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ORIGINAL ARTICLE

In search of selective 11β-HSD type 1 inhibitors without nephrotoxicity: An approach to resolve the metabolic syndrome by virtual based screening

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PASS toxicity;
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ADME

Abstract Over expression of 11β-HSD 1 in key metabolic tissues is related to the development of type 2 diabetes, obesity, hypertension and metabolic syndrome. Nephrotoxicity of corosolic acid (selective inhibitor of 11β-HSD 1) is recently reported, which is one of the major drawback. Therefore, it is of great interest to find out the selective 11β-HSD 1 inhibitors without nephrotoxicity. Using crystal structures of 11β-HSD 1 in complex with inhibitors as a source of structural information, a combined structure-based virtual screening approach followed by PASS toxicity prediction, Lipinski's rule and ADME prediction was implemented to find out the potent and selective 11 β-HSD 1 analog of corosolic acid without nephrotoxicity. Two compounds with NCBI compound identification number CID59752459 (Genins of Asiatic acid) and CID 119034 (Asiatic acid) were found to be selective for the 11β-HSD 1 enzyme without nephrotoxicity which comply with

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Lipinski's rule and ADME parameter defined for human use. However, none of the hits inhibited 11β-HSD 2 at 100 μM indicating their selectivity against 11β-HSD 1.

1. Introduction

The prevalence of obesity and its metabolic complications has been increasing rapidly over the past two decades. Obesity is associated with an increased risk of type 2 diabetes, metabolic syndrome, cardiovascular disease, stroke and certain cancers (Zimmet et al., 2001). However, the medicine for treatment of type 2 diabetes is far from sufficient. So the development of novel agents with good therapeutic index and low side effects is still in urgent demand (Skyler, 2004). Metabolic syndrome is a prediabetic state, which features with abdominal obesity, impaired glucose tolerance, dyslipidemia, low-levels of high density lipoprotein (HDL), and hypertension (Jarrett et al., 1996). When metabolic syndrome progresses to diabetes, complications associating with this disorder, including cardiovascular disease, kidney failure and diabetic retinopathy, become prominent. Recent investigations revealed that aberrant glucocorticoid receptor (GR) signaling was closely associated with metabolic syndrome. Glucocorticoid hormones, including cortisone and cortisol in human, are important regulators of glucose and lipid homeostasis (Tomlinson et al., 2004). Elevated level of glucocorticoids can lead to insulin resistance by decreasing insulin-dependent glucose uptake, enhancing hepatic gluconeogenesis, and inhibiting insulin secretion from pancreatic cells. Patients with sustained glucocorticoid excess will develop dyslipidemia, visceral obesity, and other metabolic syndromes (Arnaldi et al., 2003). 11β-hydroxysteroid dehydrogenase (11β-HSD) catalyzes the interconversion of the glucocorticoids, cortisone and cortisol, in human (Fig. 1) (Stewart et al., 1993). 11β-HSD has two isoform, 11β-HSD 1 and 11β-HSD 2. 11β-HSD 1, which is primarily found in liver, adipose and brain, converted the inactive cortisone to the active cortisol. Its counterpart 11β-HSD 2, which is mainly expressed in kidney, catalyzes the reverse conversion. Both 11β-HSD 1 and 11β-HSD 2 are involved in maintenance of the balance of glucocorticoid hormones as shown in Fig. 1. Evidence from the homozygous 11 β-HSD 1 knock-out mice model revealed that impaired function of 11β-HSD 1 could result in reducing of gluconeogenesis and lipophilia in liver, and increasing of insulin sensitivity. However, inhibition of 11β-HSD 2 would lead to sodium retention, hypokalemia and hypertension (Walker et al., 1995; Kotelevtsev et al., 1999). Therefore, selective inhibition of 11β-HSD 1 will be a therapeutic strategy to combat type 2 diabetes and obesity (Masuzaki et al., 2001; Davani et al., 2004; Stulnig and Waldhaeusl, 2004; Deng et al., 2013).

Based on this view 11β-HSD 1 will be the better target for treating the metabolic disorder and hence the multinational pharmaceutical companies, such as Merck, Pfizer, Amgen, and Abott are extensively working in discovering the 11β-HSD 1 inhibitors for treating the diabetes (Fig. 2). Among the 11β-HSD 1 inhibitors, carbenoxolone (CBO) is one of the most commonly used, which is a semisynthetic derivative of 18 β-glycyrrethinic acid a type of triterpene found in several plants (Classen-Houben et al., 2009). However, the 11β-HSD 2 inhibitory activity of carbenoxolone was a limiting factor because it induces renal mineralocorticoid excess at higher doses (Walker et al., 1995). Other triterpenic acids, particularly those with ursane or oleanane skeleton, are good inhibitors of this enzyme but also inhibit the 11β-HSD 2 isoform, the one that performs reverse reaction (Blum et al., 2009). Nephrotoxicity of corosolic acid is recently reported, which is one of the major drawbacks of this selective 11β-HSD 1 inhibitor. Therefore, it is of great interest to find out the selective 11β-HSD 1 inhibitors without nephrotoxicity (Zheng et al., 2010).

The *in silico* evaluations, including virtual screening of compound libraries are extensively studied for synthetic compounds but the application of these tools to natural product libraries or particular class of phytoconstituents is underestimated (Goel et al., 2011). A number of potent and selective 11β-HSD 1 inhibitors was reported up to now, some of which are progressing to clinical trial (Fig. 2) (Ge et al., 2010; Xiang et al., 2007). So unearthing the selective 11β-HSD 1 inhibitors from the inexhaustible natural product reservoir will be a promising project and hence current research work deals with virtual screening of corosolic acid analogs in order to get more selective and potent 11β-HSD 1 inhibitor without nephrotoxicity.

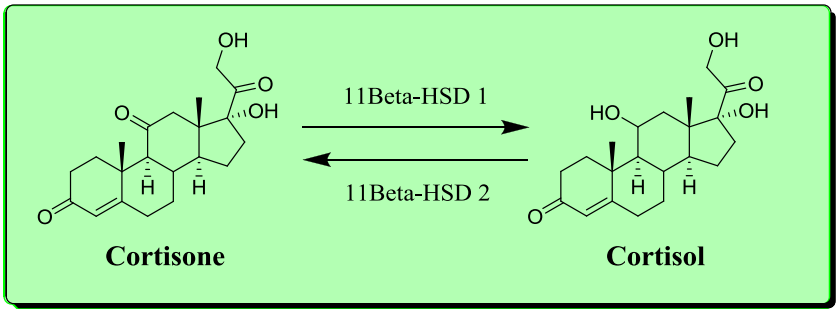

Fig. 1 Interconversion of cortisone and cortisol by 11β-HSD type 1 and 2 enzymes.

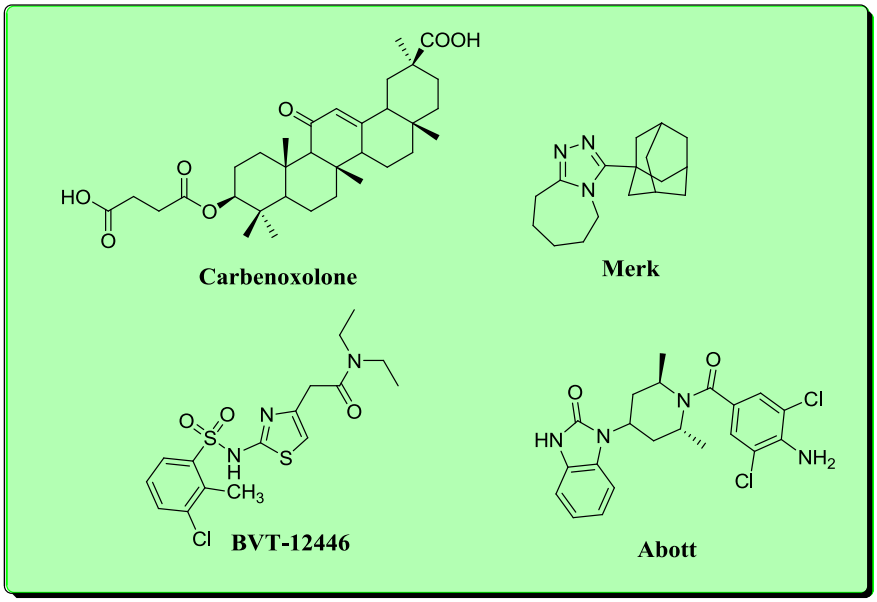

Fig. 2 Representative 11β-HSD 1 inhibitors.

2. Virtual screening protocol

In the present study, we have tried to find out the potent and more 11β-HSD 1 selective analog of corosolic acid without nephrotoxicity by structure based virtual screening approach. The workflow of the virtual screening campaign is outlined in Fig. 3. Very first structurally similar analogs of corosolic acid were collected from NCBI database by performing similarity search since corosolic acid is the structural analog of carbenoxolone (non-selective) having selective 11β-HSD 1 inhibitory activity. Docking study of Corosolic acid analogs was performed by Maestro 8.0 to find out affinity against 11 β-HSD1 enzyme. Those compounds showing highest docking score were further subjected to the toxicity prediction by PASS. Further we analyzed all the compounds for Lipinski's rule of five to evaluate drug likeness and established *in silico* ADME parameters using QikProp to find out the selective 11 β-HSD1 inhibitors. *In vitro* enzyme inhibitory assay further confirms our hypothesis.

2.1. Computer hardware and software

Structure based virtual screening is performed on Apple workstation (8-core processor) using Glide and QuickPro module of Schrodinger, LLC, New York, USA, 2008, on the windows XP operating system. The crystal structure of 11β-HSD1 is retrieved from the Protein Data Bank (PDB) with the accession code 2BEL (http://www.rcsb.org/pdb/explore/explore.do?StructureId=2BEL). Toxicity prediction was carried out by PASS version (Version 9.1, http://www.ibmc.msk.ru/PASS). This software estimates the predicted toxicity spectrum of a compound as probable activity (Pa) and probable inactivity (Pi). Prediction of this spectrum by PASS is based on SAR analysis of the training set containing more than 205,000 compounds exhibiting more than 3750 kinds of biological activities (Poroikov et al., 2009).

2.2. Database building

3234 similar analogs of corosolic acid were collected from NCBI database (http://pubchem.ncbi.nlm.nih.gov/search/search.cgi) by performing similarity search option in the NCBI database. These compounds (3234 corosolic acid analogs) were converted into Mol file by using ChemBiodraw Ultra 11.0. Three-dimensional (3D) conversion and minimization were performed using LigPrep (MMFFs force field) (Halgren et al., 2004). Conformers were generated using a rapid torsion angle search approach followed by minimization of each generated structure using the MMFFs force field, with an implicit GB/SA solvent model using LigPrep 2.2. A maximum of 1000 conformers was generated per structure using a pre-process minimization of 1000 steps and post-process minimization of 500 steps. Each minimized conformer was filtered through a relative energy window of 50 kJ mol^{-1} and a minimum atom deviation of 1.00 Å (Evans et al., 2007). This value (50 kJ mol^{-1}) sets an energy threshold relative to the lowest-energy conformer. Conformers that are higher in energy than this threshold are discarded. All distances between pairs of corresponding heavy atoms must be below 1.00 Å for two conformers to be considered identical. This threshold is applied only after the energy difference threshold, and only if the two conformers are within 1 kcal mol^{-1} of each other.

2.3. Docking study

The molecular docking tool, GLIDE (Schordinger, USA) was used for ligand docking studies into the 11β-HSD 1 enzyme binding pocket. The crystal structure of 11β-HSD 1 was obtained from the protein data bank (PDB: 2BEL). The protein preparation was carried out using 'protein preparation wizard' in Maestro 8.0 in two steps, preparation and refinement. After ensuring chemical correctness, hydrogens were added, where they were missing. Using the OPLS 2005 force field energy of

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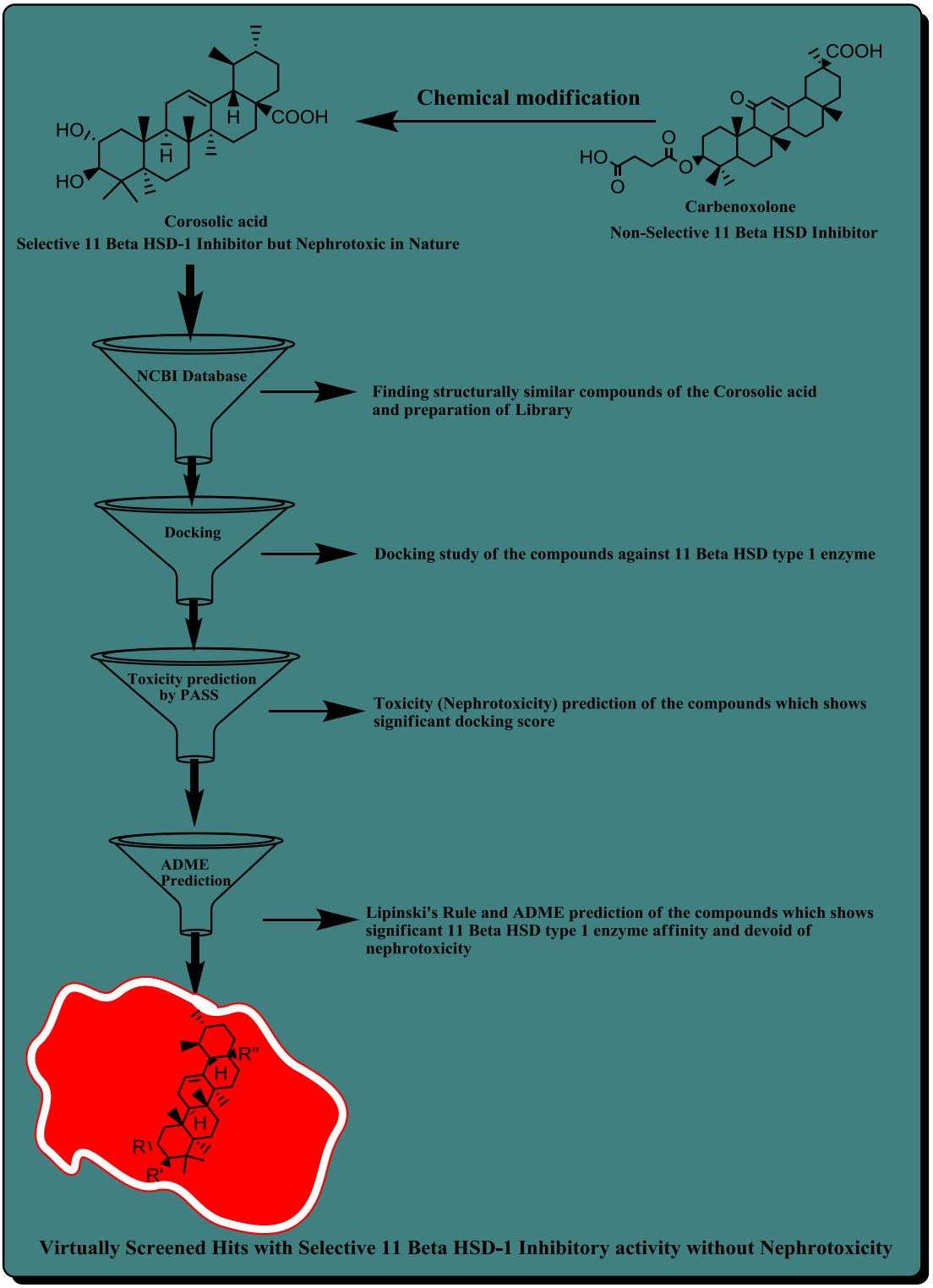

Fig. 3 Virtual screening protocol.

crystal structure was minimized (Zhong et al., 2009). Grid was defined centering them on the ligand in the crystal structure using the default box size. The ligands were built using maestro build panel and prepared by LigPrep 2.2 module, which produce the low-energy conformer of ligands using OPLS 2005 force field. The low-energy conformation of the ligands was selected and was docked into the grid generated from protein structures using standard precision (SP) docking mode. The final evaluation is done with glide score (docking score) and single best pose is generated as the output for a particular ligand.

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2.4. PASS toxicity prediction

Toxicity of chemical compound is a complex phenomenon that may be caused by its interaction with different targets in the organism. Two distinct types of toxicity can be broadly specified: The first one is caused by the strong compound's interaction with a single target (e.g. AChE inhibition), while the second one is caused by the moderate compound's interaction with many various targets. Computer program PASS predicts about 2500 kinds of biological activities based on the structural formula of chemical compounds. Prediction is based on the robust analysis of structure–activity relationships for about 60,000 biologically active compounds. Mean accuracy exceeds 90% in leave-one-out cross-validation. In addition to some kinds of adverse effects and specific toxicity (e.g. carcinogenicity, mutagenicity), PASS predicts approximately 2000 kinds of biological activities at the molecular level, providing an estimated profile of compound's action in biological space. Such profiles can be used to recognize the most probable targets, interaction with which might be a reason of compound's toxicity. Applications of PASS predictions for analysis of probable targets and mechanisms of toxicity are discussed (Poroikov et al., 2007).

2.5. Drug likeness (Lipinski's Rule of Five) and in silico ADME study

Pharmacokinetic property optimization is a rather complex undertaking that is likely to require changes in those molecular determinants that are responsible for binding affinity and specificity like hydrogen bonds. It is well known that numerous drug candidates have failed during clinical tests because of problems related to absorption, distribution, metabolism and excretion (ADME) properties. We analyzed Lipinski's rule and *in silico* ADME properties using QikProp 2.3 module of the Maestro 8.0. QikProp is a quick, accurate, easy-to-use absorption, distribution, metabolism, and excretion (ADME) prediction program designed by Professor William L. Jorgensen. QikProp predicts physically significant descriptors and pharmaceutically relevant properties of organic molecules, either individually or in batches (Lipinski et al., 2001; Vistoli et al., 2008; Ertl et al., 2000).

2.6. Determination of half maximum inhibitory concentrations (IC_{50})

2.6.1. 11β-HSD1 assay in rat microsomes

Six male adult rat testes were used for preparation of rat testis microsomes to measure 11β-HSD1 activity, as they contained a large amount of 11β-HSD1 enzyme (Ge and Hardy, 2000). The preparation of microsomes was performed as described previously (Guo et al., 2012). In brief, rat testes were homogenized in 0.01 mM PBS buffer containing 0.25 M sucrose. The nuclei and large cell debris were removed by centrifugation at 1500×*g* for 10 min. The post-nuclear supernatants were centrifuged twice at 105,000×*g*. The resultant microsomal pellets were resuspended. Protein contents were measured by NanoDrop 2000 (Thermo Scientific). The microsomes (20 mg/mL) were used for measurement of 11β-HSD1 or 11β-HSD2 activities. The rat testes microsomes (10 mg) were incubated with substrate, 0.2 mM NADPH and 0.5 mM glucose-6-phosphate (G6P) and various concentrations of the hit compounds 37 °C for 60–90 min. The inhibitory potency of hits was measured relative to control (only DMSO). The hit compounds were dissolved in DMSO with final DMSO concentration of 0.4%, at which DMSO did not inhibit this enzyme activity. The reaction was stopped by adding 1 mL of ice-cold ether. The steroids were extracted, and the organic layer was dried with N_2. The steroids were separated chromatographically on the thin layer plate in chloroform and methanol (90:10,v/v), and the radioactivity was measured using a scanning radiometer (System AR2000, Bioscan Inc., Washington, DC) as described previously (Ge et al., 1997). The percentage conversion of 11DHC to CORT, or cortisone to cortisol was calculated by dividing the radioactive counts identified as 11-OH-steroids by the total counts.

2.6.2. 11β-HSD2 assay in rat microsomes

Six male adult rat kidneys were used for preparation of rat kidney microsomes to measure 11β-HSD2 as high levels of 11βHSD2 expression and activity (Klusonova et al., 2008). Preparation of kidney microsomes was carried out as previously described (Guo et al., 2012). 11β-HSD2 activity assay tubes contained 25 nM (within the range of physiological levels of CORT). [^{3}H] cortisol and [^{3}H] CORT were used as substrates to measure rat 11β-HSD2 oxidase activity. Kidney microsomes were incubated with substrates, NADP. The reactions were stopped by adding 1 mL of ice-cold ether. The steroids were extracted, and the organic layer was dried with nitrogen. The steroids were separated chromatographically on thin layer plates in chloroform and methanol (90:10), and the radioactivity was measured using a scanning radiometer (System AR2000, Bioscan Inc., Washington, DC). The percentage conversion of CORT to 11DHC and cortisol to cortisone was calculated by dividing the radioactive counts identified as 11DHC (or cortisone) by the total counts associated with both substrate and product.

3. Results and discussion

The virtual screening protocol used in this study is based on the application of sequential filters to find out a selective 11β-HSD 1 inhibitor. The virtual screening campaign conducted to screen novel 11β-HSD 1 inhibitors started with search of 3234 similar analogs of corosolic acid from NCBI database (http://pubchem.ncbi.nlm.nih.gov/search/search.cgi) by performing similarity search option in the NCBI database since corosolic acid is structural analog of carbenoxolone (non-selective) having selective 11β-HSD 1 inhibitory activity. Virtual screening of the compound libraries was carried out with Schrodinger's ligand docking tool in a two-step process, consisting of first high-throughput virtual screening (HTVS) and later standard precision (SP) docking. The molecular docking tool, GLIDE (Schordinger Inc., USA) was used for ligand docking studies into the 11β-HSD1 enzyme binding pocket. The crystal structure of 11β-HSD1 was obtained from the protein data bank (PDB: 2BEL). After HTVS, a Glide score cutoff of -8.0 was used, and compounds with values greater were discarded, leaving a total of 1562 ligands. Additionally, for the compound to be included for SP docking, at least two of three hydrogen bonds must be present, one to Tyr 183 and one of two possible to Ser 170. This resulted in

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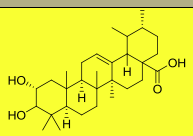
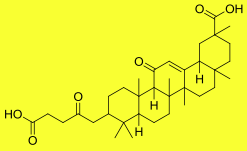
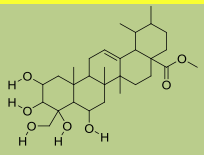
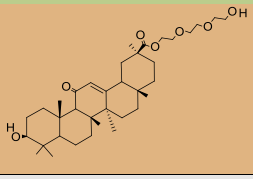
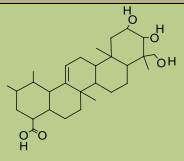
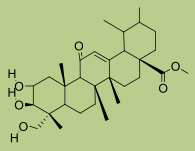
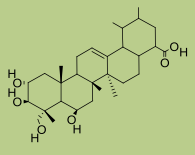
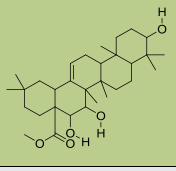
Table 1 Glide docking results (SP docking method) and toxicity prediction by PASS of selected hits.

NCBI Compound Identification Number (CID)	Structures	Hydrogen Bond Interaction	RMSD	Glide Score	Glide evdw	Glide Energy	Glide emodel	H Bond	TOXICITY		
									Pa	Pi	Toxicity
Corosolic acid CID6918774 Selective Inhibitor		TYR-183	0.002	-7.74	-39.074	-40.39	-19.71	1	0,680	0,032	Nephrotoxic
Carbenoxolone CID21913 Non-Selective Inhibitor		ILE-46, GLY-47, ASN-119	0.028	-7.805	-27.24	-35.43	-50.61	3	0,822	0,010	Nephrotoxic
CID21669127		GLY-216, TYR-183, SER-170	0.008597	-11.8467	-28.4839	-41.15	18.05165	3	-	-	-
CID11548915		ASN-119, ALA-42, GLY-47, SER-170	0.028694	-10.8643	-43.0415	-50.952	-38.5649	4	0,950	0,003	Nephrotoxic

NCBI Compound Identification Number (CID)	Structures	Hydrogen Bond Interaction	RMSD	Glide Score	Glide evdw	Glide Energy	Glide emodel	H Bond	TOXICITY		
									Pa	Pi	Toxicity
CID59752459		LYS-44, LYS-44, THR-222, SER-170	0.003929	-10.5962	-34.3363	-49.447	-53.5505	4	-	-	-
CID5319746		ASN-119, LYS-187, THR-183, SER-170	0.00738	-10.3903	-13.0297	-33.708	50.78204	4	-	-	-
CID12073158		TYR-183	0.046985	-10.315	-32.7476	-37.039	-21.9332	1	-	-	-
CID612532		TYR-183, GLY-216	0.002658	-10.2775	-22.6495	-31.748	13.70799	2	-	-	-

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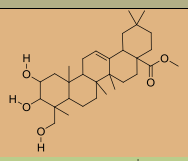
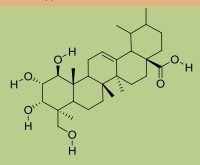
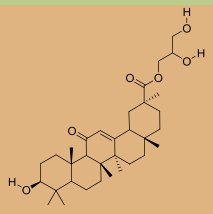
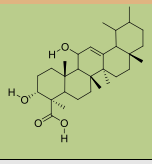
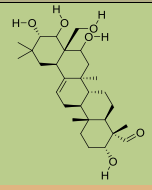
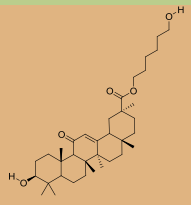
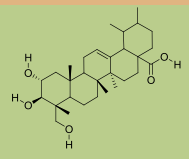
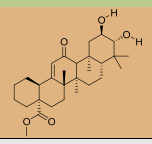
NCBI Compound Identification Number (CID)	Structures	Hydrogen Bond Interaction	RMSD	Glide Score	Glide evdw	Glide Energy	Glide emodel	H Bond	TOXICITY		
									Pa	Pi	Toxicity
CID609112		TYR-183	0.003357	-10.2135	-32.3994	-37.250	-26.8141	1	0,804	0,013	Nephrotoxic
CID44470116		SER-170	0.0127	-10.2295	-35.8396	-42.061	-17.3579	2	-	-	-
CID57110617		TYR-177, SER-170, GLY-216	0.047592	-10.1085	-29.14	-40.182	13.78735	3	0,951	0,003	Nephrotoxic
CID11648525		GLY-216, SER-170, THR-220	0.008033	-9.9218	-28.7561	-41.051	6.160157	3	-	-	-

NCBI Compound Identification Number (CID)	Structures	Hydrogen Bond Interaction	RMSD	Glide Score	Glide evdw	Glide Energy	Glide emodel	H Bond	TOXICITY		
									Pa	Pi	Toxicity
CID5315633		TYR-183	0.000644	-9.77286	-33.6092	-40.105	-30.5426	1	-	-	-
CID70696198		GLY-47, GLY-41, ASN-119, SER-170, MET-233	0.002792	-9.64109	-41.8672	-52.753	-51.8224	5	0,922	0,004	Nephrotoxic
CID119034		TYR-183, SER-170	0.000955	-9.63596	-24.7625	-30.820	-25.6706	2	-	-	-
CID11605753		SER-170, SER-169	0.03209	-9.54355	-29.2385	-32.700	31.51918	2	0,758	0,019	Nephrotoxic

(*continued on next page*)

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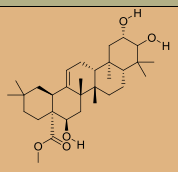
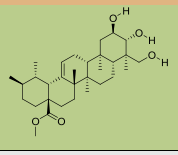
NCBI Compound Identification Number (CID)	Structures	Hydrogen Bond Interaction	RMSD	Glide Score	Glide evdw	Glide Energy	Glide emodel	H Bond	TOXICITY		
									Pa	Pi	Toxicity
CID14432544		TYR-183	0.034414	-9.52721	-43.0948	-44.621	19.79395	1	0,765	0,018	Nephrotoxic
CID44272564		TYR-183	0.006191	-9.51601	-34.167	-39.117	-31.2415	1	-	-	-

RMSD: Root Mean Standard Deviation; Pa: Coefficient of Activation; Pi: Coefficient of Inhibition; (–) indicates no toxicity; Yellow Color: Known inhibitor; Green Color Hits: Non-Nephrotoxic; Brick Red Color Hits: Nephrotoxic

544 compounds for SP docking of the previous total. After SP docking, compounds with a docking score greater than −9.50 were automatically excluded, and a total of 16 compounds were finalized by considering each of the compounds' hydrogen bonding to the active site residues, particularly Tyr 183 and Ser 170 as shown in Table 1. Figs. 4 and 5 revealed that all ligands binds to a common site located close to the nicotinamide ring in NADPH, forming hydrogen bond interaction with Tyr 183 and Ser170 residues that have been reported as essential for catalysis (Sun et al., 2008; Thomas and Potter, 2011). These 16 compounds were further passed through the next filter of toxicity prediction by PASS (Version 9.1, http://www.ibmc.msk.ru/PASS) (Table 1). This software estimates the predicted toxicity spectrum of a compound as probable activity (Pa) and probable inactivity (Pi). Prediction of this spectrum by PASS is based on SAR analysis of the training set containing more than 205,000 compounds exhibiting more than 3750 kinds of biological activities. Pass toxicity prediction shows the nephrotoxicity of corosolic acid and carbenoxolone, and hence we checked all the 16 ligands for nephrotoxicity, and we got 10 hits devoid of this.

We employ the next filter of "Lipinski's rule of five" for further filtering of the hits obtained from the previous filter, i.e. PASS toxicity filter (10 hits). As discussed by Lipinski, molecular properties are closely related to the oral bioavailability of a drug. Lipinski's rule of five is a rule of thumb to evaluate drug likeness, or determine whether a chemical compound with a certain pharmacological or biological activity

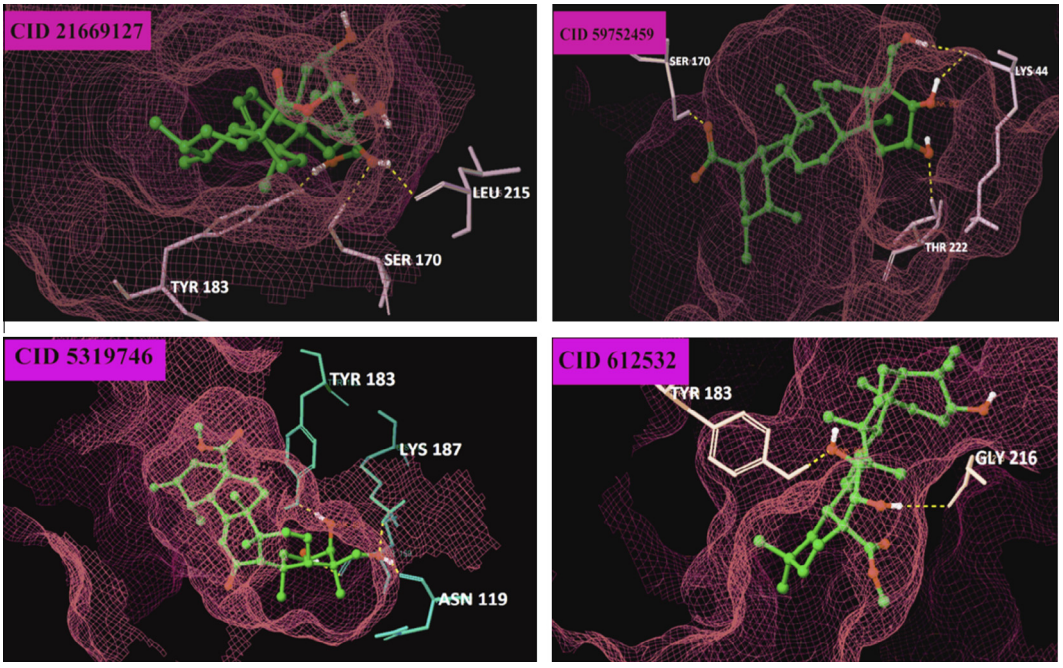


Fig. 4 Binding mode of **CID 21669127, CID 59752459, CID 5319746** and **CID 612532** with Tyr 183 and Ser 170 residues in the X-ray crystal structure of 11β-HSD 1 (PDB code: 2BEL).

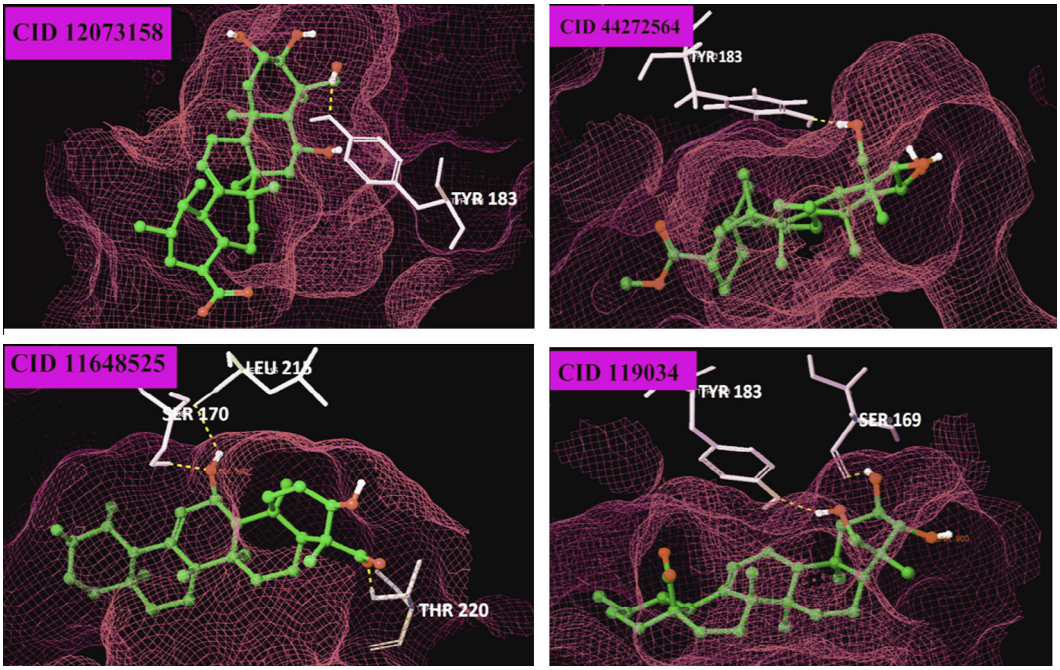


Fig. 5 Binding mode of **CID 12073158, CID 44272564, CID 11648525** and **CID 119034** with Tyr 183 and Ser 170 residues in the X-ray crystal structure of 11β-HSD 1 (PDB code: 2BEL).

has properties that would make it a likely orally active drug in humans. The rule describes delicate balance among the molecular properties of a compound that directly influence its pharmacodynamics and pharmacokinetics and ultimately affect their absorption, distribution, metabolism, and excretion in the human body like a drug (Vistoli et al., 2008). In general, these parameters allow to ascertain a poor oral absorption, or membrane permeability, that occurs when the evaluated molecules present values higher than five H-bond donors (HBD), 10 H-bond acceptors (HBA), molecular weight (MW) >500 Da and LogP (cLogP) >5 (Lipinski's 'rule-of-five') (Lipinski et al., 2001). The QikProp 3.2 was used to analyze drug likeness (Lipinski's Rule of Five) and *in silico* ADME evaluation; the results are given in Table 2 and it was found that among the 10 hits only two comply with these rules [CID59752459 (Genins of Asiatic acid) and CID 119034 (Asiatic acid)] and others are showing violation for Lipinski's Rule of Five.

However, it is important to note that there are many violations of this rule among existing drugs and vice versa, and therefore, qualifying the "rule of five" does not guarantee that a molecule is "drug-like" (Vistoli et al., 2008). Topological polar surface area (TPSA) is now being recognized as a good indicator of drug absorbance in the intestines, Caco-2 monolayer's penetration, and blood brain barrier crossing (Ertl et al., 2000). Topological polar surface area (TPSA), i.e., surface belonging to polar atoms, is a descriptor that was shown to correlate well with passive molecular transport through membranes and, therefore, allows prediction of transport properties of drugs in the intestines and blood brain barrier crossing (Ertl et al., 2000). The percentage of absorption (% ABS) was calculated using TPSA. Caco-2 cells are a model for the gut-blood barrier. Caco-2 permeability is good indicators of drug absorbance in the intestine. MDCK cells and log BB are good markers to determine blood–brain barrier crossing ability of compounds. Plog Khsa shows the prediction of binding to human serum albumin. The result for ADME prediction is shown in Table 2. Human Intestinal Absorption (HIA) and Caco-2 (QPPCaco) permeability are good indicators of drug absorbance in the intestine and Caco-2 monolayer penetration, respectively. Human Intestinal Absorption data are the sum of bioavailability and absorption evaluated from the ratio of excretion or cumulative excretion in urine, bile and feces (Zhao et al., 2001). The predicted percentage of intestinal absorption is 76.997% and 85.169% for CID59752459 (Genins of Asiatic acid) and CID 119034 (Asiatic acid) (obeys the Lipinski's rule of five). The same compounds present good permeability values in Caco-2 (QPPCaco) cells, i.e. 34.151–77.32 respectively and hence theoretically these two compounds should present good passive oral absorption. The partition coefficient (QPlogPo/w) and water solubility (QPlogS), critical for the estimation of absorption and distribution of drugs within the body for hit CID59752459 (Genins of Asiatic acid) is 4 and -5.333 respectively and for another hit with CID 119034 (Asiatic acid) it is 4 and -5.118. Cell permeability (QPPCaco), a key factor governing drug metabolism and its access to biological membranes, is 34.151 and 77.32 for CID59752459 (Genins of Asiatic acid) and CID 119034 (Asiatic acid) respectively. We similarly studied the number of violations of Jorgensen's rule of three. The three rules are QPlogS >-5.7, QPCaco >22, Primary Metabolites <7. Compounds with fewer (and preferably no) violations of these rules are more likely to be orally available. Hits with a compound identification number CID59752459 (Genins of Asiatic acid) and CID 119034 (Asiatic acid) are following this rule, showing best candidate for oral bioavailability. Similarly, CID 5319746 and CID 44470116 also follow Jorgensen's rule but fail for the Lipnski's

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Table 2 Lipinski's rule of five and ADME prediction of those Hits which are devoid of nephrotoxicity.

Criteria →		Lipinski's Rule of Five (Drug Likeliness)					*In Silico* ADME by QikProp, Schordinger 9.0						
Sr.No.	Compounds	Molecular Weight	QPlogPO/W [a]	H-bond donor	H-bond acceptor	Violation of Lipinski's Rule	QPlogS[b]	QPlogHERG[c]	QPPCaco [d]	QPMDCK [e]	QPlogKhsa[f]	% Human Oral Absorption [g]	Violation of Rule of Three
1	CID21669127	520.705	5	9.55	2.912	1	-4.654	-3.577	184.15	79.449	0.447	71.581	1
2	CID59752459	474.679	4	7.1	3.861	0	-5.333	-1.927	34.151	16.352	0.56	76.997	0
3	CID5319746	516.717	3	9.1	3.494	1	-5.393	-3.699	250.074	110.594	0.686	77.366	0
4	CID12073158	504.706	5	8.8	3.232	1	-5.049	-2.038	19.375	8.861	0.343	55.952	2
5	CID612532	502.733	2	6.1	5.208	2	-6.352	-3.573	966.756	476.957	1.273	84.953	1
6	CID44470116	504.706	5	8.8	3.209	1	-4.523	-1.539	34.97	16.776	0.288	60.403	0
7	CID11648525	472.707	2	4.4	5.691	1	-6.612	-1.675	118.579	62.792	1.263	84.428	1
8	CID5315633	504.706	4	9.5	2.759	1	-4.624	-3.68	144.899	61.314	0.396	68.819	1
9	CID119034	488.706	4	7.1	4.172	0	-5.118	-1.568	77.32	39.552	0.596	85.169	0
10	CID44272564	502.733	3	7.1	4.467	1	-6.404	-4.07	381.159	174.412	1.037	86.34	1

[a] Predicted octanol/water partition co-efficient log p (acceptable range: −2.0 to 6.5).
[b] Predicted aqueous solubility in mol/L (acceptable range: −6.5 to 0.5).
[c] Predicted IC_{50} value for blockage of HERG K+ channels (concern below −5.0).
[d] Predicted Caco-2 cell permeability in nM/s (acceptable range: <25 is poor and >500 is great).
[e] Predicted apparent MDCK cell permeability in nM/s.
[f] Prediction of binding to human serum albumin.
[g] Percentage of human oral absorption (<25% is poor and >80% is high).

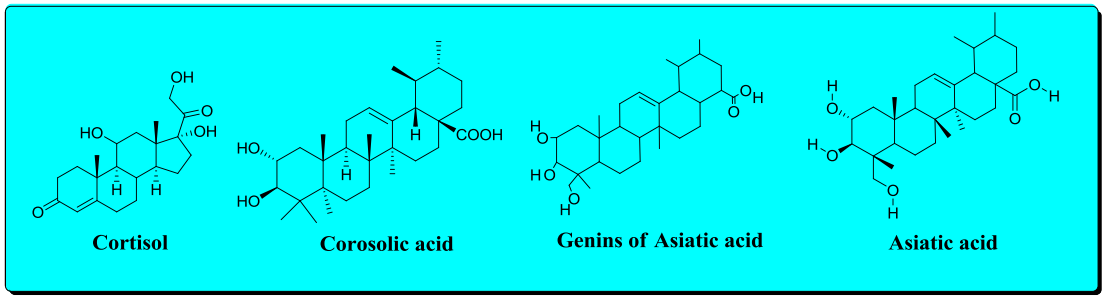

Fig. 6 Structural comparison of Cortisol, Corosolic acid, Genins of Asiatic acid and Asiatic acid.

rule because of higher molecular weight exceeding 500. All these pharmacokinetic parameters are within the acceptable range for CID59752459 (Genins of Asiatic acid) and CID 119034 (Asiatic acid) defined for human use (see Table 2 footnote), thereby indicating their potential as a drug-like molecule. The inhibitory effects on 11β-HSD1 of the hit compounds CID59752459 (Genins of Asiatic acid) and CID 119034 (Asiatic acid) (shown in Table 3) were evaluated using

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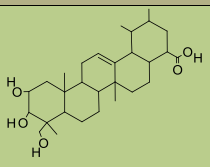
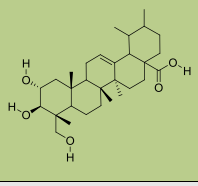
Table 3 Rat 11β-HSD1 and 11β-HSD2 inhibition of virtually screen hits.

NCBI Compound Identification Number (CID)	Structures	Rat 11β-HSD 1 IC_{50} (nM)	Rat 11β-HSD 2 IC_{50} (nM)	Nephrotoxicity
CID 59752459 (Genins of Asiatic Acid)		240	>10,000	Nephroprotective (Xu et al., 2013).
CID119034 (Asiatic acid)		215	>10,000	Nephroprotective (Xu et al., 2013).

the methods described previously (Guo et al., 2012). The IC_{50} was determined by adding 25 nM substrate, 0.2 mM co-factor and various concentrations of hit compounds CID59752459 (Genins of Asiatic acid) and CID 119034 (Asiatic acid) in 250 mL reaction buffer (0.1 mM PBS) containing rat microsomal protein. Significant 11β-HSD1 enzyme inhibitory effects in rats were exhibited by CID59752459 (Genins of Asiatic acid) and CID 119034 (Asiatic acid), with IC_{50} values of 240 and 215 nM. However, none of the hits inhibited 11β-HSD2 at 100 μM indicating their selectivity against 11βHSD1.

Due to the structural similarities of both CID 59752459 and CID119034 with the glucocorticoid receptor substrate, the potential for cross-reactivity of these compounds with the glucocorticoid receptor has been predicted using the PASS software and *in silico* result shows no cross-reactivity with glucocorticoid receptor.

However, small changes to the structures of the inhibitors could generate compounds with high affinity for type 1 isoform, earlier reported by Rollinger et al., such as corosolic acid which has an ursane structure and two hydroxyl groups on positions 2 and 3 that result on determinant factors for its inhibitory and specific activity (Rollinger et al., 2010). These compounds are examples of the so-called selectivity cliffs, because they show a closely related structural similarity but large changes in biological activity (Medina-Franco, 2012). Structural comparison of corosolic acid and virtually screened hit indicates that the presence of hydroxymethyl group at 4th position favors the selectivity toward 11β-HSD 1 (see Fig. 6). It is well reported that Asiatic acid is nephroprotective in nature thus confirming our goal of the study (Xu et al., 2013).

4. Conclusion

In summary, we performed a successful virtual based screening on 3234 structurally similar analogs of corosolic acid collected from NCBI database. Corosolic acid is the structural analog of carbenoxolone (non-selective) having selective 11 β-HSD1 inhibitory activity with adverse effect of nephrotoxicity. Therefore, it is of great interest to find out the selective 11β-HSD 1 inhibitors without nephrotoxicity. From 3234 structurally similar analogs of corosolic acid, two hits with NCBI compound identification number CID59752459 (Genins of Asiatic acid) and CID 119034 (Asiatic acid) were found to be selective for the 11β-HSD1 enzyme without nephrotoxicity which comply with Lipinski's rule and ADME parameter defined for human use. Significant 11β-HSD 1 enzyme inhibitory effects were exhibited by CID59752459 (Genins of Asiatic acid) and CID 119034 (Asiatic acid), with IC_{50} values 240 and 215 nM. However, none of the hits inhibited 11β-HSD 2 at 100 μM indicating their selectivity against 11β-HSD1. The identified hits represent a very promising starting point for the development of potent and selective 11β-HSD1 inhibitor with potential for treatment of important diseases such as type 2 diabetes, obesity, hypertension and metabolic syndrome without nephrotoxicity.

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