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RESEARCH ARTICLE

Chemistry behind the Synergism of Diclofenac and Vitamin B1

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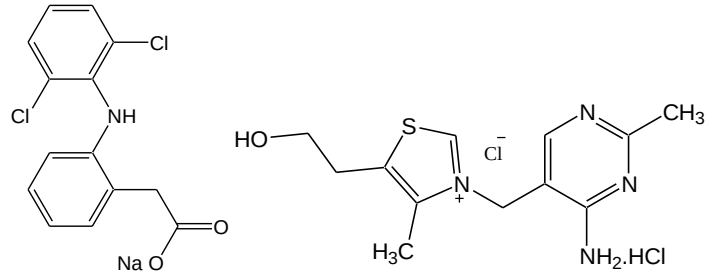
Abstract

A thorough study of the synergism of diclofenac and vitamin B1 was performed using different spectroscopic tools and molecular modeling. We aim to clarify the type of interaction of the studied drugs and how they act together by studying the chemistry of their synergism. The drugs were found to react with each other by non-covalent interactions. The predicted structure by molecular simulation is online with IR and ^{1}H-NMR results. Mass spectrometry was used to confirm the interaction of the drugs. Molecular docking studies were performed to investigate the interaction of the individual drugs and their interaction product with the human cyclooxygenase-2 enzyme (COX-2). The potential energy of the docking results shows that the affinity of the vitamin to COX-2 protein is greater than that of diclofenac. Moreover, vitamin B1 was found to share the active site of COX-2 with diclofenac. As a result, the attachment of the substrate will be hindered more effectively due to blocking of possible active sites and conformational changes of the enzyme structure. Consequently, the drugs act synergistically to inhibit the biosynthesis of prostaglandins inside the human body.

INTRODUCTION

Synergism is a well–known drug interaction in pharmacological sciences. It is the joint action of agents so that their combined effect is greater than the algebraic sum of their individual parts (http://medical-dictionary.thefreedictionary.com/synergism). It was found in previous studies)Mibielli et al., 2009; Brüggemann et al., 1990; França et al., 2001; Reyes-Garcia et al., 2002; Rocha-González et al., 2004) that vitamin B1, known as thiamine hydrochloride (TH), 3-[(4-Amino-2-methylpyridin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium chloride hydrochloride, enhances the anti-inflammatory and analgesic effects of diclofenac sodium (DIC), sodium [2-[(2,6-dichlorophenyl)amino] phenyl] acetate. Moallem et al. studied the acute and chronic anti-nociceptive and anti-inflammatroy effects of TH in mice (Moallem et al., 2008). However, it was not clear how they work together to inhibit prostaglandins synthesis.



DIC.Na TH Chloride HCl

It was proved that the anti-inflammatory and analgesic functions of DIC and its analogues are due to COX-2 inhibition (Viegas et al., 2011) although Singh et al. proved the specific binding of DIC to phospholipase A_2)Singh et al., 2006). The first aim of this study is to investigate the interaction product of DIC and TH by spectroscopic techniques and molecular modeling studies. In addition, the synergistic relationship between the selected drugs will be investigated by studying the binding of the individual drugs to COX-2 with the aid of molecular modeling. This work will uncover the role of TH in COX-2 inhibition. Pharmacologists may depend on this study in designing new potent analgesic and anti-inflammatory drug formulations. In addition, vitamin B1 may be prescribed in combination with DIC for analgesia. This will allow the physicians to limit their use of corticosteroids which have harmful side effects and require withdrawal in case of long-term treatment.

COX-1 and COX-2 are related to cyclooxygenase enzymes. They catalyze the synthesis of prostaglandins from arachidonic acid during inflammation (Vane et al., 1998), **Figure 1.** Vane et al. described the arachidonic acid cascade in their review (Vane et al., 1998). After the discovery of COXs crystal structures, different researchers investigated the mechanism of COXs inhibition (Viegas et al., 2011; Loll et al., 1995; Kurumbail et al., 1996; Price and Jorgensen, 2000). It was found from these studies that some non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin, indomethacin and phenylbutazone, inhibit both COX-1 and COX-2 enzymes. Other drugs like diclofenac and celecoxib can selectively inhibit COX-2 which is inducible in a limited number of tissues in response to products of and activated immune and inflammatory cells.

In this work, the interaction of DIC and TH at the molecular level was studied. Additionally, the binding of the individual drugs and their interaction product was investigated with molecular modeling softwares to unearth the role of TH in enhancing the anti-inflammatory effect of DIC. It was found that DIC reacts with TH through non-covalent interactions which can be easily destroyed in aqueous solutions to enable the individual binding of TH to the active site of the enzyme thus enhancing the action of the other drug.

Material and Methods

DIC sodium raw material was supplied by Delta Pharma, Egypt, while TH Chloride HCl raw material and absolute ethanol were obtained from Sigma-Aldrich, Germany. Distilled water was prepared using a glass distillation instrument.

Equivalent amounts of DIC and TH were weighed accurately and dissolved in the same amounts of distilled water to prepare the 1:1 (DIC:TH) product. The product was crystallized three times from ethanol. The obtained needle-shaped crystals were sent to the Micro-Analytical Center, Faculty of Science, Cairo University and subjected to IR spectroscopy, ^{1}H-NMR spectroscopy and mass spectrometry. IR spectra were recorded using the potassium bromide disc method in a test scan Shimadzu FTIR spectrometer. ^{1}H-NMR spectra were recorded using a Varian 300 MHz NMR Spectrometer and tetramethylsilane as an internal standard. Moreover, the mass spectra of the reaction product were recorded with the aid of a Q 1000 EX GC-MS Shimadzu spectrometer (Japan) at 70 eV and 100 μA energy using a direct insertion probe at temperature 90-110 °C.

Molecular modeling was performed using MOE software (Chemical Computing Group Inc MOE 2010.01, http://www.chemcomp.com). The molecules were drawn and subjected to energy minimization using MMFF94x. For docking, the crystal structure of human COX-2 was downloaded from the Protein Data Bank (PDB). The Molecular Operating Environment of docking was used to calculate the docking energies between the ligands and

the COX-2 active sites. The results were sorted according to energy and the lowest energy result was selected as the best. All the molecular modeling studies were carried out in the gas phase.

Result and Discussion

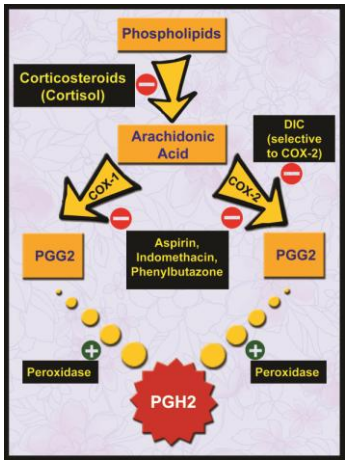


Figure 1. Biosynthesis of prostaglandins. DIC acts selectively on COX-2 to inhibit PGH2 synthesis. Steroidal anti-inflammatory drugs inhibit the formation of arachidonic acid. NSAIDs act only on COXs. PGG2: hydroperoxy endoperoxide; PGH2: hydroxy endoperoxide (Vane et al., 1998).

Mass Spectrometry. Mass spectrometric analysis was performed for the DIC-TH adduct (**Figure 2**) to confirm that the two drugs interacted with each others. The molecular ion peak appears at m/z 596. According to the chemical structures of DIC (molar mass 296.0 g/mol) and TH (molar mass 300.8 g/mol) and the fragmentation patterns it was found that the peaks at m/z 43, 57, 71, 151, 389, 406 and 574 are referred to DIC-TH. The molecular ion peak together with the shown fragments peaks elucidates the reaction between the two molecules.

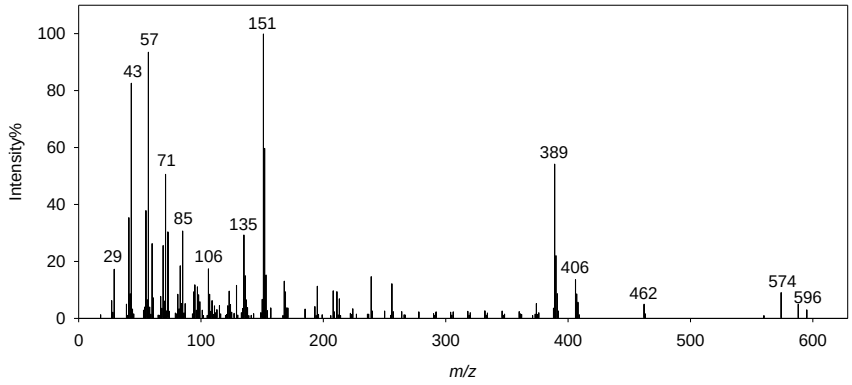


Figure 2. Mass spectrum of DIC-TH.

IR and ^{1}H-NMR. By studying the IR and ^{1}H-NMR spectra of TH, DIC and DIC-TH, the results shown in **Table 1** were obtained. It is obvious from the IR spectrum of DIC that the band observed at 3437 cm^{-1} and assigned to the intramolecular hydrogen-bonded NH in the DIC molecule disappeared upon reaction with TH to form the DIC-TH adduct. In addition, the bands of the carboxylate group in the range 1404-1624 cm^{-1} in DIC spectrum were highly shifted to a lower wave number (1151-1500 cm^{-1}) which confirms the reaction of DIC with TH through its carboxylate group. Moreover, the OH-stretching band of TH appears within the range 3200-3500 cm^{-1}. On the other hand, the band of stretching frequency of NH in DIC appears at 3251 cm^{-1}. These two bands were observed in the IR spectrum of DIC-TH at 3200-3500 and 1099 cm^{-1}, respectively, verifying the interaction between the studied molecules (Colthup et al., 1968).

The ^{1}H-NMR data of DIC-TH show that the NH_3^+ peak appears at 12.7 ppm as a broad peak which is not found in the ^{1}H-NMR spectrum of TH. In addition, the amino group of TH shows a singlet peak at 5.6 ppm which disappeared in the DIC-TH spectrum. Moreover, the aromatic proton of the 2-pyrimidine ring of TH observed as a singlet peak at 8.8 ppm was shifted to another region between 6.3 and 7.5 ppm in the DIC-TH spectrum. IR and ^{1}H-NMR data suggest that the interaction between DIC and TH occurs via the —NH_3^+ of TH and the —COO^- of DIC forming two hydrogen bonds between the amino nitrogen atom of TH and the two carboxylate oxygen atoms of DIC. The reaction does not occur on the nitrogen atom of the thiazole ring because of steric hindrance. However, there may be another type of interaction due to presence of aromatic π-electrons which will be proved by molecular modeling of the reaction product (Kemp, 1993).

Table 1. Assignment of IR and ^{1}H-NMR data.

	Assignment	TH	DIC	DIC-TH
		Wave number (cm^{-1})		
IR	NH_2 symmetric stretch	2040-2264	—	2075-2117
	O—H stretch	3200-3500 broad	—	3200-3500
	C—H aromatic	fused with O—H	—	fused with O—H
	C—H aliphatic	2978	—	2920
	C=N in the heterocycle	1631	—	1631
	Pyrimidine	1527	—	—
	Intramolecular H-bond N—H—O	—	3437	—
	N—H stretching	—	3251	3244
	—COONa	—	1404-1624	1151-1500
	Meta Ar 2Cl shifted due to N—H	—	1095	1099
	Assignment*	**TH**	**DIC**	**DIC-TH**
			δ (ppm)*	
^{1}H-NMR	O—H	2.3(s)	—	—
	CH_3—	2.5(s)	—	2.5(s)
	CH_2—CH_2—OH	2.8(t)	—	3.2(t)
	CH_2—CH_2—OH	3.5(t)	—	3.7(t)
	NH_2	5.6(s)	—	—
	Aromatic proton of 2-pyrimidine	8.8(s)	—	6.3-7.5(m)
	CH_2COO—	—	3.4(s)	3.6(t)
	Aromatic protons of the benzene rings	—	6.2-7.5(m)	6.3-7.5(m)
	Aromatic N—H proton	—	10.4(s)	—
	NH_3^+	—	—	12.7(s), broad

* (s): singlet; (t): triplet; (m): multiplet. The ^{1}H-NMR chemical shifts are assigned by the bold functional groups in the assignment column.

Molecular Modeling. The geometries of DIC, TH and DIC-TH were minimized using MMFF94x forcefield in the MOE software (**Figure 3**). An intramolecular H-bond appears between the nitrogen atom and the carboxylate oxygen atom of DIC which confirms the results obtained from IR and ^{1}H-NMR spectra of DIC. The angle between the carboxylate group and the benzene ring is 113˚ enabling the formation of the H-bond. The two benzene rings of DIC are non-coplanar with a dihedral angle of -47.7˚. Similarly, the 2-pyrimidine and thiazole rings of TH make a dihedral angle of 25.8˚ with each other. The tetravalent nitrogen atom of the thiazole ring bears a partial positive charge. The presence of an amino group enables the creation of another positively charged site in solution. These two sites can be the origin of interaction with the DIC molecule. The amino hydrogen atoms of TH are positioned midway between the nitrogen atom and the two carboxylate oxygen atoms of DIC in DIC-TH. This participates in the binding of the two molecules by two hydrogen bonds. Another hydrogen bond can be noticed between one of the thiazole hydrogen atoms of TH and the π-system of one of the DIC benzene rings.

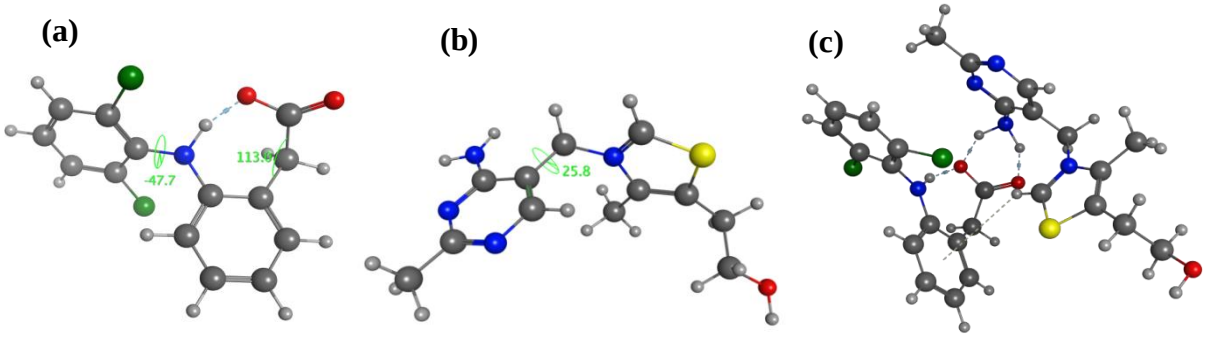


Figure 3. Minimized geometries of **(a)** DIC, **(b)** TH and **(c)** DIC-TH.

Docking of DIC with COX-2. The binding of DIC to COX-2 was studied by molecular docking. The carbonyl oxygen atom of DIC acts as a H-bond acceptor for the Lys 83, Tyr 122 and Lys 79. One of the thiazole hydrogen atoms interact with the non-chlorinated benzene ring of DIC via extra H-bond in which the arene group acts as H-bond acceptor due to its π-electron system. This site is the most appropriate site for interaction with DIC on the basis of total potential energy. **Figure 4** shows the results of molecular docking of DIC with human COX-2.

Docking of TH with COX-2. TH interacts with COX-2 in a different fashion. One H-bond is formed between the highly electronegative hydroxyl oxygen atom (H-bond donor) and the basic Arg 44 residue (H-bond acceptor). Another hydrogen bond is formed between the sulfur atom of the thiazole ring and Glu 46. **Figure 5** illustrates the results of TH docking with human COX-2 enzyme.

Docking of DIC-TH with COX-2. It is well-known that non-covalent interactions such as hydrogen bonding and Van der Waals forces are much weaker than covalent bonds (Gautam and Thomas, http://www.oxfordscholarship.com). Consequently, they can be easily broken during the formation of a protein-ligand complex. Based on the above results, TH and DIC cannot interact as a single molecule with the active site of the enzyme. So that docking studies could be made for DIC with TH-COX-2 complex to investigate the effect of TH on the binding affinity of DIC to COX-2 pocket. The results show that DIC does not change its binding mode to the protein molecule, **Figure 3**, panel **(c)**. The potential energies of protein-ligand complexes indicate that DIC has a higher affinity to COX-2 in case of subsequent binding of TH to the protein. This result can be interpreted by the conformational changes made in the protein active site upon interaction with TH which makes DIC fits better into its binding site. This is also confirmed by the fact that the two pockets are very close to each other, **Figure 6**, panel **(a)**.

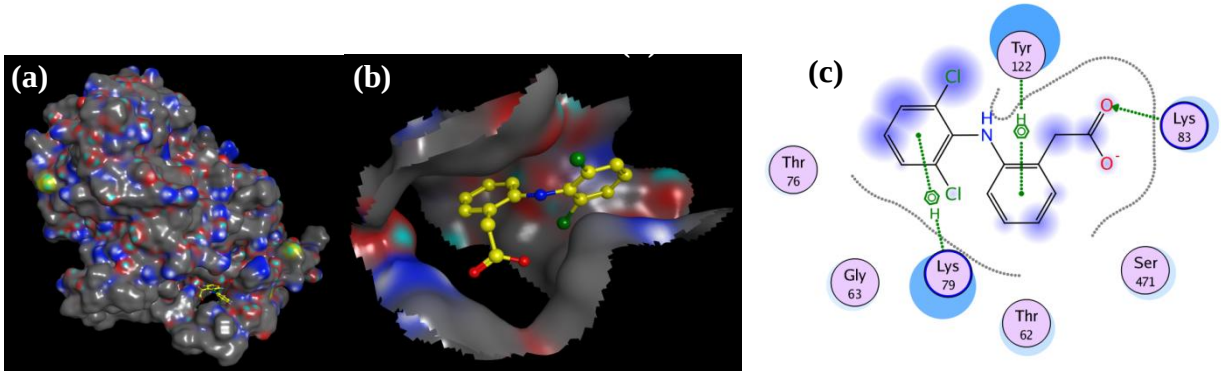


Figure 4. Interaction of DIC with its appropriate human COX-2 active site. Panel **(a)**: A zoomed out view of the active site position on the protein domain; Panel **(b)**: DIC bound to the isolated pocket; Panel **(c)**: 2D ligand interactions of DIC with human COX-2.

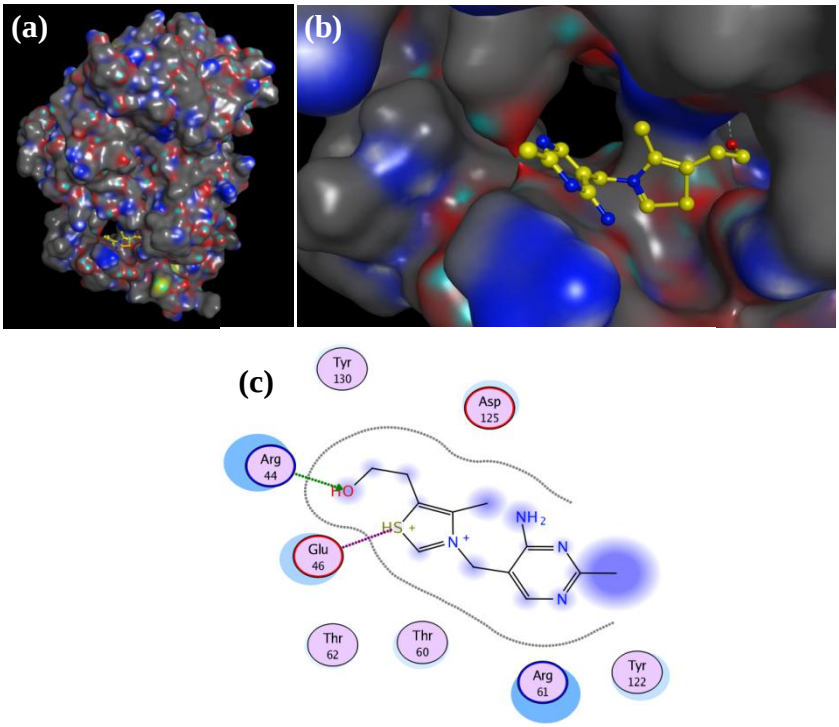


Figure 5. Interaction of TH with human COX-2 active site. Panel **(a)**: A zoomed out view of the active site with TH; Panel **(b)**: TH inside the pocket; Panel **(c)**: 2D ligand interactions of TH with human COX-2.

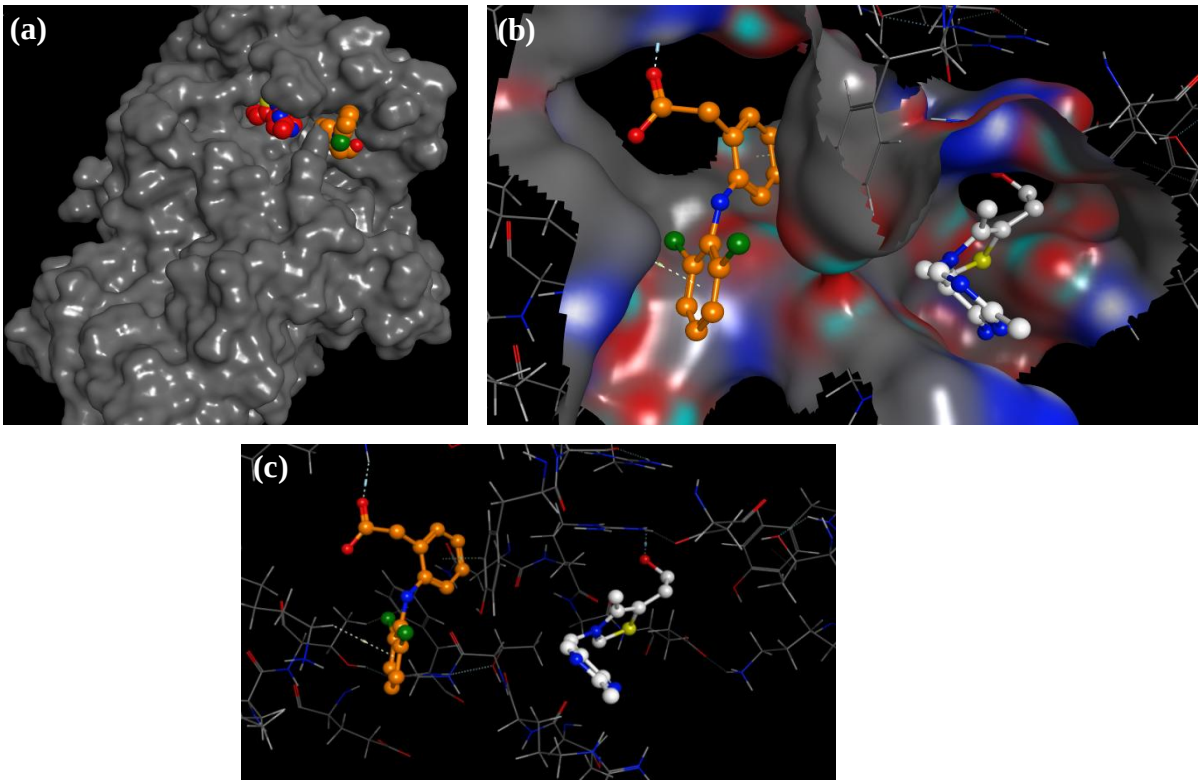


Figure 6. Mode of bonding of TH and DIC with their appropriate human COX-2 active sites. Panel **(a)**: A zoomed out view of the active sites with TH (left) and DIC (right); Panel **(b)**: TH (right) and DIC (left) inside their COX-2 pockets; Panel **(c)**: H-bonding of TH (right) and DIC (left) to the residues of their COX-2 pockets.

Conclusions

This work gives a comprehensive study of the chemical basis behind the enhancement of the anti-inflammatory effect of DIC by vitamin B1. The data obtained from spectroscopic studies and molecular modeling show the interaction of TH and DIC with each other to form the DIC-TH adduct. These data indicate that the two drugs interact by non-covalent interactions (different types of H-bonds). The spectroscopic data was online with the energy minimized forms of the studied molecules. Molecular docking was performed to indicate the interaction of the drugs with the specific protein COX-2. Docking results confirm that TH makes conformational changes in a site near to the binding site of DIC on COX-2 molecule. These conformational variations change the size of the DIC pocket and this leads to better fitting of DIC inside the pocket. This hypothesis was built based on the potential energy of the docked molecules obtained from MOE software which reflect the improvement of the binding affinity of DIC to COX-2 in presence of TH. This work suggests prescribing TH with DIC for the treatment of inflammatory disorders. On the other hand, pharmaceutical companies may depend on this work to synthesize new formulations containing TH and DIC in a combined tablet form. Finally, we recommend decreasing the usage of steroidal anti-inflammatory drugs for the treatment of inflammation due to their harmful side effects and expensive costs. In addition, they require tapering or dose adjustment in all cases of treatment.

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