

In silico assessment of the druggability of two novel germacranolides isolated from *Capparis decidua* (Forsk.) as carbonic anhydrase II inhibitors

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ABSTRACT

Novel naturally derived molecules are frequently isolated from different plants with endowed biological activities. Nevertheless, druggability studies for most of these molecules have been put on hold due to, in part, the limited quantity of material available from isolation and the expensive lengthy drug development process in the other part. *In silico* drug discovery has always been an effective approach to reduce the cost and time needed to bring a drug to the market. In the current study, Autodock 4.0 software along with different chemoinformatic tools, namely, PharmMapper, Molinspiration, Metaprint2D and AdmetSAR servers were used to assess the druggability of two novel germacranolides isolated from *Capparis decidua* (Forsk.). Our findings revealed that these two isolates are potential carbonic anhydrase II (CA II) inhibitors. Interestingly, inhibition of CA II is known to have a vital role in curing different diseases, e.g. neuropathic pain, bipolar disorder, migraine, obesity, osteoporosis and most interestingly tumor. Thus, the two germacranolides could be envisioned as promising drug candidates for curing these aforementioned disorders.

INTRODUCTION

Conventional drug discovery is challenging, time consuming, expensive and requires consideration of many aspects that have a negative impact on the pharmaceutical industry (Earm and Eram, 2014). Interestingly, *In silico* drug discovery has nowadays surpassed the conventional approach where potential biological activity and druggability of a new lead could be estimated computationally at an early stage, shortening the time needed to bring the drug to the market (Sliwoski *et al.*, 2014). As an important source of drug discovery, medicinal plants have always played a key role in curing diseases. In this context, *Capparis decidua* Family (Capparidaceae) is not an exception where different parts of this plant were used by ancient people for medicinal purposes (Nizar *et al.*, 2011). The plant is

widely distributed in Sudan and other countries where it has many traditional uses for the control of various ailments (Al-Yahya 1986; Shah *et al.*, 1989; Elamin 1990; Elkamali and Elkhaliifa 1999; Atiqur *et al.*, 2004; Khalid *et al.*, 2012). Most importantly, the plant has also been used as a diuretic (Verma *et al.*, 2011). Diuretics are drugs used extensively for the treatment of various medical disorders (Brater, 2000). All diuretics act mainly by impairing Na⁺ re-absorption in the renal tubules. However, they differ significantly in their chemical structures, mechanism and site of action. Carbonic anhydrases are highly expressed and distributed through the nephrons where they play a fundamental role in the renal acid–base homeostasis control, bicarbonate re-absorption and ammonium excretion (Hassan *et al.*, 2012). Thus, carbonic anhydrase inhibitors such as acetazolamide produce their diuretic action through interference with the enzyme activity both within the brush border and inside the cell, resulting in impaired NaHCO₃ and water re-absorption (Carta *et al.*, 2014).

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Mohammed and co-workers have isolated and characterized two novel germacranolides (**1** and **2**; Fig. 1) from *C. decidua* (Mohammed *et al.*, 2012; Mohammed *et al.*, 2014). Being involved in drug discovery from medicinal plants (Dirar *et al.*, 2014; Dirar *et al.*, 2016; Mohamed *et al.*, 2016), we herein report the *In silico* druggability of these novel isolates as CA II inhibitors.

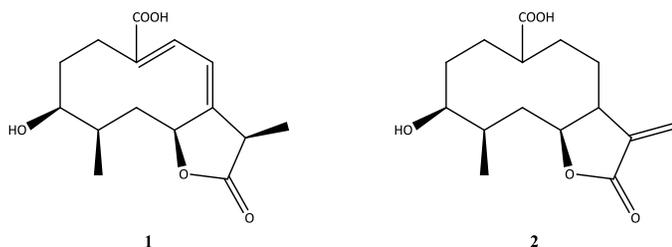


Fig. 1

MATERIALS AND METHODS

General

ChemDraw Ultra 12 software was used to prepare the compounds' 3D structures which were saved as PDB format. On the other hand, MDL.sdf and SMILE formats were generated using Open Bable software (O'Boyle *et al.*, 2011). The manually optimized protein 3D structure was retrieved from protein data bank. Manual energy minimization was conducted on the Swiss PDB viewer V.4.1.0. software (Guex and Peitsch, 1997). Molecular docking was performed using Autodock 4.0 software based on Lamarckian Genetic Algorithm (Morris *et al.*, 1998; Morris *et al.*, 2009).

Prediction of biological activity

PharmMapper server was used to predict the potential protein target for germacranolides **1** and **2**. On the other hand, Molinspiration server was used to predict drug likeness properties of these leads as G-protein coupled receptor (GPCR) ligands, ion channel modulators (ICM), kinase inhibitors (KI), nuclear receptor ligands (NRL), protease inhibitors (PI) and enzyme inhibitors (EI).

Molecular docking

The 3D structures of the two germacranolides were prepared according to the standard docking protocol. The co-crystal ligand was removed from the protein target which was then prepared according to the standard docking protocol. The UniProt databases were used to verify the active site. The target's grid map was calculated and set to 60×60×60 points with grid spacing of 0.375 Å to assure that all the active residues were included in the center. The default docking algorithms were set in accordance with the standard docking protocol.

Finally, ten independent docking runs were carried out for each ligand and results were retrieved as binding energies.

Poses that showed lowest binding energies were visualized using MOE (Available at: <http://www.chemcomp.com>) [Accessed 17 May 2016] and UCSF chimera (Pettersen *et al.*, 2004).

Physicochemical properties

Molinspiration server was used to estimate different physicochemical properties, namely LogP, Topological polar surface area (TPSA) and number of hydrogen bond donors (HBD) and acceptors (HBA) for germacranolides **1** and **2**.

Metabolic transformations and toxicity

MetaPrint2D online server was used to predict the metabolic sites and transformations for germacranolides **1** and **2**. AdmetSAR server was used to assess the potential toxicity of these isolates.

RESULTS AND DISCUSSION

PharmMapper server has predicted that carbonic anhydrase II (CA II; PDB ID: 1G48) (Kim *et al.*, 2000) is the best target, in terms of fit score, for our isolates **1** and **2** (3.887 and 3.160, respectively). The CA II is involved in many physiological and pathological processes (Supuran, 2008). For example, it facilitates electrolyte secretion in addition to transportation of CO₂ and bicarbonate between metabolic sites and lungs resulting in controlled respiration. Furthermore, CA II has a major role in pH and CO₂ homeostasis (Hassan *et al.*, 2012). Moreover, it plays a vital role in the biosynthesis of glucose, lipids and urea (Supuran, 2008).

Thus, CA II inhibitors are prescribed clinically as diuretics and for glaucoma (Supuran *et al.*, 2003). In addition, these inhibitors were proved to have potential therapeutic uses for neuropathic pain, bipolar disorder, migraine, obesity, osteoporosis, epilepsy and tumor (Scozzafava *et al.*, 2006; Thiry *et al.*, 2006; Riihonen *et al.*, 2007). With CA II, as a potential target for our germacranolides **1** and **2**, in hand, we next decided to investigate the course of the biochemical interactions of these two isolates with CA II active site which was verified from UniProt databases. Molecular docking revealed interesting interactions between the two isolates and CA II with satisfactory binding energies (−6.94 and −9.31 Kcal/mol, respectively). Germacranolide **1** forms a hydrogen bond with the key residue His 64, which is involved in the proton-shuttling processes between the active site and the environment (Fig. 2).

The interaction of germacranolides **1** with His 64 could therefore have an inhibitory role in regeneration of the basic active form of CA II (Supuran and Scozzafava, 2007). Additionally, the predicted hydrogen bonds with the residues Asn 62, Asn 67, Gln 92 and Thr 200 could potentiate such an inhibitory role as these residues have been reported to interact with some CA II inhibitors (Supuran *et al.*, 2003; Supuran and Scozzafava, 2007). Germacranolide **2**, on the other hand, forms hydrogen bonds with the key residues His 94, His 96 and His 119 whose interaction with Zn metal is crucial for enzyme's activity (Fig. 2).

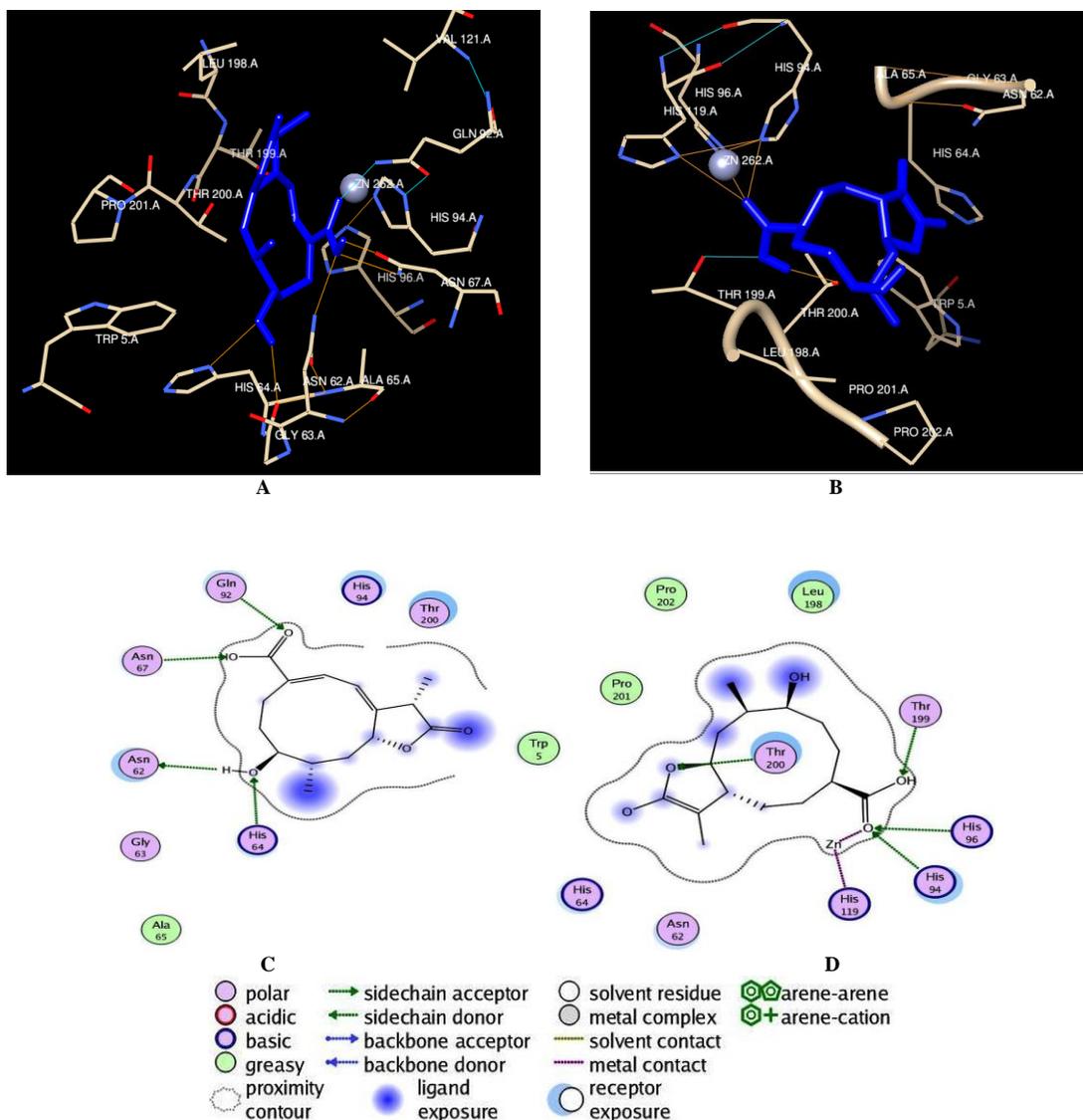


Fig. 2: Ligand-Carbonic anhydrase II interactions.

Germacranolide 1-CA II interactions visualized by (a) Chimera and (c) MOE. Germacranolide 2-CA II interactions visualized by (b) Chimera and (d) MOE.

As for the other inhibitors, interaction of germacranolide 2 with the aforementioned key residues would contribute very much to its potential CA II inhibitory effect (Supuran, 2008). In addition, germacranolide 2 could potentially inhibit bicarbonate formation via hydrogen bond interaction with the key residue Thr 199 which is known to facilitate the nucleophilic attack of water on carbon dioxide (Supuran, 2008). Moreover, germacranolide 2, binds two hydrophobic residues, namely Leu 198 and Pro 202 (Supuran and Scozzafava, 2007). It is worth noting that these two residues are part of the hydrophobic pocket where CO₂ resides before it interacts with Zn-bound water (Pastorekova *et al.*, 2004). Based on Lipinski's rule of five (Lipinski *et al.*, 2001), our isolates 1 and 2 were predicted to have good oral bioavailability, where the estimated LogP values, the number of hydrogen bond acceptors (HBA) and number of hydrogen bond donors (HBD) were found within the limited range. In addition, Total Polar Surface Area

(TPSA), total hydrogen bond count, number of rotatable bonds and molecular weights were found to be within the limit stated (Table 1) (Lipinski *et al.*, 2001; Veber *et al.*, 2002; Srimai *et al.*, 2013).

Table 1: Physicochemical parameters predicted by Molinspiration.

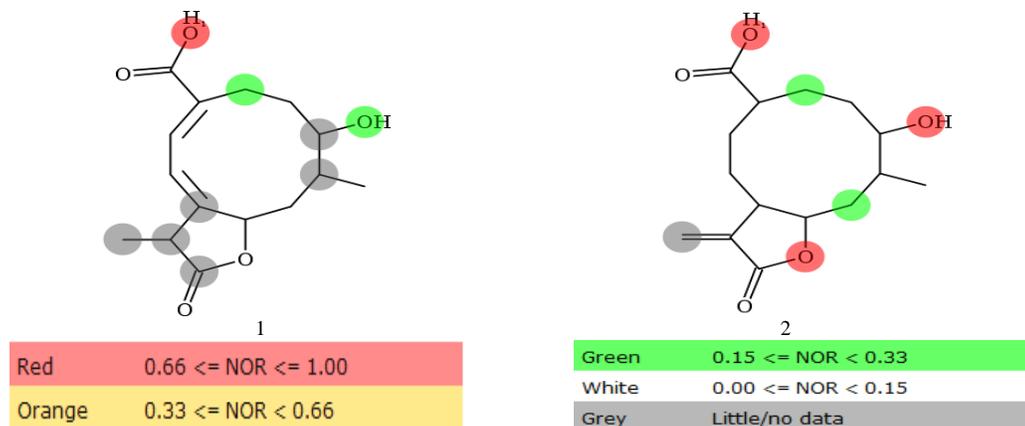
Compound	Mi logP	TPSA	MW	HBA	HBD	nrotb
1	0.30	83.83	280.32	5	2	1
2	0.51	83.83	282.34	5	2	1

Regarding drug likeliness properties, germacranolides 1 and 2 were predicted to have high activity (values > 0.00) as GPCR ligands, ICMS, NRLs, PIs and EIs (Paramashivam *et al.*, 2015). Nonetheless, both compounds were found to be moderately active as KI (values = -0.22 and -0.25, respectively) (Table 2).

Table 2: Drug likeliness property estimations by Molinspiration.

Compound	GPCR ^a	ICM ^a	KI ^a	NRL ^a	PI ^a	EI ^a
1	0.26	0.42	-0.22	0.46	0.28	0.61
2	0.31	0.17	-0.25	0.83	0.26	0.68

^avalues > 0.00 indicate high activity; values between 0.00 to -0.5 indicate moderate activity; values < -0.5 indicate inactivity.

**Fig. 3:** Metabolic predictions using Metaprint2D for tested compounds.

NOR : Normalized Occurrence Ratio. Atoms are colored according to the likelihood of a metabolic site; High: red, Medium: orange, Low: green, very low is not colored, and No data: grey.

Based on Normalized Occurrence Ratio (NOR), the Metaprint2D-predicted metabolic transformations revealed that the hydroxyl group of the carboxylic acid represents a good site for phase II metabolism in both compounds. In this context, glucuronidation, alkylation, esterification and sulfation were predicted for germacranolides **1** and **2**. In addition, glycation, dehydroxylation, phosphorylation and glutamination were predicted for **2**. Contrary to **2**, germacranolide **1** was also predicted to undergo metabolic transformation (with high NOR) at two additional sites, namely the secondary alcohol and the lactone moiety. While only dealkylation was predicted for the lactone oxygen, seven transformations were predicted for the secondary alcohol; these are glucuronidation, oxidation, sulfation, acylation, glucosidation, methylation and acetylation (Fig. 3). The toxicity of the two leads was estimated by AdmetSAR. Interestingly, neither carcinogenicity nor mutagenicity were predicted for germacranolides **1** or **2**.

CONCLUSION

The *In silico* assessment of the druggability for two novel germacranolides isolated from *Capparis decidua* (Forsk.) has been addressed in this study. Both compounds have been proved to be potential drug candidates for curing different diseases, e.g. tumors, through CA II inhibitory mechanism.

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