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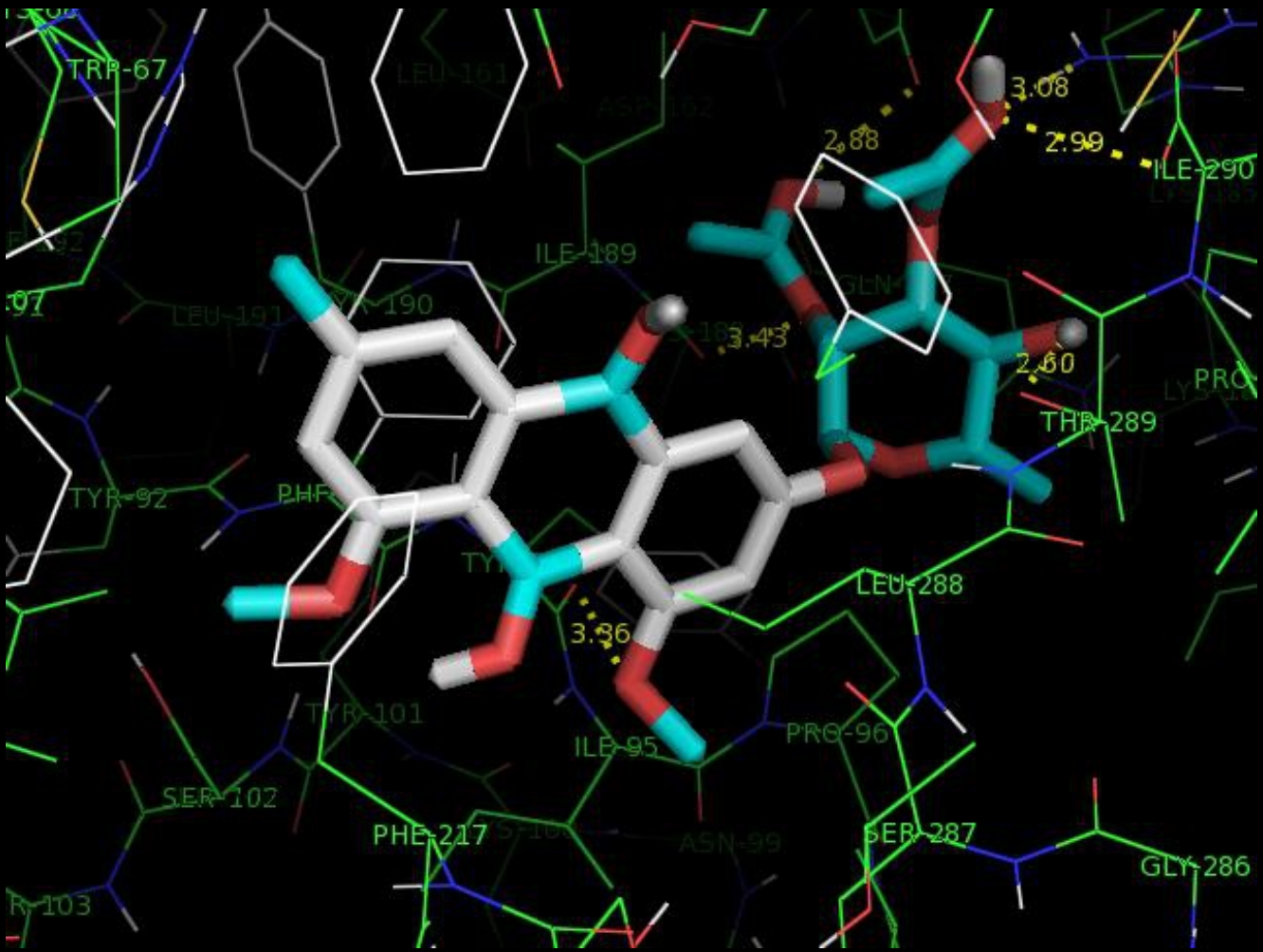

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Molecular Docking and ADMET Study of Emodin Derivatives as Anticancer Inhibitors of NAT2, COX2 and TOP1 Enzymes

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Abstract

Over the past years natural products and/or their derivatives have continued to provide cancer chemotherapeutics. Glycosides derivatives of emodin are known to possess anticancer activities. An *in silico* study was carried out to evaluate emodin derivatives as inhibitors of Arylamine N-Acetyltransferase 2, Cyclooxygenase 2 and Topoisomerase 1 enzymes, predict their pharmacokinetics and explore their bonding modes. Molecular docking study suggested that D2, D5, D6 and D9 to be potent inhibitors of NAT2, while D8 was suggested to be a potent inhibitor of TOP1. Derivatives D2, D5, D6 and D9 bind to the same pocket with different binding conformation. Pharmacokinetic study suggested that selected emodin derivatives can be potential cancer chemotherapeutic agent. Physicochemical parameters such density, balaban index, surface tension, logP and molar reflectance correlated to compounds activity. These finding provides a potential strategy towards developing NAT2 and TOP1 inhibitors.

Keywords

Human Arylamine N-Acetyltransferase 2 (NAT2), Cyclooxygenase 2 (COX2), Topoisomerase 1 (TOP1), Emodin, *In Silico* Inhibition, Pharmacokinetics, Molecular Docking

1. Introduction

Natural products have continued to be used as sources of potential anticancer agents over the years. Almost, half of the approved anticancer agents are from natural products or derivatives exhibiting broad pharmacological activities [1]. Emodin is an anthraquinone, is naturally distributed and well known to possess

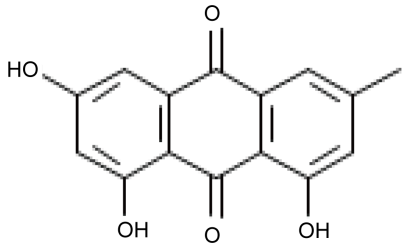
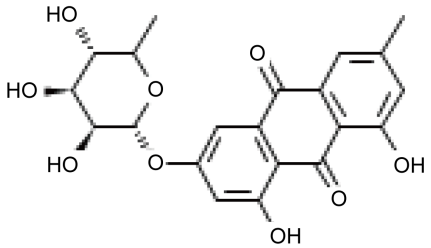
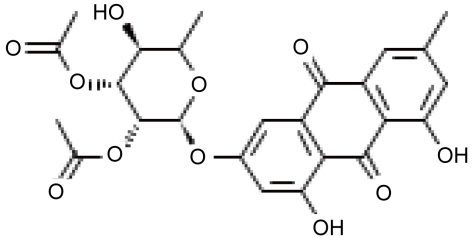
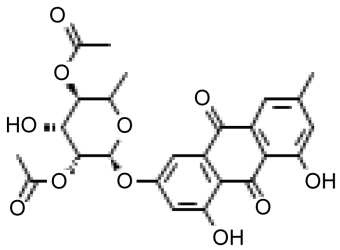
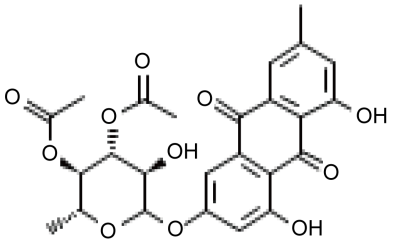
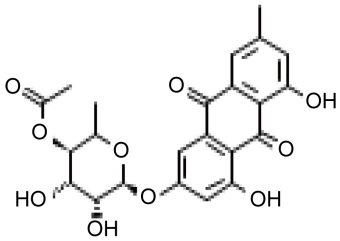
broad pharmacological activities [2]. Emodin (D1) has been reported to inhibit the proliferation of many cancer cells which include; Colon cancer [3] [4], breast cancer [5] [6], gall bladder cancer [7] [8], pancreatic cancer [9], lung cancer [10] [11] and human cervical cancer [12]. Poor bioavailability and toxicity *in vivo* have been documented to limit emodin as cancer chemotherapy [13]. Natural emodin glycoside derivatives have been reported to possess high antitumor activities than emodin [13]. It has been shown that modification of the glycoside by addition of sugar chain at carbon-3 hydroxide (C3-OH) increases solubility and antitumor activity of emodin [14].

Recently, Xing and co-workers [13], reported a novel derivatives of emodin (**Table 1**) which strongly inhibited anti-proliferative activities on different human cancer cell lines [13]. Thus, possession of broad anti-proliferative activities makes them become potential anticancer agents [13]. Despite of the fact that the anticancer activity of these derivatives on various cancer cells has been reported, their biological targets, molecular mechanisms of action and pharmacokinetic profile remain uncovered. Recently, the use of *in silico* approach has been found to help addressing the biological targets and molecular mechanism of action of small molecules with protein or enzymes. In this regard, molecular docking, modeling and pharmacokinetic (ADMET) studies were carried out on emodin derivatives specifically to three key enzymes involved in cancer formation namely; N-Acetyltransferase 2 (NAT2), Cyclooxygenase 2 (COX2) and Topoisomerase 1 (TOP1).

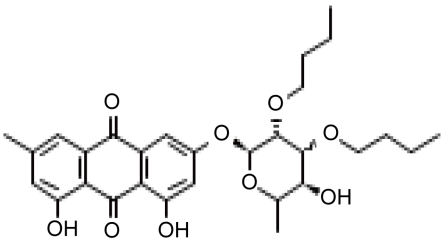
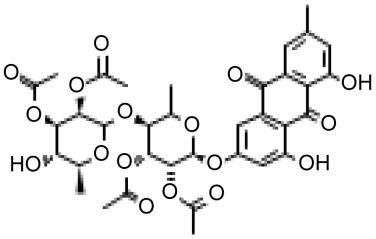
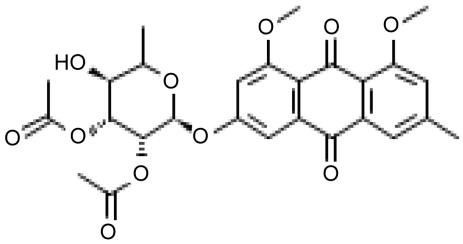
N-Acetyltransferase is an important enzyme known to catalyse the transfer of acetyl groups from acetyl CoA to Arylamines [15]. N-Acetyltransferase exist in two isozymes; NAT1 and NAT2. The two forms of enzymes are polymorphic and catalyse both *O*-acetylation (activation) and *N*-acetylation (deactivation) of aromatic and heterocyclic amine carcinogen [16]. The later isoform, Arylamine N-Acetyltransferase 2 (NAT2) is known to metabolize arylamine and hydrazine moieties present in several chemicals, carcinogens and therapeutic drugs [16]. The polymorphic gene encoding of NAT2 often results to slow or rapid acetylator phenotypes [16], which in-turn, causes drug-induced toxicities as well as risks of developing cancers of colon, bladder and lung [16]. Studies have shown a positive correlation of NAT2and rapid acetylator phenotype with colon cancer [17], while slow NAT1 has been positively correlated with urinary bladder cancer [18]. Heterocyclic aromatic amines (HAAs) are family of mutagenic compounds produced in meat subject to high temperature cooking. Consumption of HAAs is highly associated with the risk of colon, breast, lung, skin, liver cancers [16] [19]. Dietary of HAAs are transformed by the polymorphic NAT2 enzymes to carcinogen [16] [19]. Dietary NAT2 enzyme is known to express in genotypes dependent in colon epithelium. Increased NAT2 enzyme activity activates HAAs in colon, and thus, increasing the risk of colon cancer. Inhibition of the activity of NAT2 enzyme play an important role in colon cancer prevention and treatment.

Cyclooxygenase (COX) enzymes exist in two isoforms, cyclooxygenase 1

Table 1. Binding energies (kcal/mol).

Structure/Name	Derivative	Target protein		
		COX2	NAT2	TOP1
	D1	−8.8	−9.5	−6.9
	D2	−8.3	−10.6	−7.9
	D3	−7.1	−9.4	−7.7
	D4	−8.2	−8.8	−8
	D5	−8.2	−10.2	−8.4
	D6	−8.1	−10.2	−8.1

Continued

	D7	−7.4	−8.6	−7.3
	D8	−7.1	−7.2	−8.1
	D9	−6.7	−9.7	−7.2
Celecoxib		−15.4	−8.3	−9.2
Capecitabine				
Camptothecine				

(COX1) and cyclooxygenase 2 (COX2). COX is known to catalyze the conversion of arachidonic acid to prostaglandins which play a vital role in the proliferation of cancer cells [20]. COX2 overexpress in human breast tumor cells and is positively correlated to the development of breast cancer [20]. Consequently, its inhibition is essential towards treatment of breast cancer.

Human topoisomerase 1 (TOP1) enzyme is an important drug target in cancer treatment [21]. The enzyme is responsible for catalysing the breaking and re-joining of phosphodiester of the DNA strand throughout cell cycle. It relaxes super coiled DNA during DNA replication and transcription [22] [23]. TOP1 inhibitors work by blocking the ligation step of the cell cycle by generating single and double strands breaks which in turn harm the integrity of the genome; the break results to apoptosis cell death [24]. Apparently, TOP1 inhibitors play a great role towards cancer treatment.

Having showed broad anticancer activities on various cancer cells [13], emodin derivatives (**Table 1**) was therefore investigated *in silico* to reveal whether NAT2, COX2 and TOP1 enzymes are the molecular targets they exert their action. Furthermore, their pharmacokinetic profiles are predicated to unveil their ADMET properties.

2. Materials and Methods

2.1. Proteins and Ligands Accession

The three dimension (3D) crystal structure of NAT2 complexed with Coenzyme A, COX2 and TOP1 enzymes that were used for evaluation were obtained from RCSB protein data bank (PDB ID: 2PFR, PDB ID: 3NTG, PDB ID: 1A35) respectively, [25] [26] [27]. Emodin derivatives were searched from the literature [13].

2.2. Ligands and Protein Preparation

Ligand and Protein used in this study were prepared as previously reported [28] [29]. Ligand was generated using Chem3D Pro 12, geometry were optimized and energy minimized by using Vega ZZ and ArgusLab using AM1 force field [30] [31] to obtain coordinates with minimum energy and stable conformation. The optimized ligand was served in .pdb file format. The binding sites of NAT2, COX2 and TOP1 were analyzed by using 3D Ligand Site predication server [32]. The three proteins were separately prepared and energy minimized using Vega ZZ.

2.3. Docking Procedures

Docking experiments were done as reported by [28] [29]. Firstly, for NAT2 enzyme, docking experiment was validated by redocking the acetyl CoA. The acetyl CoA was extracted from the binding site and redocked again; the acetyl showed to bind in a similar pocket interacting with amino acid residue as before it was docked which validated the docking method. Docking was done by using PyRx-virtual screening tool, with AutoDockVina docking option based on scoring functions [33]. The energy interaction of NAT2, COX2 and TOP1 with ligands was assigned as grind point. A blind docking with flexible conformation was allowed to allow ligands to search the binding site. The parameters were set as default, except for energy of interaction between the derivatives and NAT2, COX2 and TOP1 which were evaluated using atomic affinity potentials computed on a grid.

2.4. ADMET Analysis

The pharmacokinetics (ADMET) were predicted using ADMET predictor ver. 8.0 [34], The predicated ADMET properties are; Blood-Brain Barrier (BBB), Human Intestinal Absorption (HIA), Caco-2 cell permeability, Pgp inhibition, CYP 450 inhibitor and substrate (CYP1A2, 2C9, 2D6, and 3A4) and hERG inhibition.

2.5. Physicochemical Descriptors and SAR Correlation Analysis

Various physicochemical descriptors (**Table 3**) were correlated to their structural activities as NAT2 inhibitors using ADMET Modeler ver. 8.0 [34] and SPSS ver.16. The observed and predicated activities (**Table 4**) were further used to

study correlated analysis of the activity. The two sets; training set, a set of data used to discover potentially predictive relationship, and a test set which was used to assess the strength and utility of a predictive relationship were used to compute the observed and predicated activities using ADMET modeler ver. 8.0. The correlation analysis was computed using SPSS ver. 16.

3. Results and Discussion

3.1. Molecular Docking Studies

Drug design and development are costly and time consuming. Computer Aided Drug Design (CADD) provides a valuable alternative to time and cost in designing and developing drugs [16]. Emodin derivatives were docked to NAT2, COX2 and TOP1 enzymes to evaluate their inhibition. Docking results of emodin derivatives and standard drugs against the three enzymes are presented in Table 1. These derivatives were selected for docking studies due to their broad anticancer activities they have shown [13]. Possession of broad activities motivated us to further investigate their interaction with selected enzymes (NAT2, COX2 and TOP1) as target for cancer chemotherapeutics.

A docking study was done by using PyRx-virtual screening tool. The lowest docking pose energy with lower root mean square deviation was selected as the docking score with best protein affinity (Table 1). Docking study of emodin derivatives to target enzymes was done along with standard drugs of the respective enzyme inhibitor. Docking of emodin derivatives to NAT2 enzyme showed interesting results. Derivatives D2, D5, D6 and D9 strongly inhibited NAT2 enzyme with binding affinity of −10.6, −10.2, −10.2 and −9.7 kcal/mol, respectively. Derivative D9 was in agreement to reported experimental results [13] which strongly inhibited various cancer cells, NAT2 could be the target enzyme of D9 which could work in a similar manner as described by [13]. The other derivatives D2, D5 and D6 probably inhibit NAT2 in a different way as D9 work. When compared to emodin, derivatives D2, D5, D6 and D9 inhibited strongly NAT2 than emodin which had −9.5 kcal/mol. Furthermore, when compared to known NAT2 inhibitor (capecitabine), derivatives D2, D5, D6 and D9 strongly inhibited NAT2 enzymes than capecitabine which had −8.3 kcal/mol (Table 2). Docking of emodin derivatives to COX2 enzymes indicated that, emodin had high inhibition than all derivatives. Derivatives D2 (−8.3 kcal/mol), D4 (−8.2 kcal/mo), D5 (−8.2 kcal/mol) and D6 (−8.1 kcal/mol) indicated lower inhibition to COX2 enzyme compared to emodin (−8.8 kcal/mol). Further, all derivatives had lower inhibition when compared to celecoxib a known COX2 potent inhibitor (Table 1). Docking results for these derivatives suggests that COX2 is not their target enzyme. Emodin derivative did not strongly inhibit TOP1 enzyme when compared to camptothecin a known TOP1 inhibitor. Docking results further suggested that, derivative D5, D6 and D8 can inhibit TOP1 enzyme. Experimental results [13] have shown that D8 and D9 strongly inhibit various human cancer cell lines in vitro. In the present study, molecular docking results are agreement to experimental finding [13]. It can further be suggested that D8 is an

Table 2. Pharmacokinetics (ADMET) profile of selected emodin derivatives.

Properties	Emodin derivatives				
	D2	D5	D6	D8	D9
MlogP[a]	0.173	0.233	0.206	−0.994	0.128
S + logP[a]	1.804	2.401	2.254	1.99	2.12
S + logD[a]	1.804	2.256	2.103	1.904	2.12
S + MDCK[b]	9.845	13.164	15.138	9.323	81.294
Perm Skin	1.704	5.602	3.351	5.896	1.803
S + Sw[a]	0.568	0.3	0.386	0.483	0.03
BBB Filter	Low	Low	Low	Low	Low
LogBB[c]	−0.531	−0.842	−0.683	−1.435	−0.983
HIA[d]**	60.677526	72.549229	67.291434	45.032	91.58083
PPB[e]**	77.929576	82.389526	80.018728	76.73368	83.92445
Caco2[f]**	17.0761	15.6549	16.6035	11.5246	18.1515
PrUnbnd	5.96	5.249	4.743	9.359	8.758
CYP2D6 Inh	No (70%)	No (79%)	No (83%)	No (83%)	No (95%)
CYP2D6 km	Non substrate	Non substrate	Non substrate	Non substrate	Non substrate
CYP2D6 Vmax	Non substrate	Non substrate	Non substrate	Non substrate	Non substrate
CYP2D6 Clint	Non substrate	Non substrate	Non substrate	Non substrate	Non substrate
CYP3A4 Inh	Yes	Yes	Yes	Yes*	Yes*
CYP3A4 km	Non substrate	Non substrate	Non substrate	5.592	5.051
CYP3A4 Vmax	Non substrate	Non substrate	Non substrate	3.148	10.107
CYP3A4 Clint	Non substrate	Non substrate	Non substrate	62.482	222.115
hERG Filter	No (95%)	No (95%)	No (95%)	No	No (95%)
hERG pIC50[g]	3.754	3.895	3.895	3.878	3.955
Rat Acute	860.562	958.379	958.379	1771.6	1712.419
Rat TD50	24.45	24.013	24.013	1.463	2.314

*The site of metabolism is shown in Figure 4, **Properties were predicated using Pre-ADMET server. [a]Lipinski rule of five used to evaluate lipophilicity, it requires molecular a to have MlogP < 5, [b]predicated Madin-Darby canine kidney (MDCK) cell permeability recommended range (<25 poor and >500 great), [c]Blood brain barrier recommended range (−3 to 1). [d]Predicated Human intestinal absorption recommended range. [e]Plasma protein binding, [f]Permeability to carcinoma cell recommended range (<5 low and >100 high), [g]Predicated inhibition concentration to human ether a-go-go-related gene, the potential risk for inhibitors ranges 5.5 - 6.

inhibitor of TOP1 enzyme, while D9 is an inhibitor of NAT2 enzyme. Docking analysis of all emodin derivatives to three key cancer chemotherapeutic target suggest that, NAT2 is the target enzyme for these derivatives with D2, D5, D6 and D9 having better binding energy (Table 1). Thus, derivatives D2, D5, D6, D8 and D9 were further investigated for their pharmacokinetic properties.

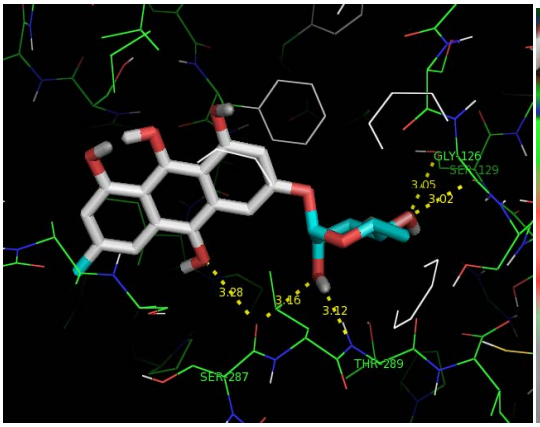
Emodin derivatives D2, D5, D6, D8 and D9 were further analysed for their

interaction with NAT2 and TOP1 enzymes, as they strongly inhibited these enzymes compared to COX2 enzyme. Interaction analyses are presented in **Figures 1(a)-(i)**. Interaction of D2 with NAT2 involved five hydrogen bonds (**Figure 1(a)**, **Figure 1(b)**), the bond formed involved Ser287---OH (3.28 Å), the hydroxyl group (OH) which formed the hydrogen bond with amino acid Ser287 was from the ring B (**Figure 2(a)**). The other four hydrogen bond were contributed from the rhamnoside moiety which includes; Ser287---OH (3.16 Å), Thr289---OH (3.12 Å), Ser129---OH (3.02 Å) and Gly126---OH (3.05 Å). Interaction of D5 with NAT2 formed seven hydrogen bond (**Figure 2(c)**, **Figure 2(d)**), all hydroxyl groups in the rings formed hydrogen with NAT2 (**Figure 2(b)**). The residues involved in forming hydrogen bonds includes; Ser216---OH (3.19 Å), Phe93---OH (3.34 Å), Ser125---OH (3.05 Å) of ring near sugar moiety (ring C), Thr289---OH (3.36 Å) and Gly125---OH (3.38 Å) both from ring B (**Figure 2(b)**), Gly125---OH (2.98 Å) and Ser129---OH (2.86 Å) both from ring A (**Figure 2(b)**). Derivative D6 formed five hydrogen bonds with NAT2, the amino acid residues involved in forming hydrogen bonds were; Lys188---OH (3.08 Å) from sugar moiety, Tyr94---O (2.87 Å) from ring B (**Figure 2(c)**), Gln163---O (3.10 Å), Ile---OH (3.07 Å) and Arg165---OMe (3.09 Å). Derivative D9 formed six hydrogen bonds with NAT2 enzyme, one hydrogen bond was from ring C (**Figure 2(d)**). Other hydrogen bonds were from the sugar moiety which contributed to its increase in activity. The interaction of D9 with NAT2 by forming hydrogen bond with amino acid residue Try94 could further explain the increased activity of D9. The hydrogen bond interaction with amino acid residue was Tyr94---OH (3.36 Å) from ring C, Lys188---MeOMe (3.43 Å), Ile290---OH (2.99 Å), Thr289---OH (2.60 Å) (**Figure 1(g)**, **Figure 2(d)**). The interaction of D8 with TOP1 involved several hydrogen bond, the sugar moiety were responsible in forming many hydrogen bonds (**Figure 1(h)** and **Figure 1(i)**). It was interesting to note that, like known TOP1 inhibitors such as camptothecin which interacts and forms hydrogen bonds with Arg364, Lys532 and Asn722 [22], D8 also interacted and formed hydrogen bonds with such amino acid residues. Other amino acid residue formed hydrogen bond with D8 are Asp440---OH (3.14 Å) from ring A, Lys443---O (2.86 Å) from ring B and Lys443---OH (3.04 Å) ring C. Derivative D8 could inhibit TOP1 enzymes via a similar mechanism of action as camptothecin.

Docking analysis further showed that D2, D5 D6 and D9 with different conformation bind to similar binding pocket at different conformation of NAT2 (**Figure 3**). Binding of ligands at the same pocket with different conformation may be due to changes of receptor specific conformation as explained by [35]. Binding to same pocket may further suggest similar mechanism of action for these derivatives.

3.2. Pharmacokinetic (ADMET) Analysis

Many drugs fail to enter into clinical markets due to poor pharmacokinetics. In the present study, we explored the pharmacokinetics of the selected compounds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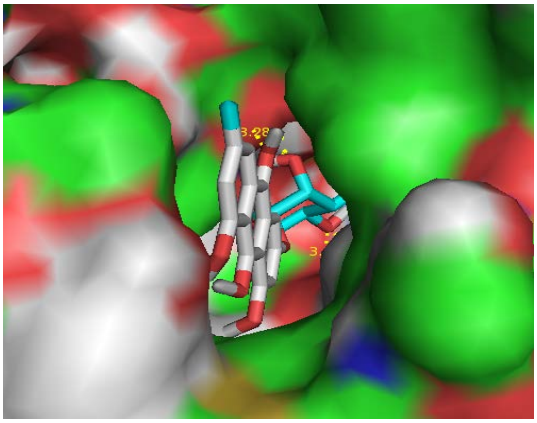
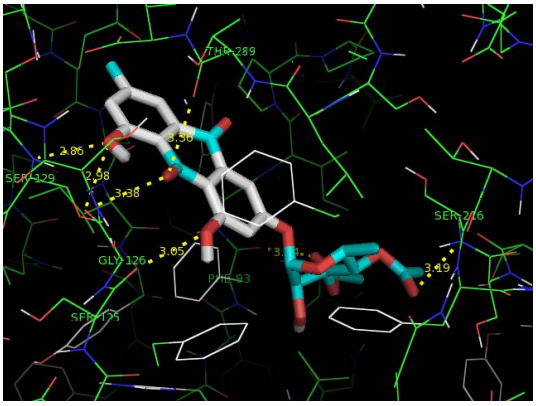
SER-129
3.05
3.02
3.08
3.16
3.12
SER-287
THR-289

(a)



(b)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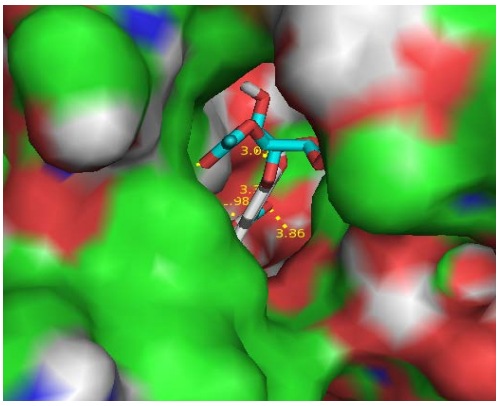
SER-129
2.98
3.38
SER-216
GLY-126
3.05
3.19
PHE-93
SER-125

(c)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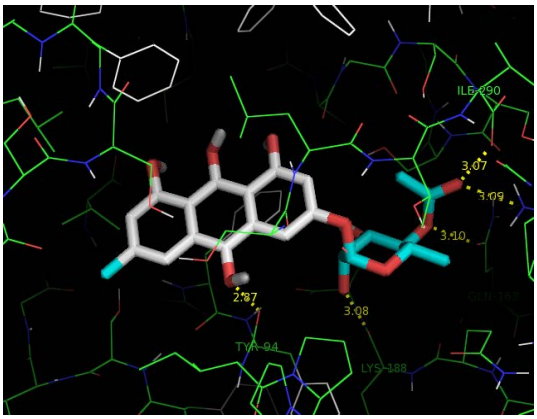
(d)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3.10
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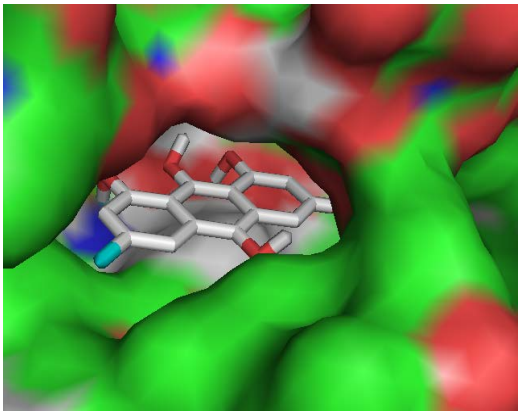
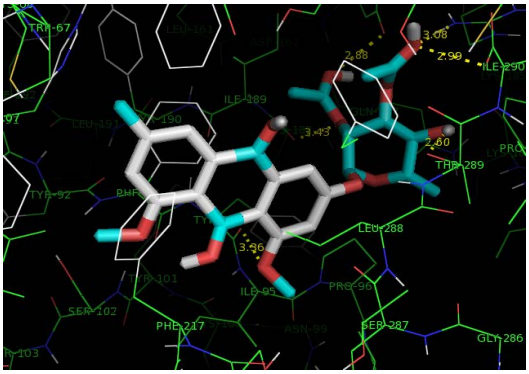
LYS-188

(e)



(f)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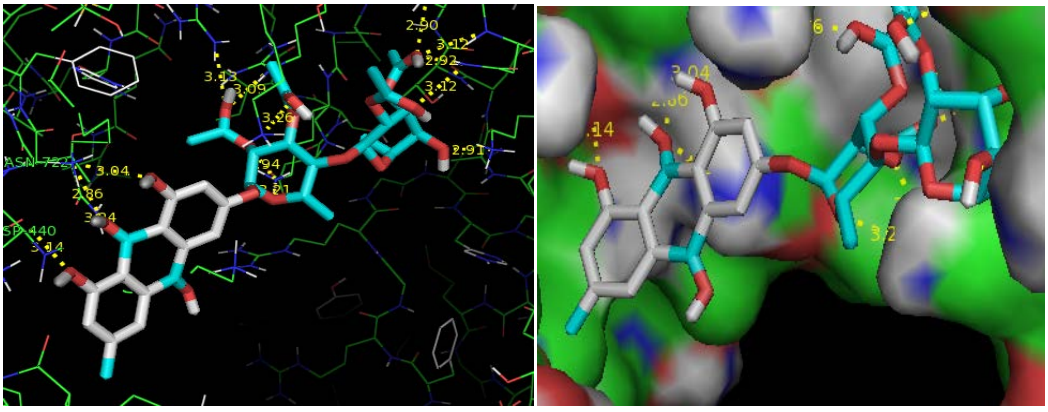
3.08
2.88
2.99
ILE-290
ILE-189
3.43
2.60
THR-289
LEU-288
3.36
PRO-96
SER-102
PHE-217
SER-287
GLY-286

(g)



(h) (i)

Figure 1. (a)-(g) molecular interaction of D2, D5, D6 and D9 with NAT2 enzyme, (h)-(i) interaction of D8 with TOP1 as viewed by PyMol. Interaction was stabilized by presence of hydrogen bonds indicated by yellow dotted line.

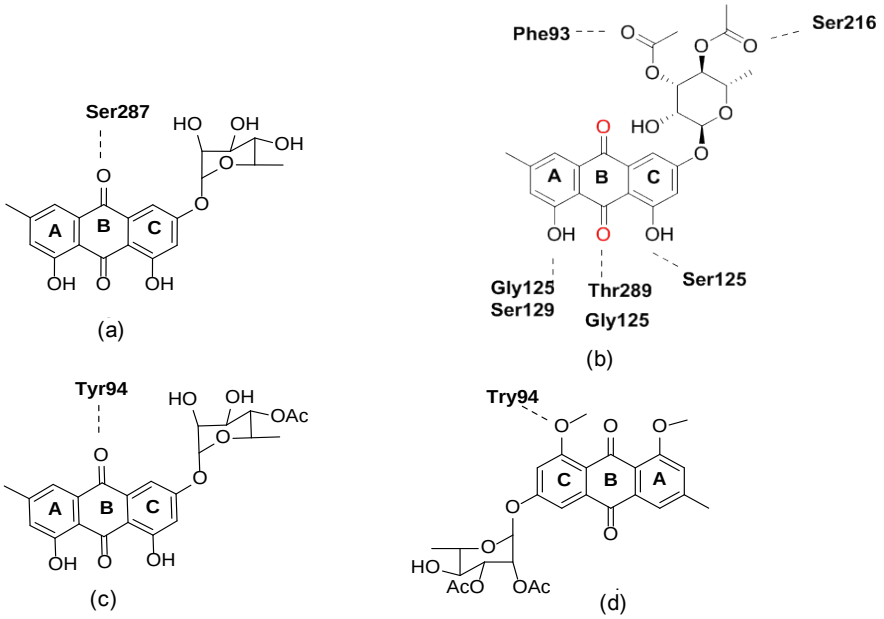


Figure 2. 2D representations of the interaction of D2, D5, D6 and D9 with NAT2 shown in figure (a), (b), (c) and (d), respectively.

using ADMET Predictor™. The pharmacokinetics parameters (Table 2) analyzed includes: MlogP, S + logP, S + logD, BBB, Caco-2, HIA, S + MDCK, PPB, Pgp inhibition, CYP 450 inhibitor and substrate (2D6 and 3A4), hERG inhibition, S + Sw, skin permeability and rat acute toxicity, CYP 3A4 intrinsic clearance, CYP 2D6 intrinsic clearance and kinetic parameters for CYP 2D6 and 3A4 enzymes. Lipophilicity defined as the ability of a chemical compound to dissolve in fats, oils, lipids and non-polar solvents, is a physicochemical property which affects drug transport through lipid structure and drug interaction and with the target protein [36]. Lipophilicity and water solubility was predicated using different models: Moriguchi model of octanol-water partition coefficient (MlogP), octanol-water partition coefficient (S + logP) and octanol-water distribution

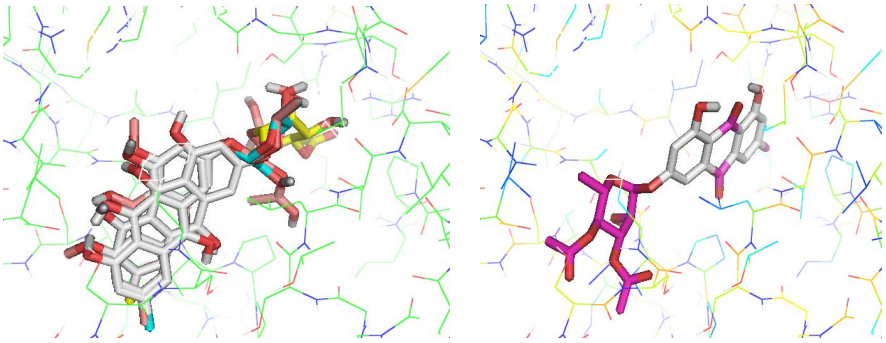


Figure 3. Positions of derivatives in NAT2 active site after docking, (a) Superposing of D2, D6 and D9 showing similar position (b) D5 showing different binding position in the same pocket. Binding in different position could be explained by the acetyl chain on the sugar ring causing it to bind in different position, unlikely for D2, D6 and D9.

coefficient (S + logD) calculated from S + *pKa* and S + logP. Water solubility was predicated using native water solubility model (S + Sw, (mg/mL)).

All compounds showed lipophicility and aqueous water solubility to be in an acceptable range (**Table 2**). The Lipinski rule of five was further used to evaluate lipophilicity and water solubility, the rule requires that, for a compound to have good lipophilicity it should have no more than 5 MlogP value (MlogP ≤ 5). The predicated BBB filter indicated low for all derivatives suggesting that they may not permeate to the brain and thus not causing damage to the central nervous system, the computed log BB fell with the recommended range (−3 to 1) for log BB [37]. Oral bioavailability was predicated by using Madin-Darby canine kidney (MDCK) cell permeability, the recommended range for MDCK is <25 poor and >500 great. Results indicated that, except derivative D9 all other derivatives were indicated to have poor oral bioavailability (**Table 2**). Permeability to carcinoma cell (Caco-2) was studied (**Table 2**), all the derivatives indicated moderately permeability with values falling within the recommended range (<5 low and >100 high). The predicated plasma protein binding was below 90 (<90%) suggesting that even though they bind to protein, there is available fraction of molecules required for exerting therapeutic effects (**Table 2**). It is well acknowledged that, metabolism plays an important role in drug-drug interaction and bioavailability. Cytochrome CYP 450 is known to metabolize drugs during phase I metabolism [38]. Metabolism of the selected compounds was evaluated using the developed model for cytochrome P450 (CYP) site of metabolism, kinetic parameters (estimated Michael-Menten Km constant for predicated sites of metabolism (μM), estimated Michael-MentenVmax constant for predicated sites of metabolism (nmol/min/nmol enzyme) and estimated intrinsic clearance (Clint) for predicated sites of metabolism (μL/min/mg HLM protein) and inhibition. CYP 2D6 and CYP 3A4 are important isoform enzymes involved in drug metabolism, CYP 3A4 is known to metabolize half of the drugs. Thus, prediction of CYP inhibitor is of great importance in drug development. In this study, the kinetic parameters and inhibition of CYP 2D6 and CYP 3A4 was predicated using the model developed in ADMET Predictor™ (**Table 2**).

Results showed that, all compounds were non inhibitors of CYP 2D6 and non-substrate for all kinetic parameter (Table 2). Results suggest that, these compounds could be well metabolized by CYP 2D6, easy cleared from the body and thus causing little toxicity. However, it was further noted that, D8 and D9 inhibited CYP 3A4 enzyme (Table 2), while D2, D5, and D6 were nonsubstrate to CYP 3A4 Km, Vmax and Clint, suggesting to be cleared without causing significant toxicity. Further analysis showed that, derivatives D8 and D9 were predicted to be substrate to CYP 3A4 (Figure 4). Human toxicity was evaluated using the model human ether-a-go-go related gene inhibition (hERG_pIC50). hERG blockers are known to prolong the QT interval and results to fatal cardiac arrhythmia known as *Torsades de pointes.* Thus, potential blockers of hERG potassium channel need to be investigated early during drug development and has recently been a major concern in pharmaceutical industries. The model filter provides Yes or NO to question "is the compound potential hERG inhibitor?", the model further predicts hERG_pIC50 (Table 2). The hERG risk value for hERG_pIC50 starts at 5.5 to 6. The pIC50 for all compounds was below 5.5 (<5.5) indicating compounds to be non-blocker of the hERG, thus non-toxic. Generally, the predicated ADMET properties for derivatives suggest that these derivatives can be possible inhibitors of NAT2 and TOP1 enzymes with desirable pharmacokinetic properties.

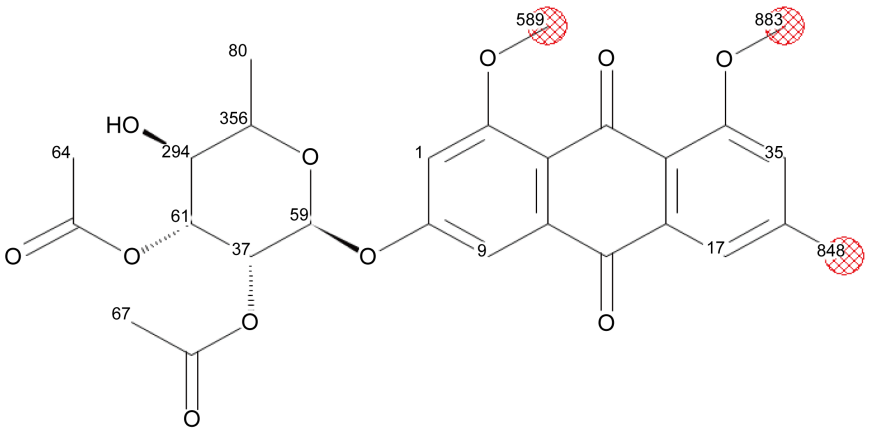
3.3. SAR Correlation Analysis

An attempt to correlate the activity of compounds with their structure or property descriptors as NAT2 inhibitors was done using computational approaches. Computational approaches are nowadays used to classify compounds and correlate their activities using physicochemical descriptors. In the present study, different physicochemical descriptors (Table 3) were calculated using computational tools and online software and correlated to their activities pIC50 (Table 3). Predicated and observed values (Table 4) were also correlated which gave r^2 = 0.56 upon removal of the outlier (D4 and D8) from the training set. The correlation results of the physicochemical descriptors with the pIC50 are presented in Table 5. The index of reflectance (IoR) correlated positively and significantly contributed to enhancement of activities ($P < 0.05$) however, the lipophilicity (logP) as well as molar reflectance (MR) negatively correlated to activities of the compounds.

4. Conclusion

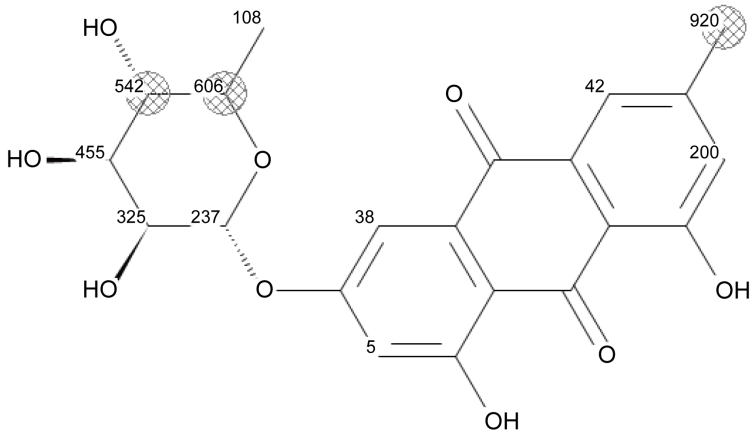
In the present study, emodin derivatives were *in silico* valuated for their inhibitory activities and pharmacokinetics on NAT2, COX2 and TOP1 enzymes as agents for colon and other forms of cancer. Docking studies suggested that D8 to be a target inhibitor of TOP1 while D5, D6 and D9 targets inhibitors of NAT2 enzymes. Pharmacokinetics suggested that these compounds can be potential anticancer agents. Physicochemical parameter correlated to the compounds activities.

D9


589
883
80
HO
356
294
64
1
35
61
59
37
9
17
848
67

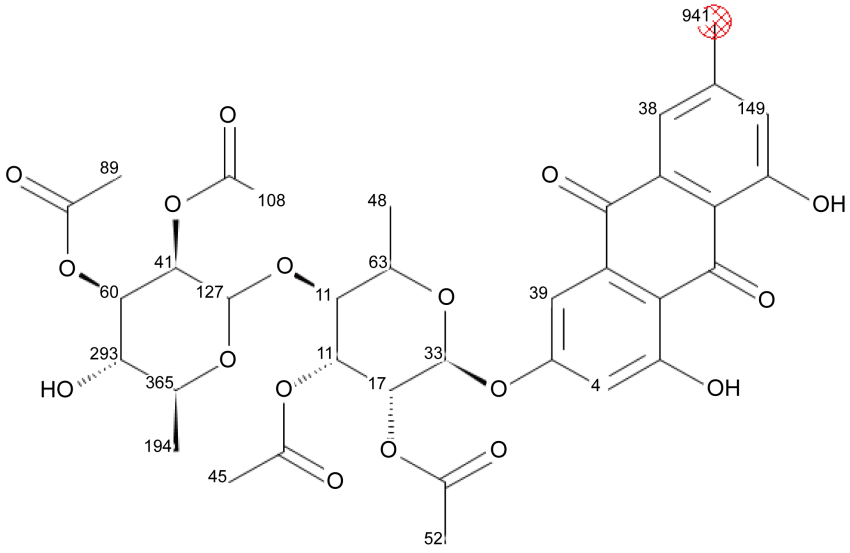
(a)

D2

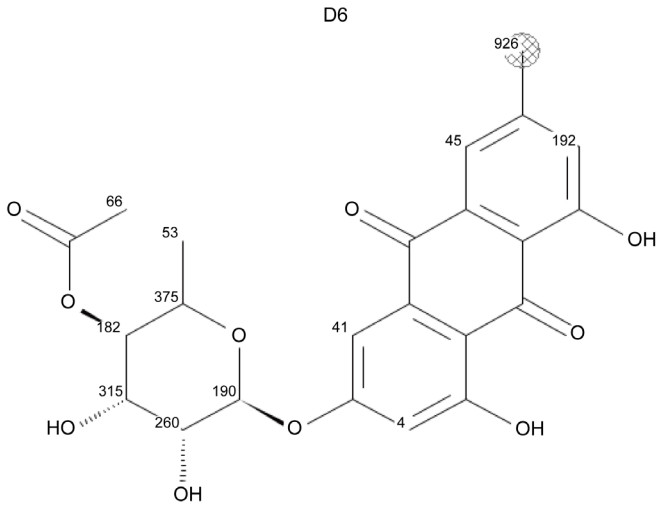

HO
108
920
542
606
42
HO
455
200
325
237
38
HO
OH
5
OH

(b)

D8


941
38
149
89
108
48
OH
41
63
60
127
11
39
293
11
33
HO
365
17
4
OH
194
45
52

(c)



(d)

Figure 4. CYP 3A4-Site of metabolism for D2, D6, D8 and D9. Display of atomic properties for the first step of metabolic oxidation by a CYP 450. Results are generated for likely substrate (sites in red) and likely non-substrate (sites in grey).

Table 3. Physicochemical descriptors.

Derivatives	IC50[a]	D[b]	ST[c]	IoR[d]	logP[e]	MR[f]	J[g]	J_MSD[h]
D1	19.54	1.583	85.4	1.744	3.064	69.13	1.899	4.05
D2	20	1.597	86.1	1.706	1.804	101.44	1.486	6.236
D3	20	1.52	76.7	1.648	2.265	119.45	1.522	7.001
D4	9.27	1.52	76.7	1.648	2.486	119.45	1.497	7.161
D5	3.16	1.52	76.7	1.648	2.401	119.45	1.479	7.27
D6	2.25	1.57	85.3	1.68	2.254	109.87	1.45	6.96
D7	2.78	1.32	62.4	1.606	4.703	137.75	1.49	7.519
D8	2.6	1.49	71.4	1.614	1.99	170.81	1.383	9.076
D9	3.63	1.39	61.5	1.599	2.12	129.13	1.556	7.167

[a]IC50 in μg/mL of A549 cells [13], data for this cell type were chosen because emodin derivatives showed better inhibition to this cell unlikely for other cancer cells. [b]Density it related the size and bulk of the substituent, it is calculated by ACD/Lab, [c]Surface tension calculated by ACD/Lab, [d]Index of reflectance calculated by ACD/Lab, [e]ThelogP was calculated using ADMET Predictor™. [f]Molar reflectance was calculated using ACD/Lab, [g]Balaban distance connectivity index of the hydrogen suppressed molecular graph, [h]Balaban mean square distance index of the hydrogen-suppressed molecular graph.

Table 4. Comparison of predicted and observed activities for the training and test set.

Derivatives	Predicted	Observed	Residual[j]	SET
D1	19.419	19.54	0.121	Training
D2	20.982	20	−0.982	Training
D3	12.799	20	7.201	Training
D4	−0.867	9.27	10.137	Test
D5	10.055	3.16	−6.895	Training
D6	13.553	2.25	−11.303	Test
D7	3.869	2.78	−1.089	Training
D8	−7.113	2.6	9.713	Test
D9	1.903	3.63	1.727	Training

[j]Residual = Observed − Predicated.

Table 5. Correlation analysis showing correlation of physicochemical descriptors and inhibitory activities.

	pIC50**	J	J_MSD	D	ST	IoR	LogP	MR
pIC50**	1.000							
J	0.538	1.000						
J_MSD	−0.668*	−0.898	1.000					
D	0.557	0.229	−0.460	1.000				
ST	0.561	0.278	−0.555	0.960	1.000			
IoR	0.683*	0.633	−0.837	0.825	0.903	1.000		
LogP	−0.184	0.236	−0.118	−0.616	−0.402	−0.162	1.000	
MR	−0.642	−0.778	0.968	−0.571	−0.670	−0.870	−0.016	1.000

*Correlation is significant at the 0.05 level, **predicted IC50 values from the IC50 [13].

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References

[1] Newman, D.J. and Cragg, G.M. (2012) Natural Products as Sources of New Drugs over the 30 Years from 1981 to 2010. *Journal of Natural Products*, **75**, 311-335. https://doi.org/10.1021/np200906s

[2] Chun-Guang, W., Jun-Qing, Y., Bei-Zhong, L., Dan-Ting, J., Chong, W., Liang, Z., *et al*. (2010) Anti-Tumor Activity of Emodin against Human Chronic Myelocyticleukemia K562 Cell Lines *in Vitro* and *in Vivo*. *European Journal of Pharmacology*, **627**, 33-41. https://doi.org/10.1016/j.ejphar.2009.10.035

[3] Ma, Y.S., Weng, S.W., Lin, M.W., Lu, C.C., Chiang, J.H., Yang, J.S., Lai, K.C., Lin, J.P., Tang, N.Y., Lin, J.G. and Chung, J.G. (2012) Antitumor Effects of Emodin on LS1034 Human Colon Cancer Cells *in Vitro* and *in Vivo*: Roles of Apoptotic Cell Death and LS1034 Tumor Xenografts Model. *Food and Chemical Toxicology*, **50**, 1271-1278. https://doi.org/10.1016/j.fct.2012.01.033

[4] Damodharan, U., Ganesan, R. and Radhakrishnan, U.C. (2011) Expression of MMP2 and MMP9 (Gelatinases A and B) in Human Colon Cancer Cells. *Applied Biochemistry and Biotechnology*, **165**, 1245-1252. https://doi.org/10.1007/s12010-011-9342-8

[5] Huang, Z., Chen, G. and Shi, P. (2008) Emodin-Induced Apoptosis in Human Breast Cancer BCap-37 Cells through the Mitochondrial Signalling Pathway. *Archives of Pharmacal Research*, **31**, 742-748. https://doi.org/10.1007/s12272-001-1221-6

[6] Huang, Z., Chen, G. and Shi, P. (2009) Effects of Emodin on the Gene Expression Profiling of Human Breast Carcinoma Cells. *Cancer Detection and Prevention*, **32**, 286-291. https://doi.org/10.1016/j.cdp.2008.12.003

[7] Li, X.X., Dong, Y., Wang, W., Wang, H.L., Chen, Y.Y., Shi, G.Y. and Wang, J. (2013) Emodin as an Effective Agent in Targeting Cancer Stem-Like Side Population Cells of Gallbladder Carcinoma. *Stem Cells and Development*, **22**, 554-566. https://doi.org/10.1089/scd.2011.0709

[8] Li, X.X., Wang, J., Wang, H.L., Wang, W., Yin, X.B., Li, Q.W., Chen, Y.Y. and Yi, J.

(2012) Characterization of Cancer Stem-Like Cells Derived from a Side Population of a Human Gallbladder Carcinoma Cell Line SGC-996. *Biochemical and Biophysical Research Communications*, **419**, 728-734. https://doi.org/10.1016/j.bbrc.2012.02.090

[9] Liu, A., Chen, H., Wei, W., Ye, S., Gong, J., Jiang, Z., Wang, L. and Lin, S. (2011) Antiproliferative and Antimetastatic Effects of Emodin on Human Pancreatic Cancer. *Oncology Reports*, **26**, 81-89.

[10] Ko, J.-C., Su, Y.-J., Lin, S.-T., Jhan, J.-Y., Ciou, S.-C., Cheng, C.M. and Lin, Y.-W. (2010) Suppression of ERCC1 and Rad51 Expression through ERK1/2 Inactivation Is Essential in Emodin-Mediated Cytotoxicity in Human Non-Small Cell Lung Cancer Cells. *Biochemical Pharmacology*, **79**, 655-664. https://doi.org/10.1016/j.bcp.2009.09.024

[11] Lin, H., Juanjuan, B., Qian, G., Yin, Y., Xiufeng, Y. and Wei, L.J. (2011) Emodin Down-Regulates ERCC1 and Rad51 and Inhibits Proliferation in Non-Small Cell Lung Cancer Cells. *Journal of Third Military Medical University*, **33**, 2370-2375.

[12] Wang, Y., Hui, Y., Zhang, Y., Liu, Y., Ge, X. and Wu, X. (2013) Emodin Induces Apoptosis of Human Cervical Cancer Hela Cells via Intrinsic Mitochondrial and Extrinsic Death Receptor Pathway. *Cancer Cell International*, **13**, 71. https://doi.org/10.1186/1475-2867-13-71

[13] Xing, J.-Y., Song, G.-P., Deng, J.-P., Jiang, L-Z., Xiong, P., Yang B.-J. and Liu, S.-S. (2015) Antitumor Effects and Mechanism of Novel Emodin Rhamnoside Derivatives against Human Cancer Cells in Vitro. *PLoS ONE*, **10**, e0144781. https://doi.org/10.1371/journal.pone.0144781

[14] Lin, C.N., Chung, M.I., Gan, K.H. and Lu, C.M. (1991) Flavonol and Anthraquinone Glycosides from Rhamnusformosana. *Phytochemistry*, **30**, 3103-3106. https://doi.org/10.1016/S0031-9422(00)98262-1

[15] Evans, D.A. (1989) N-Acetyltransferase. *Pharmacology & Therapeutics*, **42**, 157-234. https://doi.org/10.1016/0163-7258(89)90036-3

[16] George, S., Santhlingam, K., Chandran, M., Gangwar, P. and Gururagavan, M. (2012) Docking Studies of Novel Coumarin Derivatives as Arylamine N-Acetyltransferance 2 Inhibitors. *Asian Journal of Pharmaceutical and Clinical Research*, **5**, 94-96.

[17] Borlack, J. and Reamon-Buetter, S.M. (2006) N-Acetyltransferance 2 (NAT2) Gene Polymorphisms in Colon and Lung Cancer Patients. *BMC Medical Genetics*, **7**, 58. https://doi.org/10.1186/1471-2350-7-58

[18] Hein, D.W., Doll, M.A., Fretland, A.J., Leff, M.A., Webb, S.J., Xiao, G.H., Devanaboyina, U.S., Nangui, N.A. and Feng, Y. (2009) Molecular Genetics and Epidemiology of the NAT1 and NAT2 Acetylation Polymorphisms. *Cancer Epidemiology, Biomarkers & Prevention*, **9**, 29-42.

[19] Rahman, U., Sahar, A., Khan, M.I. and Nadeem, M. (2014) Production of Heterocyclic Aromatic Amines in Meet: Chemistry, Health Risks and Inhibition. *LWT—Food Science and Technology*, **59**, 229-233. https://doi.org/10.1016/j.lwt.2014.06.005

[20] Madeswaran, M., Ummaheswari, K., Asokkumar, T., Sivashnmugam, V. and Subhadradevi, P. (2012) *In Silico* Docking Studies of Cyclooxygenase Inhibitory Activity of Commercially Available Flavonoids. *Asian Journal of Pharmaceutical Sciences*, **2**, 174-181.

[21] Ranger, G.S., Thomas, V., Jewell, A. and Mokbel, K. (2004) Elevated Cyclooxygenase-2 Expression Correlates with Distant Metastases in Breast Cancer. *Anticancer Research*, **24**, 2349-2351.

[22] Laco, G.S., Collins, J.R., Luke, B.T., Kroth, H., Sayer, J.M., Jerina, D.M. and Pommier, Y. (2002) Human Topoisomerase I Inhibitors: Docking Camptothecin and Derivatives into a Structure Based Active Site Model. *Biochemistry*, **41**, 1428-1435. https://doi.org/10.1021/bi011774a

[23] Deweese, J.E., Osheroff, M.A. and Osheroff, N. (2008) DNA Topology and Topoisomerases: Teaching a "Knotty" Subject. *Biochemistry and Molecular Biology Education*, **37**, 2-10. https://doi.org/10.1002/bmb.20244

[24] Xu, Y. and Her, C. (2015) Inhibition of Topoisomerase (DNA) I (TOP1): DNA Damage Repair and Anticancer Therapy. *Biomolecules*, **5**, 1652-1670. https://doi.org/10.3390/biom5031652

[25] Wu, H., Tempel, W., Dombrovski, L., Loppnau, P., Weigelt, J., Sundstrom, M., Arrowsmith, C.H., Edwards, A.M., Bochkarev, A., Grant, D.M. and Plotnikov, A. N (2007) Structural Basis of Substrate-binding Specificity of Human Arylamine N-Acetyltransferases. Structural Genomics Consortium. *Journal of Biological Chemistry*, **282**, 30189-30197.

[26] Wang, J.L., Limburg, D., Graneto, M.J., Springer, J., Rogier, J., Hamper, B., Liao, S., Pawlitz, J.L., Kurumbail, R.G., Maziasz, T., Talley, J.J., Kiefer, J.R. and Carter, J. (2010) The Novel Benzopyran Class of Selective Cyclooxygenase-2 Inhibitors-Part II: The Second Clinical Candidate Having a Shorter and Favorable Human Half-Life. *Bioorganic & Medicinal Chemistry Letters*, **20**, 7159-7163. https://doi.org/10.1016/j.bmcl.2010.07.054

[27] Redinbo, M.R., Stewart, L., Kuhn, P., Champoux, J.J. and Hoi. W.G. (1998) Crystal Structures of Human Topoisomerase I in Covalent and Noncovalent with DNA. *Science*, **279**, 1504-1513. https://doi.org/10.1126/science.279.5356.1504

[28] Sundarrajan, S., Lulu, S. and Arumungan, M. (2015) Computational Evaluation of Phytocompounds for Combating Drug Resistant Tuberculosis by Multi-Targeted Therapy. *Journal of Molecular Modeling*, **21**, 247. https://doi.org/10.1007/s00894-015-2785-z

[29] Muhammad, S.A. and Fatima, N. (2015) *Insilico* Analysis and Molecular Docking Studies of Potential Angiotensin-Converting Enzyme Inhibitor Using Quercetin Glycosides. *Pharmacognosy Magazine*, **11**, 123-126. https://doi.org/10.4103/0973-1296.157712

[30] Pedretti, A., Villa, L. and Vistoli, G. (2004) Vega—An Open Platform to Develop Chemo-Bio-Informatics Applications, Using Plug-In Architecture and Script Programming. *Journal of Computer-Aided Molecular Design*, **18**, 167-173. https://doi.org/10.1023/B:JCAM.0000035186.90683.f2

[31] Thompson, M.A. (2004) Molecular Docking Using ArgusLab, an Efficient Shape-Based Search Algorithm and the a Score Scoring Function. ACS Meeting, Philadelphia.

[32] Wass, M.N., Kelley, L.A. and Sternberg, M.J. (2010) 3DLigandSite: Predicting Ligand-Binding Sites Using Similar Structures. *Nucleic Acids Research*, **38**, W469-W473. https://doi.org/10.1093/nar/gkq406

[33] Trott, O. and Olson, A.J. (2010) AutoDockVina: Improving the Speed and Accuracy of Docking with a New Scoring Function, Efficient Optimization and Multithreading. *Journal of Computational Chemistry*, **31**, 455-461.

[34] ADMET Predictor. www.simulations-plus.com

[35] Othman, R., Kiat, T.S., Khalid, N., Yusof, R.E., Newhouse, I., Newhouse, J.S., Alam, M. and Rahman, N.A. (2008) Docking of Noncompetitive Inhibitors into Dengue Virus Type 2 Protease: Understanding the Interactions with Allosteric Binding

Sites. *Journal of Chemical Information and Modeling*, **48**, 1582-1591. https://doi.org/10.1021/ci700388k

[36] Rutkowska, E., Pajak, K. and Jozzwiak, K. (2013) Lipophilicity-Methods of Determination and Its Role in Medicinal Chemistry. *Acta Poloniae Pharmaceutica*, **70**, 3-18.

[37] Ntie-Kang, F. (2013) An *in Silico* Evaluation of the ADMET Profile of the Streptome DB Database. *SpringerPlus*, **2**, 1-11. https://doi.org/10.1186/2193-1801-2-353

[38] Williams, J.A., Hyland, R., Jones, B.C., Smith, D.A., Hurst, S., Goosen, T., Peterkin, V., Koup, J. and Ball, S.E. (2004) Drug-Drug Interactions for UDP-Glucuronosyltransferase Substrates: A Pharmacokinetic Explanation for Typically Observed Low Exposure (AUCI/AUC) Ratios. *Drug Metabolism and Disposition*, **32**, 1201-1208. https://doi.org/10.1124/dmd.104.000794

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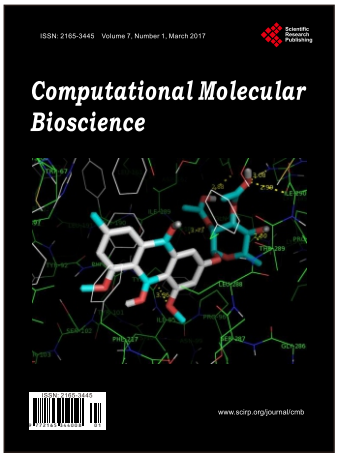

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