

Analgesic Activity of Acyl-Salicylic Acid Derivatives And *In Silico* Docking Study For Their Potency As Cyclooxygenase-2 Inhibitors

Nuzul Wahyuning Diyah^{1*}, Anindi Lupita Nasyanska², Bambang Tri Purwanto¹, Siswandono¹

¹ Pharmaceutical Chemistry Department, Faculty of Pharmacy, Universitas Airlangga, Universitas Airlangga Campus C Jl. Dr. Ir. H. Soekarno Surabaya

² Pharmacy department, Faculty of Health, University Muhammadiyah Gresik, Gresik

* Corresponding Author, Email: nuzul-w-d@ff.unair.ac.id

ABSTRACT

A series of acyl salicylic acid derivatives were screened to investigate their analgesic activities and their potency as cyclooxygenase-2 (COX-2) inhibitors. Fourteen compounds (**BS1–14**) were assayed by acetic acid induced writhing test. Their ability for interaction with COX-2 was studied through a docking simulation at the COX-2 active site (PDB. 5IKQ). The results of the analgesic activity test gave 3 compounds that produce $ED_{50} < 0.39$ mmol/kg body weight, lower than aspirin as a positive control. The compounds **BS3** and **BS4** showed excellent analgesic activity and the *tert*-butyl substituted molecule **BS3** (*O*-(4-*tert*-butylbenzoyl)-salicylic acid analog) showed the highest analgesic activity with ED_{50} of 0.26 mmol/kg. Based on *in silico* molecular docking, it is known that almost all of the tested ligands (12 compounds) showed a higher binding affinity for COX-2 than meclofenamic acid which is a COX-2 inhibitory NSAID. The results of *in vivo* analgesic activity were justified with the outcome of *in silico* investigation. Molecular docking of acyl-salicylates confirmed *in vivo* experiments and it was found that **BS3** was the most active compound as an analgesic agent and the most potent as a COX-2 inhibitor among the evaluated compounds.

Key words: acyl-salicylates, analgesic activity, writhing test, COX-2 inhibitor, docking 5IKQ

Introduction

Salicylic acid derivatives (salicylates) represent the earliest class of anti-inflammatory agents and possess analgesic and antipyretic properties. Aspirin (acetylsalicylic acid) is the most commonly used and most commonly recognized salicylate. Although replaced by newer anti-inflammatory agents in some cases, aspirin remains a common over-the-counter analgesic for humans (Kosinski, 2018).

Salicylates can be classified into two subgroups, including acetylated and nonacetylated. Aspirin and benorylate are from the acetylated group, whereas drugs in the nonacetylated group include choline salicylate, choline magnesium trisalicylate (Trilisate), and salsalate or salicylic acid (de Arriba, 1999). Nonacetylated salicylates are converted to salicylic acid and are much less potent inhibitors of Cyclooxygenase (COX) than aspirin *in vitro*. This low potency may explain why they seem to possess lower gastrointestinal toxicity and have less of an inhibitory effect on platelets. However, they exhibit comparable efficacy in the *in vivo* models of inflammation (Wu, 2003).

In developing salicylic acid derivatives as non-steroidal anti-inflammatory drugs, molecular modification of salicylic acid on the phenolic group has been carried out by attaching a substituted benzoyl group to produce an aromatic aspirin analog (Diyah et al., 2016). From some compounds tested in the study, it was found that derivatives containing a substituent on the benzoyl group could result in higher anti-inflammatory activity than the unsubstituted compound benzoyl-salicylic acid and found one derivative that showed higher anti-inflammatory activity than aspirin according to *in vivo* test on the inhibition of carrageenan-induced oedema in rat paws. Several derivatives of aspirin have been tested for analgesic and antipyretic properties in

mice and rats. *O*-(diphenylacetyl)salicylic acid (DPA) is a superior analgesic but an inferior antipyretic to aspirin. DPA has a low toxicity but it failed to show significant anti-inflammatory properties (Amann and Peskar, 2002). It is still necessary to study whether benzoyl-salicylates also have analgesic activity.

Although frequently defined as NSAIDs, salicylates do not seem to have the same mechanism of action of this medication group (Law et al., 2000; Saunders et al., 2001; Cieslik et al., 2005). Treatment of chronic inflammatory diseases needs much higher doses of salicylates than required for inhibition of COX (prostaglandin-H/PGH synthase) by covalent modification, suggesting that these drugs have additional prostaglandin-independent effects. This is supported by the finding that analogs such as salicylate, which cannot alkylate PGH synthase, retain their anti-inflammatory potential (Cronstein et al., 1999; Aronoff et al., 2003). Other nonsteroidal anti-inflammatory COX inhibitors, including acetaminophen and indomethacin, had no effect.

The poor COX inhibitors and salicylic acid as major aspirin metabolite is known to exert analgesic and anti-inflammatory effects by still unidentified mechanisms (Mitchell et al., 1997). Aspirin suppressed lipopolysaccharide (LPS)-induced COX-2-dependent synthesis of prostaglandin E2 (PGE2) in RAW 264.7 macrophages at IC_{50} of 5.35 μ M, whereas no significant inhibition was observed in the presence of sodium salicylate and its metabolite salicylic acid at concentrations up to 100 μ M. However, the metabolites gentisic acid and salicyl-coenzyme A, the intermediate product in the formation of salicylic acid, significantly suppressed LPS-induced PGE2 production at IC_{50} of 10-100 μ M and 100 μ M

Cite this as Nuzul Wahyuning Diyah^{1*}, Anindi Lupita Nasyanska², Bambang Tri Purwanto¹, Siswandono¹ (2020), Analgesic activity of acyl-salicylic acid derivatives and *in silico* docking study for their potency as cyclooxygenase-2 inhibitors 7(2), 47-54

respectively (Hinz et al., 2000). It is suggested that certain metabolites of salicylic acid may contribute to the pharmacological action of its parent compound by inhibiting COX-2-dependent PGE2 formation at sites of inflammation.

In this study, we tested the analgesic activity of a series of acyl-salicylic acid derivatives using animal models. Based on various publications which reported that salicylates have inhibitory action against COX-2, we investigated the molecular interactions of the derivatives with COX-2 by *in silico* molecular docking. The purpose of this study was to find compounds that have the highest analgesic activity and high potency as COX-2 inhibitors.

Materials and Methods

Fourteen compounds used in this study were available in the Department of Pharmaceutical Sciences, Faculty of Pharmacy Universitas Airlangga (Diyah et al, 2010; 2014; 2016). The compounds were evaluated for their *in vivo* analgesic activity by writhing test, a chemical visceral pain model. Swiss albino mice (*Mus musculus*) 20–25 g for analgesic evaluation of male sex of appropriate age were used. Experimental procedures were carried out in agreement with the Institutional Animal Care Committee. The compounds showing high potential for analgesic activity from three groups of compounds were selected as ligands in *in silico* molecular docking study. The name and structure of the test compounds are shown in Table 1.

Writhing Test

Analgesic activity was assessed by Writhing Test using the acetic acid to induced abdominal constrictions (Miranda et al. (2006). The abdominal constriction is defined as an exaggerated extension of muscle abdomen accompanied with the outstretching of the hind limbs. Test compounds were prepared in aqueous suspension using 0.5% carboxymethyl cellulose sodium (CMC Na) as suspending agent. The mice of appropriate weight were kept under controlled conditions of light and temperature. The animals were divided in several groups, each carrying 6 animals. Group-1 was treated as control, group-2 served as positive control which was treated by aspirin and group-3 to group-16 were treated by test compounds at 3 dose levels in the range of 50 – 400 mg/kg body weight. For each test compounds, the animals were divided into 3 sub-groups (a-d) consisting 6 animals to be treated with the test compound in the predetermined doses administered orally. Mice in control group were given 0.5% CMC Na solution as placebo.

Thirty minutes after the administration of placebo in group-1, aspirin in group-2 and test drugs in groups 3–16, all the groups were administered with 0.6% v/v acetic acid solution at a dose level of 1 mL/100 g of body weight intraperitoneally). The frequency of writhing was counted

for 30 minutes. Finally, the percentage of analgesic activity was calculated by the formula,

$$\% \text{ pain inhibition} = \frac{\text{mean of writhing number in (control group)} - 1}{\text{mean writhing number in control}} \times 100$$

The analgesic activity was expressed in ED₅₀ which is the dose that produces 50% pain inhibition within the range of the treatment doses. The log dose response curves permitted the calculation of ED₅₀ antinociception (Noriega et al., 2020) which can be performed by the formula,

$$50\% \text{ inhibition} = (\text{slope} \times \log \text{ED}_{50}) + \text{intercept} \quad (2)$$

In silico docking study

As the computational approach, docking is employed for locating a suitable synthetic compound against a protein target retrieved from Protein Data Bank (www.rcsb.org). Human cyclooxygenase-2 (PDB ID: 5IKQ) was selected as target protein for analgesic activity in docking study. The co-crystallized ligand bound to the COX-2 was Meclofenamic acid with PDB ID: JMS_602 (Orlando and Malkowski, 2016) which served as reference ligand in docking simulation.

The structure of the test compounds were prepared by using Chem Draw ultra 16.0 and converted to *.mol file format after optimizing geometry using MMFF94 calculation. *In silico* protein–ligand interaction of the test compounds was investigated individually using Molegro Visual Docker (MVD) 6.0 software. The docking method was validated by re-docking the reference ligand into the appropriate site of COX-2 (cavity-4). Re-docking is accepted when the root mean square value (RMSD) < 2.0 Å. The resulting score obtained by molecular docking predicts the strongest binders. Two dimension (2D) visualization of the protein–ligand interaction was carried out by Ligand Scout software. **3. Results and Discussions**

Analgesic activity

The ED₅₀ analgesic activity was calculated from the data on the % pain inhibition produced by each dose treatment of the test compound, which was calculated by formula F1. Before being used to calculate % pain inhibition for acetic acid induced writhing model, the number of writhing in each treated group by dose of test compound and the control group were analyzed by One Way ANOVA followed by LSD. F-value denotes statistical significance at p < 0.05 in comparison to control group. The ED₅₀ value of all test compounds are displayed in Table 2, which also lists some of the physicochemical properties of the compounds.

Table 1. Structure of acyl salicylic acid derivatives

Code	Name	Type	R ₁	R ₂	R ₃	R ₄
BS1	Benzoylsalicylic acid	I	C ₆ H ₄	H	H	H
BS2	O-(4-methylbenzoyl) salicylic acid	I	C ₆ H ₄	H	H	CH ₃
BS3	O-(4-t-butylbenzoyl) salicylic acid	I	C ₆ H ₄	H	H	C-(CH ₃) ₃
BS4	O-(4-methoxybenzoyl) salicylic acid	I	C ₆ H ₄	H	H	OCH ₃
BS5	O-(4-chlorobenzoyl) salicylic acid	I	C ₆ H ₄	H	H	Cl
BS6	O-(3-chlorobenzoyl) salicylic acid	I	C ₆ H ₄	H	Cl	H
BS7	O-(2,4-dichlorobenzoyl) salicylic acid	I	C ₆ H ₃	Cl	H	Cl
BS8	O-(3,4-dichlorobenzoyl) salicylic acid	I	C ₆ H ₃	H	Cl	Cl
BS9	O-pentanoyl salicylic acid	II	C ₄ H ₉			
BS10	O-hexanoyl salicylic acid	II	C ₅ H ₁₁			
BS11	O-octanoyl salicylic acid	II	C ₇ H ₁₅			
BS12	2-(4'-t-butylbenzoyloxy)-5-chlorobenzoic acid	III	C ₆ H ₄	H	H	C-(CH ₃) ₃
BS13	2-(3'-chlorobenzoyloxy)-5-chlorobenzoic acid	III	C ₆ H ₃	H	Cl	H
BS14	2-(3',4'-dichlorobenzoyloxy)-5-chlorobenzoic acid	III	C ₆ H ₃	H	Cl	Cl

Table 2. ED₅₀ analgesic activity on acetic acid induced writhing and some physicochemical properties of acyl-salicylic acid derivatives

Compound	MW ^a (g/mol)	ED ₅₀ (mmol/kg)	Log P	pKa	MR ^b (cc/mol)
BS1	242	0.3867	3.07	3.301	63.42
BS2	256	0.6301	3.56	3.299	69.32
BS3	298	0.2628	4.78	3.298	83.09
BS4	272	0.3017	2.95	3.295	70.67
BS5	277	0.5319	3.63	3.296	68.02
BS6	277	0.6651	3.63	3.273	68.02
BS7	311	0.6143	4.19	3.119	72.63
BS8	311	0.3849	4.19	3.267	72.63
BS9	222	0.5786	2.67	3.293	57.23
BS10	236	0.7311	3.08	3.292	61.83
BS11	264	0.7100	3.92	3.291	71.03
BS12	333	0.3177	5.34	2.931	87.70
BS13	311	0.4275	4.19	2.906	72.63
BS14	346	0.4380	4.75	2.901	77.24
ASA ^c	180	0.3941	1.18	3.264	43.29

Notes: a) MW= Molecular weight; b) MR= molar refractivity; c) ASA= aspirin as positive control for writhing test

According to the ED₅₀ value (Table 2), there are 5 compounds that show higher activity than aspirin (ASA) on the molar basis, which include BS1, BS3, BS4, BS8, and BS12. The compound that produced the highest activity was BS3 which contained tert-butyl as substituent at the benzoyl moiety, followed by BS4 which contained methoxy substituent. Compound BS1 which contained no substituents in benzoyl showed comparable activity to BS8 (O-(3,4-dichlorobenzoyl) salicylic acid) and ASA. Benzoyl derivatives (type I and III) have higher analgesic potency than aliphatic acyl derivatives (type II), except for BS9 which contained pentanoyl as acyl group. Among the aliphatic acyl derivatives, only BS9 which has an ED₅₀ below 0.7 mmol/kg. The attachment of chlorine atoms at 5-position on the benzene core of salicylic acid from BS3 and BS8 (type I compounds) to BS12 and BS14 (type III) respectively, resulted in a decrease in analgesic activity.

In the benzoyl-salicylates (type I), compounds containing two chlorine atoms at 3-position and 4-position of the benzene ring (BS8) showed higher activity than compounds substituted with one chlorine (BS5, BS6) or 2 chlorines at 2-position and 4-position (BS7). In aliphatic acyl derivatives (type II), the increase in the alkyl chain of the acyl group tends to decrease the potency. This is also supported by the ED₅₀ data for aspirin (acetylsalicylic acid) which is 0.39 mmol/kg. Aspirin is an aliphatic acyl derivative which has only one alkyl carbon in the acyl group. The longer the alkyl chain, the larger the molecular size as seen from MR value in Table 2, so that there was an influence of steric effect. In this acyl homolog, the increased lipophilic nature does not support the increase in analgesic activity. It seems that the effect of steric properties was more dominant than lipophilicity against analgesic activity of aliphatic acyl-salicylates. In previous studies it was reported that these two properties had an effect on anti-inflammatory activity of the benzoyl salicylates (Diyah et al., 2016).

Among benzoyl-salicylates (type I), those with high potency was the most lipophilic compound (BS3) as well as those containing methoxy substituent which has high electronic effect (BS4). The presence of a chlorine

substituent in the benzoyl group (BS5) which result in increased electronic effect and can also increases lipophilic properties seems to be sufficient to support the increase in analgesic activity compared to the methyl substituent (BS2) which only increases lipophilic properties. Based on the result of in vivo analgesic activity test, there were indications that the electronic properties of the substituents on the benzoyl group can affect the analgesic activity. One of the structural characteristics of the NSAIDs group of drugs is that the compound needs to have a pKa value between 3–6 (Bell et al., 2003), whereas all test compounds have pKa value in the range of 2.9–3.3. The tert-butyl-benzoyl derivatives of 5-chlorosalicylate (BS12) in the type III which was more acidic (pKa ≈2.9) showed lower activity than corresponding compounds from benzoyl-salicylate derivatives (BS3) in type I. pKa is one of the parameters of the electronic properties in the study of structure-activity relationship, so that the electronic properties can play a role in modifying the analgesic activity of this derivatives.

Docking Result

Docking is widely used in modern drug discovery process and is an effective tool capable of quickly and accurately predicting biomolecular conformations with binding energies of protein–ligand complexes which is represented in MVD program by docking score (MolDock Score=MDS). MolDock score is generated from a molecular docking algorithm which is based on a new heuristic search algorithm that combines differential evolution with a cavity prediction algorithm. The docking scoring function of MolDock is an extension of the piecewise linear potential (PLP) including new hydrogen bonding and electrostatic terms (Thomsen and Christensen, 2006). The molecular docking is aimed at finding out the potential compounds against target protein COX-2 and to explain the mode of binding between the test compounds of acyl-salicylates and COX-2. In silico interaction of ligands in active site of COX-2 in three dimension (3D) was shown in Figure 1, the docking energies and amino acid involved in interaction with the test ligands were displayed in Table 3.

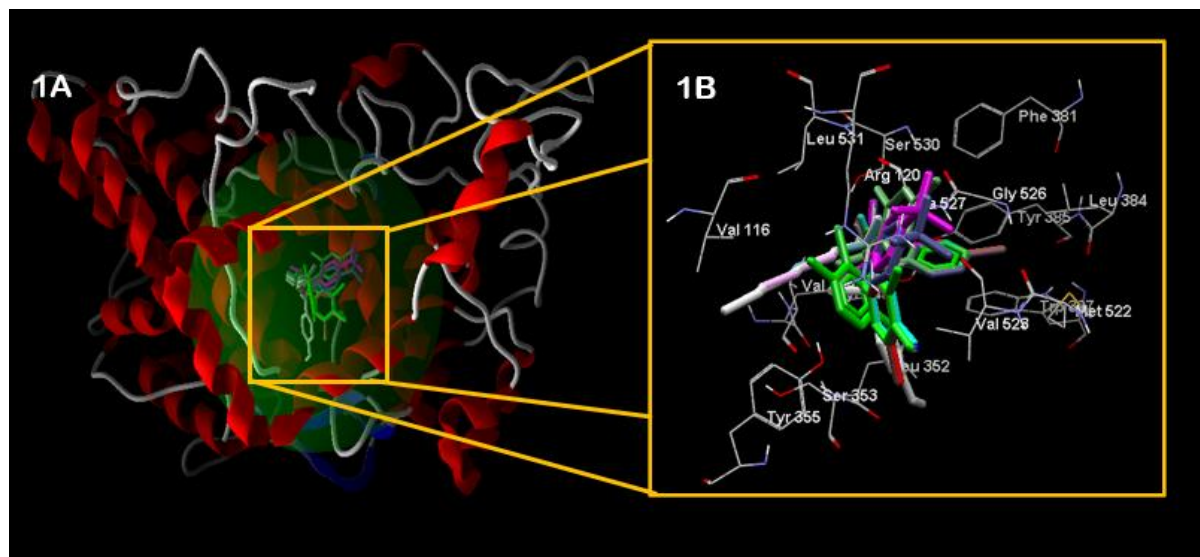


Figure 1. Three-dimensional structure of protein–ligand interaction of acyl-salicylates against COX-2 (PDB ID: 5IKQ); (1A) ligands within active site of protein in secondary structure, (1B) interactions of ligands with amino acids in active site (cavity-4) of the COX-2

Figure 1 showed that the test ligands interacted with amino acids in one of the active sites of COX-2 which has a volume of 78.848 Å. Each test ligands were docked against COX-2, then the docking energies of the salicylic acid

congeners were obtained in negative value out of which **BS3** and **BS4** were potent inhibitors of COX-2 (Table 3).

Table 3. Docking score of the ligand interaction with COX-2 (PDB ID: 5IKQ) and the interactive amino acids

Ligand	MolDock Score (kcal/mol)	Interactive amino acids	
		in hydrogen bond	in steric interaction
BS1	-108.754	Ser530, Tyr385	Ala527, Val349, Val523
BS2	-121.324	Ser530, Tyr385	Ala527, Val349, Val523
BS3	-132.924	Ser530, Tyr385	Ala527, Arg120, Glu 524, Gly528, Pro528, Ser530, Val349, Val523
BS4	-130.206	Ser530, Tyr385	Ile517, Leu352, Ser353, Ser530, Val349
BS5	-121.282	Ser530, Tyr385	Ala527, Val349, Val523
BS6	-124.708	Ser530, Tyr385	Gly526, Pro528, Ser530, Val349, Val523
BS7	-127.302	Ser530, Tyr385	Ala527, Val349, Val523
BS8	-124.929	Ser530, Tyr385	Ala527, Val349
BS9	-98.191	Ser530, Tyr385	Ala527, Ser530, Val116, Val349
BS10	-106.98	Ser530, Tyr385	Ala527, Ser530, Val116, Val349
BS11	-115.544	Ser530, Tyr385	Ala527, Ser530, Val116, Val349
BS12	-130.466	Ser530, Tyr385	Ala527, Arg120, Glu 524, Leu384, Pro528, Ser530, Val349, Val523
BS13	-123.009	Ser530, Tyr385	Ala527, Leu384, Val349, Val523
BS14	-122.094	Ser530, Tyr385	Ala527, Arg120, Leu384, Val349, Val523
JMS_602	-107.814	Ser530, Tyr385	Ser530 Tyr

The data in table 3 showed that almost all acyl-salicylic acid derivatives have lower MDS than meclofenamic acid (JMS_602) against COX-2 (PDB ID: 5IKQ), except for BS9 and BS10, so it is predicted that these ligands have high potency as COX-2 inhibitors because showed a higher binding affinity than meclofenamic acid, a reference ligand

that capable of inhibiting COX-2 (Orlando and Malkowski, 2016). All ligands bind to the amino acids of COX-2 protein by two hydrogen bonds, where the hydrogen atom of hydroxyl (OH) in acid moiety served as H-bond donor for Ser530 and the oxygen of carbonyl (C=O) acted as H-bond acceptor for Tyr385. There were little differences in the

Cite this as Nuzul Wahyuning Diyah 1*, Anindi Lupita Nasyanska2, Bambang Tri Purwanto1, Siswandonol (2020), Analgesic activity of acyl-salicylic acid derivatives and in silico docking study for their potency as cyclooxygenase-2 inhibitors 7(2), 47-54

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

type of amino acids involved in steric interactions among type I, II, and III derivatives, although in general all ligands can interact with Ala527 and Val349. Compounds BS3 and BS12 which contain the *tert*-butyl group bound to the benzoyl moiety were able to interact with more amino acids, resulting in lower docking energies and the highest binding affinity of BS3 against COX-2.

The protein–ligand interaction of all compounds against COX-2 with target surface in 3D was displayed in Figure 2 showing the difference for compounds containing *tert*-butyl groups (BS3 and BS12). Majority of molecule compounds were within the surface while the *tert*-butyl group were outside of the surface.

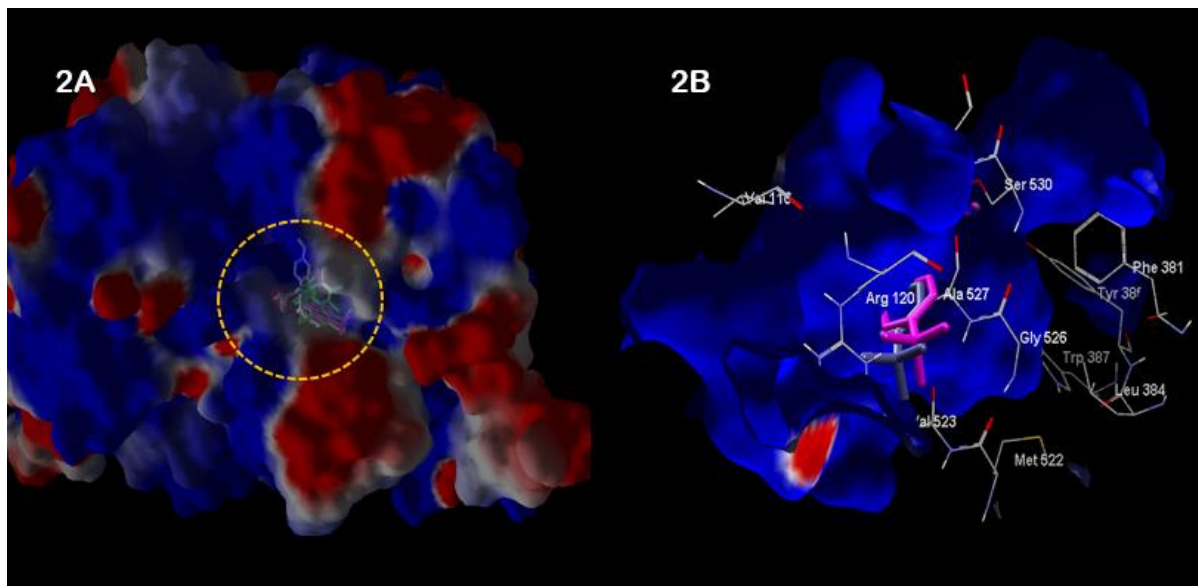


Figure 2. Three-dimensional structure of protein–ligand interaction of acyl-salicylates against COX-2 (PDB ID: 5IKQ) within target surface in 50% transparency (2A), and the same protein–ligand interaction showing *tert*-butyl group of ligand BS3 (purple) and BS12 (dark grey) are at the surface (2B).

The protein–ligand interaction of selected compounds against COX-2 in two dimension (2D) was displayed in Figure 3. The interactions shown were compounds of type I, II, and III, which showed the highest binding affinity in each type while the BS1 compound was a type I compound that did not contain any substituents bound to benzoyl moiety.

In Figure 3 it can be seen that there is only one aromatic ring in JMS-602 (mefenamic acid) that interacted with the amino acids in COX-2. Likewise, almost all ligands of the acyl-salicylate derivatives, except for BS3 and BS12. The attachment of a *tert*-butyl group to the second aromatic ring could increase the interaction with the amino acids in COX-2. This was consistent with the docking results which showed that the two compounds had the lowest docking score against COX-2. When it was related to the results of

analgesic assay, it appeared that there was a match between the docking energies of the compounds BS3 and BS4 against COX-2 with their ED₅₀ of pain inhibition. The *tert*-butyl group could increase the binding affinity for COX-2 and also improved the analgesic activity.

The results of analgesic activity of salicylic acid congeners revealed that the substituted *tert*-butyl-compound (BS3) has highest analgesic activity, followed by methoxy-compound (BS4). The *in silico* investigation of acyl-salicylates also predicted that the 4-*tert*-butyl substituted benzoyl-salicylic acid analog has highest binding affinity with energy score $-132,924$ kcal/mol against Cyclooxygenase-2 which also supported the results obtained by acetic acid induced method.

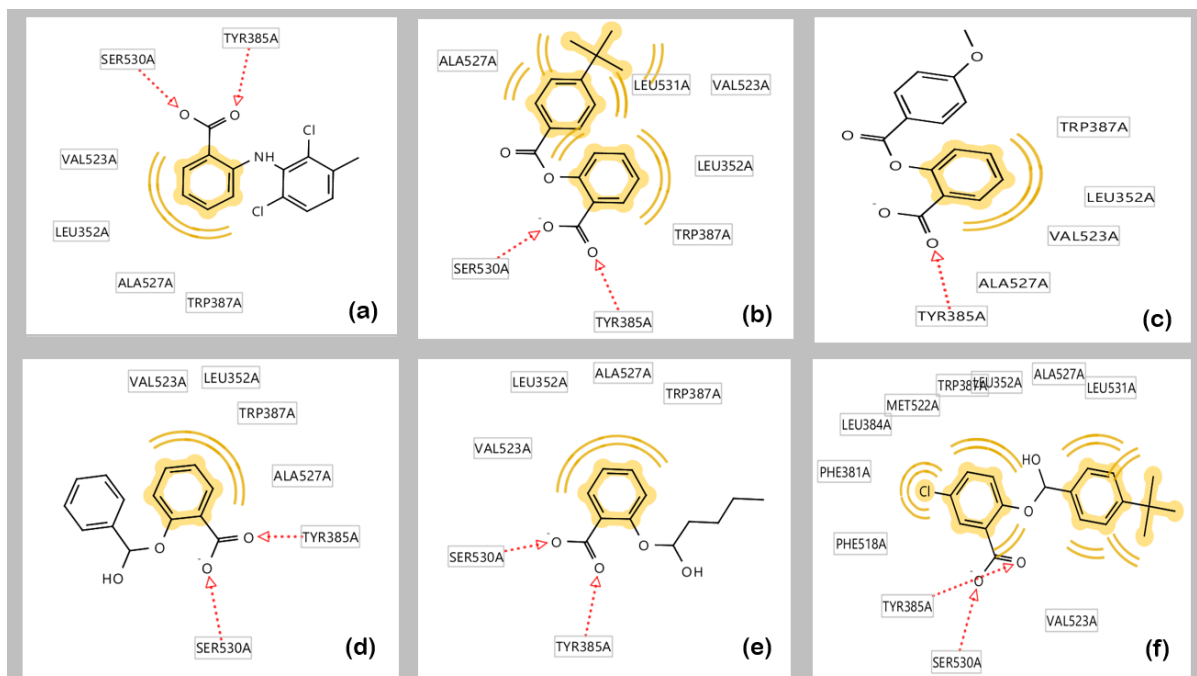


Figure 3. Protein–ligand interaction of selected ligands against COX-2 (PDB ID: 5IKQ) in two-dimension, showing hydrogen bonds (red dashed-line): (a) reference ligand JMS-602, (b) best docked ligand BS3 (compound type I), (c) BS4 (compound type I), (d) BS1 (unsubstituted compound type I), (e) BS9 (compound type II), (f) BS12 (compound type III).

Conclusion

This study has succeeded in finding a compound that has higher analgesic activity than aspirin on the molar basis and has a higher potency as a COX-2 inhibitor compared to meclofenamic acid, namely BS3 (O-(4-*tert*-butylbenzoyl)-salicylic acid). In addition to the BS3 compound, there were two compounds (BS4 and BS12) which showed higher analgesic activity than aspirin and a compound (BS1) whose activity was comparable to aspirin. Moreover, there were 12 compounds that have a higher binding affinity for COX-2 than meclofenamic acid. However, their ability to inhibit COX-2 need to be quantified through further *in vitro* and *in vivo* assays.

References

- Aronoff, D.M., Boutaud, O., Marnett, L.J., and Oates, J.A. (2003). Inhibition of Prostaglandin H₂ Synthases by Salicylate is Dependent on the Oxidative State of the Enzymes. *J Pharmacol Exp Ther*, 304 (2), 589-595.
- Amann, R., and Peskar, B.A. (2002). Review Anti-inflammatory effects of aspirin and sodium salicylate, *Eur. J. Pharmacol.*, 447 (1), 1-9.
- Bell, R., Harris, R.R., Stewart, A.O. (2003) COX-2 Inhibitors and Leucotriene Modulators. In: Abraham, D.J. (ed.) *Burger's Medicinal Chemistry and Drug Discovery 6th Vol. 4: Autocoids, Diagnostics, and Drugs from New Biology* John Wiley and Sons, Inc. pp. 229 – 246.
- Cieslik, K.A., Zhu, Y., Shtivelband, M., Wu, K.K. (2005). Inhibition of p90 Ribosomal S6 Kinase-mediated CCAAT/Enhancer binding Protein Activation and Cyclooxygenase-2 Expression by Salicylate., *J. Biol. Chem.*, 280 (18), 18411–18417.
- Cronstein, B.N., Montesinos, M.C., Weissmann, W. (1999), Salicylates and sulfasalazine, but not glucocorticoids, inhibit leukocyte accumulation by an adenosine-dependent mechanism that is independent of inhibition of prostaglandin synthesis and p105 of NFκB. *Proc. Natl. Acad. Sci.*, 96, 6377-6381.
- Diyah, N.W., Susilowati, R., Hardjono, S. (2010). Kinetics of Degradation of Benzoysalicylic Acid Derivatives in an Alkaline Solution. *Majalah Farmasi Airlangga*, 8 (2), 31-35.
- Diyah, N.W., Siswandono. (2014). Docking Molekul Dan Sintesis Turunan Asam Benzoil Salisilat Tersubstitusi Klor Sebagai Penghambat Siklooksigenase-2. *Berkala Ilmiah Kimia Farmasi*, 3 (1), 7–15.
- Diyah, N.W., Nofianti, K.A., Hakim, L. (2016). Aktivitas Antiinflamasi Turunan Asam O-Benzoilsalisilat. *Berkala Ilmiah Kimia Farmasi*, 5 (1), 14 – 17.
- de Arriba, A.F., Fernando Cavalcanti, F., Agustí Miralles, A., Yolanda Bayón, Y., Andrés Alonso, A., Merlos, M., García-Rafanell, J., Forn, (1999). Inhibition of Cyclooxygenase-2 Expression by 4-Trifluoromethyl Derivatives of Salicylate, Triflusal, and Its Deacetylated Metabolite, 2-Hydroxy-4-trifluoromethylbenzoic Acid. *J Mol. Pharmacol.*, 55 (4), 753-760.
- Hinz, B., Kraus, V., Pahl, A., Brune, K. (2000). Salicylate Metabolites Inhibit Cyclooxygenase-2-Dependent Prostaglandin E₂ Synthesis in Murine Macrophages. *Biochem. Biophys. Res. Commun.*, 274 (1), 197 – 202.
- Kosinski, P., Sarzynska-Nowacka, U., Fiolna, M., Wielgos, M. (2018). The practical use of acetylsalicylic acid in the era of the ASPRE trial. Update and literature review. *Ginekol Pol.* 2018, 89 (2), 107-111.

Cite this as Nuzul Wahyuning Diyah 1*, Anindi Lupita Nasyanska2, Bambang Tri Purwanto1, Siswandono1 (2020), Analgesic activity of acyl-salicylic acid derivatives and in silico docking study for their potency as cyclooxygenase-2 inhibitors 7(2), 47-54

- Law, B.K., Waltner-Lawi, M.E., Entingh, A.J., Chytil, A., Aakre, M.E., Nürgaard, P., Moses, H.L. (2000). Salicylate-induced Growth Arrest is Associated with Inhibition of p70 s6k and Down-regulation of c-Myc, Cyclin D1, Cyclin A, and Proliferating Cell Nuclear Antigen. *J. Biol. Chem.*, 275 (49), 38261-38267.
- Miranda, H.F., Sierralta, F., Aranda, N., et al. (2016) Pharmacological profile of dexketoprofen in oofacial pain. *Pharmacol Rep*, 68, 1111–1114.
- Mitchell, J.A., Saunders, M., Peter J., Barnes, P.J., Newton, R., Belvisi, M.G. (1997). Sodium Salicylate Inhibits Cyclo-Oxygenase-2 Activity Independently of Transcription Factor (Nuclear Factor κ B) Activation: Role of Arachidonic Acid. *Mol. Pharmacol.*, 51 (6), 907-912.
- Noriega, V., Sierralta, F., Poblete, P., Aranda, N., Sotomayor-Zarate, R., Cprieto, J., Miranda, H. F. (2020). Receptors involved in dexketoprofen analgesia in murine visceral pain. *J Biosci*, (2020) 45 (94), 1–6.
- Orlando, B.J., Malkowski, M.G. (2016). Substrate-selective Inhibition of Cyclooxygenase-2 by Fenamic Acid Derivatives Is Dependent on Peroxide Tone. (2016) *J Biol Chem* 291: 15069-15081.
- Saunders, M.A., Sansores-Garcia, L., Gilroy, D.W., Wu, K.K. (2001). Selective Suppression of CCAAT/Enhancer-binding Protein Binding and Cyclooxygenase-2 Promoter Activity by Sodium Salicylate in Quiescent Human Fibroblasts. *J. Biol. Chem.*, 276 (22), 18897-18904.
- Thomsen, R., Christensen, M.H. (2006). MolDock: a new technique for high-accuracy molecular docking. *J Med Chem*, 49(11), 3315-3321.
- Wu, K. (2003). Aspirin and other cyclooxygenase inhibitors: new therapeutic insights. *Semin Vasc Med*, 3 (2), 107-12.