Can Tho University Journal of Science
website: sj.ctu.edu.vn

DOI: 10.22144/ctu.jen.2020.027

In vitro anti-inflammatory resorcinol derivatives and their *in silico* analysis

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Article info.

Received 11 Jun 2020
Revised 10 Sep 2020
Accepted 30 Nov 2020

Keywords

Anti-inflammatory, docking, nitrobenzoic acid, resorcinol

ABSTRACT

Resorcinol with its two hydroxyl groups was derivatized in laboratory to observe the anti-inflammatory potential in vitro. Subsequently, in silico docking analysis was done for observing the binding modes in cyclooxygenase enzyme to have idea about the subsequent possible developments. At the doses of 200 μg/mL and 400 μg/mL, the compounds showed the anti-inflammatory property. Among them, 1,3-phenylene bis(2-chloro-4-nitrobenzoate) also offered dose dependent 51% and 70% of inhibition of heat-induced hemolysis respectively. The scaffold thus poses as an interesting pharmacophore suitable for further development for managing the inflammatory disorders.

Cited as: Mahmuda, R., Islam, S., Shoshi, N.S., Poly, K.A., Saha, P., Akhter, S., De, T.Q. and Bepary, S., 2020. *In vitro* anti-inflammatory resorcinol derivatives and their *in silico* analysis. Can Tho University Journal of Science. 12(3): 80-84.

1 INTRODUCTION

Cyclooxygenase (COX) is responsible for formation of prostanoids (Jane, 2019) including thromboxane and prostaglandins like prostacyclin, from arachidonic acid. The two types, namely COX-1 and COX-2 having minor variations in structure and distribution pattern in the human body, are responsible for different types of human chronic inflammatory disorders like cancer, cardiovascular diseases, diabetes, obesity, osteoporosis, rheumatoid arthritis, inflammatory bowel disease, asthma, depression and Parkinson's disease, etc. Though there are diversified classes of non-steroidal anti-inflammatory drugs (NSAIDs), most of them results in gastrointestinal complications (da Silva Guerra *et al.*, 2011; dos Santos *et al.*, 2012; Elhenawy *et al.*, 2014; Suryawanshi *et al.*, 2014, Goldstein *et al.*, 2015) including primary local irritation to gastrointestinal ulceration. These agents are not suitable for long term applications in managing the chronic inflammatory disorders, thereby demanding the better alternatives. Thus, this scaffold has been considered for study and the results have been reported here.

2 EXPERIMENTAL SECTION

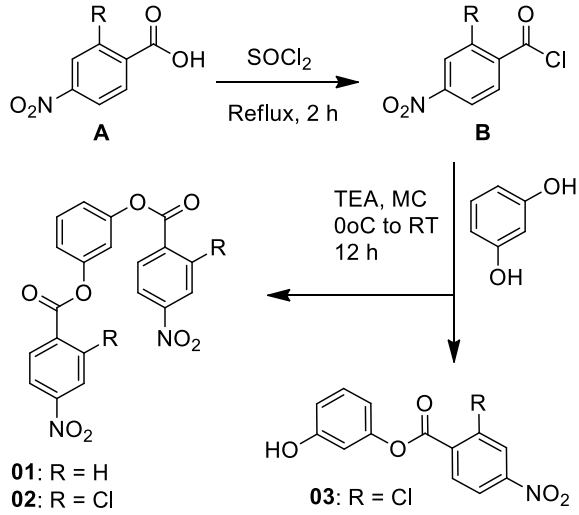
2.1 Materials and methods

The necessary reagents were purchased from Sigma-Aldrich (USA) and TCI (Japan). Methanol and dichloromethane were collected from Duksan Pure Chemicals Co. Ltd, South Korea. Solvents were collected from Daejung Chemical & Metal Co. Ltd. Derivatives were synthesized in the laboratory and then were purified by flash column chromatography using silica gel (45-100 μ). The reaction end

points were checked by the TLC using Sigma-Aldrich Glass plates having silica gel coated with fluorescent indicator F254. The compounds were characterized by ^{1}H NMR by Bruker 400 MHz.

2.2 Synthesis of resorcinol derivatives

To the stirred solution of appropriate benzoic acid (**A**) was added 3.0 equivalent of thionyl chloride and the resulting solution was refluxed for 2 hours (Scheme-1). After subsequent removal of excess thionyl chloride by vacuum evaporation, dry dichloromethane was added. Under an ice bath system then were added triethylamine and resorcinol. Stirring was continued for overnight and reaction end point was confirmed by using of TLC. After subsequent addition of distilled water, the organic layer was collected by a separating funnel, washed successively by brine and water. The organic layer was then dried with sodium sulfate before it was subjected to filtration by using Whatman filter paper to collect the filtrate. The solvent was evaporated *in vacuo* to afford crude esters which were then purified by flash column chromatography to get the desired products (**01**-**03**, 82-88% yield).



Scheme 1: Synthesis of resorcinol derivatives

2.3 Observation of the *in vitro* anti-inflammatory activity

Anti-inflammatory activity was done by following the reported methods (Okoli *et al*., 2008) with some minor modifications. Isotonic buffer solution (5 mL) was taken in centrifuge tubes containing 200 and/or 400 µg/mL of the test drugs. The centrifuge tubes were taken in 4 sets (per concentration). Tubes containing 5 mL of the vehicle only were taken for control. Reference standards were taken as 2 sets of 5 ml buffer solution containing 100 µg/mL of ibuprofen. Erythrocyte suspensions (0.05 mL) were added to each tube. After gentle mixing, one set of the tubes was incubated at 54°C for 20 minutes and the other pair was maintained at 0–4°C in a freezer for 20 minutes. The reaction mixture was then centrifuged at 3,000 rpm for 3 minutes at the end of the incubation and absorbance (OD) of the supernatant was measured at 540 nm using isotonic buffer solution as the blank.

The level of hemolysis was calculated by using the following equation:

Percentage of hemolysis = $100 \times (OD_2\text{-}OD_1)/(OD_3\text{-}OD_1)$

Where, OD_1 = absorbance of test sample unheated; OD_2 = absorbance of test sample heated;

OD_3 = absorbance of control sample heated.

The percentage inhibition of hemolysis was calculated by using the following relation:

Inhibition of hemolysis (%) = 100 – hemolysis (%)

2.4 Docking analysis

The compounds were subsequently docked into the cyclooxygenase-2 enzyme. The enzyme protein Data Bank (PDB) was collected from the internet (Orlando *et al*., 2015). The original PDB was having the reference drug ibuprofen docked into the ligand binding site of cyclooxygenase-2 enzyme. However, after getting the original PDB file, the ligand

was separated out and the receptor PDB was processed for the docking studies.

2.4.1 Preparation of the ligand file of the synthesized compounds

The compound structures were drawn by using the ChemDraw software. The structures thus obtained were in 2-D from and thus were converted to 3-D which were then transformed to PDBQT files by using the Python Molecular Viewer (PMV).

2.4.2 Docking of the ligand PDBQT in the receptor PDBQT file

The ligand PDBQT files were then docked in the receptor PDBQT by using the Autodock Vina software (Trott *et al*., 2010). The output PDBQT files were then viewed by using the PyMOL software. The binding modes with lowest energy were taken under consideration for interaction analysis. This selected ligand PDBQT files were taken along with the ligand PDB to find out the binding interactions and next possible improvements.

3 RESULTS AND DISCUSSION

3.1 Synthesis of resorcinol ester of benzoic acids

To the benzoic acid, thionyl chloride, triethylamine, resorcinol, and other necessary chemicals, reagents and utilities were collected from local suppliers. The laboratory condition was sufficient for getting very good yields. The process was reproducible and thus can be used for further derivatizations if desired. Compounds were characterized by using the ^{1}H NMR available in the Bangladesh Council of Scientific and Industrial Research (BCSIR).

3.2 Proton NMR data of the synthesized compounds

Comound (**01**) 1,3-Phenylene bis(4-nitrobenzoate) is an orange solid with yield of 77% and ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.20-7.24 (m, 2H), 7.54 (t, J = 8.0 Hz, 1H), 8.26-8.39 (m, 9H).

Comound (**02**) 1,3-Phenylene bis(2-chloro-4-nitrobenzoate) is a pale orange solid with yield of 81% and ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.23-7.29 (m, 3H), 7.54 (t, J = 8.0 Hz, 1H), 8.17-8.20 (m, 2H), 8.23-8.25 (m, 2H), 8.39 (m, 2H).

Comound (**03**) 3-Hydroxyphenyl 2-chloro-4-nitrobenzoate is a yellow solid with yield of 79% and ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.76-6.82 (m, 3H), 7.28 (t, J = 8.0 Hz, 1H), 8.13-8.15 (m, 1H), 8.21-8.26 (m, 1H), 8.37-8.38 (m, 1H).

3.3 Biological evaluation of resorcinol ester of benzoic acids

The compounds were observed for the anti-inflammatory property by using the *in vitro* methods where the efficacy in preventing the heat induced hemolysis of the human red blood cells (HRBC) was observed. The results obtained have been given in the Table 1.

Table 1: Efficacy of compound 01, 02 and 03 in preventing the heat induced hemolysis

Sample	Strength (µg/ml)	Absorbance		Lysis (%)	Inhibition (%)
		Hot	Cold	(Heat-Cool)/ (Control-Cool)*100	(100-Lysis%)
01	200	0.060	0.048	44	56
02	200	0.052	0.031	49	51
	400	0.043	0.029	30	70
03	200	0.064	0.038	72	27
Ibuprofen	100	0.038	0.030	17	83
Control	Blank	0.075	-	-	-

The compound **03** was found to be comparatively weak (27% inhibition) in preventing the lysis of HRBC. Whereas, compound **02** having di-substitution, showed significantly higher inhibition (51%). There was a good dose-depended increase in the efficacy as shown by 400 µg/mL (70% inhibition). The compound **01**, having di-substitution but no halogen, was comparable with **02**. Ibuprofen was used as the reference compound in this study.

3.4 *In silico* analysis for the binding pattern

Docking analysis was done by using the Autodock Vina software published from The Scriffs Research Institute. Compound **01** and **02** showed similar types of inhibition and so the former was taken for this study. In this study, various modes were observed from the output PDBQT files having different energy levels. The lowest energy mode was taken into consideration for further prediction.

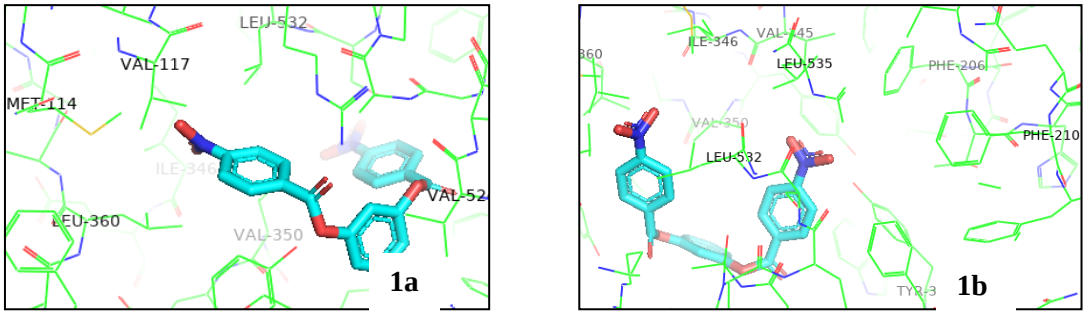


Figure 1: Projection of nitrobenzene groups to a non-polar pocket of the COX enzyme

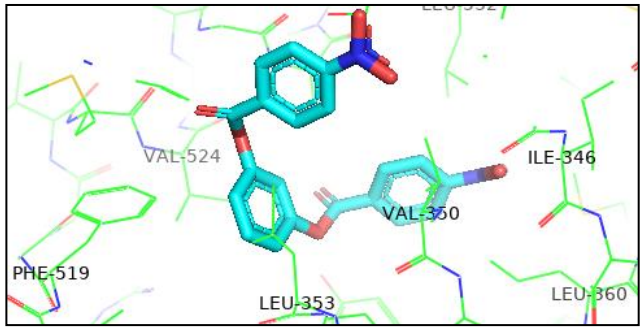


Figure 2: Access of the central phenyl to a non-polar site of the COX enzyme

One of the nitrobenzene moieties was found to be projected to a side pocket having the non-polar characters due to the dominance of the side chains from Val-117, Met-114, Leu-532, and Leu-360 as shown in Figure 1. Thus more groups can be tried based on this study. The other nitrobenzene ring was also found to be projected to another non-polar site though was staying little far. Accordingly, this ring can be replaced to ensure proper fit in this pocket. On the other hand, the central phenyl ring has projected its unsubstituted part to another site having non-polar residues like, Phe-529, Val-523, Leu-353. But as shown in Figure 2, there is little more blank space which can be utilized for introducing additional non-polar groups and/or atoms on this central ring.

4 CONCLUSIONS

It is obvious that the resorcinol derivatives possessed encouraging potency for inhibiting the heat-induced lysis of the HRBC membrane which may be significant while targeting the chronic inflammatory disorders. Moreover, since this scaffold does not bear any carboxylic acid group, this may be better tolerated option for the long-term managements. Thus further study should be done with this scaffold to develop more derivatives and thereby to make a comprehensive structure-activity-relationship study with this pharmacophore.

ACKNOWLEDGMENTS

We are thankful to Jagannath University and University Grants Commission of Bangladesh for providing the required laboratory facilities and complementary financial supports for this project.

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