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Virtual screening of 2,3-disubstituted-4(3H)-quinazolinones possessing benzenesulfonamide moiety for COX-2 inhibitor

Hayun[1]*, Arry Yanuar[1], Muhammad Hanafi[3], Sumi Hudiyono PWS[2]

[1]Department of Pharmacy, Faculty of Natural Sciences, University of Indonesia, Depok, Indonesia; [2]Department of Chemistry, Faculty of Natural Sciences, University of Indonesia, Depok, Indonesia; [3]Research Center for Chemistry, Indonesian Institute of Sciences, Tangerang, Indonesia. Hayun - E-mail: hayun.ms@ui.ac.id; *Corresponding author

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Abstract:
COX inhibitors which selectively inhibits the inducible COX-2 is an oenzyme that causes inflammation. They are clinically effective anti-inflammatory agents with less gastrointestinal and renal toxicity. However, they lack anti-thrombotic activity and hence lead to increased incidences of adverse cardiovascular trombotic events such as myocardial infarction. Therefore, there is still a need to develop better therapeutic effect and tolerability COX-2 inhibitor. The majority of COX-2 inhibitors are diaryl heterocycles. For optimum COX-2 selectivity and inhibitory potency a $-SO_3CH_3$ or a- SO_2NH_2 substituent at the *para*-position of phenyl ring was essential. A wide variety of heterocycles can serve as central ring system of the diaryl heterocycles structures. We report the screening of various 2,3-disubstituted-4(3H)-quinazolinones possessing benzenesulfonamide moiety, directly or indirectly bound to the ring system, using the Protein-Ligand ANT System (PLANTS) docking software against the COX-2 enzyme. Various molecular structures of ligands were docked and scored to identify structurally similar ligands to SC-558 (reference ligand) in binding interaction to COX-2 binding site. The results show that 2,3-disubstituted-4(3H)-quinazolinones possess *p*-benzenesulfonamide moiety at C-2, and phenyl moiety at N-3 binds directly or indirectly to the ring system with high binding affinity. The docked ligand has orientations similar to that observed with SC-558 satisfying Lipinski's rule of five.

Keywords: cyclooxygenase, 4(3H)-quinazolinonebenzenesulfonamide, virtual screening, PLANTS.

Background:
The Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are profoundly used in the treatment of a wide variety of inflammation conditions. They exhibit their effect by inhibiting cyclooxygenase (COX) activity. The enzyme is involved in the biosynthesis of prostaglandins, prostacyclins and thromboxanes from arachidonic acid **[1]**. It has been shown recently that the COX enzyme exists in three isoforms: COX-1, COX-2 and COX-3. COX-1 isoform is expressed constitutively and believed to play a role in physiological process such as gastroprotection and vascular homeostasis, COX-2 isoform in its inducible form is associated with inflammation **[2]**, whereas COX-3 isoform has no importance in the development of inflammation **[3, 4]**. The first two COX isoforms are about 60% homologues. Despite the great similarity in the sequence data, detailed examination of structure of the catalytic sites revealed the substrate binding channel in the two enzymes to be quite different. A single amino acid change, from the comparatively bulky isoleucine in COX-1 to valine at position 523 in COX-2, and the conformational changes it produces, resulted in enhanced access to a 'side pocket' that allowed the binding of COX-2 specific inhibitors by providing a docking site for bulky phenylsulfonamide residue of drugs such as SC-558 **[5, 6]**. The classical NSAIDs produce their adverse effects via inhibition of COX-1 isoform, hence many investigations have been directed to find compounds able to act as selective COX-2 inhibitors. The COX inhibitors such as celecoxib, valdecoxib and rofecoxib selectively inhibit COX-2 isoenzyme and clinically effective

anti-inflammatory agents with less gastrointestinal and renal toxicity. However, there is now convincing evidence that higly selective COX-2 inhibitors alter the balance in the COX pathway resulting in decrease in the level of vasodilatory and anti-aggregatory prostacyclin (PGI2), relative to an increase in the level of the prothrombotic thromboxane (TxA2), leading to increase incidences of an adverse cardiovascular thrombotic event such as myocardial infarction [7, **8**]. Thus, there is still a need for novel, selective, and potent COX-2 inhibitors with an improved profile, compared to current COX-2 inhibitors based on structural templates modification. The majority of COX-2 inhibitors are diarylheterocycles. A $-SO_3CH_3$ or a- SO_2NH_2 substituent at the *para*-position of phenyl ring was essential for optimum COX-2 selectivity and inhibitory potency. However, heterocycles ring system can be used a wide variety of heterocycles, in general a five membered or six membered ring [**9**]. Some of quinazolin-4-one derivatives have been synthesized and evaluated to discover more potent and tolerable anti-inflammatory agent and reported to exhibit mild COX-2 inhibition and anti-inflammatory activity [**10, 11**]. Traditional synthesis of new quinazolin-4-one derivatives and bioactivity evaluation can be carried out for optimization activity. However, those processes are of high cost and also time consuming. On the other hand, screening of the small molecules of novel compounds represents an alternative process. Docking various ligands to the protein of interest followed by scoring to determine the binding affinity and to reveal the strength of interaction has become extensively used in virtual screening of large databases and lead optimization [**12-14**]. We report screening of various 2,3-disubstituted-4(3H)-quinazolinones possessing benzenesulfonamide moiety bound directly or indirectly to ring system against the COX-2 enzyme extracted from protein data bank, by utilizing the Protein-Ligand ANT System (PLANTS) v1.1 docking software [**14**]. Various molecular structures of the ligands were docked and scored to identify the ligands that bind similar to reference ligand binding for COX-2 and to estimate the ligands binding affinity for its target. The molecular properties of the docked ligands were also to be analyzed to predict the oral bioavailability of the ligands.

Methodology:

Preparation of Target Protein X-ray Structure

The crystal structure of murine COX-2 in complex with SC-558 inhibitor (PDB code: 6COX, http://www.pdb.org/) [**5**] was selected as the protein target model in this virtual screening study. Using YASARA software [**15**] hydrogens were added and enzyme structure was subjected to a refinement protocol in which the constraints on the enzyme were gradually removed, and converted into SYBYL mol2 format. The crystal structures of human COX-2 bound to 4 ligands were solved in 2010 and publicly available (PDB code: 3LN0, 3LN1, 3MQE, 3NTG) **[16]**. Nonetheless, we used the structure in PDB ID: 6COX for further analysis and experiment.

Ligands Preparation

The various structures of 2,3-disubstituted 4(3H)-quinazolinones possessing benzensulfonamide moiety bound directly or indirectly to the ring system, were drawn and prepared for docking using Chemaxon's Marvin software (http://www.chemaxon.com) [**17**] **(Table 1, see supplementry materials).** The ligands for docking input were prepared using combination of 10 conformations structure and converted into SYBYL mol2 format.

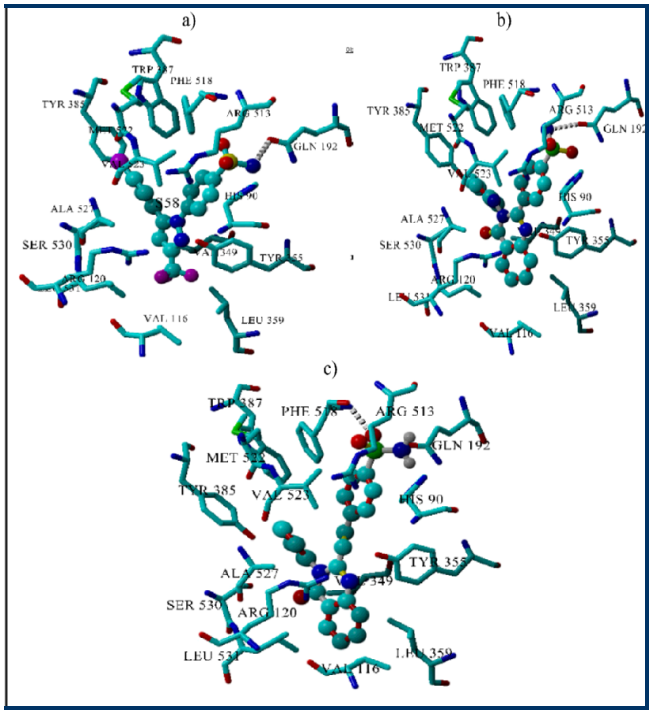

Figure1: Orientation of docked pose of (a) SC- 558, (b) compound 3d, and (c) compound 7d (ball & stick) respectively in the active site of COX-2. All of hydrogen atoms have been removed to improve clarity.

Protein-Ligand Docking

The docking of the target protein with the ligand was performed using the PLANTS v1.1 docking software (http://www.tcd.uni-konstanz.de/research/plants.php). The docking algorithm PLANTS is based on a class of stochastic optimization algorithms called ant colony optimization (ACO) with empirical scoring function ($PLANTS_{CHEMPLP}$). The docking tool generated 10 conformation for each docked ligand. The goal of this docking is to find a low energy ligand conformation in the protein's binding site [**14**]. The virtual screening technique employed in this study was identifying the ligands that bind in comparable manner similar to SC-558 (reference ligand) binding for COX-2. YASARA v10.1.8 software was utilized to visualize molecular docked poses. Before screening the ligands, the docking protocol was validated by redocking SC-558 ligand into it's binding pocket within the COX-2 crystal to obtain the docked pose and RMSD. The result showed that the first ranked cluster contained 10 conformations in the same orientation within the binding site, and the RMSD of the first conformation of the ligands with respect to reference ligand was 1.3758 Å. Thus, the protocol is good in reproducing the X-ray crystal structure in complex forms for further docking experiments.

Lipinski's Rule of 5 Screening

Lipinski's rule of 5 is widely implemented to analyze the "drug-likeness" of the proposed ligand. It states that poor absorption or permeation are more likely when a ligand molecule violates

these rules. [**18**]. The properties of the ligands are calculated for the screening using Chemaxon's Marvin software [**17**].

Results and Discussion:

The protein-ligand interaction score values obtained during docking (total $PLANTS_{CHEMPLP}$ score values), the docked poses obtained from visualization, and the properties of the ligands are given in **Table 1 (see supplementry material)**. In general, the obtained scores are in between -61.491 and -101.373, except compound 7c (-13.576). As a comparison, the $PLANTS_{CHEMPLP}$ score obtained for SC-558 and celecoxib were -75.3077 and -67.7373 respectively. All the ligands docked deeply within the binding pocket region suggest their shape complimentarity with COX-2. However, only 16 of the ligands (50.0%) make docking pose similar to SC-558 satisfying Lipinski's rule of five. The molecular weight of the molecules are in between 377.08 to 448.12 with ClogP value between 2.33 to 3.62, hydrogen bond donor between 1 to 2 and hydrogen bond acceptor between 4 to 6. This results indicate that the above mentioned molecules are predicted to be orally bioavailable. SC-558 is a diaryl heterocyclic inhibitor with a 1,900-fold selectivity for COX-2 over COX-1. It has a central pyrazole ring and a sulfonamide substituent bound to one of the aryl rings [**5**]. The crystal structure of COX-2 with SC-558 (S-58 in YASARA) reveals that the bromophenyl ring of SC-558 is bound in a hydrophobic cavity formed by Phe381, Tyr385, Trp387, Phe518, Met522, Val523, Ala527 and Ser530 and the trifluoromethyl group of the pyrazole ring binds in an adjacent pocket formed by Met113, Val116, Arg120, Val349, Tyr355, Leu359 dan Leu531. The benzenesulfonamide moiety extends into a relatively polar region and interacts with His90, Gln192, Leu352, and Ser353. One of *H*-atom of SO_2NH_2 forms hydrogen bond interaction to the backbone carbonyl oxygen (OE1) of Gln192 (distance ≈ 2.1 Å). The second H-atom of SO_2NH_2 is positioned about 3.48 Å from the nitrogen (NE2) of His90. The distance between O-atom of SO_2NH_2 and the NH group of Phe518 is about 2.76 Å. The second O-atom of SO_2NH_2 is positioned about 3.15 Å from the NH_2 (guanidino group) of Arg513 (**Figure 1a).** The latest mentioned interaction is in line with previously published study [**19**]. All of 2,3-disubstituted-4(3)-quinazolinone compounds possessing *p*-benzenesulfonamide moiety at C-2 and phenyl ring at N-3 directly or indirectly bound to ring system are oriented in a similar way to that of SC-558. Accordingly, the compounds possess *m*-benzenesulfonamide moiety at C-2 (62.5%), *p*-benzenesulfonamide moiety at N-3 (25.0%) and *m*-benzenesulfonamide moiety at N-3 (12.5%). The docked pose of compound 3d and 7d are presented in **Figure 1a** and **1b**. The molecules of compound 3d and compound 7d occupied all the three pocket regions and similar binding modes as observed with SC-558. The phenylamino group at N-3 and the *p*-benzenesulfonamide moiety at C-2 of the quinazolin-4-one ring of compound 3d, and the unsubstituted phenyl ring at N-3 and *p*-[(*E*)-2-ethenyl]benzenesulfonamide moiety at C-2 of the quinazolin-4-one ring of compound 7d are oriented towards *p*-bromophenyl group and the *p*-benzenesulfonamide moiety of the SC-558, respectively. While the A ring of quinazolin-4-one is oriented towards the triflurometyl group of the pyrazole ring of the SC-558. The majority of interacting residues of SO_2NH_2 of compound 3d and 7d are similar with those of SC-558. One of them is Arg513. This is in line with previously published study [**19**]. Compound 3d forms hydrogen bond interaction between its *H*-atom of SO_2NH_2 with carbonyl oxygen (OE1) of Gln192 (distance ≈ 2.37 Å). The second *H*-atom of SO_2NH_2 is positioned about 3.31 Å from carbonyl oxygen of Phe518. The distance between *O*-atoms of SO_2NH_2 and N*H* group (HE2) of Gln192 is about 3,37 Å. The second *O*-atoms of SO_2NH_2 is positioned about 3.39 Å from NH_2 (guanidino group) of Arg513. Compound 7d form hydrogen bond interaction between its *O*-atoms of SO_2NH_2 with N*H* group of Phe518 (distance ≈ 1.53 Å). The second *O*-atoms of SO_2NH_2 is positioned about 3.61 Å from N*H* group of Arg513. The distance between *H*-atoms of SO_2NH_2 and nitrogen (NE2) of His90 is about 2.64 Å. The other *H*-atoms of SO_2NH_2 is positioned about 3.71 Å from carbonyl oxygen of Arg513. The distance between *O*-atoms of quinazolinone ring (compound 3d and 7d) and O*H* group of Ser530 are about 5.27 and 5.12 Å, respectively. The interaction between the ligand with Ser530 is important for inhibition of COX-2 by several compounds besides aspirin [**20**], and it was suggested to be considered in compounds optimization for COX-2 inhibitor [**19**].

Conclusion:

Thirty two molecular structures of 2,3-disubstituted-4(3H)-quinazolinones possessing benzenesulfonamide moiety bound directly or indirectly to the ring system have been docked and scored to identify the ligands that bind similar orientation as observed with SC-558 binding for COX-2. The result show that 2,3-disubstituted-4(3H)-quinazolinones possessing *p*-benzenesulfonamide moiety at C-2 and phenyl ring at N-3 showed equal to higher binding affinity than that of SC-558 with similar orientation to SC-558 ligand. The majority of interacting residues of SO_2NH_2 of compound **3d** and **7d** are similar with those of SC-558. The *O*-atoms of quinazolinone ring have the potential to interact with Ser530 satisfying Lipinski's rule of five. These compounds could be considered as potent COX-2 inhibitors.

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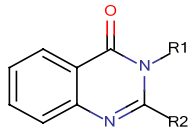
Supplementary material:

Table 1: Structures, Properties and Docking Results of 2,3-Disubstituted-4(3H)-Quinazolinones Possessing Benzenesulfonamide Moiety, SC-558 and Celecoxib.



Compd	Substituents*)		Lipinski's Rule of 5				Score**)	Docked Pose ***)
	R1	R2	Mol Weight	No of H-Donors	No of H-Acceptors	ClogP		
1a	*p*-BSA	Ph	377.08	1	4	3.12	-77.3232	×
1b	*m*-BSA	Ph					-70.9226	×
1c	Ph	*m*-BSA					-76.0898	✓
1d	Ph	*p*-BSA					-78.676	✓
2a	*p*-BzSA	Ph	391.10	1	4	3.18	-89.2743	×
2b	*m*-BzSA	Ph					-91.1331	×
2c	Bz	*m*-BSA					-92.1175	✓
2d	Bz	*p*-BSA					-79.6776	✓
3a	*p*-ABSA	Ph	392.09	1	6	3.34	-88.5913	×
3b	*m*-ABSA	Ph					-88.9771	×
3c	PhA	*m*-BSA					-93.7915	✓
3d	PhA	*p*-BSA					-88.0979	✓
4a	N-4-SBA	Ph	420.09	2	5	2.50	-74.8437	✓
4b	N-3-SBA	Ph					-84.7734	×
4c	N-BA	*m*-BSA					-93.9921	✓
4d	N-BA	*p*-BSA					-91.5505	✓
5a	N-4-SPhAc	Ph	434.10	2	5	2.37	-74.6049	✓
5b	N-3-SPhAc	Ph					-89.4398	×
5c	N-PhAc	*m*-BSA					-100.349	×
5d	N-PhAc	*p*-BSA					-93.5586	✓
6a	N-E-4-SBA	Ph	448.12	2	5	2.33	-74.4486	×
6b	N-E-3-SBA	Ph					-87.6545	×
6c	N-EBA	*m*-BSA					-88.9119	✓
6d	N-EBA	*p*-BSA					-100.129	✓
7a	*p*-BSA	2-Ph-(*E*)-Etn	403.10	1	4	3.62	-85.8609	×
7b	*m*-BSA	2-Ph-(*E*)-Etn					-80.3884	×
7c	Ph	[(*E*)-Etn]B-3-SA					-13.5760	×
7d	Ph	[(*E*)-Etn]B-4-SA					-61.491	✓
8a	*p*-BSA	2-PhE	405.11	1	4	3.54	-101.373	×
8b	*m*-BSA	2-PhE					-91.1976	✓
8c	Ph	EB-3-SA					-90.4981	×
8d	Ph	EB-4-SA					-79.3338	✓
	SC-558 (reference ligand)		446.24	1	7	4.16	-75.3077	
	Celecoxib		381.37	1	6	3.83	-67.7373	✓

*) Substituents: Ph = Phenyl; BSA = Benzenesulfonamide; BzSA = Benzylsulfonamide; ABSA = Aminobenzenesulfonamide; PhA = Phenylamino; SBA = Sulfamoylbenzamide; BA = Benzamide; SPhAc = Sulfamoylphenylacetamide; PhAc = Penylacetamide; E-SBA = Ethyl-sulfamoylbenzamide; EBA = Ethylbenzamide; PhEtn = Phenylethenyl; EtnBSA = Ethenylbenzenesulfonamide; PhE = Phenylethyl; EBSA = Ethylbenzenesulfonamide.

**) Total $PLANTS_{CHEMPLP}$ score

***) in comparison with reference ligand, SC-558; ✓ = similar; × = unsimilar.