

Full Length Research Paper

Investigative study on the angiotensin converting enzyme (ACE) inhibiting properties of the terpenoid extract of *Crataegus monogyna* using *in silico* models

D. L. Farrugia^{1*}, C. M. Shoemake¹, E. Attard², L. M. Azzopardi¹ and S. J. Mifsud¹

¹Department of Pharmacy, Faculty of Medicine and Surgery, University of Malta, Msida, Malta.

²Division of Rural Sciences and Food Systems, Institute of Earth Systems, University of Malta, Msida, Malta.

Accepted 11 February, 2013

Crataegus monogyna is mainly used in the treatment of cardiac and circulatory system disorders. *In vitro* and clinical studies are indicative of the fact that the hydroethanolic extract of *C. monogyna* has angiotensin converting enzyme (ACE) inhibitory activity. This study sought to support these claims through the use of *in silico* modelling techniques. Possible binding conformations for β -amyrin, oleanolic acid and ursolic acid were generated using captopril, as well as enalaprilat and lisinopril, as template ligands. The ligand binding affinity (LBA) of each was calculated and the best binding conformation of each triterpene was established. Results indicate that these naturally occurring terpenes possess *in silico* predicted ligand binding affinities that are superior to both the small molecule captopril and the larger molecules enalaprilat and lisinopril.

Key words: *Crataegus monogyna*, hydroethanolic extract, angiotensin converting enzyme (ACE) inhibition, *in silico*.

INTRODUCTION

Hypertension is recognised as being one of the most preventable causes of premature morbidity and mortality. The World Health Organization (WHO) statistics indicate that hypertension affects approximately 40% of all individuals aged 25 years and over. It is prevalent worldwide in both developed and developing countries (WHO, 2012). The majority of hypertensive patients currently rely on the use of angiotensin converting enzyme (ACE) inhibitors for management of their condition (Sweileh et al., 2009).

In silico models are of value in that they allow virtual screening for desirable properties and further optimization

optimization of identified lead candidate molecules, thus drastically lowering financial and time requirements for the chemical synthesis and biological testing of promising leads. They also minimise animal testing; a factor that should not be considered superficial in the highly regulated and ethically conscious scenario in which contemporary drug discovery operates (Kapetanovic, 2008).

In vitro and clinical studies have indicated that the triterpenic extract of *Crataegus monogyna* is capable of exerting an inhibitory effect on the ACE (Attard and Attard, 2006). Subsequent to this work, we hereby report the results of a static *in silico* investigation of the ACE and of the molecular and conformational bases for the ACE inhibitory effect exerted by the terpenoid extract of *C. monogyna*, which to our knowledge has as yet not been described.

*Corresponding author. E-mail: dfar0021@um.edu.mt. Tel: +35679496191.

MATERIALS AND METHODS

Protein data bank (PDB) deposition selection

Three Protein data bank (Bernstein et al., 1977) depositions were selected as templates for this study. These were PDB IDs 1UZF (Natesh et al., 2004), 1UZE (Natesh et al., 2004) and 1O86 (Natesh et al., 2003), describing the bound co-ordinates of testicular ACE and the small molecules captopril (resolution 2.00 Å), enalaprilat (resolution 1.82 Å) and lisinopril (resolution 2.00 Å).

PDB deposition visualisation and modelling

Molecular visualisation and modelling were carried out using SYBYL[®] (SYBYL 7.3, Tripos International, 1699 South Hanley Rd., St. Louis, Missouri, 63144, USA.) (SYBYL 7.3, Tripos International, Cartera[™]). The selected PDB depositions were treated identically during this phase of the study. Specifically, each crystallographic deposition 1UZF, 1UZE and 1O86 was read into SYBYL[®] (SYBYL 7.3, Tripos International, Cartera[™]) with precautions being taken to preserve the bound co-ordinates and consequently the bioactive conformation of each. All moieties considered as superfluous to binding were edited. This means that in the case of 1UZF, two chlorine atoms, 2 N-acetylglucosamine molecules and all water molecules lying at a distance ≤ 5 Å were removed. In the cases of 1UZE and 1O86, two chlorine atoms and two glycine molecules were removed. The result of this editing process consequently was *holo*-ACE bound to captopril, enalaprilat and lisinopril, respectively with water molecules at a radius ≤ 5 Å being retained.

Retention of these water molecules was carried out on the premise that crystallographic data was suggestive of the fact that their proximity to the bound small molecules and the ligand binding pocket (LBP) could give rise to a situation in which bound ligands could be stabilised within the LBP through the formation of water bridges. This editing process was performed in preparation for molecular dynamics studies which will be carried out during subsequent stages of this study.

The small molecules captopril, enalaprilat and lisinopril were subsequently extracted, using SYBYL[®] (SYBYL 7.3, Tripos International, Cartera[™]), from their respective ACE ligand binding pockets (ACE-LBP). Each small molecule was saved in PDB and mol2 formats while the *apo*-ACE with the retained water molecules ($n = 143, 455$ and 569 , respectively) were saved in pdb format. The choice of file format in which to save each moiety was a function of software requirements of subsequent phases of the study. Specifically, ligand binding affinity (LBA) and *de novo* design were carried out using the algorithms of Wang and co-workers in SCORE[®] (Wang et al., 1998) and LigBuilder[®] (Wang et al., 2000), respectively. These require that small molecules be saved in mol2 format and that the protein receptor be saved in pdb format. VMD[®] (Humphrey et al., 1996) was used for image generation and this required that all molecules be read in pdb format.

Analysis of the ligand binding pocket

The extracted bioactive conformations of the small molecules captopril, enalaprilat and lisinopril were used as probes in order to generate ACE-LBP maps of each bound conformation of the ACE. This was done using the pocket module of LigBuilder[®] (Wang et al., 2000) which exploited the contacts forged between the small molecule and the ACE-LBP in each case to generate a 3D bond type specific map of the ACE-LBP as described in each deposition being studied. This process also generated proposed

pharmacophores for each deposition. Elucidation of ligand specific LBP maps and pharmacophores was carried out based on the fact that it is known that receptor tertiary structure and LBP conformation is specific ligand driven. This process consequently served to highlight the conformational differences that the 3 ligand probes induced within the ACE-LBP. At the end of this process, consequently, 2 output files were generated for each deposition. These were:

1. The `key_site_file` depicting the key interaction sites between the amino acid side chains lining the ACE-LBP and the resident small molecule. By convention, hydrogen bond donor sites were represented in blue, hydrogen acceptor sites were represented in red, while hydrophobic sites were represented in cyan.
2. The `pharmacophore_file` depicting the proposed pharmacophoric model. Colour conventions were assigned in the same manner as described for the `key_site_file`.

Both files were saved in pdb format such that they could be later used in the visualization software VMD[®] (Humphrey et al., 1996).

Estimation of the ligand binding affinity (LBA) of the template ligands for the ACE receptor

The *apo*-ACE files which were generated when the selected pdb depositions were edited together, with their respective extracted small molecules saved in mol2 format, which were read into and processed in SCORE[®] (Wang et al., 1998). Through its static algorithm, the *in silico* provided LBA (pK_d) of each of the small molecules captopril, enalaprilat and lisinopril and was calculated for the respective cognate receptor.

Superimposition of the triterpene molecules onto the bound coordinates and 3D volume of the template ligands

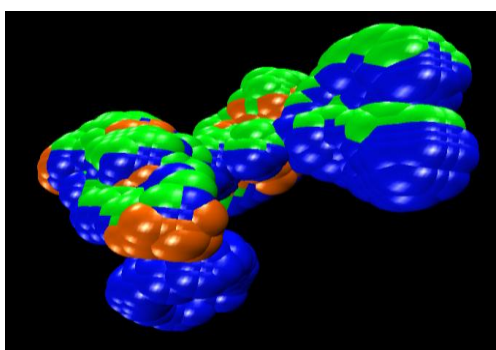
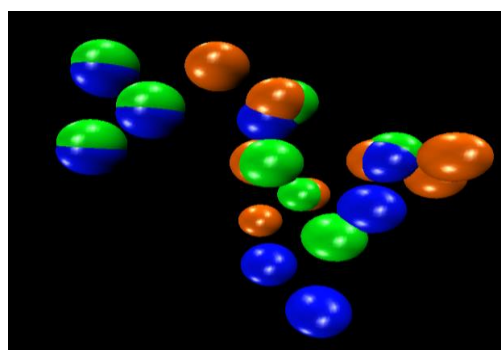
In this part of the study, the bioactive conformations of the small molecules captopril, enalaprilat and lisinopril together with the 3D volumes which they occupy within their respective ACE-LBP conformations were used to guide β -amyrin, oleanolic acid and ursolic acid, all of which are constituents of *C. monogyna*, into the 3 conformations of the ACE-LBP. This was done in order to identify the highest affinity conformers for each molecule, and to utilise these during subsequent stages of the rational drug design process. Identification of the optimally binding conformations of the triterpenic small molecules was carried out using the *Similarity Suite* algorithm in SYBYL[®] (SYBYL 7.3, Tripos International, Cartera[™]). This algorithm facilitated initial positioning of each small molecule within the ACE-LBP based on the conformation of its cognate ligand. Subsequent to this process the triterpenic molecules were allowed conformational rotation within each ACE-LBP conformation. The 21 conformers for each molecule which exhibited optimal binding characteristics were selected and their *in silico* LBA (pK_d) was then quantified in SCORE[®] (Wang et al., 1998).

RESULTS

The *in silico* LBA (pK_d) of captopril, enalaprilat and lisinopril for their cognate receptor was predicted to be 5.36, 6.44 and 6.53, respectively. Significant differences in ACE-LBP occupation by captopril, enalaprilat and lisinopril could be seen when the LBP maps generated by

Table 1. The LBA (pK_d) of the 21 optimally binding conformers of each terpene ligand based on the three different template ligands employed in this study as calculated in SCORE[®].

Captopril			Enalaprilat			Lisinopril		
β -amyryn	Oleanolic acid	Ursolic acid	β -amyryn	Oleanolic acid	Ursolic acid	β -amyryn	Oleanolic acid	Ursolic acid
6.56	6.95	6.74	7.03	7.04	7.26	6.98	6.78	7.37
6.56	6.95	6.74	7.00	7.13	7.23	7.05	6.66	7.34
6.30	7.04	6.82	7.24	7.14	7.35	6.81	7.19	7.41
6.55	6.62	6.68	7.10	7.21	7.21	7.09	6.93	6.95
6.56	6.70	6.69	7.04	7.17	7.15	7.11	6.94	6.85
6.62	6.64	7.18	7.15	7.13	7.59	7.00	7.00	6.96
6.74	6.68	7.14	7.18	7.20	7.29	6.64	7.04	7.02
6.63	6.94	6.53	7.03	7.20	7.19	6.89	6.99	7.30
6.60	6.99	6.94	6.94	7.19	7.36	6.62	6.89	7.34
7.25	6.53	7.27	7.30	7.41	7.25	6.53	7.35	6.85
6.57	6.94	7.30	7.25	7.05	7.31	7.25	6.59	7.27
6.67	6.89	7.24	7.20	7.42	6.93	6.88	6.87	7.08
6.61	6.64	7.29	7.20	7.43	7.42	6.89	6.90	7.05
6.64	6.69	7.69	6.69	7.44	7.43	6.94	6.90	7.17
6.56	6.98	7.84	6.67	7.46	7.26	6.94	6.52	7.07
6.47	6.36	7.11	6.68	7.25	7.08	6.55	6.52	6.62
6.47	6.94	7.79	6.77	7.29	7.21	6.74	6.37	6.81
6.47	6.47	7.15	7.05	7.11	7.20	6.39	6.73	6.63
6.82	6.61	7.41	6.80	7.02	6.67	6.68	6.74	6.80
6.86	6.17	6.76	6.77	7.05	6.58	6.74	6.11	6.55
6.56	6.95	6.83	7.03	7.04	7.26	6.98	6.78	7.37

**Figure 1.** Key interaction sites of captopril (in orange), enalaprilat (in green) and lisinopril (in blue) rendered in VMD[®].**Figure 2.** Proposed pharmacophores of captopril (in orange), enalaprilat (in green) and lisinopril (in blue) rendered in VMD[®].

each molecule were compared (Figure 1). Similarly, the proposed pharmacophores in each case were also dissimilar (Figure 2). The LBAs of the 21 optimally binding conformers of β -amyryn, oleanolic acid and ursolic acid for the captopril-, enalaprilat- and lisinopril- bound conformations of the ACE were calculated and are shown in Table 1. The most salient finding in this case was that the predicted *in silico* LBAs of all 3 experimental

triterpenes (pK_d 7.25, 7.46 and 7.84) exceeded those calculated for captopril, enalaprilat and lisinopril (pK_d 5.36, 6.44 and 6.53, respectively).

DISCUSSION

This *in silico* study further corroborates the hypothesis

of Attard and Attard (2006) that the triterpenic extract of *C. monogyna* has ACE inhibitory activity. All three molecules β -amyrin, oleanolic acid and ursolic acid exhibit binding affinities (7.25, 7.46 and 7.84, respectively) that are superior to those of captopril, enalaprilat and lisinopril (5.36, 6.44 and 6.53, respectively), implying a superior inhibitory activity when compared to the ACE inhibitors that are currently in widespread clinical use. Although this study utilised algorithms that were static, it was still possible to infer the importance of understanding the dynamic nature of both the ACE and its cognate small molecules. This is borne out by the fact that ACE-LBP maps and proposed pharmacophores were obtained that differed according to resident ligand. This was indicative of the fact that LBP conformation is essentially ligand driven. The fact that for example, ursolic acid had a LBA which was highest when captopril was used as a template may be taken to imply that ursolic acid adopts a captopril-like conformation within the ACE-LBP. A similar conclusion may be drawn for oleanolic acid whose best LBA was observed when its conformation within the ACE-LBP was modelled on that of enalaprilat. It was also interesting to note that in the case of β -amyrin, the *in silico* calculated LBA for its optimally binding conformation remained constant irrespective of which ACE inhibitor it was modelled on. This conformation of β -amyrin is consequently very interesting from a rational drug design point of view.

The results obtained from this study consequently open new avenues for the rational design of ACE inhibitors based on the triterpenic scaffold. A molecular dynamics simulation through which the dynamic interactions between the ACE and the ACE inhibitors captopril, enalaprilat and lisinopril may be compared with those between the ACE and the triterpenic molecules β -amyrin, oleanolic acid and ursolic acid represents the next step in this direction.

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