Tropical Journal of Pharmaceutical Research March 2021; 20 (3): 579-583
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v20i3.20

Original Research Article

Aspirin-aromatic amino acid conjugates as selective Cox-2 inhibitors: A docking study

Mahmood HM Jasim[1,2], Mohammed N Abed[1,2], Mohannad E Qazzaz[3], Mohanad Alfahad[1,4], Fawaz A Alassaf[1,5]*

[1]Department of Pharmaceutical Chemistry, [2]Department of Pharmacognosy and Medicinal Plants, [3]Department of Pharmaceutics, [4]Department of Pharmacology and Toxicology, College of Pharmacy, University of Mosul, Mosul, Nineveh Province, Iraq

For correspondence:* ***Email:* *Fawaz.Alassaf@uomosul.edu.iq;* ***Tel:*** *+964-7502274465*

Sent for review: 25 November 2020 *Revised accepted: 24 February 2021*

Abstract

Purpose: *To examine eight compounds in which aspirin was conjugated with aromatic amino acids virtually using docking studies for their ability to inhibit cyclooxygenase 2 (Cox-2) enzyme.*
Methods: *The compounds were drawn, energy minimised and then docked into the active site of Cox-2 along with celecoxib for comparison using GOLD docking program.*
Results: *Five of the designed compounds docked into the active site with a bent conformation producing a pose similar to that of celecoxib, with the aromatic amino acid moiety facing the outside of the active site. The interactions were mainly hydrophobic with some hydrogen bonds formed between the compounds and the key residues in the active site. Although the obtained scores were less than that of celecoxib, they were the top ranked poses in the solutions generated for each compound.*
Conclusion: *The conjugation of aspirin with amino acids may offer a potential for the development of selective, but safe, Cox-2 inhibitors.*

Keywords: *Aspirin, Cox-2 inhibitors, Docking, GOLD, Scoring*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) represent one of the most used classes of drugs whether for their anti-inflammatory or pain-relieving properties. Their non-selectivity for their target enzyme isoforms (cyclooxygenase) is the reason behind their most important and common adverse reaction, the gastrointestinal problems [1]. Many attempts at designing selective inhibitors of cyclooxygenase-2 (Cox-2)-a favourable target for NSAIDs, have been made with some successful drugs reaching the market. These selective inhibitors are either modifications of original NSAIDs or entirely new molecules, but generally they are larger than the non-selective Cox inhibitors for them to accommodate the larger active site of Cox-2. Removal of the carboxylic acid group, ester and amide linkages represent additional techniques for enhancing the selectivity of known NSAIDs to Cox-2 [2].

Amino acids are linked to traditional NSAIDs such as naproxen to improve its selectivity and

reduce side effects. Amino acids offer the advantage that they are safe in case of their release upon possible cleavage of the amide linkage with the NSAID within the body. This linkage will mask the free carboxylic acid group of the NSAID and should therefore enhance its selectivity. The bulky size of the amino acid residues additionally provides the increase in molecular size required for selective Cox-2 inhibitors [3].

Aspirin represents one of the smallest and oldest NSAIDs, with its anti-inflammatory action greatly affected by gastrointestinal adverse effects [4]. Therefore, this work aims to explore potential selective Cox-2 inhibition by aspirin linked to the following aromatic amino acids: tyrosine (**1** in Figure 1), tryptophan (**2** in Figure 1) and phenylalanine (**3** in Figure 1) using an *in silico* approach. Eight compounds were designed having aspirin linked by an amide bond to the *L*- and *D*- forms of the three amino acids above. In addition, two compounds possessing aspirin linked to glycine and then to *L*- and *D*-phenylalanine (**4** in Figure 1) were designed. Their potential to inhibit Cox-2 was tested by molecular docking.

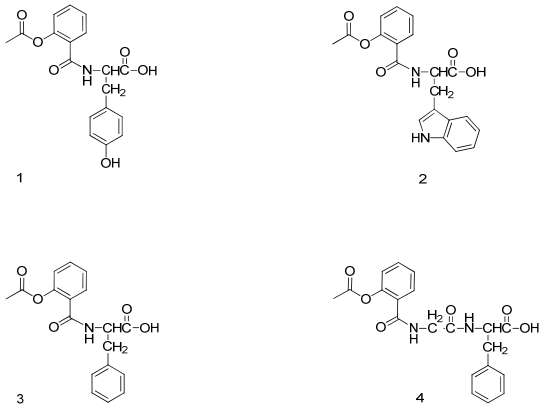

Figure 1: Chemical structures of the designed compounds used in this work

METHODS

Programs

Avogadro software version 1.2.0 [5] was used for the drawing and minimisation of the designed compounds. Docking of the designed compounds into Cox-2 active site was performed using GOLD (Genetic Optimisation for Ligand Docking) software version 5.7.3 [6–11]. Accelrys Discovery Studio (DS) Visualizer version 4.0 [12] was used for visualisation and analysis of the generated docked complexes and for the production of the pictures.

Preparation of the compounds

The designed compounds used in this work were sketched using the Draw Tool of the Avogadro software and their valences checked. With the Auto Optimization Tool of the same program, the compounds were then energy minimised using the UFF force field [13] and the steepest descent algorithm. The compounds were saved as MOL2 files to be used for docking. The eight compounds were designated **1***L*, **1***D*, **2***L*, **2***D*, **3***L*, **3***D*, **4***L* and **4***D*, where the number represents the conjugated amino acids (TYR, TRP, PHE and PHE-GLY) respectively and the letter is the conformation of the conjugated amino acid.

Preparation of the protein

A crystal structure for Cox-2 in complex with celecoxib was obtained from the RCSB Protein Data Bank (https://www.rcsb.org/) with the PDB entry 3LN1 [14] at 2.4 Å resolution. This structure contains 4 chains of Cox-2, each complexed with one molecule of celecoxib. With DS Visualizer, three chains were deleted and hydrogen atoms were added to the remaining chain.

Docking

Default setting of GOLD were applied for the docking. The active site of Cox-2 was defined by the residues within 6 Å of the bound celecoxib. The latter was then extracted from the active site and added to the list of compounds to be docked. Twenty GA (genetic algorithm) runs were set for each compound and early termination was turned off so that all the possible solutions will be generated. ChemPLP [15] was used as the scoring function.

RESULTS

The top 3 ranked poses for celecoxib showed scores ranging between 95.3 and 97.9 with RMSD values between 0.3 Å and 0.6 Å. Interestingly, the pose with the best RMSD had the lowest score (GOLD uses positive values for score, and best ranks have the highest scores). Figure 2 presents the pose of celecoxib with the best RMSD.

The docked celecoxib displayed a network of interactions roughly categorized into hydrogen bonds and van der Waals interactions. Hydrogen bonds were observed between the sulphonamide moiety of celecoxib and the side chains (and the backbone carbonyl group) of the residues entrance to the active site (side pocket). The remainder of the docked celecoxib molecule was involved in a grid of hydrophobic interactions with

deeper active site residues. These interactions are presented in Figure 3.

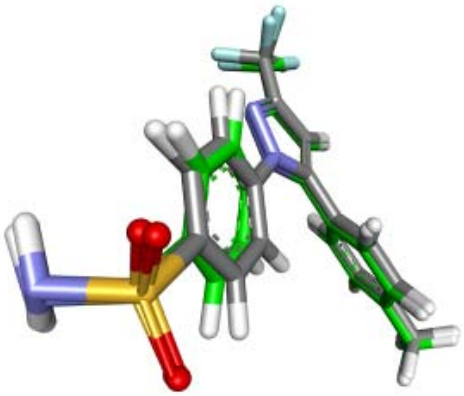

Figure 2: Celecoxib pose generated by GOLD (coloured by element with grey carbon atoms) with the best RMSD compared to the pose from the crystal structure (coloured by element with green carbon atoms)

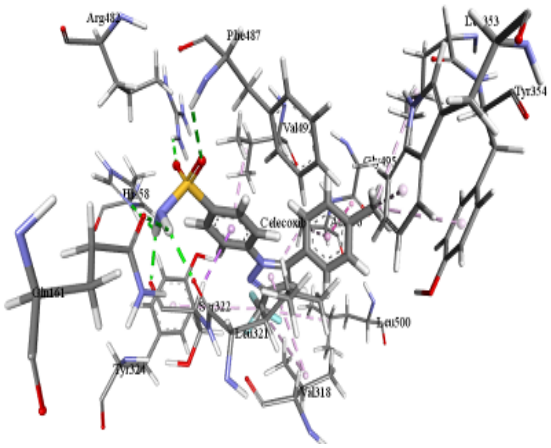

Figure 3: Interactions observed between the docked celecoxib and active site residues of TG2. Green dashed lines represent hydrogen bonds while hydrophobic interactions are represented by purple dashed lines. All interacting amino acid residues are labelled

Five of the eight designed compounds (**1***L*, **1***D*, **2***L*, **2***D*, **3***L*) docked into the active site of Cox-2 in a similar manner where the acetyl salicylic acid (ASA) moiety in the top ranked solutions of the 5 compounds was aligned within the deep region of the active site (Figure 4 A). The aromatic side chains of the amino acid moieties of the compounds positioned themselves toward the side pocket of the active site. This orientation of the designed compounds resulted in an overall bent conformation that was comparable to the conformation of celecoxib within the active site of Cox-2 (Figure 4 B).

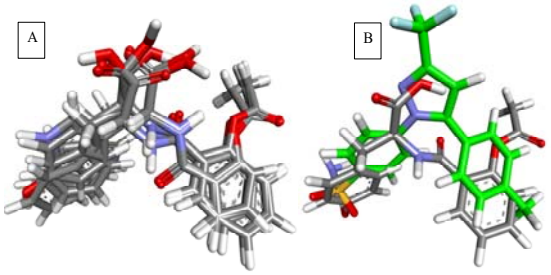

Figure 4: A: The poses generated by docking for **1***L*, **1***D*, **2***L*, **2***D*, **3***L*. **B:** The pose **1***D* with the pose of celecoxib from the crystal structure (celecoxib has green carbon atoms).

The interactions that the five (5) compounds displayed with the active site residues were mainly hydrophobic in nature. Hydrogen bonds with the side chains of the amino acid moieties of the compounds were observed with **1***L*, **1***D* and **3***L*. However, with **2***L* and **2***D*, there were hydrophobic interactions involving the indole moiety. Moreover, in the latter 2 compounds and in **3***L*, there were hydrogen bonds involving the ASA moiety. There was an additional intramolecular hydrogen bond observed in **1***L* and **2***L* between the backbone amino group hydrogen and the ester oxygen of the ASA moiety. This hydrogen bond may have contributed to the bent pose of these compounds. The above-mentioned poses for the five compounds were ranked first among the generated solutions for each compound. However, their scores were all lower than that of celecoxib with scores ranging between 79.1 for **3***L* and 87.8 for **2***L*. The scores obtained for the remaining three compounds were all lower than 79.1. The scores are presented in Table 1.

Table 1: Scores and ranks of the best posed solutions of **1***L*, **1***D*, **2***L*, **2***D*, **3***L*

Compound	Score*	Rank
Celecoxib	95.3	3rd
1*D*	83.7	1st
1*L*	81.3	1st
2*D*	80.9	1st
2*L*	87.8	1st
3*L*	79.1	1st

**The numbers in the scores' columns do not represent actual energies, and higher numbers represent better scores*

DISCUSSION

The docking protocol applied in this work was able, despite its simplicity, to capture the correct binding mode of the reference ligand and produce a docking pose that is very similar to the

original crystal pose as shown by the RMSD value. The general shapes of the designed compounds were closely comparable to that of celecoxib. The central pyrazole ring in celecoxib probably acts as an anchor to help position the peripheral rings and maintain the bent shape of celecoxib within the active site of Cox-2. In the designed compounds, this role was taken by the backbone atoms of the amino acid moiety to produce a similar orientation.

When compared to celecoxib, the designed compounds showed less, or even absent, hydrogen bonds involving the part of the molecule positioned in the side pocket. This is probably attributed to celecoxib sulfonamide moiety having both hydrogen bond donor and acceptor groups, whereas the designed compounds had less hydrophilic groups in the side chains of the amino acid moieties. However, all the 5 compounds that docked well within the active site of Cox-2 showed hydrophobic interactions involving the aromatic rings in this region of the molecule, which might not be enough to compensate for a hydrogen bond but could contribute to the stabilisation of the bent pose. The reduced ability of the compounds to form hydrogen bonds may be the reason behind the lower scores of the compounds when compared to celecoxib.

The designed compounds have an ASA moiety which was involved in hydrogen bonds in 3 compounds (**2***L*, **2***D* and **3***L*) within the deep region of Cox-2 active site. This feature was not observed with celecoxib due to the lack of hydrogen bond forming groups in the part of its molecule located deep within the active site. The fact that celecoxib still scored higher than the designed compounds despite the lack of such hydrogen bonds may signify that the bonds within the side pockets are more important for good docking. However, the presence of hydrogen bonds with deeper region of the active site could be considered a sign of the ability of these compounds to accommodate well within the active site.

The *L*-amino acids offered the advantage of forming an intramolecular hydrogen bond within the centre of the compounds' molecule, and thus allowing more stability of the docked bent pose. This was seen twice with **1***L* and **2***L*. In the latter, this bond has resulted in the best score achieved with any of the designed compounds.

CONCLUSION

The findings of this study indicate that the designed compounds offer the increase in size required for selectively inhibiting Cox-2, and although they scored less than celecoxib, they may offer the potential for safer alternatives.

DECLARATIONS

Acknowledgement

The authors would like to acknowledge the role of the University of Mosul represented by the College of Pharmacy in completing this work.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Mohanad Alfahad designed the study along with Mahmood H. M. Jasim and was responsible for setting up the compounds. Mahmood H. M. Jasim was involved in designing methodology, running the programs and writing the first draft of the paper. Mohammed N. Abed was involved in the writing of the final draft and will take the responsibility of responding to the reviewers. Mohannad E. Qazzaz took on the responsibility of visualising the results and investigating their significance. Fawaz A. Alassaf supervised the execution of the work and the writing and ascertained the validity of the methods employed. The final draft to be submitted was approved by all the authors and they declare responsibility for the contents and the similarity index of the manuscript.

REFERENCES

1. *Harirforoosh S, Asghar W, Jamali F. Adverse effects of nonsteroidal anti-inflammatory drugs: An update of*

gastrointestinal, cardiovascular and renal complications. J Pharm Pharm Sci 2013; 16(5): 821–847.

2. *Zarghi A, Arfaei S. Selective COX-2 inhibitors: A review of their structure-activity relationships. Iran J Pharm Res IJPR 2011; 10(4): 655–683.*
3. *Elhenawy AA, Al-Harbi LM, Moustafa GO, El-Gazzar MA, Abdel-Rahman RF, Salim AE. Synthesis, comparative docking, and pharmacological activity of naproxen amino acid derivatives as possible anti-inflammatory and analgesic agents. Drug Des Devel Ther 2019; 13: 1773–1790.*
4. *Subramanian B, Kuo F, Ada E, Kotyla T, Wilson T, Yoganathan S, Nicolosi R. Enhancement of anti-inflammatory property of aspirin in mice by a nano-emulsion preparation. Int Immunopharmacol 2008; 8(11): 1533–1539.*
5. *Hanwell MD, Curtis DE, Lonie DC, Vandermeersch T, Zurek E, Hutchison GR. Avogadro: An advanced semantic chemical editor, visualization, and analysis platform. J Cheminform 2012; 4(1): 17.*
6. *Verdonk ML, Cole JC, Hartshorn MJ, Murray CW, Taylor RD. Improved protein-ligand docking using GOLD. Proteins 2003; 52(4): 609–623.*
7. *Jones G, Willett P, Glen RC. Molecular recognition of receptor sites using a genetic algorithm with a description of desolvation. J Mol Biol 1995; 245(1): 43–53.*
8. *Jones G, Willett P, Glen RC, Leach a R, Taylor R. Development and validation of a genetic algorithm for flexible docking. J Mol Biol 1997; 267(3): 727–748.*
9. *Nissink JWM, Murray C, Hartshorn M, Verdonk ML, Cole JC, Taylor R. A new test set for validating predictions of protein-ligand interaction. Proteins 2002; 49(4): 457–471.*
10. *Verdonk ML, Chessari G, Cole JC, Hartshorn MJ, Murray CW, Nissink JWM, Taylor RD, Taylor R. Modeling water molecules in protein-ligand docking using GOLD. J Med Chem 2005; 48(20): 6504–6515.*
11. *Hartshorn MJ, Verdonk ML, Chessari G, Brewerton SC, Mooij WTM, Mortenson PN, Murray CW. Diverse, high-quality test set for the validation of protein-ligand docking performance. J Med Chem 2007; 50(4): 726–741.*
12. *Accelrys Software Incorporation. Accelrys Discovery Studio Visulizer version 4.0.100.13345, California, USA. California: Accelrys Software Incorporation; 2013.*
13. *Rappe AK, Casewit CJ, Colwell KS, Goddard WA, Skiff WM. UFF, a full periodic table force field for molecular mechanics and molecular dynamics simulations. J Am Chem Soc 1992; 114(25): 10024–10035.*
14. *Wang JL, Limburg D, Graneto MJ, Springer J, Hamper JRB, Liao S, Pawlitz JL, Kurumbail RG, Maziasz T, Talley JJ, et al. The novel benzopyran class of selective cyclooxygenase-2 inhibitors. Part 2: The second clinical candidate having a shorter and favorable human half-life. Bioorg Med Chem Lett 2010; 20(23): 7159–7163.*
15. *Korb O, Stützle T, Exner TE. Empirical scoring functions for advanced protein-ligand docking with PLANTS. J Chem Inf Model 2009; 49(1): 84–96.*