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IN SILICO PHARMACOKINETIC AND MOLECULAR DOCKING STUDIES OF *N*-CINNAMOYLTETRAKETIDE DERIVATIVES AS INHIBITORS OF CYCLOOXYGENASE-2 ENZYME

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ABSTRACT

Recent phytochemical analysis of Toussaintia orientalis leaves yielded series of novel bioactive N-cinnamoyltetraketide derivatives namely toussaintines A-G (t_1 - t_8) some portraying cytotoxicity against the triple negative aggressive human breast cancer cell line (MDA-MB-231) among other potencies. Despite having broad bioactivity spectrum, their general drug-likeness profiles and mode of action (simulated or actual) targeting any enzyme remains uninvestigated. In silico pharmacokinetic, drug-likeness descriptors and molecular docking of the compounds t_1-t_8 targeting inhibition of cyclooxygenase-2 (COX-2) enzyme were evaluated. The Lipinski Rule of Five heralded the pharmacokinetic properties of the studied metabolites. The studied compounds were docked with COX-2 following already established protocol. ADMET descriptors fell within the recommended range, except for compound t_3 that was predicted to potentially have positive blood brain barrier (BBB+) penetration. Docking studies indicated N-cinnamoyltetraketide derivatives as potential inhibitors of COX-2 enzyme. Compounds t_3 and t_5 showed lower binding energy of -13 and -12.3 kcal/mol, respectively, being closely comparable to celecoxib (-12.3 kcal/mol) indicating compatibility with the protein receptor. The findings provide baseline information on drug or lead-likeness and potential mode of action of the studied molecules towards inhibition of COX-2 enzyme.

Keywords: *N*-cinnamoyltetraketide derivatives; molecular docking, ADMET, *in silico*, COX-2.

INTRODUCTION

Drug discovery and development are time consuming and expensive multistep processes. A number of drug candidates usually face limitations to enter into clinical trials and pharmaceutical markets (Darvas et al. 2002), whereas the few that enter into the market are often banned or stopped shortly after commencement of their clinical use. Poor pharmacokinetics and toxicity of the efficaciously promising drug candidates are the main obstacles among other factors for development of drug into clinical applications (Waterbeemd and Gifford 2003; Boobis et al. 2002). For economic reasons therefore, poor drug-like candidates need to be eliminated earlier during drug development (Boobis et al. 2002). Today, the use of computational approach in drug discovery and development provides a safer and the fastest means of reducing cost related to experimental studies (Boobis et al. 2002, Ntie-Kang 2013). Thus, *in silico* approach is preliminarily used in drug discovery to evaluate drug-likeness properties such as absorption, distribution, metabolism, elimination and toxicity (ADMET) of compounds at early stages of

their development (Boobis et al. 2002). Such evaluation facilitate selection of compounds with high probability of becoming lead molecules or drugs while eliminating those with less probabilities (Boobis et al. 2002, Lagorce et al. 2008).

Recently, phytochemical analysis of *Toussaintia orientalis* Verdc (Annonaceae), a medicinal plant endemic to Tanzania yielded *N*-cinnamoyltetraketide derivatives namely toussaintines A-G (t_1 - t_8, respectively, Figure 1) (Samwel et al. 2011, Nyandoro et al. 2015) some having cytotoxic properties against the triple negative aggressive human breast cancer cell line (MDA-MB-231) (Nyandoro et al. 2015). The compounds are also reported to possess antitubecular activities against *Mycobacterium tuberculosis* (H37Rv strain) (Nyandoro et al. 2015), antiviral efficacy against Infectious Bursal Disease and Newcastle Disease Viruses (Nyandoro et al. 2014) and other antimicrobial activities (Samwel et al. 2011). Different extract preparations from *T. orientalis* are used in traditional medicine for remedy of respiratory infections and inflammation associated with irritating skin rashes (Samwel et al. 2011). Despite the reported bioactivities of the *N*-cinnamoyltetraketide derivatives, their general drug-likeness profiles and mode of action (simulated or actual) targeting any enzyme remains uninvestigated. In the present study, *in silico* pharmacokinetic drug-likeness properties of these bioactive metabolites (t_1 - t_8) prefigured by Lipinski Rule of Five followed by molecular docking targeting inhibition of cyclooxygenase-2 (COX-2) enzyme were evaluated.

Cyclooxygenase (COX) enzymes exist in two isoforms, cyclooxygenase 1 (COX-1) and cyclooxygenase 2 COX-2). The two isoenzymes convert arachidonic acid to prostaglandin, resulting in pain and inflammation (Madeswaran et al. 2012). They exert metabolism roles in different physiological situations and disease processes ranging from inflammatory, pyretic, thrombotic, neurodegenerative to oncological illnesses. COX-1 is known to be present in most tissues including the gastrointestinal tract where it maintains the normal lining of the stomach. It is also involved in kidney and platelet function. On the other hand, COX-2 is primarily present at the sites of inflammation (Madeswaran et al. 2012) and positively linked to proliferation of cancerous cells including human breast cancer cells (Ranger et al. 2004). Thus, due to COX enzymes other functions, preferential inhibition of COX-2 over COX-1 is considered desirable. A number of cinnamic acid derivatives have been reported to possess interesting antioxidant, anti-inflammatory, cytotoxic, lipoxygenase inhibition properties (Pontiki et al. 2014). Consequently, *N*-cinnamoyltetraketide derivatives having demonstrated cytotoxicity against the human breast cancer cells (Nyandoro et al. 2015), are hereby investigated *in silico* to reveal whether COX-2 enzyme could be their molecular targets as they exert their cytotoxic action.

MATERIALS AND METHODS

Sources of data and chemical structure

Information regarding structure and bioactivities of *N*-cinnamoyltetraketide derivatives were obtained from the previously published work (Samwel et al. 2011, Nyandoro et al. 2015) and the compounds are herein referred to as t_1 - t_8. The structures were initially drawn in ChemDraw Pro Software 12.0 and served as .sdf and .mol file format.

ADMET descriptor calculations

ADMET related descriptors were calculated by using MedChem Designer, ACD/iLab ver 2.0 and Pre ADMET online server. The

software generated descriptors used in ADME prediction. Pharmacokinetic profiles of the *N*-cinnamoyltetraketide derivatives were assessed by using the ADMET drug-likeness parameters. The calculated descriptors for drug-likeness based on the Lipinski's Rule of Five (Lipinski et al. 1997) included molecular weight (MW), hydrogen bond donor (HBD), hydrogen bond acceptor (HBA), lipophilicity (logP) and topological polar surface area (TPSA). The other parameter calculated was the number of rotatable bonds (nRB). Other predicted properties based on the method described by Jorgensen and Duffy (2002) includes logarithm of binding constant to human serum albumin (log K_{HSA}) range of -1.5 to 1.2 (Colmenarejo et al. 2001), logarithm of blood/brain barrier (BBB) partition coefficient (log $_{B/B}$) range of -3 to 1 (Luco 1999) and apparent Caco-2 cell membrane permeability (caco-2 permeability), (Stenberg et al. 2001). Other descriptors were: apparent Madin-Darby canine kidney (MDCK) cell permeability in nm s^{-1}range <25 poor, and >500 great (Irvine et al. 1999), IC_{50} value for blockage of hERG K^+channel (log $_{hERG}$, conc<-5) (Cavalli et al. 2002).

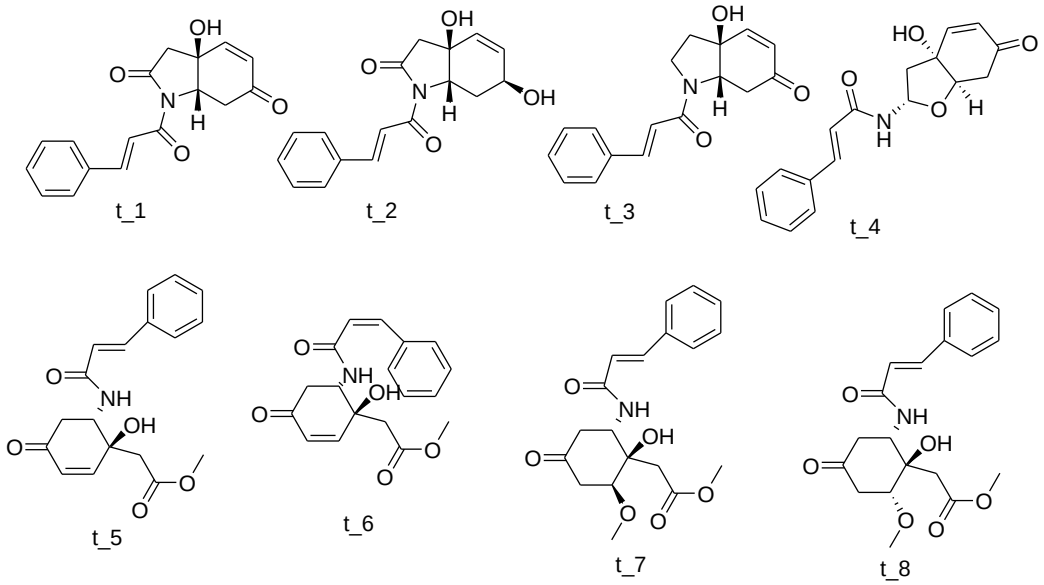


Figure 1: *N*-cinnamoyltetraketide derivatives: Toussaintine A – G (t_1 – t_8) (Samwel et al. 2011; Nyandoro et al. 2015)

Molecular docking studies

Protein and ligands preparation

The three dimension structure (3D) of target COX-2 enzyme was retrieved from RCSB protein data bank (PDB ID: 3NTG) with 2.19 Å resolution (Wang et al. 2010). Hetero atoms and water were removed from the protein binding sites. Protein structure was prepared using Chimera software (http://www.cgl.ucsf.edu/chimera). Furthermore, the protein was checked for allowed and disallowed regions using the Ramachadran plot and the ProSA was used to display the Z-score energetic plots. Molecular framework of the cytotoxic *N*-cynnamoyltetraketide derivatives, herein,

now referred to as ligands (t_1, t_2, t_3, t_4, t_5, t_6, t_7 and t_8) originated from the literature (Samwel et al. 2011, Nyandoro et al. 2015). Their structures were drawn in ChemDraw Pro 12.0 software and exported as .mol and .pdb file. Hydrogen was added and structures were energy minimized.

Molecular docking

Docking experiments were done following established procedure (Muhammad and Fatima 2015, Shadrack et al. 2016) using PyRx virtual screening tool, with AutoDock Vina docking option based on scoring functions. The energy interaction of 3NTG with ligands was assigned as grind point. The parameters were set as default, except for energy of interaction of ligands and 3NTG which was evaluated using atomic affinity potentials computed on a grid. Docking of the ligands was validated using celecoxib drug. PyMol or Malegro Molecular Viewer software was used to analyze the binding sites and orientations of ligands and COX-2 enzyme.

RESULTS AND DISCUSSION

The Lipinski Rule of Five Evaluation of Drug-likeness

The Lipinski's Rule of Five including its extended metrics (Lipinski et al. 1997, Lipinski 2000) was used to predict the drug-likeness of the natural products under investigations prior to further ADMET and other pertinent pharmacokinetic descriptors assessment. The parameters used to assess drug-likeness of the studied compounds includes molecular weight (MW), number of hydrogen bond acceptors (HBA), number of hydrogen bond donor (HBD), octanol-water partition coefficient (lipophilicity, log *P*), the topological polar surface area (TPSA) and the number of rotatable bonds (RB). Compound which obey the Lipinski Rule of Five should have MW < 500 Da, TPSA ≤ 140 Å^2, HBA < 10, HBD <5, log *P* < 5 and RB < 10. All *N*-cinnamoyltetraketide derivatives investigated were in agreement with the rule (Table 1). The MW analysis of the compounds showed the highest mass of 361.397 Da for t_6 and t_7 (Table 1). Thus, the molecular weights were in agreement with the Lipinski Rule of Five for small molecules. The number of HBA (which includes number oxygen and nitrogen) and HBD calculated were found to be <7 and <2, respectively for all compounds, being in compliance with the rule. All *N*-cinnamoyltetraketide derivatives showed log *P* <5 and TPSA greater than 60, but less than 100 qualifying the rule requirement for these criteria. The log *P* < 5 indicates good permeability across cell membrane in human body as well as low toxicity. According to the rule, the molecules having a TPSA value >140 Å^2 have a minimal chance to permeate the cell membrane. The computed number of rotatable bonds was found to be ≤ 7 for all compounds, being in agreement with the rule as for other analyzed descriptors.

Pharmacokinetics Predictions

Blood-brain barrier (BBB) penetration prediction

Drugs which are too polar and those with high TPSA value (60-90 Å^2) have poor or no BBB permeation. BBB permeability is measured by two parameters, log B/B (concentration of drug in blood/brain) and log PS (permeability surface area product) (Suenderhauf et al. 2012, Carpenter et al. 2014). The predicated log B/B penetration (Table 2) was used further to predict whether compounds can access the central nervous system (CNS). The predicated CNS activity (+) and CNS inactivity (-) were computed in a +1(activity) to -1 (inactivity) scale. None of the *N*-cinnamoyltetraketide derivatives showed activity to the CNS (Table 2). The computed log B/B fell within the recommended range for blood brain barrier predication (-3 to 1) (Carpenter et al. 2014). However, unlike other *N*-

cinnamoyltetraketide derivatives, t_3 was indicated to be BBB positive based on support vector machine (SVM) prediction (Table 2), the outcome that correlates to its low TPSA value (57.61 $Å^2$) and MW (283.329 Da). The predicated TPSA values were ≥ 60 $Å^2$ for all *N*-cinnamoyltetraketide derivatives, except for t_3 whose TPSA value was slightly < 60 $Å^2$ and correspondingly the lowest MWt. Generally, compounds with TPSA < 60-70 $Å^2$ have higher chances for BBB permeation (Jouyban and Soltani, 2012).

Table 1: Physicochemical descriptors of compounds t_1 – t_8

Name	MlogP	S+logP	S+logD	RuleOf5	[a]MWt	[b]HBA	[c]TPSA	[d]HBD	[e]nRB
t_1	1.094	0.903	0.903	0	297.313	5	74.68	1	2
t_2	1.171	0.785	0.785	0	299.328	5	77.84	2	3
t_3	1.553	1.107	1.107	0	283.329	4	57.61	1	2
t_4	1.171	0.932	0.931	0	299.328	5	75.63	2	3
t_5	0.559	0.917	0.916	0	329.355	6	92.7	2	6
t_6	0.559	0.917	0.916	0	329.355	6	92.7	2	6
t_7	0.11	0.786	0.785	0	361.397	7	101.93	2	7
t_8	0.11	0.786	0.785	0	361.397	7	101.93	2	7

[a]Molecular weight (MWt), [b]number of hydrogen bond acceptor (HBA), [c]topological polar surface area (TPSA) [d]number of hydrogen bond donor (HBD), [e]number of rotatable bonds.

Table 2: Blood-Brain Barrier Permeability Properties of the compounds t_1 - t_8

Compounds	**log BB**	**[c]log PS**	**[d]log (PS*$f_{u,\,brain}$)**	**[b]BBB+/-**	**[a]CNS+/-**
t_1	-0.74	-2.1	-2.4	-	-
t-2	-0.63	-2.0	-2.4	-	-
t_3	-0.53	-1.7	-2.2	+	-
t_4	-0.5	-2.1	-2.5	-	-
t_5	-0.5	-2.2	-2.6	-	-
t_6	-0.49	-2.2	-2.6	-	-
t_7	-0.22	-2.4	-2.7	-	-
t_8	-0.22	-2.4	-2.7	-	-

[a]Central Nervous System active(+) and inactive(-), [b]Predicated on support vector machine (SVM) with pubchem fingerprint, [c]Rate of brain penetration, [d]Brain/plasma equilibrium rate.

Predication of the plasma protein binding (PPB)

Drugs bind reversibly to human plasma protein with varying degree of association. The binding affects the pharmacokinetic properties in terms of volume of distribution, metabolism and clearance as well as the pharmacological effects of the drugs (Ghafourian and Amin 2013). Drugs with high protein binding normally have high

half-life in contrast to those with lower value. Large number of drugs bounded to plasma protein result into a small fraction of the drug being available for therapeutic effects, thereby reducing the quantity of drug in the blood circulation. Drugs bind to protein plasma such as human serum albumin, lipoprotein, glycoprotein, α, β, and γ globulins (Ntie-kang 2013). Herein, the plasma protein binding (PPB) was predicated by computing the predicated human serum albumin (log K_{HSA}). The predicated log K_{HSA} values (Table 3) suggest the compounds to bind to human serum albumin (HSA). The predicated PPB for all *N*-cinnamoyltetraketide derivatives were compared to the value in the literature as classified by discovery studio (Reddy et al. 2012). The predicated value fell within level 0. Though the compounds bind to plasma protein, the recorded value suggests that they can exert the intended therapeutic effect (Zhivkova and Doytchinova 2012).

Table 3: Predicated plasma protein binding properties of compounds t_1 – t_8

Compounds	**log K_{HSA}[a]**	**% PPB[a]**	**Level**
t_1	3.37	89.05	0
t-2	3.4	88.84	0
t_3	3.67	89.65	0
t_4	3.59	87.62	0
t_5	3.64	86.73	0
t_6	3.64	86.73	0
t_7	3.53	70.87	0
t_8	3.53	70.87	0

[a]Values were predicated by using ACD/Labs and compared to the value in the literature (Reddy et al. 2012) as classified by discovery studio 2.5 level 0 = PPB < 90 %, level 1 = PPB > 90 % and level 2 = PPB > 95 %.

Predicting the inhibition of human ether-a-go-go-related gene (hERG) channel

The investigation on hERG channel inhibition is always needed in drug development in order to reduce expenses and cardiotoxicity, which may result when left unverified. The inhibition of the hERG channel results into drug-causing delay in ventricular repolarization [induce Q and T wave (QT) prolongation], which is a side effect of non-cardiovascular therapeutic agents (Aronov 2005, Song and Clarck 2006). hERG is known to be associated with the modulation of some nervous system cell function (Aronov 2005), thus, hERG inhibitors are potentially toxic and need to be investigated earlier. In this study, hERG inhibition constant (Ki) was predicated to be <10 µM for all studied compounds (Table 4). The blockage of hERG is known to decrease significantly for compounds having the calculated Clog $P < 1$. The limitation for potent hERG inhibitor is Clog $P \geq 3.7$ (Buyck 2002). *N*-cinnamoyltetraketide derivatives evaluated in this study had Clog $P < 1$ implying that they could be non-hERG blockers (Table 4).

Predicted inhibition of the human cytochrome P450 enzymes (CYP) 2C19, 2C9, 2D6, 3A4

Studying the inhibition potency of drug-like and lead-like molecules against cytochrome P450 (CYP) enzymes is crucial for determining their drug-drug interactions and drug toxicity. These enzymes are involved in biotransformation of drugs and xenobiotics. CYP 3A4 is known to metabolize almost half of the drugs used today (Reddy et al. 2012). This enzyme is also concerned with oral bioavailability of drugs and hence the inhibition of such enzymes needs to be assessed earlier. In this study, the ability of compounds t_1 – t_8 to inhibit four human CYP 450 enzyme isoforms was predicated. It was found that all *N*-cinnamoyltetraketide derivatives under study were non-inhibitors to all four isoforms of human P450 enzymes (Table 5). According to the reported standard level of CYP 450 inhibitor (Reddy et al. 2012), all compounds were in level 0, which is the non-inhibition level.

Table 4: Prediction of the inhibition of hERG channels by compounds t_1 – t_8

Compounds	**hERG Ki (μM)**	**Inhibition probability (Clog *P*)**	**Reliability**
t_1	<10	0.02	Not reliable
t_2	<10	0.02	Not reliable
t_3	<10	0.10	Not reliable
t_4	<10	0.09	Not reliable
t_5	<10	0.02	Not reliable
t_6	<10	0.02	Not reliable
t_7	<10	0.01	Not reliable
t_8	<10	0.01	Not reliable

Table 5: Predicted inhibition of CYP450 enzymes by compounds t_1 – t_8

Compounds	**Predicated Levels of Inhibition**[a]			
	CYP 2C19	**CYP 2C9**	**CYP 2D6**	**CYP 3A4**
t_1	0	0	0	0
t-2	0	0	0	0
t_3	0	0	0	0
t_4	0	0	0	0
t_5	0	0	0	0
t_6	0	0	0	0
t_7	0	0	0	0
t_8	0	0	0	0

[a]Values were predicated in Pre ADMET online sever and results were compared to the standard value in the literature (Reddy et al. 2012) as classified in discovery studio 2.5, level 0 = non inhibitor, level 1= inhibitors.

Bioavailability predications

One among several reasons for drug failure in clinical application is their poor bioavailability. Therefore, oral bioavailability assessment should be done earlier during drug development. However, there are limited *in vitro* high throughput screening assays for oral bioavailability and hence other alternative approaches are necessary. Recently, *in silico* model to predict oral bioavailability has been developed (Ntie-Kang et al. 2013). In the present study, oral bioavailability of the *N*-cynammoyltetraketide derivatives was carried out by *in silico* model for predictions. The predicated percentage fraction (% F) of the drug dose that reaches circulation after oral administration was studied by employing the number of endpoints that affect oral bioavailability which include solubility (dose/solubility ratio), stability in acidic media, intestinal membrane permeability (IMP) by passive or active transport, likelihood of P-gp efflux, first pass metabolism in the liver, Madin-Darby canine kidney (MDCK) cell and colon carcinoma cell (Caco-2) permeability (Table 6). All compounds were predicted to possess good solubility and stability in acidic media as established at pH < 2. The compounds demonstrated also good absorption by passive transport. Compounds with more than 50% F are known to have high probability of oral bioavailability (Mandagere et al. 2002). Thus, the investigated compounds could be considered to have great chance of oral bioavailability. The predicated first pass metabolism (FPM) in the liver was in compliance except for compound t_5, t_6, t_7 and t_8. Similarly, P-gp efflux was predicated to be good for compounds t_1 and t_3, while the rest exhibited moderate response. On the other hand, all compounds showed poor intestinal membrane permeability (IMP) by active transport in contrast to passive transport. Furthermore, Madin-Darby canine kidney (MDCK) cell permeability, an important parameter mostly used to predict oral absorption was also predicated at a recommended range (<25 poor, >500 great) (Irvine et al. 1999). The results further ruled out the compounds to have poor absorption (Table 6). All compound were predicated to have moderate permeability to colon carcinoma cell (Caco-2 permeability), recording values within the recommended range of <5 low, >100 high (Stenberg et al. 2001).

Table 6: Predicated oral bioavailability of compounds t_1 – t_8

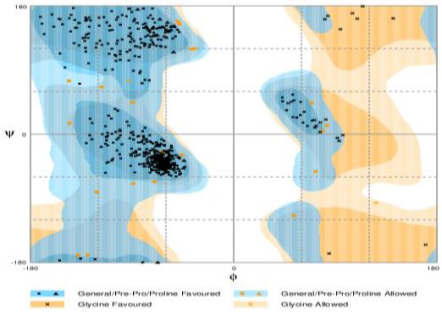
Cpds	[a]% F (OB)	[b]P-gp efflux	[c]FPM in the liver	[d]IMP by passive	[d]IMP by active	[e]Solubility	Stability (pH<2)	MDCK	Caco-2
t_1	>70	Good	Moderate	Good	Poor	Good(3)	Good	6.09	19.53
t_2	>70	Moderate	Moderate	Good	poor	Good(3)	Good	3.653	19.46
t_3	>70	Good	Moderate	Good	Poor	Good(3)	Good	10.63	21.97
t_4	>70	Moderate	Good	Good	Poor	Good(3)	Good	17.93	20.64
t_5	>70	Moderate	Problem	Good	Poor	Good(3)	Good	17.1	18.94
t_6	>70	Moderate	Problem	Good	Poor	Good(3)	Good	17.1	18.94
t_7	>70	Moderate	Problem	Good	Poor	Good(3)	Good	4.59	18.17
t_8	>70	Moderate	Problem	Good	poor	Good(3)	Good	4.59	18.17

[a]Percentage fraction of oral bioavailability of drug that reaches circulation after oral administration, [b]Likelihood of P-gp efflux, [c]First pass metabolism in the liver, [d]Intestinal membrane permeability (absorption) by passive or active transport. [e]predicated solubility, the level numbers in brackets are in accordance with the discovery studio classification for solubility as reported in literature (Reddy et al. 2012).

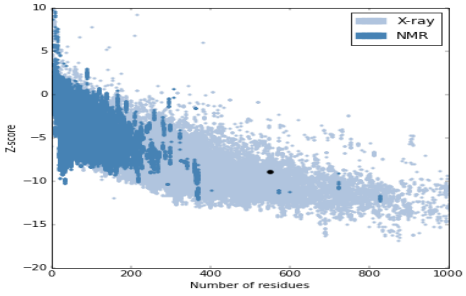
Molecular docking studies on COX-2

COX-2 enzyme was docked by the *N*-cinnamoyltetraketide derivatives (t_1 - t_8) that were used as ligands to predict binding energy (inhibition) and interaction to the enzyme. Before docking was carried out, the protein was analyzed for its residues to establish whether they are in the allowed or disallowed regions. The Ramachadran plot (Figure 2a) shows that number of residues in the favoured region was 96 per cent and the number of residues in allowed region was 4 per cent as expected. Further analyses of the protein with ProSA showed a good Z-score of -8.91 (Figure 2b) indicating quality of the protein. Z-score measures the deviation of total energy of the structure to an energy distribution obtained from random conformation. The energy diagram Figure 2c further indicated the quality of the protein with respect to its plotted knowledge-based energies against amino acid sequence. The docked compounds showed excellent binding pose energy to COX-2, with the best pose energy ranging between -6.5 and -13 Kcal/mol (Table 7). Ligands t_3, t_4 and t_5 showed low binding energy of -13, -9.2 and -12.3 kcal/mol, respectively, in comparison to other ligands. Since the binding energy is proportional to the inhibition constant, the obtained best pose binding energy implies that these molecules could potentially possess inhibition activities on COX-2 enzyme. Lower binding energy indicates good compatibility with COX-2 protein receptor. When compared to a standard drug, celecoxib (Madeswaran et al. 2012, Adinarayana et al. 2012), ligands t_3 and t_5 had the best pose energy close to that of celecoxib (-15.4 kcal/mol) (Table 7). Furthermore, the docking experiments were collated with the cytotoxic experimental values (Table 7, Figure 3) against the human breast cancer cells (Nyandoro et al. 2015). The experimental values correlated well with the docking experiments with r^2 = The binding modes of ligands with best pose energy (t_3, t_4 and t_5) are presented in

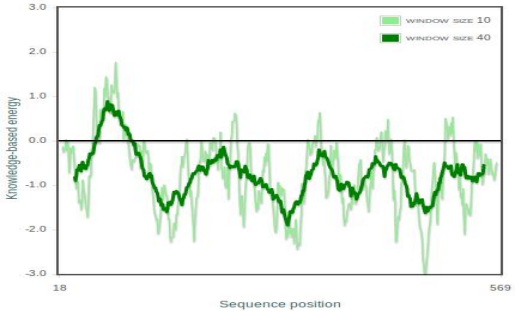
Figure 4. Ligand t_3 was found to bind to COX-2 with the binding pocket comprising of Asn1786, Glu1782, Arg1858, Tyr1789, Tyr1776, Lys1857, Phe1862, His1775, Gly1859, Val1774, Asp1799 and Glu2099 amino acid residues. No hydrogen bonding was formed (Figure 4a). Docking of ligand t_4 indicated bounding to COX-2 pocket involves Phe526, Lys527, Asn530, Ile528, Phe331, Trp515 Glu523, Thr531, Lys312 and Val524 residues. The ligand formed hydrogen bonding with Lys312 and Asn530 amino acid residues (Figure 4b). The binding of ligand t_5 to COX-2 as viewed in Pymol indicated interaction with Asp1313, Gly522, Leu335, Ile523, Arg1314, Pro517 and Lys330. Hydrogen bonding was formed between the ligand and amino acid residues Lys330 (Figure 4c). The observed hydrogen bonds and other interactions suggest possible targets of ligand t_5 to the COX-2 enzyme that could lead to inhibition activities. Docking of celecoxib as a control drug interacted with amino acid residues Glu523, Gly522, Val524, Phe526, Lys527, Asn530, Thr531, Ile528 and Ile529. Amino acid residue Val524 formed hydrogen bonding with celecoxib. The X-ray structure of celecoxib complex (Figure 4d) was further compared with the docked ligand. It was observed that, the docked ligand bound in the same pocket as celecoxib bind (Figure 4d).0.7627 indicating inhibition activities.



(a)



(b)



(c)

Figure 2: (a) Ramachandran Plot analysis of 3NTG. The plot statistics are; number of residue in favoured regions 528 (96 %) and number of residue in allowed regions 22 (4 %), the number of residues in the disallowed region was o (0 %). (b) ProSA web service analysis of NTG. In ProSA web the Z-score is determined by X-ray crystallography (light blue) or NMR spectroscopy (dark blue) with respect to their length. The Z score of the protein is highlighted as large dot. (c) Energy plot of 3NTG obtained from the ProSA.

Table 7: Best pose energies of the ligands as indicators of the potential for inhibition of COX-2 enzyme

Ligands	Best pose energy (kcal/mol)	[b]ED_{50} (µM)	-logED_{50}	Number of H-bond formed	Residues forming H-bond
t_1	-7.2	105.7	-2.02	-	-
t_2	-8.2	NT			
t_3	-13	23.6	-1.37	-	-
t_4	-9.2	70.6	-1.86	2	Lys330, Asp332
t_5	-12.3	15.5	-1.19	3	Lys512, Asn530
t_6	-7.8	NT			
t_7	-6.5	NT			
t_8	-7.3	NT			
Celecoxib	-15.4	-	-	2	Val524, Gly522

[b]Experimental values as reported (Nyandoro et al. 2015), NT = not tested (experimentally) for breast cancer cell lines

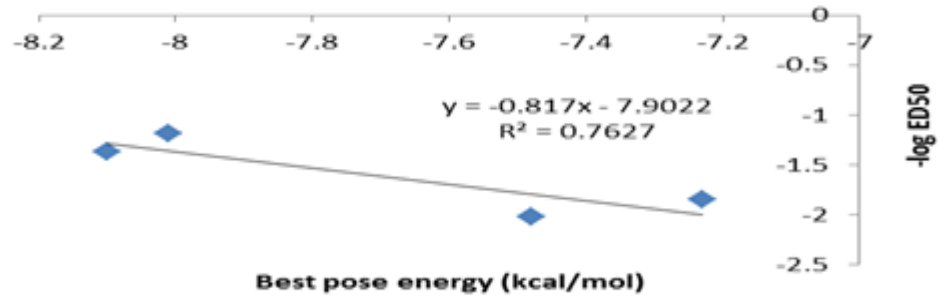


Figure 3: Predicated binding pose energy correlated to cytotoxic experimental value reported with r^2=0.7627

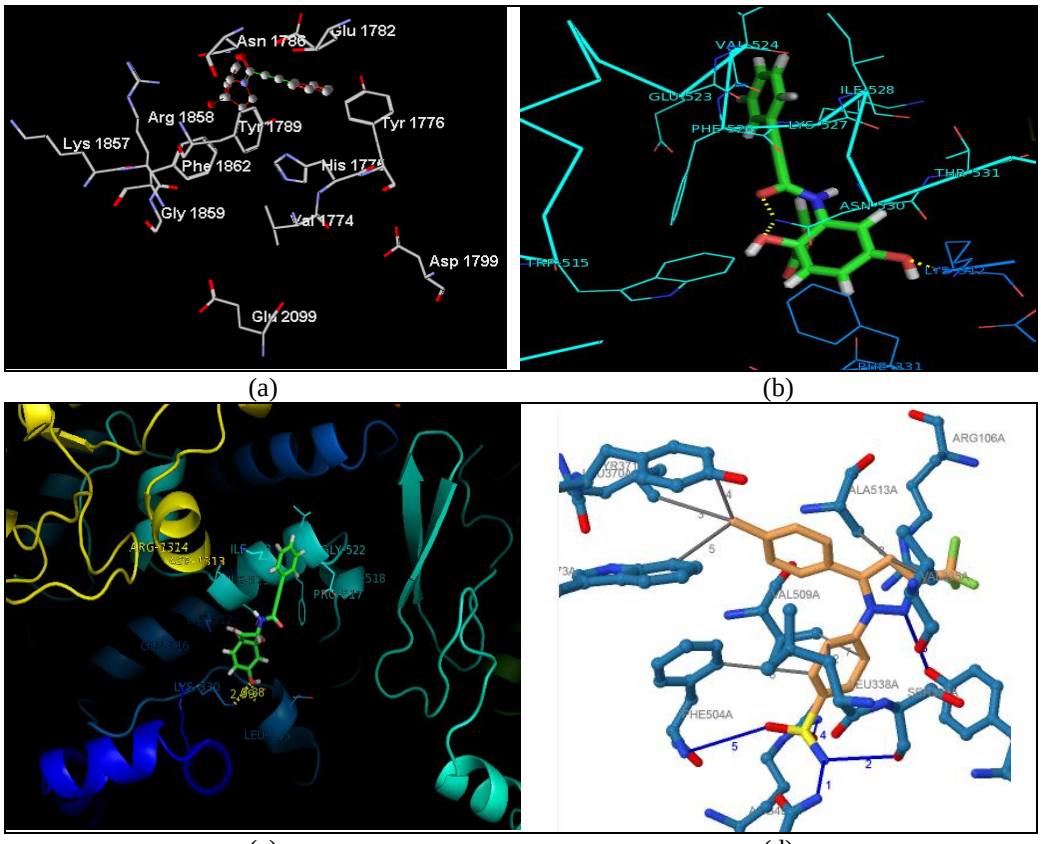


Figure 4: (a) Interaction of the amino acid residues of COX-2 enzyme with t_3 ligand as viewed by Molegro Molecular Viewer, (b) t_4 interacting with COX-2 amino acid residues as viewed in Pymol. Hydrogen bond was formed with Lys512 and Asn530 residues, hydrogen bonds are indicated by yellow dotted lines (c) Binding sites of t_5 to COX-2 enzyme indicating the amino acid residues of COX-2 enzyme. Yellow dotted lines indicate hydrogen bonding, (d) X-ray crystal structure of celecoxib-COX-2 complex. Blue lines indicated hydrogen bond and grey line indicates hydrophobic interactions. Celecoxib is shown in orange color.

Generally, the docking studies showed that the investigated ligands exhibit different binding orientations and to some extent, different or similar binding energies. Whereas ligand t_4 and t_5 were found to bind to similar pockets, ligand t_1 and t_3 were bound to other pockets and oriented differently from t_4 and t_5. This may be explained by the fact that COX-2 possesses three pockets of active site notably hydrophilic and hydrophobic pockets, and a third pocket lined with His90, Arg513, and Val523 residues (Llorens et al. 2002). Docking of the *N*-cinnamoyltetraketide derivatives depicted different binding and orientation in COX-2 enzyme. The predicted binding and orientation of t_4 and t_5 were similar to celecoxib, a known COX-2 inhibitor. However, compounds t_1 and t_3 bound to a different pocket of the COX-2

incompatible to celecoxib. The orientation of t_1 and t_3 in the pocket allowed their interactions to be closer to the amino acid residues Arg1858, Try1789, Asn1785 and Val524, Ile285, Glu523 Lys527, respectively. Docking analysis showed *N*-cinnamoyltetraketide derivatives to possess different binding patterns, phenomena reported for other known synthetic Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) that are COX-2 inhibitors (Llorens et al 2002). Formation of H bonding for ligands t_4 and t_5 as for celecoxib suggests COX-2 inhibitory properties for these compounds. Though, ligand t_1 and t_3 did not indicate H-bonding with any COX-2 amino acid residue, they showed a different mode of interaction with Val524, a residue found to interact with celecoxib. These results further suggest that *N*-cinnamoyltetraketide may inhibit COX-2 and therefore act as lead compounds towards development of suitable COX-2 inhibitors.

CONCLUSION

Pharmacokinetic profile and Lipinski Rule of Five evaluations of *N*-cinnamoyltetraketide derivatives indicate potential drug or lead likeness. *N*-cinnamoyltetraketide derivatives t_4 and t_5 have shown a comparable binding pocket and interaction to the known COX-2 inhibitor celecoxib *in silico*, unlike t_1 and t_3 that bound differently. The binding energy was in the order celecoxib (-15.4) > t_3 (-13) > t_5 (-12.3) > t_2 (-8.2) > t_6 (-7.8) > t_8 (-7.3) > t_1 (-7.2) > t_7 (-6.7) further suggesting the compounds to possess inhibition properties, some being comparable to the standard drug. These *in silico* results serve as pilot information that could be extended to *in vitro* and *in vivo* studies for possible discovery of novel COX-2 inhibitors possessing different mechanistic pathways.

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