



## Acid Property of Carboxylic Acid Functionality of Diclofenac in Cyclooxygenase Inhibitory Activity

Phurit Thanarangsarit, Padungpong Namwong, Pornchai Sudthanaphun and Ruengwit Kitbunnadaj\*

Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok 65000

\*Corresponding author. E-mail address: rkitbunna@hotmail.com

### Abstract

Diclofenac [2-(2,6-dichloranilino) phenylacetic acid] is one of the most widely used traditional nonsteroidal anti-inflammatory drugs (NSAIDs). Many studies indicated that the carboxylic acid group involve in serious side effect occurrences such as gastric damage and hepatotoxicity, thus removal of the acid group might alleviate the aforementioned side effects. X-ray crystal structures of diclofenac/COX (COX-1 and COX-2) complex, however, show that the carboxylic moiety plays a role by interacting with tyrosine-385 and serine-530 of COX via hydrogen bonds. Surprisingly, an ionic interaction is not observed through this acid functionality. The finding suggested that, in order to remain the key hydrogen-bond interactions, the carboxylic group can be replaced by non-acid functionalities. In this study, compounds such as esters, amides, nitriles, and alcohol derivatives were synthesized and evaluated pharmacologically for COX inhibitory activities. The results showed that diclofenac is the most potent COX-1 and COX-2 inhibitor with %inhibition of 65 and 77, respectively at 10  $\mu$ M concentration. In general, replacement of the carboxylic group of diclofenac with non-acid functionality leads to the dramatic decrease in inhibitory activity against COX-2, but not COX-1. The ester (RKNU070), secondary and tertiary amide (RKNU076-082), nitrile (RKNU072), and alcohol (RKNU071) derivatives are inactive against COX-2 but with %inhibition ranging from 12 to 44 against COX-1, whereas the primary amide derivative (RKNU074) possessed %inhibition of 59 and 59 against COX-1 and COX-2, respectively. Taken all together, it can be concluded that the acid property of diclofenac is essential for binding interaction against COX-2 but less important against COX-1.

**Keywords:** Diclofenac, cyclooxygenase, nonsteroidal anti-inflammatory drugs, carboxylic acid

### Introduction

Cyclooxygenase (COX) is a group of enzyme that plays an important role in inflammatory responses by catalyzing prostaglandins (PGs) biosynthesis from a substrate arachidonic acid (AA). So far, COX has been classified into two isoforms named as COX-1 and COX-2. COX-1 is constitutively expressed isoform found in most tissues and involved with the production of PGs that regulate homeostasis in the body, so-called 'housekeeping' enzyme, whereas COX-2 is primarily inducible and expressed at the site of inflammation by activation of various mediators. Thus, inhibition of COX-2

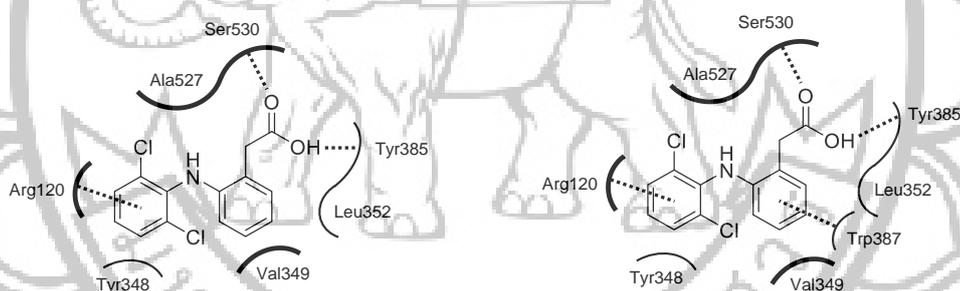
activity is a desirable pharmacological target for the treatment of inflammatory diseases (Vane, Bakhle, & Botting, 1998, pp. 97-120). Structural and molecular studies showed that COX-1 and COX-2 are heme-containing protein with about 63% identical amino acid sequence homology within species. Despite both of the COXs shares a high overall similarity, few differences are observed according to X-ray crystal structures of murine COX-1 and COX-2. The active site of COX-1 is much smaller than COX-2, due to the replacement of isoleucine in COX-1 into valine in COX-2 at the position 434 and 523 (Blobaum, & Marnett, 2007, pp. 1425-1441).



Nowadays, a so-called nonsteroidal anti-inflammatory drugs (NSAIDs) have been commonly used as anti-inflammatory drugs due to their ability to inhibit PGs production by competing with AA at the active site of the enzymes (Vane & Botting, 1998, pp. 97–120). Activities of both isoforms can be inhibited with varying degree by different NSAIDs depending on their affinities. One of most widely used traditional NSAIDs is diclofenac [2-(2, 6-dichloranilino) phenylacetic acid], it has been approved for the treatment of muscle pain, rheumatoid arthritis, osteoarthritis, or ankylosing spondylitis. *In vitro* studies showed that diclofenac is a potent COX inhibitor against human COX-1 and COX-2 with  $IC_{50}$  of 0.26 and 0.01  $\mu$ M, respectively (Cryer, & Feldman, 1998, pp. 413–421). X-ray crystal structures of complexes between

diclofenac and either COX-1 or COX-2 showed that the carboxylic group of diclofenac interacts with tyrosine-385 and serine-530 via hydrogen bonds (Figure 1). Surprisingly, the acid functionality does not generate an expected ionic interaction with amino acids nearby. Although the carboxylic group involves in binding interactions, it is believed as a major cause of severe side effects including gastric ulcer and hepatotoxicity (Aithal, 2011, pp. 139–150; Wolfe, Lichtenstein, & Singh, 1999, pp. 1888–1899).

In order to avoid all aforementioned side effects, the carboxylic group of diclofenac was replaced with various non-acid functionalities using a so-called bioisostere technique (Meanwell, 2011, pp. 2529–2591). Functional groups including esters, amides, nitrile, and alcohols were selected in this study.



**Figure 1** 2D diagram represents X-ray crystal structure of diclofenac/ovine COX-1 complex (left) and diclofenac/murine COX-2 complex (right) obtained from PDB: 3N8Y and 1PXX, respectively. For clarity, some amino acids are omitted.

## Materials and methods

### Materials

All reagents were purchased from commercial suppliers and were used without further purification. Solvents were used either commercial or AR grade. TLC was performed on silica gel plates (Merck Kieselgel 60 F<sub>254</sub> precoated). Column chromatography was carried out using Biotage Isolera One purification system with silica gel SNAP cartridge (porosity 44Å,

particle size 30–90  $\mu$ m).  $^1$ H NMR spectra were recorded on a Bruker Advance 400 spectrometer at 400 MHz using the residual undeuterated solvent peak as reference. High-resolution mass spectra was obtained on an Agilent 6540 UHD Accurate-Mass Q-TOF mass LC/MS spectrometer using electrospray ionization (ESI). FT-IR spectra were recorded using a PerkinElmer Spectrum GX. Melting points were determined on a Buchi B-535 melting point apparatus and are uncorrected.

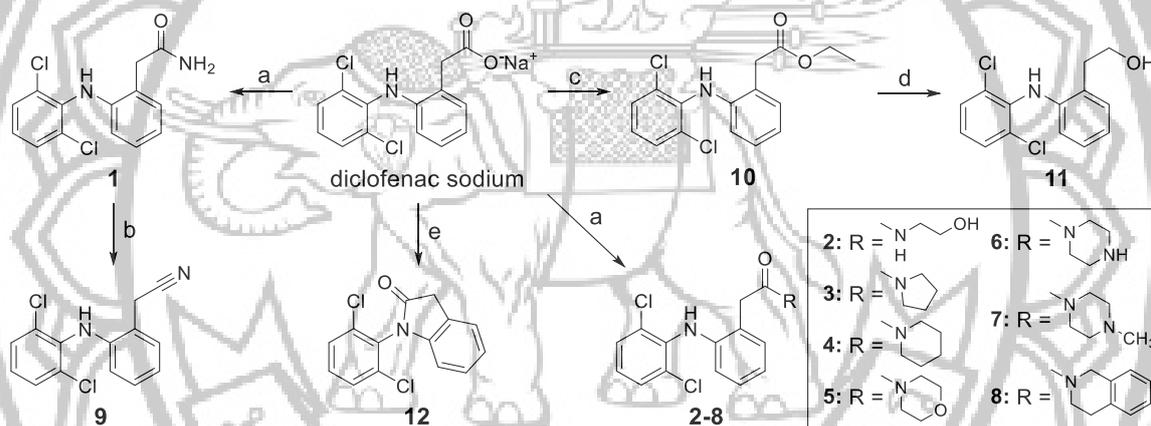


## Methods

Compound **1–12** were synthesized according to scheme 1. First, diclofenac sodium [sodium 2-(2-((2,6-dichlorophenyl)amino)phenyl)acetate] was reacted with corresponding amines in the presence of ethyl chloroformate to give a series of amide derivatives (**1–8**) in a low to moderate yield. The primary amide derivative (**1**) was undergone dehydration reaction by a treatment with cyanuric chloride in a presence of dimethylformamide to give the nitrile derivative (**9**) in a high yield. In order to synthesize the ester derivative (**10**), a so-called

‘acid-catalyzed esterification’ was applied. Diclofenac was dissolved in absolute ethanol in the presence of concentrated sulfuric acid under reflux condition. Unfortunately, the desired ester product was not observed but a cyclic compound (**12**) was obtained in a quantitative yield. Thus, an alternative method was used, diclofenac sodium was treated with iodoethane and potassium carbonate to give an ester derivative in a high yield. The ester was subsequently reduced using a common reducing agent, lithium aluminium hydride, to give an alcohol derivative (**11**) in a moderate yield.

Scheme 1 Synthetic pathway of diclofenac derivatives.



Reagents and conditions: (a) ethyl chloroformate, corresponding amines, THF, 0 °C to r.t.; (b) cyanuric chloride, DMF, r.t.; (c) iodoethane, K<sub>2</sub>CO<sub>3</sub>, acetone, reflux; (d) LiAlH<sub>4</sub>, THF, 0 °C to r.t.; (e) conc. H<sub>2</sub>SO<sub>4</sub>, abs. EtOH, reflux.

### General procedures for synthesis of amide derivatives (**1–8**).

To a solution of diclofenac sodium (1.00 mmol) in dried THF (20 mL) under a stream of N<sub>2</sub>, ethyl chloroformate (4.00 mmol) was added dropwise and stirred at 0 °C for 15 min. Corresponding amines (5.00 mmol) was then added in one portion to the suspension and subsequently stirred at 0 °C to room temperature for 16–24 h. Upon completion of reaction, the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography on silica gel and/or recrystallization.

### 2-(2-(2,6-Dichlorophenylamino)phenyl) acetamide (**1**).

A white solid (yield 17%). mp = 185.0–186.0 °C (dec.); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 7.26–7.34 (d, *J* = 8.1 Hz, 2H), 7.11–7.19 (dd, *J* = 7.5, 4.0 Hz, 3H), 6.93–7.02 (dd, *J* = 7.5, 0.2 Hz, 1H), 6.51–6.53 (d, *J* = 7.5 Hz, 1H), 3.67–3.82 (s, 2H); HRMS *m/z*: 295.0405 [M + H]<sup>+</sup>.

### 2-(2-(2,6-Dichlorophenylamino)phenyl)-N-

(2-hydroxyethyl)acetamide (**2**). A white solid (yield 18%). mp = 151.0–151.5 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 7.37–7.47 (d, *J* = 8.3 Hz, 2H), 7.21–7.28 (dd, *J* = 7.5, 4.4 Hz, 3H), 7.03–7.21 (dd, *J* = 7.5, 0.2 Hz, 1H), 6.39–6.61 (d, *J*



= 7.5 Hz, 1H), 3.81–3.91 (s, 2H), 3.70–3.73 (d,  $J = 5.5$  Hz, 2H), 3.53–3.54 (d,  $J = 5.5$  Hz, 2H); HRMS  $m/z$ : 339.0669 [M + H]<sup>+</sup>.

**2-(2-(2,6-Dichlorophenylamino)phenyl)-1-(pyrrolidin-1-yl)ethanone (3)**. A white solid (yield 8%). mp = 156.2–157.1 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 7.26–7.33 (d,  $J = 8.4$  Hz, 2H), 7.06–7.16 (dd,  $J = 7.5, 4.0$  Hz, 3H), 6.86–6.97 (dd,  $J = 7.5, 0.2$  Hz, 1H), 6.49–6.51 (d,  $J = 7.5$  Hz, 1H), 3.77–3.83 (s, 2H), 3.34–3.37 (m, 4H), 1.83–1.98 (m, 4H); HRMS  $m/z$ : 349.0884 [M + H]<sup>+</sup>.

**2-(2-(2,6-Dichlorophenylamino)phenyl)-1-(piperidin-1-yl)ethanone (4)**. A white solid (yield 13%). mp = 120.0–123.0 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 7.25–7.34 (d,  $J = 8.1$  Hz, 2H), 7.05–7.15 (dd,  $J = 7.5, 4.0$  Hz, 3H), 6.86–6.98 (dd,  $J = 7.5, 0.2$  Hz, 1H), 6.48–6.50 (d,  $J = 7.46$  Hz, 1H), 3.80–3.87 (s, 2H), 3.59–3.62 (t,  $J = 5.69$  Hz, 4H), 1.52–1.64 (m, 6H); HRMS  $m/z$ : 363.1000 [M + H]<sup>+</sup>.

**2-(2-(2,6-Dichlorophenylamino)phenyl)-1-morpholinoethanone (5)**. A white solid (yield 9%). mp = 85.0–88.0 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 7.25–7.35 (d,  $J = 8.1$  Hz, 2H), 7.08–7.13 (dd,  $J = 7.5, 4.0$  Hz, 3H), 6.88–7.00 (dd,  $J = 7.5, 0.2$  Hz, 1H), 6.50–6.52 (d,  $J = 7.5$  Hz, 1H), 3.67 (m, 8H), 3.02 (s, 2H); HRMS  $m/z$ : 365.0833 [M + H]<sup>+</sup>.

**2-(2-(2,6-Dichlorophenylamino)phenyl)-1-(piperazin-1-yl)ethanone hydrochloride (6)**. A white solid (yield 7%). mp = 124.0–127.0 °C; <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O): δ 7.48–7.53 (d,  $J = 8.1$  Hz, 2H), 7.18–7.28 (dd,  $J = 7.5, 4.3$  Hz, 3H), 7.03–7.07 (dd,  $J = 7.5, 0.2$  Hz, 1H), 6.57–6.59 (d,  $J = 8.0$  Hz, 1H), 4.78 (s, 2H), 3.84–4.02 (t,  $J = 4.81$  Hz, 4H), 3.33–3.29 (t,  $J = 5.6$  Hz, 4H); HRMS  $m/z$ : 364.0968 [M + H]<sup>+</sup>.

**2-(2-(2,6-Dichlorophenylamino)phenyl)-1-(4-methylpiperazin-1-yl)ethanone (7)**. A white solid (yield 48%). mp = 70.0–75.0 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 7.20–7.30 (d,  $J = 8.0$  Hz, 2H), 7.07–7.14 (dd,  $J = 7.5, 4.3$  Hz, 3H), 6.87–6.99 (dd,  $J = 6.6, 7.4$  Hz, 1H), 6.51 (d,  $J = 7.5$  Hz, 1H), 3.84 (s, 2H), 3.67–3.70 (t,  $J = 4.9$  Hz, 4H), 2.37–2.40 (t,  $J = 4.9$  Hz, 4H), 2.29 (m, 3H); HRMS  $m/z$ : 378.1156 [M + H]<sup>+</sup>.

**2-(2-((2,6-Dichlorophenylamino)phenyl)-1-(3,4-dihydroisoquinolin-2(1H)-yl)ethan-1-one (8)**. A white solid (yield 11%). mp = 144.0–147.0 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 7.31–7.33 (d,  $J = 8.0$  Hz, 2H), 7.15–7.19 (d,  $J = 6.7$  Hz, 4H), 7.07–7.11 (dd,  $J = 6.4, 6.5$  Hz, 3H), 6.94 (dd,  $J = 8.0, 7.4$  Hz, 1H), 6.50 (d,  $J = 7.5$  Hz, 1H), 4.80 (s, 2H), 3.90–3.96 (m, 4H), 2.87–2.89 (t,  $J = 5.8$  Hz, 2H); HRMS  $m/z$ : 411.1041 [M + H]<sup>+</sup>.

#### Synthesis of 2-(2-(2,6-dichlorophenylamino)phenyl)acetonitrile (9)

To a stirred solution of compound **1** (4.05 g, 13.72 mmol) in anhydrous DMF (20 mL), cyanuric chloride (5.06 g, 27.44 mmol) was slowly added and stirred at room temperature for 15 min. Upon completion of reaction, the mixture was slowly poured into 5% NaHCO<sub>3</sub> solution (250 mL) and EtOAc (200 mL) was then added. The organic layer was washed with H<sub>2</sub>O (2 × 200 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated under reduced pressure. The crude extract was purified by flash column chromatography on silica gel (CH<sub>2</sub>Cl<sub>2</sub>), and recrystallized in Et<sub>2</sub>O/hexane to give a white solid. (3.16 g, 83%). mp = 80.2–80.5 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 7.35–7.39 (d,  $J = 8.1$  Hz, 2H), 7.18–7.34 (dd,  $J = 7.5, 4.0$  Hz, 3H), 7.00–7.09 (dd,  $J = 7.5, 0.2$  Hz, 1H), 6.54–6.62 (d,  $J = 7.5$  Hz, 1H), 3.82–3.85 (s, 2H); IR (KBr, cm<sup>-1</sup>):



2250 (C≡N, stretch); HRMS  $m/z$ : 277.0296 [M + H]<sup>+</sup>.

#### Synthesis of ethyl 2-(2-((2,6-dichlorophenyl)amino)phenyl)acetate (10).

To a stirred solution of diclofenac sodium (5.00 g, 15.72 mmol) in acetone (20 mL) at 0 °C, iodoethane (1.89 mL, 23.57 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 15 h, then refluxed for additional 5 h. Upon completion of reaction, the solvent was removed under reduced pressure. Et<sub>2</sub>O (50 mL) was added and then washed with H<sub>2</sub>O (3 × 50 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (CH<sub>2</sub>Cl<sub>2</sub>) to give a white solid (4.60 g, 90%). mp = 70.0–72.0 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 7.30–7.41 (d,  $J$  = 8.1 Hz, 2H), 7.24–7.30 (dd,  $J$  = 7.5, 4.0 Hz, 3H), 6.99–7.11 (dd,  $J$  = 7.5, 0.2 Hz, 1H), 6.43–6.52 (d,  $J$  = 7.5 Hz, 1H), 4.21–4.24 (m, 2H), 3.94 (s, 2H), 1.24–1.28 (d,  $J$  = 7.2 Hz, 3H); HRMS  $m/z$ : 324.0551 [M + H]<sup>+</sup>.

#### Synthesis of 2-(2-((2,6-dichlorophenyl)amino)phenyl)ethan-1-ol (11).

To a stirred suspension of LiAlH<sub>4</sub> (0.14 g, 3.70 mmol) in dried THF (25 mL) under a stream of nitrogen gas at 0 °C, a solution of compound 10 (1.00 g, 3.08 mmol) in dried THF was added dropwise and the reaction mixture was stirred at room temperature for additional 5 h. Upon completion of reaction, the reaction mixture was cooled down on an ice bath and saturated Na<sub>2</sub>CO<sub>3</sub> solution was added dropwise until H<sub>2</sub> gas was absent. The resulting grey suspension was filtered through Celite and the solvent was removed under reduced pressure. Et<sub>2</sub>O (50 mL) was added and then washed with brine (3 × 50 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated under reduced pressure. The crude product was purified by recrystallized in Et<sub>2</sub>O/hexane to give a white solid

(0.44 g, 50%). mp = 107.0–107.2 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 7.26–7.35 (d,  $J$  = 8.1 Hz, 2H), 7.08–7.25 (dd,  $J$  = 7.5, 4.0 Hz, 3H), 6.94–7.00 (dd,  $J$  = 7.5, 0.2 Hz, 1H), 6.48–6.50 (d,  $J$  = 7.5 Hz, 1H), 4.00–4.03 (d,  $J$  = 5.8 Hz, 2H), 3.00–3.03 (d,  $J$  = 5.9 Hz, 2H); HRMS  $m/z$ : 282.0430 [M + H]<sup>+</sup>.

#### Pharmacological evaluation

COX inhibitory activity was evaluated using a Colorimetric COX Inhibitor Screening Assay Kit from Cayman Chemical Company. The assay was performed using *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD) as co-substrate with AA. TMPD will not turn over without the presence of prostaglandin G<sub>2</sub> (PGG<sub>2</sub>), a hydroperoxide substrate, and oxidized TMPD is monitored spectrophotometrically at 590 nm.

#### Results

According to X-ray crystal complex between diclofenac and either COX-1 or COX-2, hydrogen bonds between the carboxylic acid group of diclofenac and tyrosine-385 and serine-530 seem to play a crucial role for the binding interactions. In order to investigate the role of carboxylic acid functionality of diclofenac whether the acid and/or hydrogen bond donor/acceptor properties of the carboxylic acid is important for the binding interactions. The carboxylic acid group was replaced with non-acid, but maintain hydrogen-bond formation property, functionalities such as amides (RKNU074-082), nitrile (RKNU072), ester (RKNU070), and alcohol (RKNU071). In comparison with diclofenac, the non-acid derivatives generally exhibit no inhibitory activity against COX-2, but still retain inhibitory activity against COX-1 in low to moderate levels ranging from 12 to 59% inhibition at concentration of 10 μM (Table 1). The



secondary amide (RKNU076) possessed 44 and 0% inhibition against COX-1 and COX-2, respectively. The result was in agreement to what was observed for the tertiary amides (RKNU077–082), nitrile (RKNU072), ester (RKNU070), and alcohol (RKNU071). Surprisingly, RKNU074 which is a

primary amide congener of diclofenac expressed the inhibitory activity against both COX-1 and COX-2 with 59 and 59% inhibition, respectively. The %inhibition of RKNU074 is, however, much lower than diclofenac (%inhibition of 65 and 77 against COX-1 and COX-2, respectively).

**Table 1** Percent inhibition of diclofenac and its derivatives against ovine COX-1 and human COX-2

| No. | RKNU | R | %Inhibition |        | No. | RKNU       | R | %Inhibition |        |
|-----|------|---|-------------|--------|-----|------------|---|-------------|--------|
|     |      |   | oCOX-1      | hCOX-2 |     |            |   | oCOX-1      | hCOX-2 |
| 1   | 074  |   | 59          | 59     | 7   | 081        |   | 22          | 0      |
| 2   | 076  |   | 44          | 0      | 8   | 082        |   | 12          | 0      |
| 3   | 077  |   | 41          | 0      | 9   | 072        |   | 20          | 0      |
| 4   | 078  |   | 28          | 0      | 10  | 070        |   | 43          | 0      |
| 5   | 079  |   | 18          | 0      | 11  | 071        |   | 27          | 4      |
| 6   | 080  |   | 32          | 0      |     | Diclofenac |   | 65          | 77     |

Percent inhibition against COX-1 and COX-2 of compounds were measured at 10  $\mu$ M concentration using Colorimetric COX Inhibitor Screening Assay Kit. The values were presented as mean of three independent experiments.

### Discussion and conclusion

Emphasizing the carboxylic acid group of diclofenac, the loss of inhibitory activities of the alcohol (RKNU071) and nitrile (RKNU072) against COX-2 are probably due to the lower number of hydrogen bond donor/acceptor. RKNU071 and RKNU072 contain only one hydrogen bond donor/acceptor functionality ( $-OH$  and  $-C\equiv N$ , respectively). For the ester (RKNU070), secondary and tertiary amides (RKNU076–082) derivatives which consist of an equal number of hydrogen bond donor/acceptor functionality to the carboxylic acid, their inhibitory activities against COX-2 are still lost. The results were in contrast to what was observed for the primary amide derivative since RKNU074 still

remain a moderate inhibitory activity against COX-2. Thus, the loss of inhibitory activity of RKNU070 and RKNU076–082 may due to steric hindrance of the additional substituent(s). In comparison to the carboxylic acid moiety, the primary amide which has an equal number of hydrogen bond donor/acceptor functionality, has no substituent, but no acid property still resulted in a lower inhibitory activity against COX-2; RKNU074 exhibited a 59% inhibition for COX-2. These findings indeed suggested that acid property seems to be more important factor for the binding interactions between diclofenac and COX-2 than hydrogen bonds. On the other hand, replacement of the carboxylic acid of diclofenac with non-acid functionalities provided less effect for COX-1.



RKNU070-082 still remain inhibitory activity even lower than diclofenac.

Taken all together, it can be concluded that the acid property of diclofenac is considered as more important factor for binding interaction against COX-2 than the hydrogen-bond formation ones. Loss of acid property and/or addition of the substituent, however, does not affect the binding interaction for COX-1 as much as seen for COX-2.

Thus, for further design of selective COX-2 inhibitors, the carboxylic acid of diclofenac which is the major causes of gastric ulcer and hepatotoxicity may be replaced by other groups with retained acid property such as hydroxamic acid, hydrazide, or tetrazole.

#### Acknowledgement

This research is partly granted by graduate student's scholarship, Faculty of Pharmaceutical Sciences, Naresuan University. The authors would like to thank Assoc. Prof. Dr. Tasana Pitaksuteepong, Assist. Prof. Dr. Sutthatip Markmee, and Dr. Nitra Nuengchamnong for valuable technical supports.

#### References

Aithal, G. P. (2011). Hepatotoxicity related to antirheumatic drugs. *Nature Reviews Rheumatology*, 7(3), 139-150.

Blobaum, A. L., & Marnett, L. J. (2007). Structural and functional basis of cyclooxygenase inhibition. *Journal of Medicinal Chemistry*, 50(7), 1425-1441.

Cryer, B., & Feldman, M. (1998). Cyclooxygenase-1 and cyclooxygenase-2 selectivity of widely used nonsteroidal anti-inflammatory drugs. *The American Journal of Medicine*, 104(5), 413-421.

Meanwell, N. A. (2011). Synopsis of some recent tactical application of bioisosteres in drug design. *Journal of Medicinal Chemistry*, 54(8), 2529-2591.

Vane, J. R., Bakhle, Y. S., & Botting, R. M. (1998). Cyclooxygenases 1 and 2. *Annual Review of Pharmacology and Toxicology*, 38, 97-120.

Vane, J. R., & Botting, R. M. (1998). Mechanism of action of nonsteroidal anti-inflammatory drugs. *The American Journal of Medicine*, 104(3a), 2S-8S; discussion 21S-22S.

Wolfe, M. M., Lichtenstein, D. R., & Singh, G. (1999). Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. *New England Journal of Medicine*, 340(24), 1888-1899.