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RESEARCH ARTICLE

Design of acyl salicylic acid derivates as COX-1 inhibitors using QSAR approach, molecular docking and QSPR analysis

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Abstract

Background: Acetylsalicylic acid (aspirin), widely used as an antiplatelet agent, is more likely to inhibit COX-1. Along with discovering the cardioprotective role of COX-1 in controlling platelet aggregation, it is important to develop a selective COX-1 inhibitor. **Objective:** This study aims to design acyl salicylic acid derivatives intended as COX-1 inhibitors. **Method:** Fourteen derivatives (AcS1-14) were subjected to a quantitative structure-activity relationship (QSAR) study, and 31 QSAR models were obtained using multiple linear regression (MLR) analysis. Molecular docking was performed on COX-1 (PDB. 1PTH) using the Molecular Orbital Environment (MOE) program ver2022.02, and QSPR analysis was conducted to ascertain the contribution of physicochemical descriptors to the free energy score (S) of ligand-receptor complexes. **Results:** The QSAR-Hansch model predicted hydrophobicity (LogP) and molecular energy (Etotal) and contributed to pain inhibitory action. All derivatives displayed higher *in silico* affinity than aspirin (S= -4.33±0.00 kcal/mol), and compound AcS7 afforded the highest (S= -5.32 kcal/mol). In QSPR, E_{total} also revealed a positive contribution to the affinity. AcS1, AcS2, AcS5, AcS7, and AcS8 expressed higher drug-like properties than aspirin. **Conclusion:** Derivatives with optimum hydrophobicity and high energy would generate potent COX-1 inhibition. The five selected compounds were recommended to be developed as drug candidates for COX-1 inhibitors.

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) mainly treat inflammation, fever, and pain by inhibiting the cyclooxygenase (COX) enzyme. Two COX isoforms in mammalian cells, COX-1 and COX-2, have very similar protein sequences and catalytic mechanisms (Rouzer & Marnett, 2009; Dvorakova *et al.*, 2021). The COX plays a major role in the inflammatory process by catalysing the conversion of arachidonic acid (AA) to prostaglandins (PGs). Because thromboxane A2 (TXA2) is synthesised mostly by COX-1, and TXA2 induces platelet aggregation, pharmacological inhibition of TXA2 synthesis has a cardioprotective effect by limiting platelet aggregation (Meek *et al.*, 2013; Mitchell *et al.*, 2019). COX-2 is a dominant contributor to prostaglandin E2 (PGE2) formation and prostacyclin (PGI2).

Currently, COX-1 is considered to be responsible for the primary prostanoid response to inflammatory stimuli, especially in cells and tissues where it is constitutively expressed (Perrone *et al.*, 2015). Moreover, COX-1 upregulation has been found in several types of cancer (Pannunzio *et al.*, 2018; Perrone *et al.*, 2020) in the process of atherosclerosis or neuroinflammation. Several studies indicate that COX-1 inhibition can reverse drug resistance. Therefore, selective COX-1 inhibition may provide new opportunities in the development of anti-inflammatory compounds so that they can be useful in the treatment of cardiovascular disease, cancer, or neurodegenerative disorders

(Roodhart *et al.*, 2011; Vitale *et al.*, 2016; Dvorakova *et al.*, 2021).

Acetylsalicylic acid (aspirin) is an NSAID that inhibits COX-1 and COX-2 irreversibly, with COX-1 inhibition being more pronounced than COX-2 (Ornelas *et al.*, 2017). Aspirin inhibits COX through the acetylation of hydroxy (−OH) or amino ($-NH_2$) groups in proteins and other biological macromolecules (Kovacs *et al.*, 2014). Besides its use as an analgesic and antipyretic, aspirin is currently widely used as an antiplatelet agent at low doses to prevent cardiovascular diseases (Cofer *et al.*, 2022). Epidemiological studies have recently reported that daily low-dose aspirin consumption can reduce the incidence of cancer (Patrignani *et al.*, 2016; Loomans-Kropp *et al.*, 2021).

Certain aliphatic and aromatic acyl derivatives of salicylic acid had more analgesic action than aspirin. In contrast, several aromatic acyl derivatives of salicylic acid have been synthesised and demonstrated higher anti-inflammatory activity than aspirin (Diyah *et al.*, 2020). The aromatic acyl derivatives (benzoyl-salicylic acids) contain two benzene rings, like in the structure of potent NSAIDs, including diclofenac, and tend to be more active than the aliphatic acyl derivatives.

This study investigated the potential of designed acyl salicylic acid derivatives as COX-1 inhibitors by using the quantitative structure-activity relationship (QSAR) approach, in silico molecular docking against COX-1, which was further analysed by quantitative structure-property relationship (QSPR) method. QSAR and QSPR were employed to discover structural aspects and physicochemical factors that play a role in biological activity and molecular interactions with their receptor. Molecular docking studies were performed to select the high-potential derivatives and predict the binding mode of compounds. The selection also used consideration of drug-like properties.

Methods

Fourteen acyl derivatives of salicylic acid, including benzoyl salicylic acid (AcS1) and seven substituted-benzoyl salicylic acids (AcS2–AcS8) as type I derivatives, three aliphatic-acyl salicylic acids (AcS9- AcS11) as type II derivatives, and three benzoyl derivatives of 5-chlorosalicylic acid (AcS12–AcS14) as type III derivatives were selected on the basis of diversity in the structures.

QSAR analysis

The bioactivity data for all compounds regarding pain-inhibitory activity were retrieved from a previous study (Diyah *et al.*, 2020). Hansch QSAR method was used to find the correlation between physicochemical descriptors and the negative logarithm of $1/ED_{50}$ of the bioactivity (pD_2). Physicochemical descriptors were set as independent variables, and the pD_2 was specified as a dependent property.

Physicochemical descriptors were obtained by using the Chemoffice program. The two-dimensional (2D) structures were constructed in ChemDraw Pro16.0 and then converted to three-dimensional (3D) structures with Chem3D. Energy minimisation was carried out using MMFF94, and the structure was saved in *.mol2 format. The energy of molecules in optimised geometry was recorded as E_{total} (electronic feature), and the other physicochemical descriptors were hydrophobic (LogP), electronic (pKa), steric (MR), and geometric-topologic (tPSA).

Molecular docking

The 3D structure of ligands was processed using the Molecular Operating Environment (MOE) program version 2022.02. The COX-1 (PDB ID.1PTH) containing co-crystalized ligand SAL (salicylic acid) was downloaded from www.rcsb.org. The protein structure was prepared by MOE using the default parameter, i.e. RMS gradient 0.1kcal/mol/Å^2, with Amber10:EHT as the force field.

The co-crystalized ligand was self-docked to the binding pocket of COX-1 to validate the docking procedure. The docking process is valid if the root means square deviation (RMSD)<2.0Å. The docking performed using MOE was run in a ligand atom site with 10 poses, and other parameters were set as default. The processes were done in triplicates, and the docking results included binding affinity (S-score), ligand-receptor interactions, and amino acids interacting with the ligands.

QSPR study of ligand interactions in COX-1

QSPR method was used to correlate between physicochemical descriptors and ligand-receptor affinities (S-score). The physicochemical descriptors were the same as in the QSAR analysis. The analysis also used MLR.

Results

QSAR model

The data set used in the calculation for QSAR model development is shown in Table I, and the regression equation was derived using statistical multiple linear regression (MLR). The significance of every model was determined based on statistical values such as r-value

(correlation coefficient), which must be greater than table-r, $r^2 > 0.6$, *F*-value > table-F, *P*-value < 0.05, and s-value, which ought to be minimum.

The QSAR analysis of the Hansch model yielded 54 equations, of which 31 met the statistical requirements. From those equations, the best equation was the following:

(eq.1) $pD_2 = 0.107Log^2P - 0.818LogP + 0.004E_{total} + 1.597$

(P=0.011, r=0.811, r^2=0.681, F=6.401, s=0.098)

It was selected as the QSAR model. It predicted that hydrophobicity (quadratic LogP) and molecular energy (E_{total}) contributed to the biological activity.

Table I: Physicochemical descriptors and some drug-like properties of acyl salicylic acid derivatives

Compounds	Physicochemical properties							Drug-like properties			
	MW	pD_2	Log P	pKa	MR	tPSA	E_{total}	Drug-likeness	Drug score	MT	TM
AcS1	242	0.4126	3.07	3.301	63.42	63.6	80.0674	0.52	0.71	Yes	Yes
AcS2	256	0.2006	3.56	3.299	69.32	63.6	82.0292	-0.23	0.48	No	No
AcS3	298	0.5804	4.78	3.298	83.09	63.6	90.9251	-3.66	0.28	No	No
AcS4	272	0.5204	2.95	3.295	70.67	72.83	75.0955	-1.53	0.41	No	No
AcS5	277	0.2742	3.63	3.296	68.02	63.6	79.4562	3.07	0.62	No	No
AcS6	277	0.1771	3.63	3.273	68.02	63.6	70.3573	-1.18	0.49	No	No
AcS7	311	0.2116	4.19	3.119	72.63	63.6	57.9105	2.17	0.51	No	No
AcS8	311	0.4147	4.19	3.267	72.63	63.6	80.4358	0.51	0.55	No	No
AcS9	222	0.2376	2.67	3.293	57.23	63.6	19.6546	-7.59	0.45	No	No
AcS10	236	0.1360	3.08	3.292	61.83	63.6	19.5416	-15.30	0.16	No	No
AcS11	264	0.1487	3.92	3.291	71.03	63.6	19.2096	-25.09	0.39	No	High
AcS12	333	0.4980	5.34	2.931	87.70	63.6	81.2435	-5.06	0.28	No	No
AcS13	311	0.3691	4.19	2.906	72.63	63.6	59.3132	-2.67	0.36	No	No
AcS14	346	0.3585	4.75	2.901	77.24	63.6	69.5891	-1.06	0.34	No	No

Notes: MW=Molecular Weight; PD_2=negative log of ED50; MR=Molar Refractivity; tPSA=topological Polar Surface Area; MT=mutagenic; TM=tumorigenic; High=high-risk. Compounds name and structure were attached in Table_name-structure-acylsalicylic acid derivatives_031_IGSCPS2023.pdf

Docking results

Docking results showed the 3D interaction of ligands in the binding site of COX-1 (Figure 1). Figure 2 compares docking interactions in 2D between SAL, ASA and three types of acetylsalicylic acid derivatives. The S-score representing ligand-receptor affinity and the type of amino acids interacting with the ligands are displayed in Table II. The results showed that almost all acyl salicylic acids have a higher affinity (lower S-score) than aspirin. AcS7 was the ligand with the highest affinity against COX-1, while AcS4 and AcS9 ligands exhibited a lower affinity than aspirin (ASA).

QSPR model

In the MLR analysis to select the QSPR model, 57 regression equations were obtained, where only 17 were statistically significant. Based on further analysis of some statistical parameters, the selected models were the following equations:

(eq.2) $S=0.006E_{total}+0.102tPSA -0.030MR -9.571$

(P=0.021, r=0.778, r^2=0.605, F=5.097, s=0.315)

(eq.3) $S=0.270Log^2P -2.555LogP +0.008E_{total} +0.409$

(P=0.021, r=0.778, r^2=0.605, F=5.097, s=0.316)

Eq.2 and eq.3, which have the same statistical parameters, indicated that the quadratic value of LogP and molecular energy (Etotal) revealed a positive contribution to the free energy (S) of ligand binding to COX-1 (PDB.1PTH).

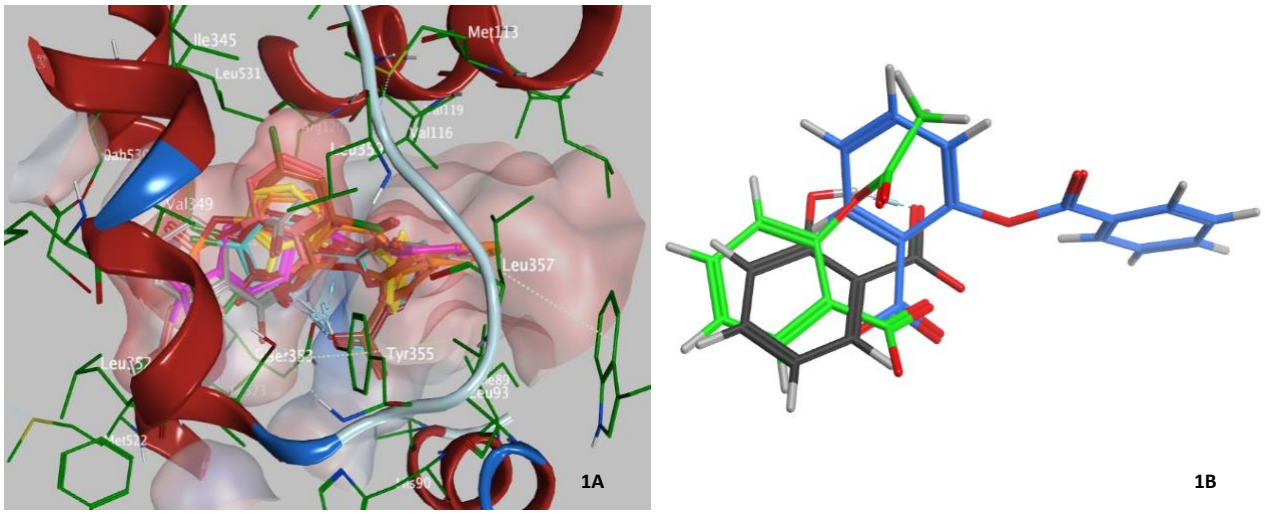

Figure 1: Docked-conformations of ligands in binding site COX-1 (1A), and overlapping view of ligands SAL (co-crystalized ligand) in grey colour, ASA (reference ligand) in green colour, and benzoyl-salicylic acid in blue colour (1B)

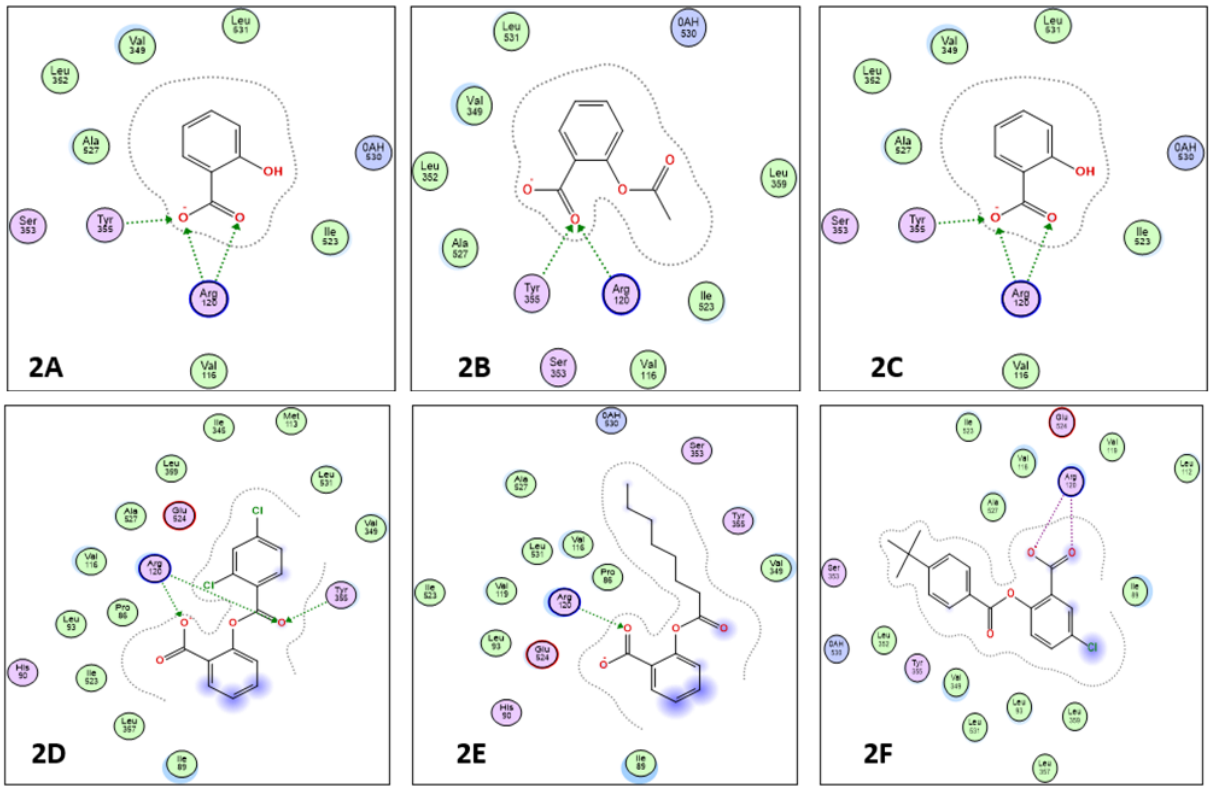

Figure 2: Comparison of docking interactions in 2D between SAL (2A), ASA (2B) and three types of acetylsalicylic acid derivatives (2C-2F)

Table II: Docking score of ligand interactions with COX-1 (PDB ID: 1PTH) and the interactive amino acids

Compounds	S-score (kcal/mol)	Interactive amino acids	
		H-bonds	Van der Waals interaction
ASA	-4.3321 ± 0.0006	Arg120, Tyr355	Val116, Val349, Leu352, Leu359, Ile523, Ala527, Leu531
AcS1	-4.3999 ± 0.0000	Arg120, Tyr355	Ile 89, Leu93, Val116, Val349, Leu359, Ile523, Ala527, Leu531
AcS2	-4.8052 ± 0.0001	Arg120, Tyr355	Ile89, Leu93, Val116, Val119, Val349, Leu359, Ile523, Ala527, Leu531
AcS3	-4.7271 ± 0.0001	Arg120, Tyr355	Ile89, Leu93, Val116, Val119, Val349, Leu352, Leu357, Leu359, Ile523, Ala527, 0AH530, Leu531
AcS4	-3.7828 ± 0.0004	Arg120, Tyr355	Ile89, Leu93, Val116, , Leu357, Leu359, Ala527, 0AH530, Leu531
AcS5	-4.4079 ± 0.0007	Arg120, Tyr355	Pro86, Ile89, Leu93, Val116, Val119, Val349, Ile523, Ala527, Leu531
AcS6	-5.0785 ± 0.2084	Arg120, Tyr355	Met113, Val116, Val349, Leu352, Leu359, Phe518, Met522, Ile523, Ala527, 0AH530, Leu531
AcS7	-5.3240 ± 0.0000	Arg120, Tyr355	Pro86, Ile89, Leu93, Met113, Val116, Ile345, Val349, Leu357, Leu359, Ile523, Ala527, Leu531
AcS8	-4.9494 ± 0.0006	Arg120, Tyr355	Pro86, Ile89, Leu93, Val116, Val119, Val349, Leu359, Ile523, Ala527, 0AH530, Leu531
AcS9	-4.2631 ± 0.3609	Arg120, Tyr355	Ile89, Leu93, Leu115, Val116, Val349, Leu359, Ile523, Ala527, Leu531
AcS10	-4.8323 ± 0.2747	Arg120, Tyr355	Pro86, Ile89, Leu93, Val116, Val119, Val349, Leu359, Ile523, Ala527, Leu531
AcS11	-5.2857 ± 0.2804	Arg120, Tyr355	Pro86, Ile89, Leu93, Val116, Val119, Val349, Ile523, Ala527, 0AH530, Leu531
AcS12	-5.2081 ± 0.0000	Arg120, Tyr355	Ile89, Leu93, Leu112, Val116, Val119, Val349, Leu352, Leu357, Leu359, Ile523, Ala527, 0AH530, Leu531
AcS13	-4.9417 ± 0.0015	Arg120, Tyr355	Pro86, Ile89, Leu93, Met113, Val116, Val119, Ile345, Val349, Leu359, Ile523, Ala527, Leu531
AcS14	-4.4603 ± 0.0002	Arg120, Tyr355	Ile89, Leu93, Leu112, Val116, Val119, Val349, Leu357, Leu359, Ile523, Ala527, 0AH530, Leu531

Discussion

Among the 31 regression equations that were statistically significant (p<0.05), equations with more than three independent variables were not selected for the QSAR model because the 14 data sets can only be used to produce significant correlations without any bias when they contain three independent variables. In linear regression analysis, for an equation containing one independent variable, a minimum of five data sets is required; for the more independent variables, in general, the r-value will increase, but the F-value may decrease (Jan & Shieh, 2019).

According to the QSAR model (eq.1), hydrophobicity and molecular energy contributed to the bioactivity. It was suggested that compounds with optimum LogP and high molecular energy would give a larger pain inhibitory activity. The presence of the quadratic value of LogP (LogP squared) in a regression equation of QSAR generates a parabolic curve indicating that the transport process, which LogP determines, is important for drugs to produce biological activity. There is a maximal value of LogP (top point in the parabolic curve). (Martin, 2010). Initially, the higher LogP will increase bioactivity due to increased penetration through the lipid bilayer membrane; however, when the maximum LogP is exceeded, the activity will decrease because the molecule is restricted from entering the hydrophilic intracellular fluid. Excessively high lipophilicity and low aqueous solubility are widespread problems for new drug molecules (Baghel *et al.*, 2020).

The QSAR model also indicated that an increase in E_{total} will increase activity, but its effect energy was smaller than that of LogP. The higher the E_{total}, the higher the molecular energy, which indicates that the molecule is less stable than those with a lower E_{total}. This energetic instability could be used to indicate reactivity, a property needed for intermolecular interaction.

Docking results (Table II) showed that almost all of the acyl-salicylic acids have higher binding affinity than aspirin as a reference ligand, except AcS9 (*O*-pentanoylsalicylic acid), which was a homolog aliphatic-acyl of aspirin. The other two homologs (AcS10 and AcS11), which have a longer carbon chain than aspirin, showed lower S-scores. This was supported by the QSAR model that stated the higher the LogP, the greater the activity.

The molecular interaction of ligands in binding site COX-1 (Figure 1) showed that all ligands formed hydrogen bonds (H-bonds) with the amino acids Tyr355

and Arg120 on the carbonyl group. The acylation of the ortho-hydroxy group in salicylic acid (parent compound) generating the aspirin and its analogue (AcS1–AcS14) could increase the binding interaction. However, the number of hydrogen bonds was not the same. This was described in Figure 2C–2F. However, all compounds formed H-bonds with the same types of amino acids as reference ligands (aspirin), indicating that the compounds have the same binding mode in COX-1.

The QSPR model on the interaction with COX-1 should be used to infer the contributing physicochemical descriptors. The two QSPR models (eq.2 and eq.3) also indicated that E_{total} supports in silico molecular interactions with COX-1 and contributes to biological activity according to the QSAR model. As seen in eq.3, increasing tPSA would increase the interaction with COX-1. Still, there was a negative effect from the steric factor (MR), which indicated that the larger the molecular size (bulky) would hinder its interaction in the binding site. Principally, for the QSAR study, this equation only applies to the same derivatives. Still, it can be used to design new acyl salicylic acid derivatives by predicting their bioactivity before the compounds are synthesised to minimise the trial and error factor.

Using the QSAR model and docking result along with the QSPR models, all compounds seemed more potent than aspirin in inhibiting COX-1. Based on druglike properties (Table I), only five compounds (AcS1, AcS2, AcS5, AcS7, and AcS8) have better drug-likeness (DL) and drug score (DS) profile than aspirin, which has a DL= -0.48 and DS= 0.14. However, almost all compounds did not show tumorigenic and mutagenic toxicity. The selected derivatives could be promoted as COX-1 inhibitors, but an in vivo assessment was still required.

Conclusion

The compounds with optimum hydrophobic character and high energy would generate potent COX-1 inhibition in designing the acyl salicylic acid derivatives. In addtion, five compounds including benzoylsalicylic acid (AcS1), *O*-(4-methyl-benzoyl)salicylic acid (AcS2), *O*-(4-chlorobenzoyl) salicylic acid (AcS5), *O*-(2,4-dichlorobenzoyl)salicylic acid (AcS7), and *O*-(3,4-dichlorobenzoyl)salicylic acid (AcS8) were recommended to be developed as drug candidates of COX-1 inhibitors.

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