

Original Article

## MOLECULAR DOCKING, PHARMACOPHORE MODELLING, AND ADME-TOXICITY PREDICTION OF CURCUMIN ANALOG COMPOUNDS AS INFLAMMATORY INHIBITOR ON RHEUMATOID ARTHRITIS

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### ABSTRACT

**Objective:** The objective of this research was to examine the activity and cytokine inhibitory mechanism of curcumin analog compound against multiple protein targets in a patient with rheumatoid arthritis (RA) and identify the absorption, distribution, metabolism, excretion and toxicity (ADME-toxicity).

**Methods:** Identification was carried out by *in silico* through pharmacophore modelling using Ligand Scout, molecular docking using iGemDock in various protein (tumor necrosis factor alpha (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ), transcription factors, signalling kinase, and cyclooxygenase enzyme) and identification of ADME-toxicity based on the physicochemical properties of the compound to simulate, predict and analyze interaction between protein and compound.

**Results:** The obtained results indicated that gamavuton (GVT-0) and penta-gamavuton (PGV) possessed high bioavailability with lower toxicity than curcumin. However, GVT-0, a curcumin analog, possessed high and specific inhibitory activity on tumor necrosis factor- $\alpha$  converting enzyme (TACE) and interleukin converting enzyme (ICE)/Caspase-1.

**Conclusion:** GVT-0 as a curcumin derivate possessed the best inhibitory activity against TNF- $\alpha$  converting enzyme and IL-1 $\beta$  converting enzyme which are the main route of inflammatory mediators in rheumatoid arthritis. In addition, GVT-0 influences less in metabolism of CYP450 enzymes, and has low toxicity.

**Keywords:** Molecular docking, Gamavuton (GVT-0), Rheumatoid arthritis, TNF- $\alpha$ , IL-1 $\beta$

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### INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease that affects the small joints of the hands and feet equally and symmetrically on the both sides. The inflammation is caused by the cytokine accumulation in the joints [1]. Cytokines induce chondrocyte apoptosis and decrease the synthesis of the main component of the extracellular matrix (ECM). It also stimulates the other inflammatory cytokines, including interleukin-1 $\beta$  (IL-1 $\beta$ ), tumour necrosis factor alpha (TNF- $\alpha$ ), interleukin-6 (IL-6), and chemokines CCL5 through autocrine or paracrine signalling. Thus, it can aggravate the condition of RA [2].

Tumor necrosis factor alpha (TNF- $\alpha$ ) and interleukin-1 (IL-1) are the cytokine immune systems that play an important role in inflammation [3]. TNF- $\alpha$  is a pro-inflammatory cytokine that is able to induce a variety of signalling pathways of the immune system and contribute to tissue inflammation. Inhibition of TNF- $\alpha$  would suppress the function of macrophages and T-cells, and prolong the inflammatory changes as well [4, 5]. IL-1 has 11 families, which play a role in the regulation of immunity and also related to the release of interleukin-2 (IL-2) and the proliferation of B-cells and T-cells. IL-1 $\beta$  is a potential hyperalgesic agent. The release of IL-1 $\beta$  can be induced by TNF- $\alpha$ -dependent and TNF- $\alpha$ -independent. Moreover, IL-1 $\beta$  is the main cytokine which stimulates the expression of cyclooxygenase-2 (COX-2) during inflammation [6, 7].

In the previous study, there was a close relationship among p38 mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK), protein kinase-C (PK-C), nuclear factor kappa B (NF $\kappa$ B), and other enzymes that regulate the RA condition

to the production of pro-inflammatory cytokine that leads to the activation of TNF- $\alpha$  and IL-1 $\beta$  receptor [8].

Curcumin is commonly used in the traditional medicine for anti-inflammatory, especially to suppress cytokine productions [9]. Gamavuton (GVT-0) and penta-gamavuton (PGV) are curcumin analog through chemical structure modification. Both of them have an anti-inflammatory activity to suppress TNF- $\alpha$  and IL-1 $\beta$  in the release of cytokine during inflammation [10, 11]. In this study, we conduct the molecular docking, which is a method that can predict the preferred orientation of one molecule to a second when bound to each other to form a stable complex [12].

The molecular docking in this study is conducted to examine the activity and cytokine inhibitory mechanism of curcumin analog compounds against multiple protein targets in patient with RA and identify the absorption, distribution, metabolism, excretion and toxicity (ADME-toxicity).

### MATERIALS AND METHODS

#### Materials

The structure of the curcumin compound and its derivatives, curcumin-keto, curcumin-enol, gamavuton (GVT-0) and penta-gamavuton (PGV), and Methotrexate and Paracetamol as comparison drug were designed in 3D using *Marvin Sketch* (ChemAxon). The ligand was made by MDL MOL format (.mol).

The macromolecular proteins were downloaded from a database of Protein Data Bank (<http://www.rcsb.org>) with different types of proteins that mediate inflammation in RA [13].

The macromolecular proteins that were included in this study are tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), (MAPK-14 [1YW2]; TNF- $\alpha$  converting enzyme [3KME]), interleukin-1 (IRAK-4 [4RMZ]; interleukin-1 beta converting enzyme [3NS7]), transcription factor (nuclear factor kappa-B [4KIK]; ERK [20]) signaling kinase (protein kinase-C [1XJD]), and cyclooxygenase enzyme (COX-1 [1EQG], COX-2 [6COX]). The selected target proteins have native ligands and were validated by calculating the RMSD < 2.0 Å.

### Instrumentation

The instruments that were used are Marvin Sketch (ChemAxon: *Academic License*), iGemDock v2.4 (BioXGEM: *Open Source*), LigandScout (Inte Ligand GmbH: *Academic License*), Chimera (UCSF: *Academic License*).

### Methods

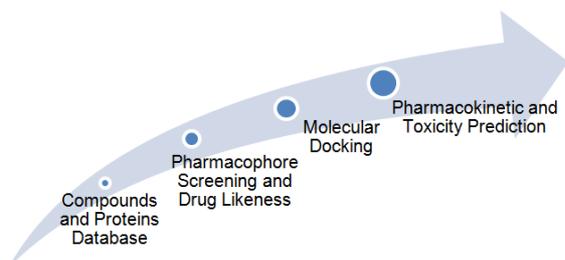


Fig. 2: *In silico*-based research road map

### Pharmacophore screening and drug likeness

Pharmacophore study was conducted to assess the part of the structure of compounds that play a role in a pharmacological effect. Moreover, it also examined the interaction between ligand and protein, including the form of energy based on Pharmacophore-Fit Score, H-bond donor (HBD), H-bond acceptor (HBA), aromatic. Additionally, the prediction of drug-likeness was conducted to

identify the compounds that possessed physicochemical characteristic 90% of the value of the general medicinal properties. Identification of drug-likeness was calculated by using the website (<http://www.scfbio-iitd.res.in/> and <https://pubchem.ncbi.nlm.nih.gov/>) [14, 15].

### Molecular docking and visualization

The testing of the ligand compound activity against the receptor was carried out by using software of iGemDock v2.4, with the size of *binding site* corresponded with the result of protein validation. The docking score result was displayed in the *fitness Score on Post Screening Analysis. Screening analysis* was carried out with the population size (300), generation (80), and the resulting solution (3).

### ADME-toxicity prediction

Pharmacokinetic analysis of curcumin derived compound related to lipophilicity and hydrophilicity compound. Prediction of its pharmacokinetic profile was carried out by using ADMETSAR server (<http://lmd.ecust.edu.cn:8000/predict/>) [16], which the prediction of absorption, metabolism, distribution, excretion, and toxicity of curcumin derivatives were included.

## RESULTS AND DISCUSSION

### Pharmacophore screening and drug-likeness

Pharmacophore group plays an important role in generating the pharmacological activity when interacting with the target protein. In pharmacophore modeling, it displays the role of steric and electronic to form the optimal interaction at a specific target, whether it is an analog or antagonist [17]. Based on the identification result, curcumin derivatives possess a group that acts as pharmacophore, including aromatic rings, H-bond donor (HBD), H-bond acceptor (HBA). In general, in (fig. 3a, 3b, and 3c) curcumin and its derivatives possess the same active group (analog), named the aromatic rings, HBD, and HBA. In addition, pharmacophore study reviewed the point of interaction on a particular group, then none of the same group showed pharmacophore.

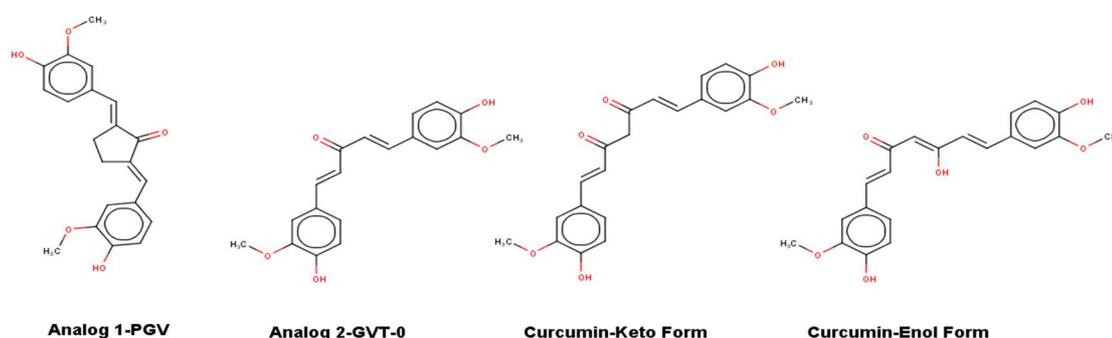
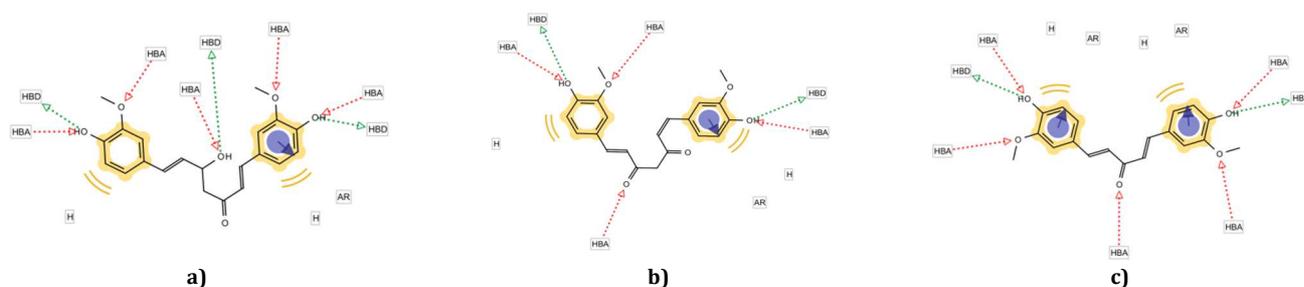
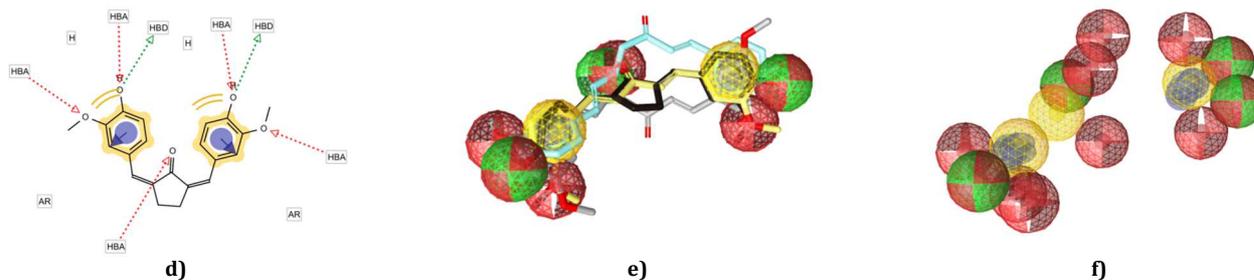


Fig. 1: Curcumin compound and its derivatives (designed using MarvinSketch)





**Fig. 3: Pharmacophore identification of curcumin and its derivatives; a) Curcumin-Enol, b) Curcumin-Keto, c) Gamavuton, d) Penta-Gamavuton, e) Ligand Pharmacophore, f) Pharmacophore model of curcumin and its derivatives (created using LigandScout)**

Each of ligands occupying the pharmacophore point in (fig. 3 e and f) indicate the compatibility level. Gamavuton and penta-gamavuton are the derivatives with similar properties, and Pharmacophore-Fit Score of gamavuton is known higher than penta-gamavuton (table 1). Curcumin has 2 tautomeric forms. The enol-tautomeric is a transition from keto-tautomeric due to the electron. But the the

difference of keto and enol tautomeric indicates the difference in the interaction of the target protein, which indicates that enol-tautomeric has the greater activity than the keto-tautomeric.

Enol tautomeric indicates more polar properties and more and more interaction with proteins [18].

**Table 1: Pharmacophore score of curcumin and its derivatives**

Compound	Pharmacophore-fit score
Gamavuton	111.58
Penta-Gamavuton	111.31
Curcumin-Enol	104.26
Curcumin-Keto	92.18

Lipinski's rule is used in the observation to assess bioavailability with various criteria. In general, curcumin and its derivatives can be absorbed well by calculating topological polar surface area (TPSA)<140 Å, molecular mass less than 500 Dalton, lipophilicity (Log P<5), HBD<5, HBA<10, and refractivity index between 40-130.

Tautomeric of keto and enol curcumin possess greater TPSA than its derivated compounds, which significantly possess lower bioavailability (table 2) [19].

Similarly, the high value of HBD and HBA in both compounds will increase the excretion.

**Table 2: Bioavailability identification through lipinski's rule**

Parameter	Compound			
	Curcumin-Keto	Curcumin-Enol	PGV	GVT-0
Formula Structure	C <sub>21</sub> H <sub>20</sub> O <sub>6</sub>	C <sub>21</sub> H <sub>20</sub> O <sub>6</sub>	C <sub>21</sub> H <sub>20</sub> O <sub>5</sub>	C <sub>19</sub> H <sub>18</sub> O <sub>5</sub>
Molecular mass (g/mol)	368.38	370.40	326.35	352.39
LogP	2.30	2.30	3.4	3.8
HBD	2	2	2	2
HBA	6	6	5	5
Molar Refractivity	102.06	103.02	92.39	98.71
TPSA (Å)	93.07	96.22	76.00	76.00

### Molecular docking

The occurrence of RA can be mediated by many inflammatory pathways mainly related to the immune systems such as TNF- $\alpha$ , interleukin-1 $\beta$ , signalling kinase, protein kinase-C, or cyclooxygenase [8]. The molecular docking result indicates that the enol tautomeric possesses higher activity than the keto tautomeric, which is consistent with the predictions using pharmacophore. In fact, these two types of curcumin generally possess greater activity than its derivatives despite possessing some differences. In TNF- $\alpha$  pathways, tumor necrosis factor- $\alpha$  converting enzyme (TACE) plays an important role in regulating TNF- $\alpha$  pathway that changes pro-TNF- $\alpha$  into active TNF- $\alpha$  that binds to TNF- $\alpha$  receptor so that it generates inflammation [20]. In this enzyme, GVT-0 possesses greater pharmacological activity than PGV and curcumin-enol. Although in the another target protein in TNF- $\alpha$  pathway, GVT-0 activity is not much different with PGV.

In the interleukin-1 $\beta$  path, the interleukin converting enzyme (ICE) processes immature pro-IL-1 $\beta$  into an active mature IL-1 $\beta$  that binds

to the receptor of interleukin (IL-1R1), which will induce multiplication of cytokines through the translation process, which is the main route bound IL-1 $\beta$  on its receptor [21]. In (fig. 5), it indicates that gamavuton possesses higher pharmacological activity than PGV. While the activities of its inhibition of cyclooxygenase enzyme-1 (COX-1) and cyclooxygenase enzyme-2 (COX-2) will reduce pain mediator. In the previous study, curcumin is known to possess selective activity against COX-1, even though it also inhibits COX-2 with a lower activity. Meanwhile, GVT-0 and PGV possess similar activities with selective COX-2 [11]. This study shows that GVT-0 and PGV play a role in the inhibition of both COX-1 and COX-2. However, GVT-0 indicates greater activity against COX-1 compared to PGV.

Thus, based on the molecular docking in variety of target proteins associated with the inflammatory cascade in RA, it is known that GVT-0 possesses specific target as the main pathway that plays an important role in RA, inhibitor of TACE in TNF- $\alpha$ , and inhibitor of ICE in IL-1 $\beta$ . It also inhibits COX-1 and COX-2 as the emergence of pain pathways. Tumor necrosis factor alpha is produced by activated

monocytes/macrophages and T-cell, and is located on the macrophage cell membrane when it is not activated. When macrophages are active then its release depends on TACE that is the enzyme containing disintegrin and metalloproteases (ADAM) which is responsible for processing inactive form of tumor necrosis factor

alpha into its active soluble form which binds to TNF- $\alpha$  so that it causes the formation of the receptor signaling pathways for apoptosis, inflammatory response or cytokine production [20,21]. Therefore, inhibition of the release of soluble TNF- $\alpha$  can suppress the inflammatory cascade in RA patients.

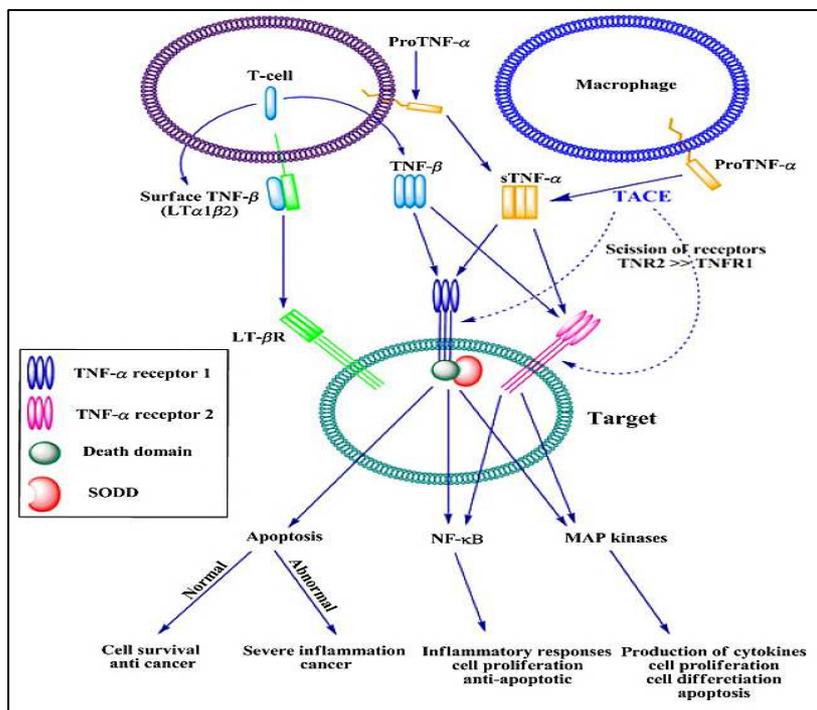


Fig. 4: Signaling and inhibition pathway of TACE [17]

In interleukin-1 pathway, there are two types of cytokines that are IL-1 $\alpha$  and IL-1 $\beta$ , primary cytokines, and both are tied to the same receptor that is IL-1R1. Attention to the IL-1 $\beta$  cytokine is emphasized because the increase in the secretion of IL-1 $\beta$  cause an auto-inflammatory disease, but the increase in secretion of IL-1 $\alpha$  does not indicate it. Activation of the pain and inflammation occur due to the IL-

1 $\beta$  binding to IL-1R1. The IL-1 $\beta$  cytokine can be secreted through a various channels to secrete pro-IL-1 $\beta$ . However, inhibition of ICE which changes pro-IL-1 $\beta$  into active IL-1 $\beta$  can block the binds of IL-1 $\beta$  to its receptor. Thus, the induction of cytokine propagation through the translation process, which is the main route where IL-1 $\beta$  binds to its receptor can be suppressed [6, 22, 23].

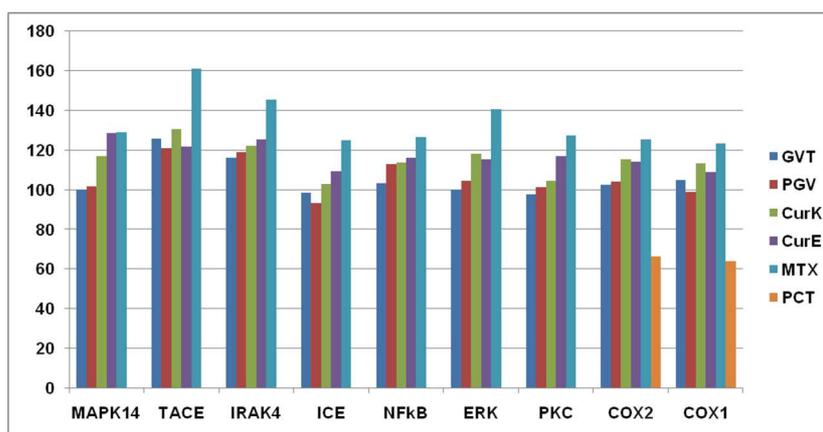


Fig. 5: Pharmacological activity of curcumin derivatives againts inflammation (Analyzed using iGemDock v2.4)

**ADMET identification**

The physicochemical properties of the compounds will provide information related to bioavailability in the body. Pharmacokinetic identification is required to predict the drug trip in the body including the absorption, distribution, metabolism,

excretion, and toxicity. Absorption is related to the active substance in any type of place of absorption.

The blood brain barrier (BBB) permeability of a compound depends on several factors such as lipophilicity, hydrogen bond desolvation potential, molecular size and pKa charge [24].

Curcumin-keto and enol undergo absorption in the BBB area but possess low permeability values in the Caco-2 cell permeability at 0.648 and 0.814 cm/s, which is caused by that curcumin-keto and enol are more hydrophilic than GVT-0 and PGV. Meanwhile, GVT-0

permeability is higher than PGV, which is 1,956 cm/s. In addition, GVT-0 possesses less inhibition in the metabolism of the CYP450 enzyme, with possessing lower toxicity than curcumin keto,-enol, and PGV.

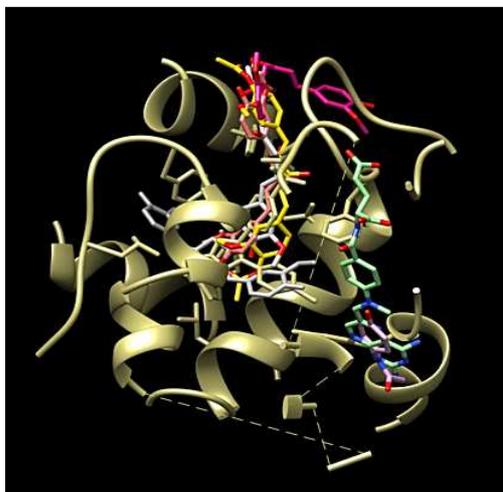


Fig. 6: Docking visualization of curcumin compound and its derivatives on the target protein (created using chimera)

Table 3: ADMET of curcumin and its derivatives (Analyzed using ADMETSAR)

Parameter	Compound			
	Curcumin-Keto	Curcumin-Enol	GVT-0	PGV
Blood-Brain Barrier	+	+	-	-
Human Intestinal Absorption	+	+	+	+
Caco-2 Permeability (cm/s)	+(0.648)	+(0.814)	+(1.956)	+(1.327)
P-glycoprotein Substrate	S	S	S	S
P-glycoprotein Inhibitor	I	I	NI	NI
Renal Organic Cation Transporter	NI	NI	NI	NI
CYP450 2C9 Substrate	NS	NS	NS	NS
CYP450 2D6 Substrate	NS	NS	NS	NS
CYP450 3A4 Substrate	NS	NS	NS	S
CYP450 1A2 Inhibitor	I	I	I	I
CYP450 2C9 Inhibitor	I	NI	I	I
CYP450 2D6 Inhibitor	I	NI	NI	NI
CYP450 2C19 Inhibitor	I	I	I	I
CYP450 3A4 Inhibitor	NI	NI	I	NI
Human Ether-a-go-go-Related Gene Inhibition	WI	WI	WI	WI
AMES Toxicity	NT	NT	NT	NT
Carcinogens	NC	NC	NC	NC
Fish Toxicity	HT	HT	HT	HT
Fish Toxicity (pLC50, mg/l)	(-0.3779)	(0.1049)	(0.5979)	(0.2833)
Honey Bee Toxicity	HT	HT	HT	HT
Biodegradation	NRB	NRB	NRB	RB
Acute Oral Toxicity	III	III	III	III

## CONCLUSION

Gamavuton (GVT-0) as a curcumin derivative possesses the best inhibitory activity against TNF- $\alpha$  converting enzyme and IL-1 $\beta$  converting enzyme which are the main route of inflammatory mediators in rheumatoid arthritis. In addition, GVT-0 influences less in the metabolism of CYP450 enzymes and possesses low toxicity.

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## AUTHOR CONTRIBUTION

We declare that all of the authors of this article have contributed since the beginning of the study process until the submission process of this article.

## CONFLICT OF INTERESTS

Declared none

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