accessibility and spatial arrangement of the functional groups involved in receptor binding, resulting in different hydrogen-bonding, electrostatic, hydrophobic, and van der Waals interactions with the CYP3A4 binding site.

When compared with the individual compounds, the paracetamol–codeine complex showed an enhancement in predicted docking energy of approximately 34.54 kcal/mol relative to codeine and 92.22 kcal/mol relative to paracetamol. Similarly, the codeine–paracetamol configuration exhibited more favorable predicted energies than both free codeine and free paracetamol. These findings suggest that the second molecule in the supramolecular assembly may contribute additional intermolecular contacts with CYP3A4 or promote a more favorable conformation for receptor binding.

Interestingly, free codeine displayed a substantially more favorable docking energy than free paracetamol, suggesting a stronger predicted interaction of codeine with CYP3A4 under the conditions of the computational model. However, the substantially lower energy obtained for the paracetamol–codeine complex indicates that the association of the two molecules may generate a binding mode that differs from those of the individual compounds.

Overall, these results support the hypothesis that supramolecular complexation can modify the predicted molecular recognition of paracetamol and codeine by CYP3A4. The difference between the two complex orientations further emphasizes that molecular arrangement and ligand-receptor assignment can significantly affect docking predictions [25-

28]. Nevertheless, docking energies should be interpreted as computational predictions rather than direct measurements of binding affinity. Experimental studies, such as enzyme inhibition or metabolic assays, would therefore be required to confirm whether the predicted increase in CYP3A4 interaction translates into a biologically relevant modification of the metabolism of paracetamol and/or codeine.

Following the evaluation of the 1:1 supramolecular assemblies, the molecular docking analysis was extended to 2:1 paracetamol–codeine systems to investigate whether increasing the proportion of paracetamol could further modify the predicted interaction with the CYP3A4 receptor. This approach was considered relevant given the frequent co-administration of paracetamol with opioid analgesics and the possibility that changes in molecular stoichiometry may influence the physicochemical properties, spatial organization, and receptor recognition of the resulting supramolecular assemblies.

Three distinct 2:1 configurations were investigated: paracetamol–paracetamol–codeine, paracetamol–codeine–paracetamol, and codeine–paracetamol–paracetamol. These arrangements were analyzed separately to assess the potential influence of molecular positioning and orientation on the predicted receptor interaction. The resulting docking energies were subsequently compared with those obtained for the individual compounds and the previously evaluated 1:1 complexes. This comparative approach was used to determine whether increasing the paracetamol-to-codeine ratio is associated with more favorable predicted receptor interactions and to establish the extent to which the organization of the molecules within the supramolecular assembly contributes to the calculated binding energy.

Table 3. The results of docking combinations of paracetamol and codeine. (2:1) with CYP3A4 [18].

Ligand	Energy(kcal/mol)
codeine_paracetamol_paracetamol	-300.21
paracetamol_paracetamol_codeine	-297.79
paracetamol_codeine_paracetamol	-269.69
codeine	-237
paracetamol	-179.32

The molecular docking results presented in Table 3 indicate that the 2:1 paracetamol–codeine supramolecular assemblies exhibit more favorable predicted interactions with CYP3A4 than the individual compounds. Among the three configurations investigated, codeine–paracetamol–paracetamol showed the most favorable docking energy, with a value of −300.21 kcal/mol, followed by paracetamol–paracetamol–codeine at −297.79 kcal/mol. The paracetamol–codeine–paracetamol configuration exhibited a less favorable energy of −269.69 kcal/mol, but it remained more favorable than both free codeine (−237.00 kcal/mol) and free paracetamol (−179.32 kcal/mol).

The particularly favorable docking energy obtained for the codeine–paracetamol–paracetamol configuration suggests that this arrangement may provide an optimal spatial organization of the three molecules within the CYP3A4 binding site. The close energy values observed for the codeine–paracetamol–paracetamol and paracetamol–paracetamol–codeine assemblies, differing by only 2.42 kcal/mol, indicate that placing codeine at either end of the supramolecular assembly results in a relatively similar predicted interaction with the receptor. In contrast, the paracetamol–codeine–paracetamol configuration produced an energy approximately 27–31 kcal/mol less favorable than the other two ternary arrangements. This difference suggests that positioning codeine between the two paracetamol molecules may lead to a less advantageous orientation of the functional groups involved in receptor recognition. Comparison with the individual compounds further supports the potential influence of supramolecular complexation on CYP3A4 interaction. Free codeine displayed a docking energy of −237.00 kcal/mol, while paracetamol showed −179.32 kcal/mol. All three 2:1

assemblies therefore exhibited substantially more negative docking energies than the individual compounds. The greatest improvement relative to free codeine was observed for the codeine–paracetamol–paracetamol complex, corresponding to a difference of approximately 63.21 kcal/mol. Compared with free paracetamol, the difference was even greater, reaching approximately 120.89 kcal/mol.

These findings suggest that the formation of higher-order supramolecular assemblies may generate additional or more favorable intermolecular contacts within the CYP3A4 binding site. The enhanced predicted interaction may result from the combined contribution of the functional groups of the three molecules and from a more extensive network of hydrogen-bonding, electrostatic, hydrophobic, and van der Waals interactions. At the same time, the differences among the three configurations demonstrate that molecular orientation and sequence within the supramolecular assembly can significantly influence the calculated docking energy, even when the stoichiometry remains identical.

Overall, the results indicate that the 2:1 paracetamol–codeine assemblies have an enhanced predicted interaction with CYP3A4 than the corresponding individual compounds, with codeine–paracetamol–paracetamol representing the most favorable configuration among those evaluated. These computational findings support the importance of considering both stoichiometry and molecular orientation when investigating supramolecular drug–drug interactions [21]. Nevertheless, docking energies represent computational predictions and should not be interpreted as direct experimental measurements of binding affinity. Experimental studies would be necessary to determine whether these predicted differences translate into biologically relevant changes in CYP3A4 activity or drug metabolism.

To further characterize the predicted interactions between paracetamol, codeine, and CYP3A4, the molecular docking poses of the individual compounds and the 1:1 supramolecular assemblies were examined. Visual analysis of the docking poses provides complementary structural information to the calculated docking energies and allows the localization and orientation of the ligands within the receptor.

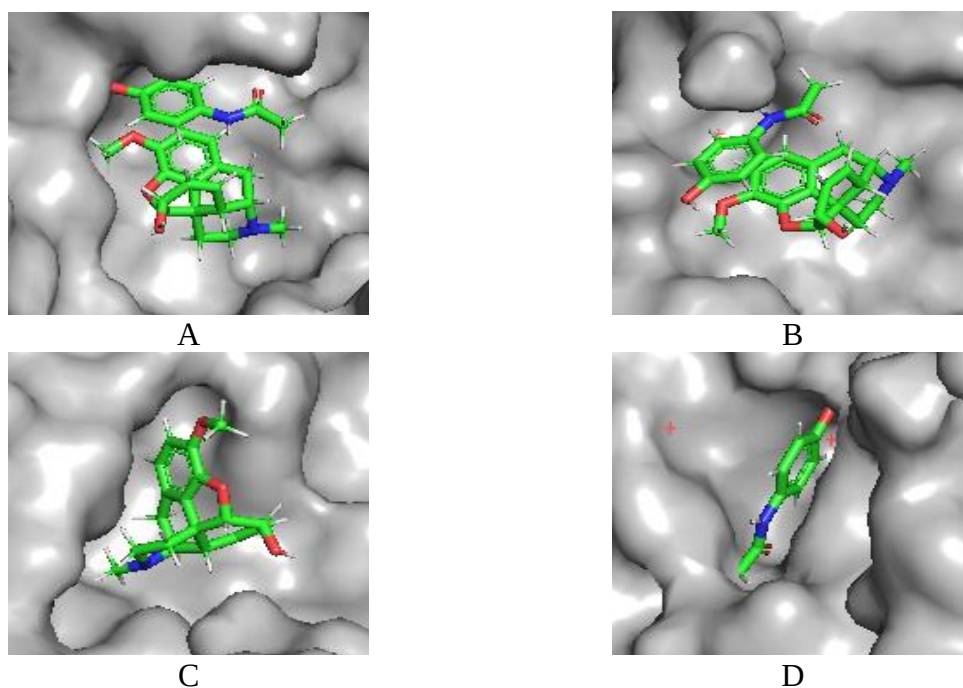


Figure 1. Predicted molecular docking poses of paracetamol, codeine, and the paracetamol–codeine supramolecular complexes within CYP3A4. (A) paracetamol–codeine complex; (B) codeine–paracetamol complex; (C) free codeine; (D) free paracetamol. The figure illustrates the predicted positioning of the ligands within the CYP3A4 binding region [29].

The docking poses presented in Fig. 1 illustrate differences in the predicted localization and orientation of paracetamol, codeine, and their 1:1 supramolecular assemblies within CYP3A4. Importantly, the ligands do not necessarily occupy the same binding site or adopt identical orientations within the receptor. The paracetamol–codeine and codeine–paracetamol assemblies are predicted to interact with CYP3A4 through distinct spatial arrangements, while the individual compounds also exhibit different binding locations and orientations. These differences provide a structural explanation for the variation in the calculated docking energies observed between the analyzed systems.

The different binding sites are particularly relevant when comparing the supramolecular complexes with the individual compounds. The formation of a paracetamol–codeine assembly may alter the spatial accessibility of functional groups and consequently favor a different region of the CYP3A4 structure. Similarly, reversing the molecular orientation within the complex may modify the way in which the assembly fits into the receptor. Thus, the docking results suggest that both complex formation and molecular orientation may influence the predicted receptor–ligand interaction.

The individual compounds also demonstrate distinct theoretical binding patterns. Free codeine and free paracetamol are predicted to occupy different regions of CYP3A4, which is consistent with their different chemical structures and interaction capabilities. The supramolecular assemblies, in turn, may establish additional contacts through the combined functional groups of both molecules. However, the visual differences between the docking poses should not be interpreted as evidence of experimentally confirmed binding sites or biological interactions.

It is important to emphasize that all interactions shown in Fig. 1 are theoretical predictions generated by molecular docking. The presence of a ligand in a particular binding site, as well as the orientation and estimated stability of the complex, reflects the assumptions and scoring functions of the computational model rather than experimentally demonstrated receptor binding. Therefore, the differences observed between the docking poses should be considered primarily as hypotheses about possible interaction modes.

Overall, Fig. 1 supports the numerical docking results by demonstrating that the analyzed compounds and supramolecular assemblies can adopt different binding orientations and occupy different regions of CYP3A4. These observations highlight the structural complexity of the paracetamol–codeine/CYP3A4 interaction and indicate that docking orientation may contribute to the differences in predicted interaction energies. Nevertheless, experimental approaches such as CYP3A4 binding, inhibition, or metabolic assays would be necessary to confirm the predicted binding modes and determine their biological relevance.

To further investigate the influence of molecular stoichiometry on receptor recognition, the docking analysis was extended to 2:1 paracetamol–codeine supramolecular assemblies. In addition to the calculated docking energies, the predicted binding poses were analyzed in order to visualize how the different ternary complexes interact with the CYP3A4 receptor. Examining the docking conformations provides structural insight into the possible orientation and localization of the supramolecular assemblies within the receptor and allows comparison between the different molecular arrangements.

The docking poses illustrated in Fig. 2 show that the three 2:1 paracetamol–codeine supramolecular assemblies interact with CYP3A4 through different predicted binding modes. Although the complexes contain the same molecular components and identical stoichiometry, their spatial organization results in distinct orientations and localization within the receptor. The codeine–paracetamol–paracetamol, paracetamol–paracetamol–codeine, and paracetamol–codeine–paracetamol assemblies are predicted to occupy different regions of CYP3A4 rather than a single common binding site. These differences suggest that the relative position of codeine and paracetamol within the supramolecular assembly may influence how the complex

is accommodated by the receptor. In particular, placing codeine at one end of the assembly or between the two paracetamol molecules produces different theoretical docking poses, indicating that the accessibility of functional groups and overall molecular geometry may affect predicted receptor interactions. Consequently, the variation in docking energies observed for these complexes is consistent with the differences in their predicted binding orientations.

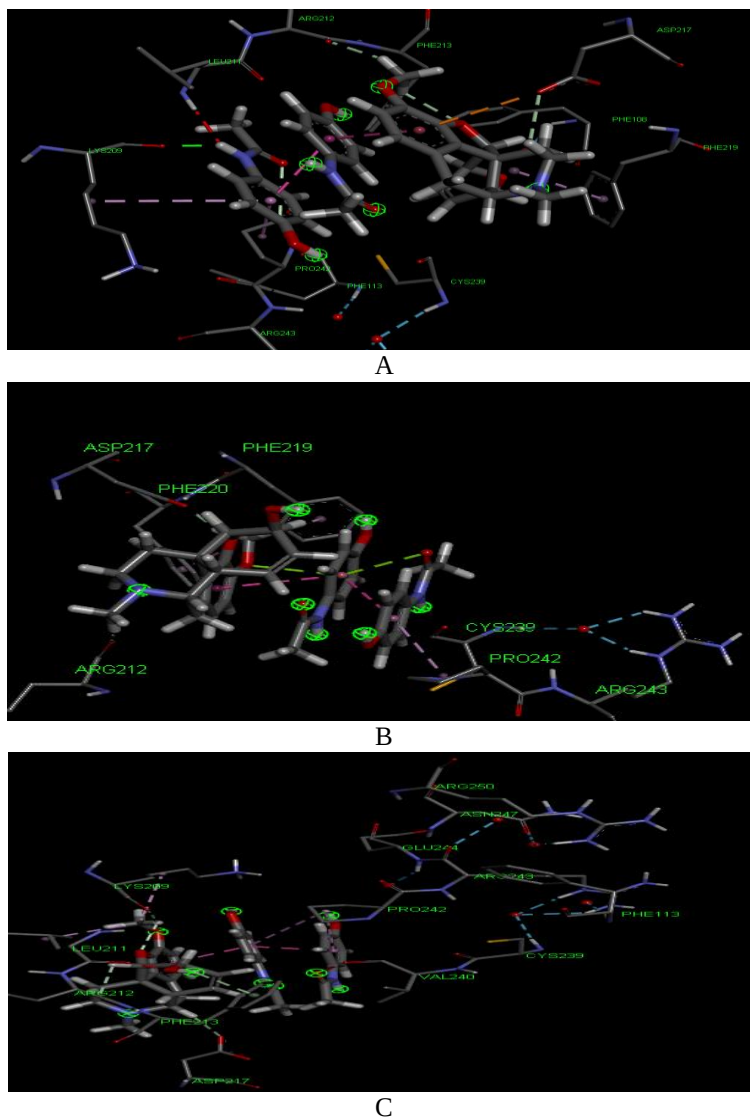


Figure 2. Predicted molecular docking poses of the 2:1 paracetamol-codeine supramolecular assemblies within the CYP3A4 receptor. (A) codeine-paracetamol-paracetamol; (B) paracetamol-paracetamol-codeine; (C) paracetamol-codeine-paracetamol. The figure illustrates the predicted localization and orientation of the ternary supramolecular complexes within CYP3A4, highlighting the differences in their theoretical binding sites and spatial arrangements [30].

It is important to emphasize that the binding sites illustrated in Fig. 2 are not experimentally confirmed. Molecular docking predicts the most favorable ligand positions according to computational scoring functions and structural algorithms; therefore, the localization of the complexes within CYP3A4 should be regarded as a theoretical model. The fact that the three supramolecular assemblies are predicted to bind in different regions of the receptor does not necessarily indicate that these interactions occur under physiological conditions.

Overall, Fig. 2 highlights that molecular orientation and supramolecular organization can influence the predicted binding behavior of ternary paracetamol–codeine assemblies. The observed differences in binding sites reinforce the importance of evaluating multiple docking configurations when studying drug–drug supramolecular interactions. Nevertheless, these results represent computational predictions only, and experimental studies would be required to verify the actual binding sites, binding affinities, and biological relevance of these proposed interaction modes with CYP3A4.

3.2. DISCUSSION

In this study, molecular docking was used to investigate the potential interaction of paracetamol and codeine with CYP3A4, with particular emphasis on the effect of supramolecular organization. The docking results for the 1:1 assemblies showed differences between the two orientations, paracetamol–codeine and codeine–paracetamol, indicating that the relative positioning of the molecules can influence their predicted interaction with the receptor. Both assemblies presented more favorable docking energies than the individual compounds, suggesting that molecular association may modify the theoretical binding behavior of the two drugs [31,32].

The analysis of the 2:1 assemblies further showed that stoichiometry and molecular arrangement influence predicted interactions with CYP3A4. Among the investigated configurations, codeine–paracetamol–paracetamol exhibited the most favorable docking energy, followed by paracetamol–paracetamol–codeine, whereas paracetamol–codeine–paracetamol showed a less favorable interaction. These differences suggest that the positioning of the molecules within the supramolecular assembly may affect the accessibility of functional groups and the intermolecular contacts established with the receptor [32].

The molecular representations shown in Figures 1 and 2 also indicate that the different assemblies adopt distinct predicted binding orientations and occupy different regions of the CYP3A4 structure. Therefore, variation in docking energy may reflect differences in molecular positioning, steric complementarity, hydrogen bonding, and electrostatic interactions. Similar observations have emphasized the importance of ligand orientation and binding configuration in molecular docking studies [18].

Importantly, these findings are purely theoretical. Molecular docking estimates favorable interaction modes but does not fully reproduce the dynamic and physiological conditions of the human body. Consequently, the present results should be regarded as computational hypotheses requiring experimental validation. These findings indicate that both stoichiometry and molecular orientation can substantially influence the predicted interaction of paracetamol–codeine assemblies with CYP3A4. The study therefore provides a theoretical basis for further investigation of potential drug–drug interactions and supramolecular organization, while highlighting the need for complementary experimental and molecular-dynamics studies to establish their biological relevance.

4. CONCLUSIONS

The paracetamol–codeine combination represents an effective pharmacological strategy for moderate acute pain because it combines two analgesics with different mechanisms of action. Nevertheless, its use requires appropriate dosing and attention to

individual risk factors, particularly opioid sensitivity, CYP metabolic variability, and cumulative paracetamol exposure. When appropriately prescribed and monitored, the combination can provide effective analgesia while limiting the need for higher opioid doses.

CYP enzymes are important determinants of the metabolic fate of both paracetamol and codeine, but their roles differ considerably. CYP3A4 has a relatively limited role in the formation of the toxic paracetamol metabolite NAPQI, but it is a major enzyme in the metabolism by *N*-demethylation of codeine to norcodeine. Understanding these pathways is therefore essential for appreciating interindividual variability, drug-drug interactions, therapeutic efficacy, and the potential toxicity of these commonly used analgesics.

The molecular docking analysis showed that the predicted interaction of paracetamol–codeine assemblies with CYP3A4 is influenced by molecular orientation and stoichiometry. Both 1:1 and 2:1 assemblies exhibited different docking energies and distinct predicted binding arrangements compared with the individual compounds. These findings suggest that the supramolecular organization of paracetamol and codeine may influence their theoretical interaction with CYP3A4.

Importantly, the docking results represent computational predictions rather than experimental evidence. The predicted binding sites, orientations, and docking energies should therefore be interpreted as theoretical models and not as confirmation of stable supramolecular complexes or altered CYP3A4 activity. Further experimental studies are required to validate the proposed interactions and to establish their potential pharmacological relevance.

Overall, the findings provide a theoretical framework for understanding how molecular organization may affect drug-enzyme interactions and support further investigation of paracetamol–codeine assemblies using complementary experimental and computational approaches.

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