โครงการประชุมวิชาการระดับชาติและนานาชาติ ครั้งที่ 8

8th National and International Conference

Theme: Research to Serve Society

(e-Conference)

PROCEEDINGS

25 June 2021

Huachiew Chalermprakiet University

Bangphli District, Samutprakarn Thailand

8th Huachiew Chalermprakiet University International Conference

Friday, 25th June 2021

Theme: Research to Serve Society

Main Host Huachiew Chalermprakiet University

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Design, Synthesis and Molecular Docking Study of Novel Celecoxib Derivatives Using Bioisosteric Replacement of Sulfonamide Moiety

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Abstract

Celecoxib, a selective COX-2 inhibitor, is one of most commonly used nonsteroidal anti-inflammatory drug for the treatment of inflammatory symptoms. However, the presence of sulfonamide group in the structure has been suspected to cause cross-hypersensitivity with antibiotic sulfonamides. To avoid this problem, the sulfonamide moiety of celecoxib was modified using bioisosteric technique to obtain novel celecoxib derivatives. Commercially available 4,4,4-trifluoro-1-(4-methylphenyl)butane-1,3-dione was reacted with 4-hydrazinobenzonitrile HCl to obtain nitrile derivative of celecoxib (**1**). In addition, carboxylic acid (**2**) and tetrazole (**3**) derivatives of celecoxib were also synthesized using compound **1** as a starting material. All synthesized compounds including corresponding bioisostere groups of sulfonamide were predicted for their COX-1 and COX-2 inhibitory activities by computer-based molecular docking method using AutoDock 4.2. The results showed that tetrazole derivative (**3**) possessed the lowest binding energy and inhibitory constant (K_i) against *m*COX-2 (-9.60 kcal/mol and 91.22 nM, respectively), but lack of selectivity due to very low calculated selectivity index (SI = 0.73). Whereas hydroxymethyl derivative (**7**) was considered to be a selective COX-2 inhibitor (SI = 6.79). Unfortunately, all designed compounds were found to be inferior to their prototype celecoxib, which exhibited the highest potency and selectivity against *m*COX-2 in this study (binding energy = -10.4 kcal/mol, K_i = 23.91 nM, and SI = 18.14).

Keywords : Celecoxib, nonsteroidal anti-inflammatory drugs, cyclooxygenase, bioisostere, molecular docking

1. Introduction

Cyclooxygenase (COX) is known as a group of enzyme that plays an important role in inflammatory responses by converting arachidonic acid (AA) to various types of inflammatory mediators called prostaglandins (PGs) or prostanoids. In general, COX exists in 2 major isozymes as COX-1 and COX-2, which functionalize in different way. COX-1, a constitutive isoform or housekeeping enzyme, is expressed in most tissues to promote and regulate homeostasis in the body, whereas COX-2, an inducible isoform, is specifically expressed at the site of inflammation by mediators or stimuli signaling (Blobaum & Marnett, 2007, pp. 1425-1441). For this reason, COX-2 is predominantly a desire target of inhibition for the treatment of various inflammatory diseases.

Currently, nonsteroidal anti-inflammatory drugs (NSAIDs) have been developed and primarily used in the treatment of inflammation or pain management. The mechanism of action of NSAIDs is to inhibit COX activities resulting to termination of PGs biosynthesis. NSAIDs are generally classified into 2 subgroups by the selectivity against COX. First, traditional or nonselective NSAIDs, such as aspirin, ibuprofen, indomethacin, and diclofenac, inhibit both COX-1 and COX-2 in varying degree. Although these medicines have been discovered and efficiently used until nowadays, major side effect of gastrointestinal (GI) tract irritation apparently occurred due to the inhibition of COX-1 activity (Vane & Botting, 1998,

pp. 2S-22S). To overcome this drawback, the development of selective COX-2 inhibitors (so-called coxibs) have been introduced.

One of most widely used marketed available coxibs is celecoxib, it has been approved by the FDA since 1998 for the relief of signs and symptoms of osteoarthritis (OA), rheumatoid arthritis (RA), ankylosing spondylitis (AS), as well as acute pain in adults with lower incidence of GI adverse effects. Nevertheless, risk of cardiovascular (CV) effects by long-term usage of celecoxib must be concerned. Celecoxib is a sulfonamide-containing 1,5-diarylpyrazole derivative, which the presence sulfonamide ($-SO_2NH_2$) moiety appeared to be essential for COX-2 blockade activity (Antoniou *et al.*, 2007, pp. 1719-1732). Unfortunately, because of this structural component, the $-SO_2NH_2$ moiety in celecoxib is also suspected to cause hypersensitivity in patients who allergy to antibiotic sulfonamides (Knowles *et al.*, 2001, pp. 239-247). Although many previous studies or case reports indicated the possibility of this cross-reactivity, it was still controversial in some studies (Wulf & Matuszewski, 2013, pp. 1483-1494). Thus, to diminish the aforementioned problem, $-SO_2NH_2$ group of celecoxib was replaced with different functionalities using a concept of bioisostere (Meanwell, 2011, pp. 2529-2591).

2. Objectives

To design and synthesize celecoxib derivatives using bioisosteric replacement of sulfonamide moiety that could avoid possible cross-hypersensitivity between antibiotic sulfonamides, and to evaluate *in silico* COX-1 and COX-2 inhibitory activities of the designed compounds.

3. Materials and methods

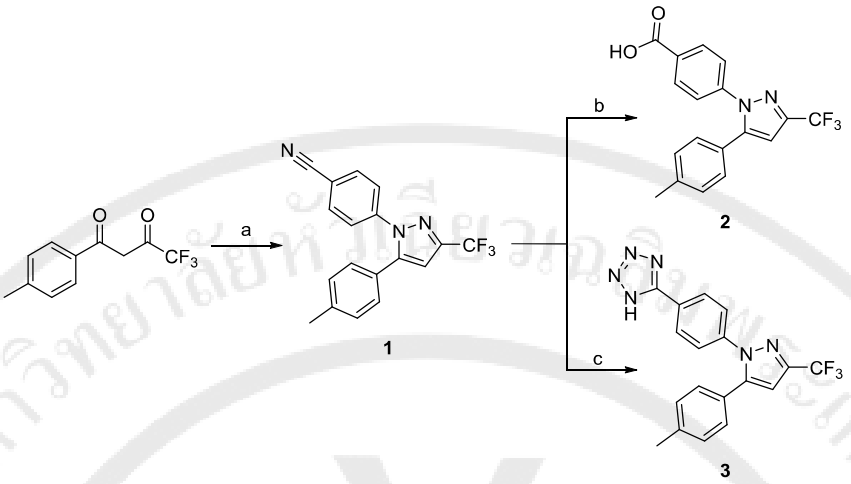
3.1 Materials

4,4,4-Trifluoro-1-(4-methylphenyl)butane-1,3-dione was purchased from Sigma-Aldrich (St. Louis, Missouri, USA) and 4-hydrazinobenzonitrile HCl was purchased from Tokyo Chemical Industry (Tokyo, Japan). Solvents were used either commercial or analytical grade. TLC was performed on Merck Kieselgel 60 F_{254} precoated silica gel plates. ^{1}H-NMR spectra were recorded using Bruker Fourier 300 (Basel, Switzerland) at 300 MHz. FT-IR spectra were recorded using PerkinElmer Spectrum 100 (Waltham, MA, USA).

3.2 Methods

Compound **1**–**3** were synthesized according to Scheme 1. Commercially available 4,4,4-trifluoro-1-(4-methylphenyl)butane-1,3-dione, a starting material was reacted with 4-hydrazinobenzonitrile HCl to give a nitrile derivative (**1**). The nitrile was used as a substrate to synthesize either carboxylic acid (**2**) or tetrazole (**3**) derivatives. To synthesize a carboxylic acid derivative, the nitrile (**1**) was undergone hydrolysis by a treatment of potassium hydroxide in a mixture of ethanol and water. In addition, the nitrile (**1**) was treated with sodium azide in the presence of copper sulfate to yield a tetrazole derivative.

Scheme 1 Synthetic pathway of celecoxib derivatives.



Reagents and conditions: (a) 4-hydrazinobenzonitrile HCl, EtOH, reflux; (b) KOH, EtOH : H_2O (1 : 1); (c) NaN_3, $CuSO_4 \cdot 5H_2O$, DMSO, reflux.

Synthesis of 4-(5-(*p*-tolyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)benzonitrile (1). To a stirred solution of 4,4,4-trifluoro-1-(4-methylphenyl)butane-1,3-dione (1 g, 4.3 mmol) in EtOH (25 mL), 4-hydrazinobenzonitrile HCl (0.8 g, 4.7 mmol) was then added and the reaction was refluxed for 20 h. On completion of reaction (monitored by TLC), the solvent was removed under reduced pressure. EtOAc (50 mL) was added and then washed with H_2O (2 × 50 mL) and brine (50 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude extract was purified by column chromatography on silica gel (hexane : EtOAc, 7 : 3) to obtain an off-white solid (yield 53%). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 8.00 – 7.91 (m, 2H), 7.60 – 7.50 (m, 2H), 7.21 (d, J = 2.5 Hz, 5H), 2.32 (s, 3H); FT-IR (ATR, υ cm^{-1}): 2229.68 (C≡N, stretch).

Synthesis of 4-(5-(*p*-tolyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)benzoic acid (2). To a stirred solution of KOH in EtOH : H_2O (1 : 1), compound **1** (0.5 g, 1.5 mmol) was added and the reaction was heated under reflux for 3.5 h. On completion of reaction (monitored by TLC), the solvent was removed under reduced pressure and cooled on ice bath. The mixture was acidified to pH of 1 by 2 N HCl and extracted with EtOAc (3 × 25 mL). The pooled organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude extract was purified by column chromatography on silica gel (EtOAc : CH_2Cl_2, 7 : 3) to obtain a white solid (yield 21%). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 8.07 – 7.94 (m, 2H), 7.53 – 7.40 (m, 2H), 7.19 (s, 5H), 2.31 (s, 3H); FT-IR (ATR, υ cm^{-1}): 1679.97 (C=O, stretch), 2920.23 (O-H, stretch).

Synthesis of 5-(4-(5-(*p*-tolyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)phenyl)-1*H*-tetrazole (3). A mixture of compound **1** (0.2 g, 0.6 mmol), NaN_3 (0.04 g, 0.6 mmol) and $CuSO_4 \cdot 5H_2O$ (0.006 g, 2 mol%) in DMSO (5 mL) was stirred under reflux for 8 h. On completion of reaction (monitored by TLC), the mixture was cooled on ice bath and 2 N HCl (20 mL) was then added. The acid mixture was extracted by EtOAc (3 × 20 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by recrystallized in EtOAc to obtain an off-white solid (yield 40%). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 8.11 (d, J = 8.5 Hz, 2H), 7.59 (d, J = 8.5 Hz, 2H), 7.21 (d, J = 2.5 Hz, 5H), 2.31 (s, 3H).

3.3 Molecular docking

For ligand preparations, the structures of celecoxib derivatives were sketched and minimized energy using MM2 protocol of ChemBio3D Ultra 13.0 software. Gasteiger charges were then assigned and non-polar hydrogens were merged using AutoDockTools 1.5.6. Three-dimension structures of wild-type ligated ovine COX-1 (*o*COX-1, PDB code: 1EQG, 1EQH and 4O1Z) and murine COX-2 (*m*COX-2, PDB code: 3LN1, 3PGH and 4PH9) were obtained from Brookhaven Protein Data Bank (https://www.rcsb.org/pdb). For protein preparations, one subunit of each homodimeric enzyme was selected and molecule of heteroatoms including water were removed. The polar hydrogen atoms were added and Gasteiger charges were also assigned. Grid box size was set to 40 × 40 × 40 Å dimension with grid spacing of 0.375 Å and located at the center of ligand. Corresponding map types were also calculated with AutoGrid.

For molecular docking, briefly, the ligands were docked onto the active site of either validated *o*COX-1 or *m*COX-2 using AutoDock 4.2 software (Morris *et al.*, 2009, pp. 2785-2791) with the Lamarckian Genetic Algorithm (LGA) method. Number of GA run was 100 and other docking parameters were set as default. One hundred independent docking poses of each ligand were generated and similar poses with less than 2.0 Å of root-mean-square deviation (RMSD) were clustered. The best conformation of the highest cluster was selected to represent the most reliable binding mode of ligand, with the analysis of binding energy and inhibitory constant (K_i). The binding modes, binding interactions and length of chemical bonds (Å) were observed and illustrated by BIOVIA Discovery Studio Visualizer 17.2.

To validate the docking method, re-docking of native ligands back to their active site of the target proteins was performed. In addition, cross-docking of native ligands against non-native proteins was also conducted. The docking validation was accepted when the re-docking and cross-docking results showed less than 2.0 Å of reference RMSD values from the highest cluster.

4. Results and Discussion

4.1 Chemistry

Using of celecoxib as a starting material cannot be directly applicable in terms of sulfonamide functionality conversion, because this group is rigid and unreactive. Thus, condensation of a diketone compound, 4,4,4-trifluoro-1-(4-methylphenyl)butane-1,3-dione with 4-hydrazinobenzonitrile was chosen to give a nitrile derivative of celecoxib (**1**) in a moderate yield (53%). Advantageously, nitrile group (-C≡N) can undergo a variety of chemical reactions such as hydrolysis, reduction, or alkylation. Compound **1** was then hydrolyzed under strong basic condition to give a carboxylic acid derivative (**2**) in a low yield (21%). Additionally, compound **1** was subsequently treated with sodium azide (NaN_3) using cupric sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) as a catalyst to give a tetrazole derivative (**3**) in a moderate yield (40%) through traditional [2 + 3] cycloaddition reaction.

4.2 Molecular docking

After the selection of *o*COX-1 and *m*COX-2 X-ray crystal structures as described in the methodology, general information of the included proteins and re-docking results were showed in Table 1 and Table 2.

Table 1 General information and re-docking results of included *o*COX-1 PDBs

PDB code	Ligand	RMSD (Å)	Cluster number	%Member in cluster	Binding energy (kcal/mol)
1EQG	Ibuprofen	1.00	1	100	-8.54
1EQH	Flurbiprofen	1.26	1	100	-9.03
3KK6	Celecoxib	0.76	1	100	-11.47
4O1Z	Meloxicam	1.21	1	100	-10.18

Table 2 General information and re-docking results of included *m*COX-2 PDBs

PDB code	Ligand	RMSD (Å)	Cluster number	%Member in cluster	Binding energy (kcal/mol)
3LN1	Celecoxib	0.95	1	100	-11.09
3PGH	Flurbiprofen	1.32	1	100	-8.91
4M11	Meloxicam	1.14	2	89	-9.70
4PH9	Ibuprofen	0.89	1	100	-8.35

The results indicated that docking of all native ligands into their active site of target protein either *o*COX-1 or *m*COX-2 exhibited acceptable outcomes due to the RMSD values from the highest cluster were less than 2.0 Å. Therefore, cross-docking of all included ligand-protein complexes was determined to confirm the validity of docking algorithm. The cross-docking results of native ligands against non-native *o*COX-1 and *m*COX-2 were displayed in Table 3 and Table 4, respectively.

Table 3 Cross-docking results of included *o*COX-1 PDBs

PDB code	Ligand	RMSD, Å (%member in highest cluster)			
		1EQG	1EQH	3KK6	4O1Z*
1EQG	Ibuprofen	1.00 (100%)	0.70 (100%)	2.14 (87%)	0.76 (86%)
1EQH	Flurbiprofen	1.22 (100%)	1.26 (100%)	1.53 (93%)	1.26 (100%)
3KK6	Celecoxib	3.98 (60%)	6.52 (33%)	0.76 (100%)	4.59 (51%)
4O1Z	Meloxicam	2.20 (54%)	2.17 (100%)	2.32 (100%)	1.21 (100%)

*Selected PDB as an *o*COX-1 template.

Table 4 Cross-docking results of included *m*COX-2 PDBs

PDB code	Ligand	RMSD, Å (%member in highest cluster)			
		3LN1	3PGH*	4M11	4PH9
3LN1	Celecoxib	0.95 (100%)	0.99 (92%)	4.84 (79%)	0.92 (52%)
3PGH	Flurbiprofen	1.15 (100%)	1.32 (100%)	1.21 (100%)	1.17 (100%)
4M11	Meloxicam	4.65 (67%)	2.15 (70%)	1.14 (89%)	4.04 (95%)
4PH9	Ibuprofen	5.23 (71%)	0.93 (100%)	0.96 (63%)	0.89 (100%)

*Selected PDB as a *m*COX-2 template.

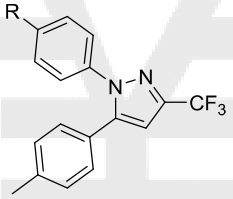
For *o*COX-1 (Table 3), unfortunately, the cross-docking results were noticed that the RMSD values obtained from docking of celecoxib (3KK6) were greater than 2.0 Å with low percent member in highest cluster which represented poor accuracy. This incidence probably happened due to the side pocket of COX-1 is smaller than COX-2, which the molecule of celecoxib could not be fitted (Blobaum & Marnett, 2007, pp. 1425-1441). Thus, the cross-docking outcomes from celecoxib were excluded and the rest of results were reconsidered.

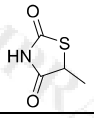
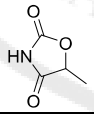
PDB 4O1Z was eventually selected as an *o*COX-1 template in this study, because the cross-docking results without celecoxib met the criteria (RMSD < 2.0 Å).

For *m*COX-2 (Table 4), some of cross-docking results were undesirable as seen in *o*COX-1. Thus, the cross-docking outcomes from meloxicam (4M11) were excluded. Finally, PDB 3PGH was selected as a *m*COX-2 template in this study, because the cross-docking results without meloxicam were acceptable. Whereas the overall results from PDB 4PH9 were also acceptable, but percent member in highest cluster obtained from celecoxib (3LN1) docking was quite low (52%).

All synthesized celecoxib derivatives (**1**–**3**) and other sketched bioisosteric molecules (**4**–**11**) were docked into the active site of *o*COX-1 and *m*COX-2. Compared with celecoxib as their prototype, molecular docking results were listed in Table 5.

Table 5 Molecular docking results of celecoxib and derivatives against *o*COX-1 (PDB code: 4O1Z) and *m*COX-2 (PDB code: 3PGH)



Compound	R	Binding energy (kcal/mol)		K_i (nM)		SI*
		***o*COX-1**	***m*COX-2**	***o*COX-1**	***m*COX-2**	
1	-C≡N	-7.96	-8.90	1470	297.15	4.95
2	-COOH	-8.23	-8.42	928.43	672.38	1.38
3		-9.79	-9.60	66.91	91.22	0.73
4	-CO-NH_2	-8.59	-8.23	503.97	930.53	0.54
5	-CO-NH-OH	-8.04	-8.10	1290	1150	1.12
6	-CO-NH-NH_2	-8.12	-7.94	1120	1510	0.74
7	-CH_2-OH	-7.93	-9.07	1530	225.34	6.79
8		-10.03	-8.43	44.62	659.02	0.07
9		-8.41	-9.00	679.09	251.75	2.70
10		-9.02	-9.38	244.9	132.86	1.84
11	-OH	-8.19	-7.61	996.38	2660	0.37
Celecoxib	-SO_2NH_2	-8.68	-10.40	433.74	23.91	18.14

*Selectivity index (SI), calculated from K_i ratio of COX-1 / COX-2.

Ligand binding energy and K_i represent the affinity and inhibitory activity (potency) of each molecule against target protein, respectively. The lower binding energy value implies the higher binding affinity, as well as K_i. In addition, selectivity index (SI) of all docked

compounds were also calculated to indicate the selectivity particularly against COX-2 as a desire target of action. In this case, higher SI compound exhibits higher selectivity against COX-2 activity. The results showed that synthesized tetrazole derivative (**3**) exhibited the lowest binding energy and K_i against *m*COX-2 (-9.60 kcal/mol and 91.22 nM, respectively). Although the affinity and inhibitory profile against COX-2 were outstanding, the selectivity of this compound was suggested to be very low with SI value of 0.73. For celecoxib derivatives, compound **7** was the most selective COX-2 inhibitor with SI value of 6.79, whereas the affinity and inhibitory activity of this compound were moderate (*m*COX-2 binding energy of -9.07 kcal/mol, and K_i of 225.34 nM). Nevertheless, all of the synthesized and sketched celecoxib derivatives (**1**–**11**) were inferior to their prototype. From the molecular docking results, celecoxib was such a very potent compound with high affinity and extremely high selectivity against COX-2 with the binding energy of -10.40 kcal/mol, K_i of 23.91 nM (4-fold more potent than compound **3**), and SI value of 18.14 (25-fold more selective than compound **3**).

To understand the binding orientation and binding interactions between ligands and active site of target proteins, the best docking pose in the highest cluster of each compound was illustrated (Figure 1). Moreover, key binding interactions and length of chemical bonds were also observed. For mentioned celecoxib derivatives, the binding orientation of compound **3** in *m*COX-2 active site cavity was different to compound **7** and celecoxib (Figure 1A). Unexpectedly, tetrazole group of compound **3** projected to hydrophobic pocket instead of allosteric pocket (side pocket), which sulfonamide moiety ($-SO_2NH_2$) of celecoxib interacted with the amino acids in this area through hydrogen bonds. Hence, the binding interactions of this compound were mostly found as hydrophobic interactions, such as the pyrazole ring interacted with Arg120 (7.43 Å) located at mouth of the active site *via* cationic-π interaction, and trifluoromethyl carbon ($-CF_3$) also exhibited hydrophobic interaction networks with Val349, Tyr355 and Leu359. Whereas tetrazole group only interacted with Gly526 backbone (2.75 Å) located deeply at hydrophobic pocket *via* hydrogen bond. These findings were not in agreement with a previous study. Navidpour, *et al.* (2006) had firstly introduced the molecular docking study of tetrazole derivative of celecoxib, the results suggested that the tetrazole ring was inserted deep into the secondary pocket (side pocket) of *m*COX-2 by interacting with Arg513 and His90 *via* hydrogen bonds (pp. 4483-4487).

Like celecoxib, hydroxymethyl group ($-CH_2-OH$) of compound **7** aligned to the side pocket of *m*COX-2 and formed a hydrogen bond with oxygen carbonyl of Leu352 backbone (5.17 Å), as well as hydrophobic interaction networks with biphenyl rings, pyrazole ring and $-CF_3$ carbon also found in a higher number (Figure 1B). Interestingly, hydrogen bonding between $-SO_2NH_2$ and Leu352 was also investigated from celecoxib–*m*COX-2 complex (4.75 and 5.25 Å). Moreover, this functionality interacted with His90 (6.52 Å) and Phe518 (5.40 Å) *via* S-π interactions (Figure 1C). This evidence indicated that number of chemical bonds, especially hydrogen bond between a molecule and amino acids around side pocket play a crucial role in terms of COX-2 inhibitory activity. For further design of novel celecoxib derivatives, not only the $-SO_2NH_2$ moiety, but also the core structure (pyrazole ring) may be simultaneously modified.

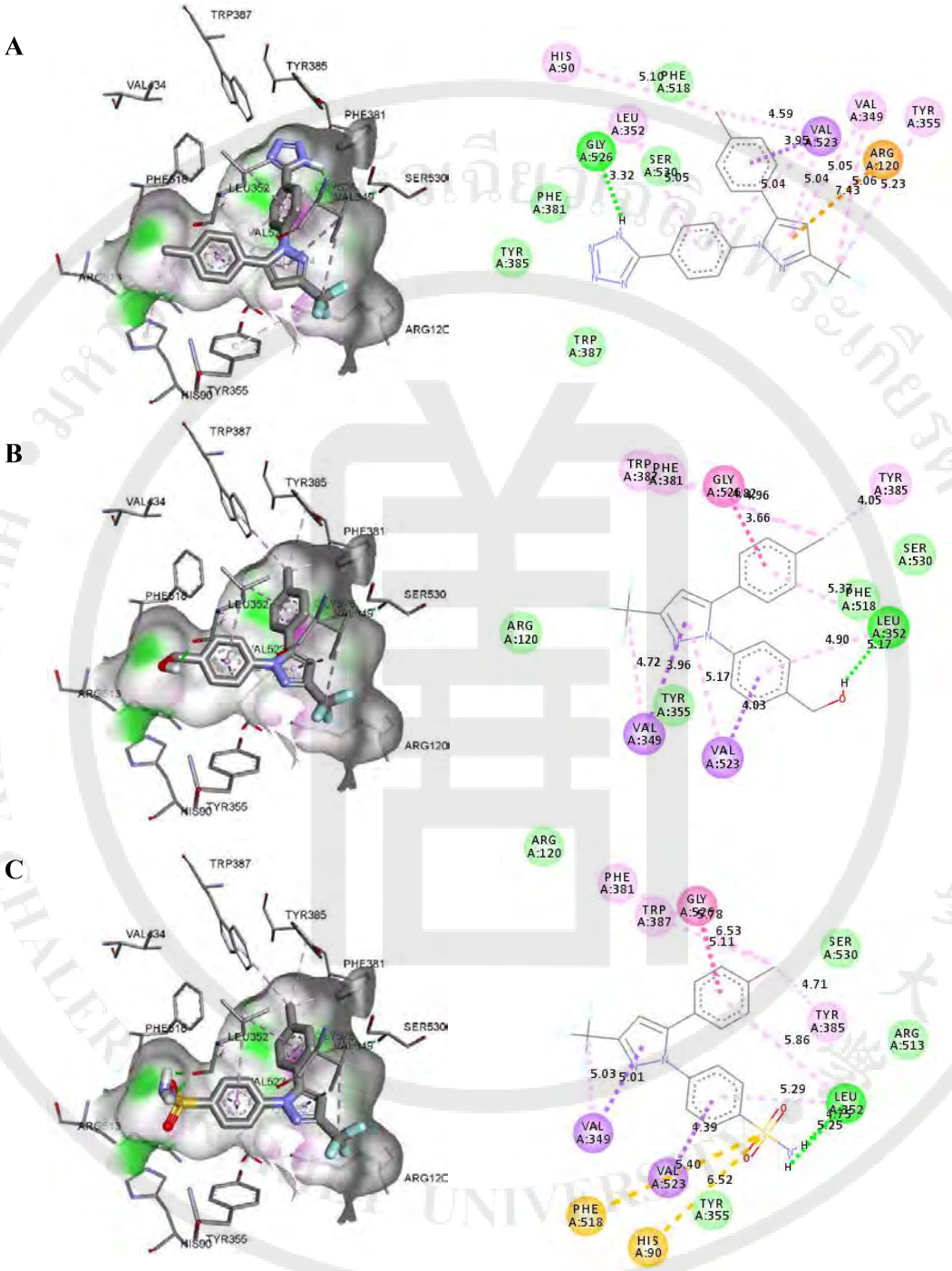


Figure 1 X-ray crystal structures obtained from molecular docking (left) and 2D binding interaction diagrams (right) of compound **3** (A), compound **7** (B), and celecoxib (C) against the active site of *m*COX-2 (PDB code: 3PGH). For clarity, only amino acids nearby the ligands are shown. All binding interactions are presented in broken lines with number as length (Å). Hydrogen bonds are displayed in green, π interactions are displayed in orange, and other hydrophobic interactions are displayed in violet or pink.

5. Conclusion

According to the design concept by using of bioisosteric technique to replace the sulfonamide moiety of celecoxib, 11 derivatives of celecoxib (3 synthesized and 8 sketched compounds) were evaluated virtually through molecular docking study. The results revealed that synthesized tetrazole derivative (**3**) possessed the highest binding affinity and inhibitory activity against COX-2, but low selectivity. Whereas hydroxymethyl derivative (**7**) was indicated as the most selective COX-2 inhibitor *in silico.* Unfortunately, all novel derivatives were still inferior to their prototype celecoxib. Hence, this study may provide the concept of drug design and useful applications of validated docking protocol for further *in silico* evaluation of well-designed COX-2 inhibitors. However, molecular docking study is advantageous to predict the binding interactions and activities of interested compound, *in vitro* screening of pharmacological activities must be conducted to obtain more reliable outcomes.

6. Acknowledgements

This research study is partly granted by the Faculty of Pharmaceutical Sciences, Huachiew Chalermprakiet University. The authors would like to thank Dr. Chirattikan Maicheen for chemicals purchasing and supply, including valuable technical supports.

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