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Molecular Docking Study of 3-Amino-2-Phenylquinazoline-4(3H)-One Derivative as A Potential COX-2 Selective Analgesic Candidate

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A B S T R A C T

Background:
Quinazoline is a group of alkaloid compounds found in several plant and animal families, such as plants in the Rutaceae family. Non-selective COX-2 inhibitors, while effective analgesics, may also inhibit COX-1 in the gastrointestinal tract, potentially disrupting protective mucus production. This research aims to assess the potential of the derivative compound 3-Amino-2-Phenylquinazoline-4(3H)-One as an analgesic agent through molecular docking. The selection of test compounds was conducted using the Topliss Tree method. The potency of the compounds was assessed based on rerank scores and interactions with amino acids in COX-2 (PDB ID 1PXX) and COX-1 (PDB ID 1EQG). The findings suggest that compound 14cpq may exhibit selective COX-2 inhibitory activity. This is supported by its lower rerank score with COX-2 (-85.2374 arb. units) compared to COX-1 (-63.9889 arb. units), as well as its interactions with amino acids Ser1530 and Met1522 within the COX-2 binding site, similar to sodium diclofenac. Furthermore, 14cpq displays distinct interaction patterns with COX-1 compared to ibuprofen, reinforcing its potential selectivity for COX-2. However, further research is required to ascertain the effectiveness of these compound as selective COX-2 analgesics.

Keywords: *3-amino-2-phenylquinazoline-4(3H)-one derivatives; analgesic; molecular docking*

INTRODUCTION

Analgesics are pharmacological agents employed to alleviate pain. These agents act on both the central and peripheral nervous systems. Analgesics are categorized into two main types: opioid analgesics and non-opioid analgesics (NSAIDs) (1). NSAIDs function by reducing the production of inflammatory mediators through the inhibition of cyclooxygenase (COX) enzymes. Non-selective COX-2 NSAIDs, in particular, can inhibit COX-1 in the gastrointestinal tract, potentially disrupting mucus secretion in the stomach and leading to gastrointestinal irritation or ulceration. One widely used non-opioid analgesic available on the market is Sodium Diclofenac (2).

The analgesic activity of Sodium Diclofenac is characterized as non-selective, with a notable inhibitory effect on the COX-2 enzyme compared to COX-1. This activity is attributed to the competition between phenylacetic acid (the chemical structure of Sodium Diclofenac) and arachidonic acid for binding to the COX enzyme (3). Despite its greater inhibition of COX-2, Sodium Diclofenac is associated with a risk of gastrointestinal adverse effects (4). Therefore, the development of COX-2 selective analgesics is essential to mitigate these adverse effects and enhance therapeutic safety.

Quinazoline is a class of alkaloids commonly found in various plant families, including the Rutaceae family (5). Chemically, quinazoline is characterized by a structure that includes a benzene ring fused to a pyrimidine ring (6). This heterocyclic structure is crucial for the biological activity of quinazoline, and it has been extensively studied for the development of biologically active compounds (7). Pharmacological activities of quinazoline derivatives that have been identified include antimalarial (8), antibacterial (9), antiviral (10), and analgesic (11) properties.

In silico research can be conducted using molecular docking computational models. Molecular docking enables the prediction of interactions between compounds and target proteins. The outcomes of molecular docking are typically represented by rerank scores, which indicate the binding affinity and specificity of a compound for a target protein (12). This study aims to evaluate the pharmacological potential of the derivative compound 3-amino-2-phenylquinazoline-4(3H)-one as a selective COX-2 analgesic through molecular docking simulations, comparing its activity to that of Sodium Diclofenac.

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MATERIALS AND METHODS

Protein Target Selection

The target proteins used in this study had resolutions ranging from 1.0 to 3.0 Å and were selected from the Protein Data Bank (www.rcsb.org). Two proteins were utilized: COX-2 (PDB ID: 1PXX) and COX-1 (PDB ID: 1EQG). The 1PXX protein represents the active site of COX-2 from *Mus musculus* with a resolution of 2.9 Å, while the 1EQG protein corresponds to the COX-1 complex from *Ovis aries* with a resolution of 2.61 Å. Protein 1PXX is bound to the natural ligand Sodium Diclofenac, whereas protein 1EQG is bound to natural ligan Ibuprofen.

Protein Target Preparation

The structure of the COX-2 protein (PDB ID 1PXX) and the COX-1 protein (PDB ID 1EQG) was downloaded from the Protein Data Bank (www.rscb.org) and saved in Protein Data Bank (.pdb) format. The protein was then imported into Molegro Virtual Docking version 5 for preparation and removal of unused molecules. Specifically, chain B of the protein and natural ligand (DIF_1701[B] for COX-2 and IBP_1701[B] for COX-1) were selected, while the cofactor and water molecules were excluded. The prepared protein was then ready for the molecular docking phase.

Ligand Preparation

The selection of ligands for testing was based on the Topliss Tree method, incorporating substituents into the phenyl structure of the 3-amino-2-phenylquinazoline-4(3H)-one compound. The derivatives of 3-amino-2-phenylquinazoline-4(3H)-one were prepared using ChemDraw Professional version 16.0 and Chem3D version 16.0. The 2D structures of the derivatives were drawn using ChemDraw and saved in ChemDraw Exchange (.cdx) format. These 2D structures were then imported into Chem3D and converted to 3D forms. In Chem3D, the 3D structures of the 3-amino-2-phenylquinazoline-4(3H)-one derivatives were energy-minimized to prepare them for molecular docking simulations (13). The structures were subsequently saved in .sdf format and were ready for use in molecular docking.

Molecular Docking Method Validation

Docking validation was performed using Molegro Virtual Docking version 5 with the prepared protein structure. The protein and the natural ligand (DIF_1701[B] for COX-2 protein and IBP_1701[B] for COX-1 protein) were subjected to docking simulations with a grid resolution of 0.3 Å. The validation of the docking method was assessed by evaluating the Root Mean Square Deviation (RMSD) values. The RMSD values were obtained from the molecular docking results and used to evaluate the accuracy of the docking simulations.

Molecular Docking

Docking simulations were conducted using Molegro Virtual Docking version 5. Prior to molecular docking, binding cavities were identified based on expanded van der Waals surface criteria, a cavity volume range of 10 - 10,000, and a grid resolution of 0.8 Å. Among the five detected binding cavities, the one occupied by the natural ligand (DIF_1701[B] for COX-2 protein and IBP_1701[B] for COX-1 protein) was selected for further molecular docking analysis. Subsequently, the prepared derivatives of 3-amino-2-phenylquinazoline-4(3H)-one were docked with the protein. The molecular docking process was carried out with a grid resolution of 0.3 Å and a binding site radius of 15 Å. Evaluation of the ligands included internal energy scores (ES), internal hydrogen bonds (H-bonds), and Sp2-Sp2 torsions. For each compound, the pose with the lowest rerank score was selected for interaction visualization with the target protein. Interaction visualization was performed using the ligand map feature in Molegro Virtual Docking version 5. The rerank scores and ligand interactions with amino acids were compared for evaluation.

RESULTS AND DISCUSSION

Root Mean Square Deviation (RMSD) is a metric used to assess the accuracy of molecular docking methods by quantifying the deviation between the positions of non-hydrogen atoms in the ligand and the target protein. An RMSD value of less than 2.0 Å indicates a high level of agreement between the docking results and the experimentally determined structures available in the Protein Data Bank (PDB). In this study, the RMSD value obtained for the molecular docking method was 1.4028 Å for COX-2 protein and 0 Å for COX-1 protein, suggesting that the method provides accurate docking results and is suitable for molecular docking applications (14).

The derivatives of 3-amino-2-phenylquinazoline-4(3H)-one selected for testing were identified using the Topliss Tree method. This approach involves selecting more potent compounds by applying substitutions to the phenyl ring (15). Using this method, a total of 21 compounds were identified as ligands for the study. **Figure 1** illustrates the Topliss Tree diagram and the ligands that were tested.

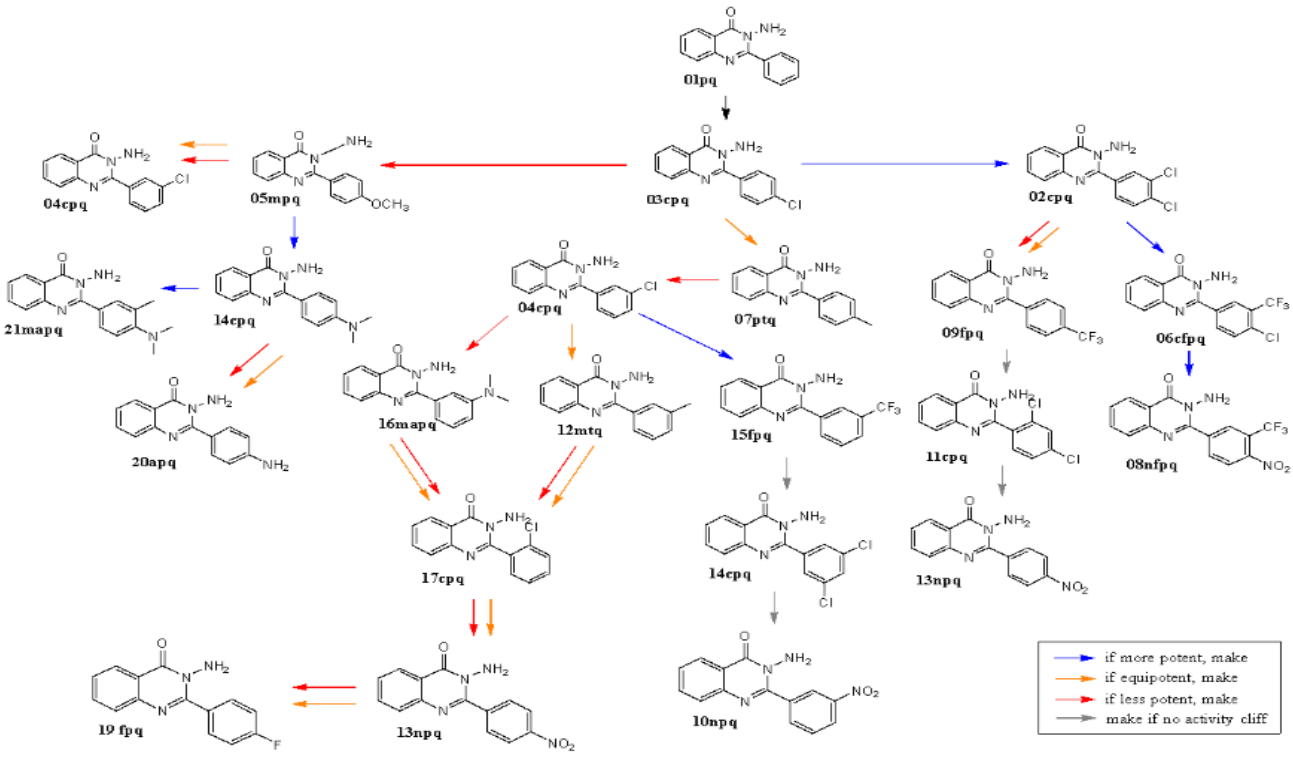

Figure 1. Topliss Tree Diagram of 3-Amino-2-Phenylquinazoline-4(3H)-One Derivatives

The results of molecular docking for the 3-amino-2-phenylquinazoline-4(3H)-one derivatives as potential selective COX-2 analgesics are presented in terms of rerank scores and ligand-protein interactions. A lower rerank score indicates a higher affinity of the ligand for the protein (16). Out of the 21 derivatives assessed, 9 compounds exhibited rerank scores that were lower than that of Sodium Diclofenac (-79.007). These compounds include 15fpq (-87.8871), 09fpq (-87.1458), 06cfpq (-86.2818), 14cpq (-85.2374), 08nfpq (-83.9039), 11cpq (-83.5682), 16mapq (-82.1640), 10npq (-79.8635), and 21mampq (-79.0522). Comprehensive molecular docking results are detailed in **Table 1**.

Table 1. Molecular Docking Results of 3-Amino-2-Phenylquinazoline-4(3H)-One Derivatives Against COX-2 Protein

Compounds	Rerank Score (Arbitrary units)	Interaction		
		Hydrogen bond	Steric Interaction	Electrostatic Interaction
01pq	-73,0055	-	Val 1523, Phe 1518	-
02cpq	-74,7511	-	Val 1523, Trp 1387, Leu 1384	-
03cpq	-73,2909	Ser 1530	Met 1522, Trp 1387	-
04cpq	-75,8820	Ser 1530	Leu 1384	-
05mpq	-77,7079	-	Phe 1518, Val 1523, Trp 1387	-
06cfpq	-86,2818	-	Leu 1531	-
07ptq	-73,2542	Ser 1530	Trp 1387	-
08nfpq	-83,9039	Tyr 1385, Tyr 1355, Ser 1530	Ser 1530, Trp 1387, Gly 1526, Ser 1353, Val 1523, Leu 1352, Phe 1518	-
09fpq	-87,1458	-	Val 1523, Leu 1352	-

10npq	-79,8635	Phe 1518	Val 1523, Ser 1353	-
11cpq	-83,5682	-	Val 1523, Phe 1518, Leu 1384	-
12mtq	-76,3502	Ser 1530	Leu 1384	-
13npq	-78,7911	Tyr 1385	Tyr 1355, Ser 1353, Leu 1352, Gln 1192, Phe 1518, Val 1523	-
14cpq	-85,2374	Ser 1530	Tyr 1385, Met 1522, His 1090	-
15fpq	-87,8871	-	Phe 1518, Val 1523	-
16mapq	-82,1640	-	Phe 1518, Val 1523	-
17cpq	-76,0704	Ser 1530	-	-
18mapq	-75,1403	Tyr 1355	Val 1523, Ser 1353, Phe 1518	-
19fpq	-75,6048	Ser 1530	Trp 1387	-
20apq	-75,8635	Ser 1530	Met 1522	-
21mampq	-79,0522	Tyr 1355	Tyr 1352, Val 1523, Phe 1518	-
Sodium Diclofenac	-79,0070	Tyr 1385, Ser 1530	Tyr 1355, Leu 1352, Met 1522	His 1386
	Similarity of interactions with amino acid **Tyr 1385** through **Hydrogen Bonds**			
	Similarity of interactions with amino acid **Ser 1530** through **Hydrogen Bonds**			
	Similarity of interactions with amino acid **Tyr 1355** through **Steric Interactions**			
	Similarity of interactions with amino acid **Leu 1352** through **Steric Interactions**			
	Similarity of interactions with amino acid **Met 1522** through **Steric Interactions**			

Ligand interactions with amino acids in a protein are crucial for the biological activity of a compound. Among the nine compounds with lower rerank scores than sodium diclofenac, only three compounds (08nfpq, 09fpq, and 14cpq) exhibited similar interactions with the COX-2 protein compared to the natural ligand (sodium diclofenac). In the inhibition of COX-2 protein, the amino acids Tyr 1385 and Ser 1530 are particularly significant for analgesic activity. These amino acids are located at the active site and can bind with the carboxylate groups of carboxylic acid-containing NSAIDs, leading to COX-2 inhibition (17). The interaction of Sodium Diclofenac with these amino acids is illustrated in **Figure 2.** Among the 9 compounds with rerank scores lower than that of Sodium Diclofenac, only 2 were found to interact with the amino acids Tyr 1385 and Ser 1530. These compounds are 08nfpq and 14cpq. The interactions of these compounds with the amino acids in the COX-2 protein are depicted in **Figures 3a and 3b**.

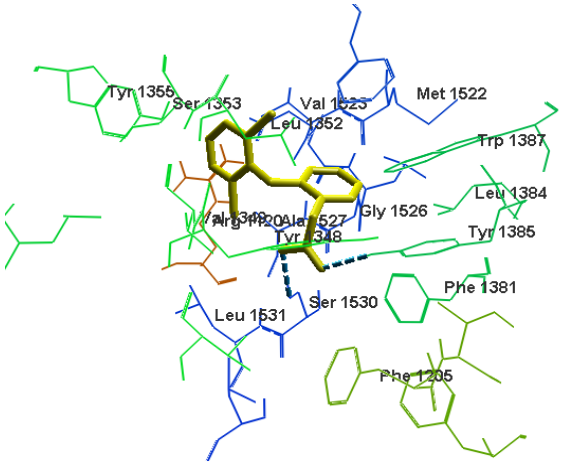

Figure 2. Visualization of Sodium Diclofenac Interactions with Protein 1PXX.
Sodium Diclofenac interacts with Tyr 1385 and Ser 1530 through hydrogen bonds

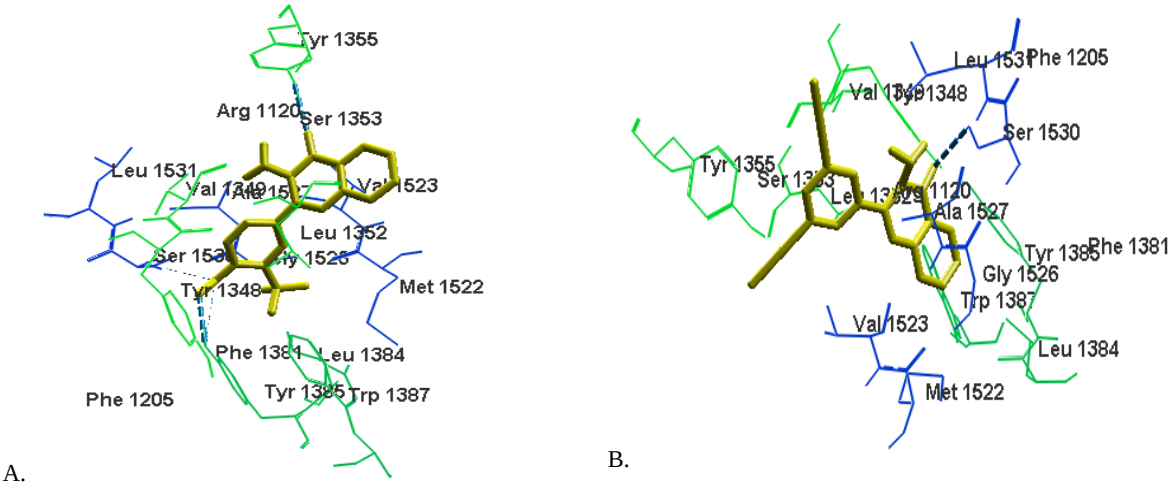

Figure 3. Visualization of Amino Acid Interactions in the COX-2 Protein with Compounds 08nfpq (A) and 14cpq (B). Compound 08nfpq interacts with Tyr 1385 and Ser 1530 via hydrogen bonds, and with Ser 1530 through steric interactions. Compound 14cpq interacts with Ser 1530 through hydrogen bonds and with Tyr 1385 through steric interactions.

Compounds 08nfpq and 14cpq interact with amino acids in COX-2 through hydrogen bonds and steric interactions, similar with Sodium Diclofenac. The hydrogen bonds between the ligands and the amino acids (08nfpq with Ser 1530 and Tyr 1385; 14cpq with Ser 1530) are the most significant. These hydrogen bonds strengthen the ligand's affinity for the amino acids by forming bonds between hydrogen atoms and electronegative atoms (18). In the case of compound 08nfpq, the carbonyl oxygen atom interacts with the hydrogen atoms of the amino acids Tyr 1385 and Ser 1530. Similarly, in compound 14cpq, the carbonyl group engages in interactions with the amino acid Ser 1530.

The interactions of both compounds with amino acids are further enhanced by steric interactions. Steric interactions are related to the influence of the ligand's volume, including its minimal and maximal dimensions, on the binding site. Ligands with bulky substituents can impact the orientation of the ligand, allowing for optimal binding within the binding site and potentially resulting in improved activity (19). Steric interaction similarities with Sodium Diclofenac were observed in compound 14cpq. Specifically, 14cpq interacts with the amino acid Met1522, which is also involved in the interaction with Sodium Diclofenac.

Table 2. Molecular Docking Results of 08nfpq and 14cpq Compounds Against COX-1 Protein

Compounds	Rerank Score (Arbitrary units)	Interaction		
		Hydrogen bond	Steric Interaction	Electrostatic Interaction
08nfpq	-107,635	-	Ala 202, **Thr 206**, Leu 390, His 388, **Phe 210**, **Asn 382**	-
14cpq	-63,9889	-	Ser 353, Phe 518, Ala 527, Arg 120, Met 113, Val 349	-
Ibuprofen	-95,4991	Thr 206	Tyr 385, His 207, **Phe 210**, **Thr 206**, **Asn 382**	His 207
	Similarity of interactions with amino acid **Thr 206** through **Steric Interactions**			
	Similarity of interactions with amino acid **Phe 210** through **Steric Interactions**			
	Similarity of interactions with amino acid **Asn 382** through **Steric Interactions**			

Docking results presented in **Table 2** suggest that compound 08nfpq has the potential to interact with the COX-1 protein. This is supported by its similarities to the natural ligand of COX-1 l (Ibuprofen) particularly in forming steric interactions with amino acids Thr206, Phe210, and Asn382. The lower rerank score of 08nfpq compared to ibuprofen indicates a stronger affinity for COX-1. Furthermore, the rerank score of 08nfpq is lower for COX-1 than for COX-2, highlighting its lack of selectivity for COX-2 and a stronger inhibitory effect on COX-1.

In contrast to compound 08nfpq, compound 14cpq exhibits a lower potential to interact with COX-1. Molecular docking results indicate that 14cpq lacks the same amino acid interactions as ibuprofen. This finding suggests that 14cpq is less likely to inhibit COX-1. The higher rerank score of 14cpq compared to ibuprofen indicates a lower affinity for the COX-1 protein. Additionally, the affinity of 14cpq for the COX-2 protein is stronger than its affinity for COX-1 based on its rerank score. Therefore, compound 14cpq has the potential to be developed as a COX-2 selective analgesic.

Among the 21 derivatives of 3-amino-2-phenylquinazoline-4(3H)-one tested, only 1 compounds, namely, were predicted to selectively exhibit COX-2 enzyme inhibition activity. Compound 14cpq exhibits a higher affinity for the COX-2 protein compared to its affinity for COX-1. Furthermore, compound 14cpq shares binding similarities with diclofenac sodium, specifically interacting with the amino acids Ser1530 and Met1522 in the COX-2 protein, but does not exhibit binding similarities with ibuprofen in the COX-1 protein. Consequently, compounds 14cpq warrant further investigation to assess their efficacy as selective COX-2 analgesics.

CONCLUSION

Molecular docking effectively predicted the potential biological activity of 3-amino-2-phenylquinazoline-4(3H)-one derivatives as selective COX-2 analgesics. Among the 21 compounds analyzed, 14cpq exhibited promising COX-2 selective analgesic activity, as evidenced by its lower rerank score with COX-2 (-85.2374 arb. units) compared to COX-1 (-63.9889 arb. units). Additionally, 14cpq formed interactions with amino acids Ser1530 and Met1522 within the COX-2 binding site, similar to sodium diclofenac, while displaying distinct interaction patterns with COX-1 compared to ibuprofen. However, further studies are required to evaluate the efficacy of both 14cpq as selective COX-2 inhibitors.

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CONFLICT OF INTEREST

The author declares that there are no conflicts of interest related to the research presented in this article.

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