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# Dihydroxy berberine from *Tinospora cordifolia: In silico* evidences for the mechanism of anti-inflammatory action through dual inhibition of Lipoxygenase and Cyclooxygenase

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The non-steroidal anti-inflammatory drugs in clinical use have been implicated with side effects on prolonged use. This implies the dearth of a safer alternative to these drugs and can be easily sourced from the huge resources available to us through Ayurveda. *Tinospora cordifolia* has been used in the preparation of traditional Ayurvedic formulations, with reported anti-inflammatory activities. Molecular docking studies have been used in an attempt to identify and elucidate the mechanism of action of the bioactive compounds in *T. cordifolia* with dual inhibition of 5-Lipoxygenase (5-LOX) and Cyclooxygenase-2 (COX-2) enzymes for identification of lead compounds. A screening of the compounds identified in the bioactive fraction of *T. cordifolia* was carried out using the drug-likeness score and the selected compounds were docked with the Glide module of Schrödinger suite 2014 with Maestro 9.3. Dihydroberberine (TC1) was found to be a potent inhibitor of both 5-Lipoxygenase and Cyclooxygenase-2 enzymes. The binding energy of the compounds to the free enzyme was better than when co-crystallized with substrate indicating preference to the enzyme active site. Dihydroberberine can be evaluated further as a promising candidate to develop a safer anti-inflammatory drug.

**Keywords**: 5-LOX, Anti-inflammatory, COX-2, Inflammation, Molecular docking, *Tinospora cordifolia*

Medicinal plants have received wide publicity in recent times as a source of bioactive constituents that are better and effective than several accepted drugs currently in clinical use. The bioactive constituents of *T. cordifolia* have been reported with several medicinal properties including anti-diabetic effect reported by alkaloids particularly palmatine, jatrorrhizine and magnoflorine[1], immunomodulatory functions of arabinogalactan as reported by Sharma *et al*.[2] and Verma *et al*.[3], immunopotentiating effects of cordioside, cordiofolioside A and cordial[4]. The adaptogenic properties of *T. cordifolia* validated with several research studies have highlighted the significance of the use of the plant extract in both traditional and modern ayurvedic preparations. Studies by the author on the anti-inflammatory activity of *T. cordifolia* have revealed dual inhibition of LOX and COX enzymes[5] with inhibition in the production of pro-inflammatory cytokines, Tumour necrosis factor (TNF-α) and Interleukin1β (IL-1β) in lipopolysaccharide-induced dendritic cells. The constituents of the bioactive fraction were identified with Quadrupole Time of Flight Mass Spectrometry (QToFMS). The compounds identified were screened for Drug-likeness properties and the selected drug-like compounds were docked *in silico* with enzymes, 5-LOX and COX-2, involved in the production of pro-inflammatory leukotrienes and prostaglandins.

*In silico* molecular docking studies predicts the preferential alignment of a target protein or nucleic acid to a ligand tso form a stable complex and these interactions are considered central to the signal transduction in biological systems[6]. Glide (Grid-Based Ligand Docking with Energetics) distributed by Schrodinger is the molecular docking software designed to investigate the best conformation of a ligand, best pharmacophoric receptor site, and parameterized scoring function[7]. Several docking studies have been carried out in medicinal plants or its bioactive compounds to identify the most efficient lead compound for drug design. The study on curcuminoids from *Curcuma longa* reveals the binding orientation of the curcumin analogues in the active sites of COX thereby identifying potential lead compounds for novel drug designing[8]. The LOX-inhibitor binding study by

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Hazai *et al*.[9] also reveals the high affinity of lycopene and lycophyll to the cleft at the interface of the β-barrel of 5-LOX indicating an allosteric mechanism in the proximity of the active center. The bioactive compound Tembetarinein *T. cordifolia* with anti-dermatophytic activity[10] has been identified in independent *in silico* studies. In the present study effective dual inhibitors of LOX and COX enzymes were identified from the compounds in the bioactive fraction of *T. cordifolia*[5] and further assessed for its Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) properties.

## Materials and Methods

### General Experimental Procedures

The plant, *T. cordifolia* was collected from Kottayam, Kerala, India and identified by Jomy Augustine, taxonomist at the Department of Botany of St. Thomas College, Pala, India. The voucher number (JA17151) was obtained on submission of the voucher specimen at Kerala Forest Research Institute. All other chemicals used were of high purity analytical grade from HiMedia chemicals, India. Structural characterization of the bioactive methanol fraction was conducted in the Waters Xevo G2 QToF. For the *in silico* studies, the structures were drawn using ChemSketch from Advanced Chemistry Development, Inc. (ACD/Labs). Drug-likeness model score was computed with MolSoft32 software[11]. Molecular docking of lead compounds was calculated using the Schrodinger Suite 2014 with Maestro 9.3,[12] with LigPrep, Protein preparation wizard and Glide[7]. The ADMET studies were analysed using the Pre ADMET software[13].

### Identification of compounds from *T. cordifolia* and evaluation of dual LOX/COX inhibition

The plant material comprising of the stem was used for the extraction of the bioactive methanol fraction from *T. cordifolia* as per the protocol described in Jacob *et.al*.[5] and was evaluated for dual inhibition of both LOX isoenzymes and COX isoenzymes as described in Jacob *et.al*.[5] was subjected to mass spectroscopic studies and the constituents of the bioactive fraction were identified as reported in Jacob *et al*.[5]. The compounds identified were labelled as TC1 to TC8 (supplementary file).

### Screening of identified compounds for Molecular Properties and Drug Likeness

The compounds, TC1-TC8, were screened for drug-likeness and molecular properties calculated using the online prediction server Molsoft. The online server calculates the drug-likeness score based on the basic Lipinski's rule of five applicable to drug candidates. The lipophilicity of the compounds is a measure of the permeability across cell membranes and is indicated by LogP. Polar surface area (PSA) is the measure of the surface area contributed by polar atoms and is calculated from the 3D structure of the compound. The molecular volume reflects on the characteristics of transport of the compounds in the body. These molecular properties can be used for screening potential drug-like candidates in the test compounds on comparison with known drugs.

### Protein structure retrieval and preparation

The target protein structures, retrieved from Protein Data Bank (http://www.rcsb.org/pdb/), were COX-2 (PDB ID: 1CVU)[14], 5-LOX (PDB ID: 3V99)[15]. Earlier enzyme kinetic studies[16] have indicated that the enzyme exhibits mixed inhibition and can thereby bind to both the enzyme-substrate complex [ES] and the free enzyme [E]. The docking procedure was thus carried out in the presence/absence of substrate arachidonic acid (ACD) for both LOX/COX enzymes to determine the binding position of the inhibitor molecule and its affinity. The COX-2 PDB entry 1CVU contains co-crystallized ligands like ACD, α-D-Mannose, N-Acetyl-D-Glucosamine etc. Docking in the presence of ACD was not possible for the 5-LOX protein (3V99) which had ACD as the only other co-crystallized ligand. Based on reported data on homology modelling by Pouplana *et al.*[17], the LOX protein co-crystallized with RS7 ligand (2P0M) was used.

### Preparation of Ligands and Proteins

The ligands were drawn using ChemSketch software from the Advanced Chemistry Development, Inc. (ACD/Labs). The LigPrep module of Schrodinger was used to prepare the ligand structures, generate variation and optimize the structures by minimizing energy levels. PDB structures of the enzymes were prepared using the Protein Preparation Wizard of Schrödinger Suite. The enzyme structure was pre-processed and assigned bond orders were included, missing hydrogen atoms added and bond lengths/ angles were corrected. The pre-processed protein was then subjected to constrained IMPACT Protein Refinement (IMPREF) minimization and to the Root Mean Squared Deviation (RMSD) of 0.3 A°.

### Receptor Grid Generation and Molecular Docking

The grid was generated using co-crystallized ligand as the center of the grid. The ligands selected were

ACD for the proteins 1CVU, 3V99 and RS7 for 2P0M. RS7 was considered as the center of the grid because as described by Choi *et al*.[18], it specifically binds to a location situated inside the substrate-binding cavity of the enzyme. So, in effect, the same area is being used for docking studies. Docking of the ligand structures identified from bioactive fractions of *T. cordifolia* (TBF) by this author as reported earlier[5] to target proteins was performed with Glide (Schrodinger). The van der Waals radii were scaled by a factor of 0.8[19]. The position of center of ligand was set as 10A° (Angstrom) docking sphere to accommodate maximum interaction of atoms. Molecular docking was performed on LOX inhibitors using the standard precision (SP) mode of Glide software. Glide scoring is based on a series of hierarchical filters for finding the possible binding locations of the ligand to the protein active site and the final scoring is done on the energy-minimized poses generated to find the Glide score. The binding energy of the ligands with the receptor was estimated using the automated Multi Ligand Bimolecular Association with Energetics (MBAE).

**ADMET studies**

The pharmacokinetic descriptors of TC1 were determined by computational methods on the Pre ADMET web server[13,20]. The descriptors were grouped into two sections as the ADME prediction and the Toxicity prediction. The predictions were calculated based on quantitative structure activity relationship (QSAR) models using the molecular descriptors of known drugs. Oral drug absorption descriptors include the Caco2-cell model and the Madin-Darby canine kidney cell models. Drug delivery both oral and transdermal is predicted by the human intestinal absorption (HIA) and the skin permeability models, respectively. The efficacy of the drug can be further explained with the blood-brain barrier (BBB) penetration and plasma protein binding calculations. The prediction of the toxicity properties of TC1were completed with Ames Salmonella mutagenicity assay and the rodent carcinogenicity assay[21].

## Results

**Molecular Properties and Drug Likeness**

Themolecular properties of all the compounds and their drug-likeness scores (TC1 to TC9) are as indicated in (Table 1). Compounds TC4A, TC4B, TC9A and TC9B have a molecular weight greater than 500Da and with no other violations for all the other parameters except for LogP of TC5.Veber's rule indicates that the PSA should not be more than 140A$^2$ for a good bioavailability of the drug candidate and all the compounds show effective PS values less than 140A$^2$[2,22,23]. The drug-likeness score was calculated by MolSoft by combining the physicochemical, pharmacodynamic and pharmacokinetic properties for

Table 1 — Molecular Properties and Drug-likeness Score of TC1 –TC9

| Descriptors | TC1 | TC2 | TC3 | TC4A | TC4B | TC5 | TC6 | TC7 | TC8A | TC8B | TC9A | TC9B |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Molecular weight | 337.13 | 268.13 | 345.32 | 522.25 | 522.25 | 417.38 | 514.26 | 468.24 | 358.2 | 358.2 | 550.36 | 550.35 |
| No of Hydrogen Bond Acceptor | 4 | 5 | 4 | 10 | 10 | 5 | 8 | 9 | 7 | 7 | 8 | 8 |
| No of Hydrogen Bond Donor | 0 | 1 | 3 | 4 | 4 | 4 | 0 | 2 | 1 | 1 | 4 | 3 |
| MolLogP | 4.19 | 1.07 | 4.96 | 0.22 | 0.44 | 5.80 | 4.60 | 0.98 | 1.43 | 1.43 | 5.17 | 5.10 |
| MolLogS Log(moles/L) | -5.92 | -1.20 | -4.78 | -2.05 | -4.02 | -6.82 | -4.96 | -3.41 | -0.85 | -0.54 | -5.09 | -4.50 |
| Molecular Polar Surface Area (A$^2$) | 34.91 | 48.22 | 54.38 | 125.75 | 128.50 | 71.94 | 76.95 | 107.27 | 61.41 | 60.69 | 99.06 | 102.73 |
| Molecular Volume (A$^3$) | 348.24 | 265.76 | 402.35 | 578.81 | 567.89 | 478.90 | 589.81 | 509.87 | 377.88 | 377.78 | 632.71 | 640.80 |
| No of stereo centers | 0 | 1 | 2 | 10 | 11 | 2 | 8 | 8 | 5 | 5 | 10 | 8 |
| Drug-likeness Score | 0.60 | -0.18 | -0.97 | 1.42 | 0.24 | -1.26 | -0.23 | -0.07 | -0.54 | -0.45 | 0.26 | 1.08 |
| Status | Active | NA | NA | Active | Active | NA | NA | NA | NA | NA | Active | Active |

NA: Not active; indicates compounds that show a drug-likeness score less than zero and are unsuitable as drug candidates.

all the compounds studied. The drug-likeness score with a value less than zero is considered as unsuitable for a drug. Five of the compounds TC1, TC4A, TC4B, TC9A and TC9B have a drug-likeness score greater than zero and have been considered for the molecular docking studies.

**Docking of ligands with COX enzyme (1CVU)**

The results obtained after docking of COX enzyme with the five selected ligands are based on the Glide score, the binding energy, the H-bonding and the van der Waals interactions of the ligand-target complex. The ligand-protein complex will havea tighter bind for more negative values of the g-score, maximum hydrogen bonds and van der Waals interactions. The results of the molecular docking of COX enzyme are as shown in (Table 2). Of the five selected compounds only one, TC1 docked in the absence of ACD. The highest glide rankis for TC1 with no hydrogen bonds formed with neighbouring amino acids in the absence of ACD. In the presence of ACD, very weak hydrogen bonds are formed with neighbouring amino acids. The presence of Met522 in both the presence and absence of ACD indicates that the inhibitor is binding at the same location of COX enzyme (substrate-binding cavity). The results of the molecular docking of LOX enzymes are as shown in (Table 2). The affinity of the ligands for the LOX enzyme active site is very low with the highest rank is for TC1. Only one ligand TC1 has docked to the binding site of RS7 and enzyme but with a greater binding affinity as compared to the ACD docked enzyme. The docked pose is as shown in (Fig. 1A & B while Table 3) shows the neighbouring amino acids present in the docking site in each protein

**ADMET Properties of TC1**

The compound TC1 which shows the highest ranking for COX and LOX in the docking studies and was further evaluated for its ADMET properties. The results of ADMET studies are as given in (Table 4)

Table 2 — Docking results for COX/LOX enzymes

| Sl. No. | Name of Ligand | Glide rank | Glide Score |
|---|---|---|---|
| COX without ACD substrate | | | |
| 1 | TC1 | 1 | -9.43 |
| COX with ACD substrate | | | |
| 1 | TC1 | 1 | -6.883 |
| 2 | TC9B | 2 | -5.13 |
| 3 | TC9A | 3 | -5.1 |
| 4 | TC4A | 4 | -4.68 |
| 5 | TC4B | 5 | -4.26 |
| LOX enzyme (3V99) without ACD | | | |
| 1 | TC1 | 1 | -5.148 |
| 2 | TC9A | 2 | -5.049 |
| 3 | TC4B | 3 | -4.932 |
| 4 | TC4A | 4 | -4.72 |
| 5 | TC9B | 5 | -4.46 |
| LOX enzyme (2P0M) without RS7 | | | |
| 1 | TC1 | 1 | -7.075 |

Table 4 — Absorption Distribution Metabolism and Excretion (ADME) and Toxicity Properties of TC1

| Name of Property | Value |
|---|---|
| BBB | 0.0342549 |
| Caco2 | 55.7322 |
| CYP_2C19_inhibition | Non |
| CYP_2C9_inhibition | Inhibitor |
| CYP_2D6_inhibition | Non |
| CYP_2D6_substrate | Non |
| CYP_3A4_inhibition | Inhibitor |
| CYP_3A4_substrate | Substrate |
| HIA | 97.867726 |
| MDCK | 12.6168 |
| Pgp_inhibition | Inhibitor |
| Plasma_Protein_Binding | 89.321948 |
| Pure_water_solubility_mg_L | 1.95863 |
| Skin_Permeability | -4.19839 |
| SKlogP_value | 3.149480 |
| Toxicity properties of TC1 | |
| Ames_test | Mutagen |
| Carcino_Mouse | Negative |
| Carcino_Rat | Negative |
| hERG_inhibition | medium_risk |
| TA100_10RLI | Positive |
| TA100_NA | Negative |
| TA1535_10RLI | Negative |
| TA1535_NA | Negative |

Table 3 — Amino Acid Interactions of Best Ligand TC1 on docking of LOX and COX

| Target | Docking Condition | Name of Ligand | Glide Score | Amino Acid Residues in the Binding Pocket |
|---|---|---|---|---|
| COX | Without ACD | TC1 | -9.43 | Met522, Gly526, Ala 527,Val523, Val349, Glu524, Met113, Leu531, Leu384, Ser530 |
| | With ACD | TC1 | -6.883 | Met522, His386, His388, Gln203, Trp387, Tyr385, Phe210, Ala207 |
| LOX | Without ACD (3V99) | TC1 | -5.41 | Asn 407, Phe 610, Gln 412, Leu188, Gln 609, Leu 179, Ile 406, Ala 410, Phe 177, His 367, Lys 409 |
| | Without RS7 (2P0M) | TC1 | -7.075 | Val 409, Leu 408, Ile 593, His 545, Ala 404, Val 594, Phe 415, Gln 357, Ile 418 |

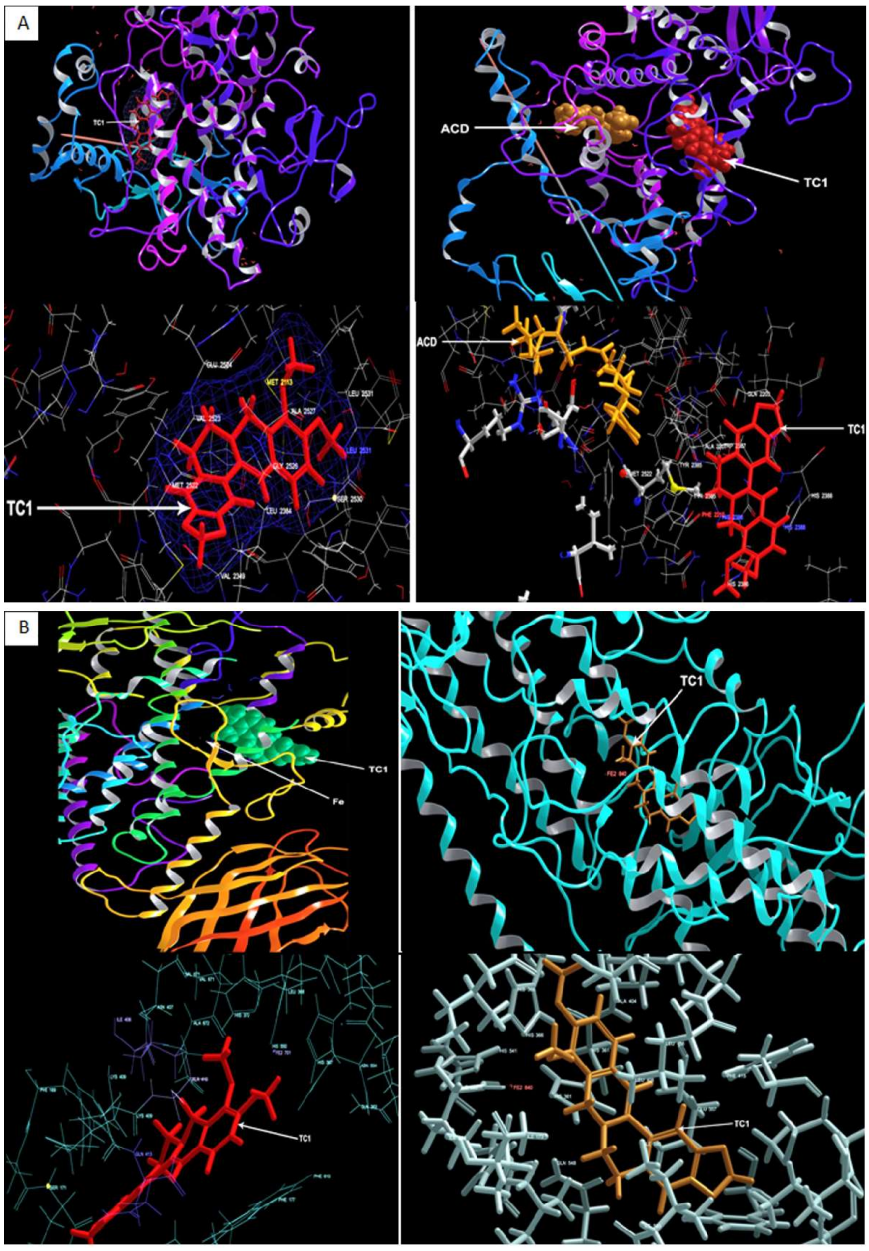


Fig. 1 — Docked pose of TC1 with COX-2 and 5-LOX protein in (A) Absence of ACD; and (B) Presence of ACD

and indicates that TC1 has the potential of mutagenicity as per the Ames mutagenicity assay but is not carcinogenic, shows P-glycoprotein inhibition, low absorption in the central nervous system (CNS), medium permeability and strong plasma protein binding properties.

## Discussion

The bioactive fraction of *T. cordifolia* was further characterized and several compounds named TC1-TC9 identified using UPLC-QToF mass spectrometry earlier[5]. The compounds identified in the bioactive fraction of *T. cordifolia* were characterized based on their molecular properties. The molecular properties were calculated based on Lipinski's rule of five that describes parameters fulfilled by successful drug candidates with no more than one exception. The molecular weight of drugs (<500 daltons), the number of hydrogen bond donors (<5) and acceptors (<10), and the Partition coefficient (octanol-water) or LogP (<5) are the factors considered desirable. The five compounds selected for molecular docking were purely based on the drug-likeness score which is based on the molecular descriptors. The molecular

docking studies were evaluated with the help of the docking grid area that allows the area for interaction of ligand and the target protein. The grid generation of the Glide program starts with the removal of the bound ligand at the center of the grid[24]. Docking strategy was performed with two objectives and in the first approach, the grid was selected with ACD as the center of the grid while in the second method alternative compounds, other than ACD were also considered in the preparation of the grid. The only PDB entry with co-crystallized ACD for 5-LOX is 3V99 which was initially used for the docking and a second PDB entry, 2P0M was used to explore the possibility of the binding of ligands to a second site on the target protein[25] with RS7 as the co-crystallized ligand. The primary structure of the catalytic domain of human 5-LOX was similar to rabbit 15-LOX based on the presence of an amino-terminal β-barrel domain (calcium-binding site) and a carboxy-terminal α-helical domain containing iron (catalytic domain) with 80% homology of the sequence to human lipoxygenase[26,27].

Based on the Glide score, TC1 (Dihydroxy berberine) was identified as the best docked ligand in both COX and LOX enzymes in the presence and absence of ACD. Comparison of the docking score for the two compounds shows a common feature where the score is higher for the COX enzyme when compared to the LOX enzyme. The docking score reported for Diclofenac with COX-2 was -10.33 on docking with Glide software[28] while for 5-LOX curcumin was reported with a score of -6.373[29]. The presence of ACD in the COX enzyme significantly lowers the docking score which indicates the possible binding of the ligand into the ACD binding site with higher affinity. The LOX enzymes docked with the ligands also exhibit a similar but opposite pattern of binding affinity. The ligand TC1 has a higher docking score for the RS7 co-crystallized protein as compared to the ACD bound enzyme. This indicates the binding of the ligand to a site distinct from the active site of the enzyme which is in accordance with experimental data[16,18]. COX enzyme inhibition usually leads to suicide inhibition in a relatively larger active site where once the inhibitor is bound the inhibition cannot be reversed. But in the case of LOX enzymes, the active site is hidden in a hydrophobic narrow pocket. The dihydroberberine has a higher affinity to the ACD binding site of LOX as demonstrated in the PDB ID 2P0M which indicates that it binds to the enzyme-substrate complex. As a result, both the enzyme and substrate are removed from the site of action.

The ligand shows no hydrogen bonds with adjacent amino acids in enzyme active sites. The structure of TC1 has no hydroxyl or amino groups for favourable hydrogen bond formation. This indicates that the binding of the ligands to the docking site depends on other hydrophobic or electrostatic interactions. The LOX enzymes have a smaller N-terminal domain and a larger iron-containing catalytic domain[30]. The iron remains in coordinate bonding with His550, His372, His367, Val671, and Asn554 while the sixth coordinate position faces an open cavity that is occupied by the docked ligand[31]. The docked poses reveal the proximity of TC1 to the iron atom and its coordinate bonds with three histidine residues at positions 550, 372, and 367 in the active site of 5-LOX.The latter amino acids are involved with the bound RS7 in the homology model.

The COX enzyme inhibitor binding has been predicted in the groove of the COX enzyme that involves Tyr 385, Trp387, Ala527, Ser530, Val349, Phe518, Leu352, Ser353, Tyr355, Val523, and Arg120 residues[32]. As the data suggests the COX binding site is larger than for the LOX enzyme[33]. The proximity of TC1 to the active site of the proteins was found to be less in the presence of ACD compared to its absence. This observation could indicate the modification of the active site by the inhibitor ligand restricts the binding of ACD to the active site and also prevents the release of product from the active site as is expected for a mixed inhibitor. The secondary binding pocket that is formed in the COX-2 due to the replacement of Ile 523 in COX-1 with the less bulky Val 523 seems to be occupied in the absence of ACD[34]. The proximity of the Trp387 and Tyr385 indicates the possibility of the hydrophobic interaction of the ligand in the presence of ACD as the bound ligand.

The ADMET study results show that the compound TC1, dihydroberberine has a Human intestinal absorption rate of 97% which indicates that it would be well absorbed. The compound also shows medium permeability in Caco2 and MDCK cell models but poor absorption from the skin. The advantage would be that dihydroberberine has low absorption to the CNS and does not breach the blood-brain barrier. The compound has a strong binding to plasma proteins and could act as a disadvantage in drug distribution[35] but recent reports[36] indicate that the plasma protein binding would not necessarily affect its efficacy. The drug efficacy is more dependent on the free drug concentration at the site of

action and not elsewhere[37] and can be increased by increasing permeation or metabolic stability or even reducing efflux. The compound shows positive for mutagenicity with a medium risk of cardiotoxicity but is largely non-carcinogenic. Zhu *et al*.[38] have reported on the induction of apoptosis and DNA damage in human osteosarcoma cells which indicates the mutagenic property of the compound but against sarcoma cells. Anis *et al*.[39] has also reported on the inhibition of carcinogenesis of berberine and its active form dihydroberberine. Dihydroberberine is found to be anti-carcinogenic as it inhibits P-glycoprotein. The P-glycoprotein is found overexpressed in cancer cells and inhibits the internalization of chemotherapeutic drugs[40]. Bansal *et al*.[41] report on the significance of flavonoid secondary metabolite in chemotherapy for cancer. Such compounds are found to enhance bioavailability. The inhibition of P-glycoprotein thereby increases the therapeutic efficacy of the treatments as compared with standard P-glycoprotein inhibition by verapamil, and cyclosporine. Dihydroberberine also exhibits properties similar to other Non-Steroidal Anti-Inflammatory drugs (NSAID) and inhibits cytochrome P (CYP) 2C9. Compounds that indicate substrate activity for CYP2C9 have been found to exhibit increased exposure leading to increased gastrointestinal and cardiovascular side effects[42]. The compound TC1 exhibits inhibition of most CYPs including CYP2C9 making it more desirable as a lead candidate for drug development. Interaction with the CYP 450 enzymes is not desirable in potential drug candidates. Dihydroberberine requires extended lead identification and pharmacophore-based modeling to develop into an effective drug candidate for anti-inflammatory effect.The fact that has to be specifically noted is that Berberine has also been identified as a potential inhibitor of COVID-19 Main Protease (Mpro)[43] and Dihydroberberine is considered to be the active form of Berberine[44]. This implicates the activity of berberine as an anti-COVID agent which has several anti-inflammatory actions[45] like inhibition of pro-inflammatory cytokines and dual LOX-COX inhibition.

## Conclusion

The compounds identified from the bioactive fraction of *T. cordifolia* were thus evaluated for their drug-likeness score and molecular properties to eliminate possible false positives in the development of suitable anti-inflammatory drug candidates. Five compounds with positive drug-likeness scores were further evaluated with *in silico* molecular docking studies with 5-LOX and COX-2 enzymes and the results indicate that TC1(dihydroberberine) shows the best binding energy as compared to the other four compounds for both enzymes. The binding pattern indicates the ability of dihydroberberine to bind to both the free enzyme and enzyme-substrate complex as evidenced earlier by kinetic studies in LOX enzymes. Dihydroberberine was then subjected to *in vitro* ADMET studies which reflect on the therapeutic potential of the compound and the need for further exhaustive studies for the development of an effective safer alternative to anti-inflammatory drugs with the possibility as a potential anti-COVID agent.

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## Conflicts of interest

All authors declare no conflict of interest.

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