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Synthesis of eugenol-derived glucosides and evaluation of their ability in inhibiting the angiotensin converting enzyme

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ABSTRACT

We report here a series of glucosides which are active as inhibitors of the angiotensin converting enzyme (ACE). They are structurally related to the natural compound eugenol and exhibited significant inhibition values. Their syntheses were expeditious and we could obtain informative docking plots of them complexed to this enzyme. A glucoside derived from eugenol, carrying a carboxylic group in the aglycone, was the most active of them (with an IC_{50} of 0.4 mM) and showed good binding energies in docking studies with ACE. Moreover, computational prediction of toxicity risks, physicochemical properties and drug score show that the glucoside derivative of eugenol is a suitable compound for optimisation studies aimed at finding new drug candidates.

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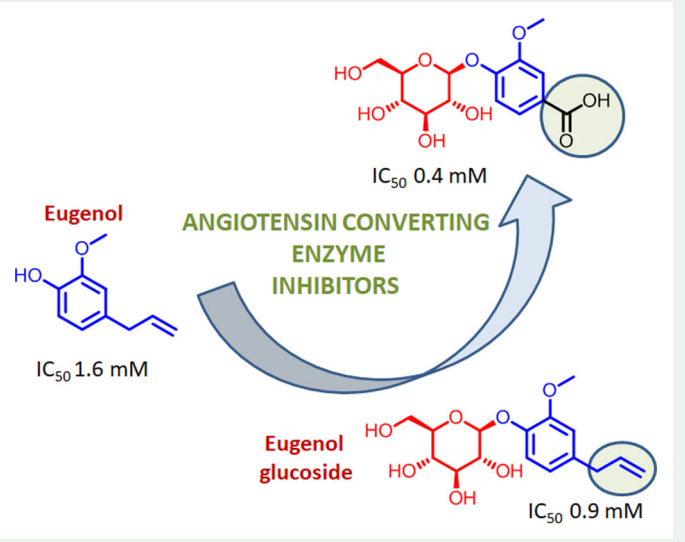

1. Introduction

Angiotensin converting enzyme (ACE) catalyses an important step in the biosynthesis of mediators that control blood pressure levels. Its hydrolytic action occurs on the peptide angiotensin I, which in turn leads to the formation of angiotensin II (Zaman et al. 2002; Atlas 2007). Drugs that act as ACE inhibitors (ACEi) have been used for more than 30 years and all of them present large numbers of polar groups, which is essential for affinity to ACE (Lima 1999; Tavares et al. 2015). Although there has been treated with ACE inhibitors for such a long time (with few side effects) there are some patient studies to understand the variability of responses to ACE inhibitors, since about 20% of patients are unable to respond to treatment. This means that in these patients, the use of ACEi is not able to return the blood pressure to normal levels (Papademetriou et al. 2004; Schilders et al. 2014; Danilov et al. 2017). These observations have also raised interest in investigating new inhibitors that have other structural profiles (Li et al. 2015). In this regard, various natural products are studied as ACE inhibitors. Some reports have shown that eugenol (**1**) (Figure 1) can inhibit ACE via *in vitro* experiments but its physical properties as the oily state, low palatability, volatility and propensity to oxidation do not encourage further studies of eugenol (**1**) as a candidate for an ACE inhibitor (ACEi) (Jeng et al. 1994). Even so, these limitations may be overcome by designing optimised derivatives, which would not present these drawbacks whilst still being active as ACEi. Our rationale for planning eugenol derivatives was based on reports that glycosylation may lead to more stable products, whilst giving better pharmacokinetic profiles (Li et al. 2015). It has been reported that some natural glucosides are active as ACEi. Figure 1 shows a number of these glucosides including (**a**) calceolarioside B (Li et al. 2015) and (**b**) junipediol A β-glucoside (Simaratanamongkol et al. 2014). In addition, Lohith et al. (2006) has shown that

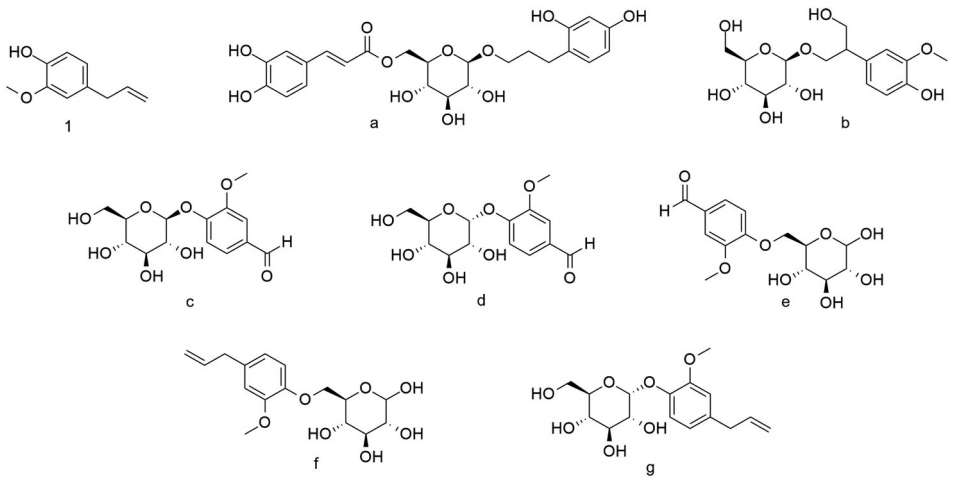

Figure 1. Structures of eugenol (1) and some natural glycosides (a-g) with ACE inhibitory activity.

separate mixtures of 1-*O*-glycosides and 6-*O*-glycosides of vanillin (**c**, **d** and **e**) and eugenol (**f** and **g**) were active as ACEi in the range of 0.5-1.0 mM. In this study, we proposed to synthesise and evaluate as ACEi, a pure eugenol β-glucoside and various analogues resulting from modifications in the aglycone unit.

2. Results and discussion

To prepare the glucosides, we followed the synthetic route depicted in Figure 2 (detailed synthesis information is described in the supplementary file available online). The purity of all products was confirmed by spectroscopic techniques and the synthesised products demonstrated analytical data in accordance with that previously described in the literature. These products were subsequently screened as ACEi according to the methodology described by Friedland and Silverstein (1976). Firstly, we performed an ACE inhibition assay with all the available compounds using the Friedland and Silverstein methodology (1976). Compounds (1), (3), (5), (8) and (10) presented at least 60% ACE inhibition at a concentration of 2 mM. Secondly, these compounds were tested via an IC_{50} assay, where the value curves were calculated and the results can be found in Table 1.

IC_{50} values for eugenol can be found in the literature, with IC_{50} values ranging from 0.3-0.8 mM (Mnafgui et al. 2013; Chaudhary et al. 2014). However, the results generated in this study showed an average IC_{50} value of 1.6 mM. It is noteworthy that the eugenol glucoside (**3**) had its potency increased as an ACEi. Whereas, the hydroxylation of the allyl chain (**5**) led to an inactive product. The shortening of the methylene chain in the benzyl derivative (**10**) reduces the IC_{50} value slightly when compared to eugenol inhibition but does not equal it to compound (**3**). The best result was found with the glucoside (**8**), wherein a carboxylic group replaced the allylic chain. In this case, the glucoside was about four times more potent than eugenol and twice as potent as the glucoside (**3**).

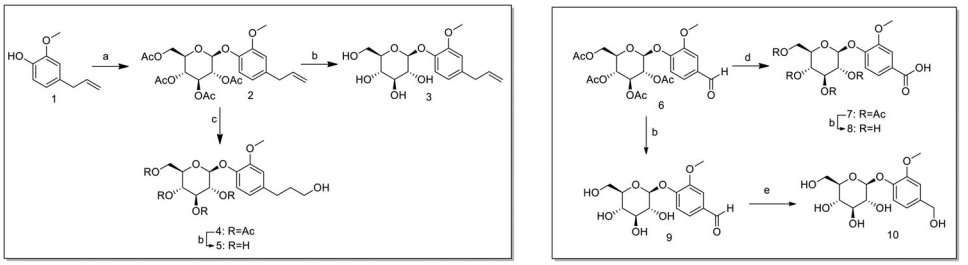

Figure 2. Reagents and conditions to the synthesis of compounds: a) 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide, K_2CO_3, TBAB, CH_2Cl_2, H_2O, r.t.; b) KOH, MeOH, 25 °C; c) $BH_3.(CH_3)_2S$, THF, 0 °C and then 25 °C, followed by 4 M NaOH, 5% H_2O_2, 0 °C to 25 °C; d) $KMnO_4$, H_2O, 80 °C; e) $NaBH_4$, aqueous NaOH, EtOH, 0 °C to 25 °C, followed by aqueous HCl, 0 °C to 25 °C.

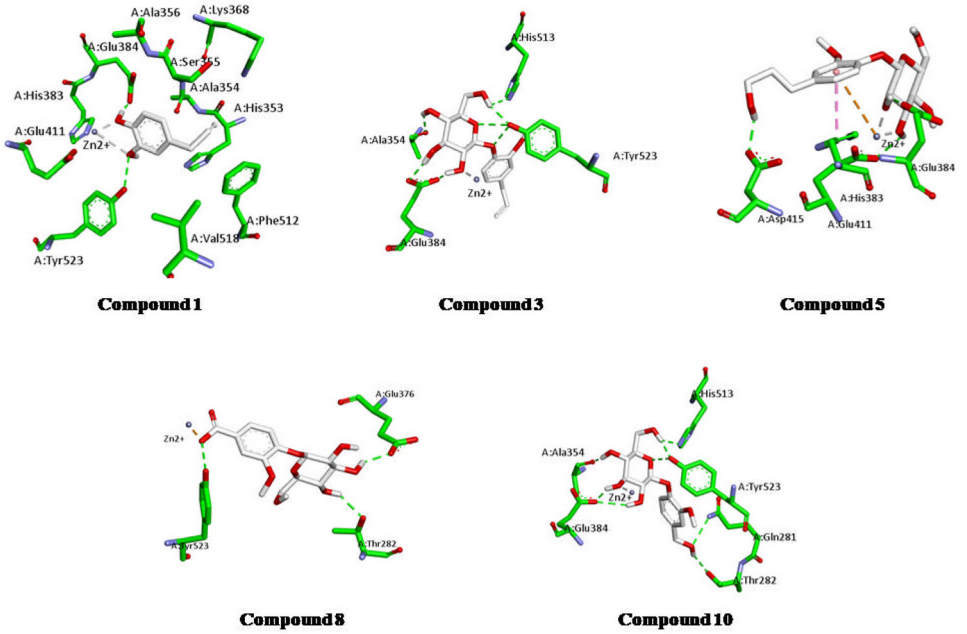

Figure 3. Interaction of compounds 1, 3, 5, 8 and 10 (white carbons) with the active site of ACE (green carbons). Caption: gray dashed lines - ion-dipole interactions; green dashed lines - hydrogen bonds; yellow dashed lines - electrostatic interactions; pink dashed lines - pi-pi stacking interaction.

Docking studies were performed to understand how these compounds interact with ACE active sites (Table 1). In general, the binding energy of more stable complexes between ACE and the evaluated compounds followed the experimental data of enzyme inhibition. Compound (**8**), which was the most active via *in vitro* evaluation, also had the greatest inhibitory potential in the docking studies which indicated that they predicted correctly the most potent compound. The main difference in the interaction of ACE and the evaluated compounds seems to be the mode of their interaction with the zinc atom present in the ACE catalytic site. For compound (**8**), electrostatic interaction between its carboxylate group and the ACE zinc is predicted, while for the others the prevalence of an ion-dipole interaction between zinc and another oxygen atom of their structure is noted.

Table 1. *In vitro* and *in silico* ACE inhibition results, predicted toxicity risks, lipophilicity and drug score for synthesised compounds and reference drugs captopril and enalapril.

		Docking Results			Toxicity risks					
Compound	IC_{50} (mM)	Binding energy (kcal/mol)	Hydrogen Bond	Interaction with Zn^{2+}	Mutagenic	Tumorigenic	Irritant	Reproductive effect	ClogP	Drug Score
1	1.60 ± 0.07	−5.38	OH:⁻OOC-GLU384 H_3CO:HO-TYR523	OH; OCH_3	+	+	+	–	2.27	0.11
3	0.90 ± 0.04	−6.96	O-Sac:HO-TYR523 O-CT:HO-TYR523 HO-C2:⁻OOC-GLU384 HO-C3-⁻OOC-GLU384 HO-C4:O = C-ALA354 HO-C6:N-HIS513	HO-C2	–	+	+	–	0.28	0.17
5	Inactive	−7.18	HO-C2:⁻OOC-GLU384 HO-C3:⁻OOC-GLU411 HO-Prop:⁻OOC-ASP415	HO-C2; HO-C3; Ar	–	–	–	–	−0.46	0.46
7	N.D.	N.D.	N.D.	N.D.	–	–	–	–	0.68	0.66
8	0.40 ± 0.10	−9.02	COO^-:HO-TYR523 HO-C3:⁻OOC-GLU376 HO-C4:HO-THR282	COO^-	–	–	–	–	−1.26	0.49
10	1.20 ± 0.10	−6.92	O-Sac:HO-TYR523 HO-C2:⁻OOC-GLU384 HO-C3:⁻OOC-GLU384 HO-C4:O = C-ALA354 HO-C6:N-HIS513 HO-C6:HO-TYR523 HO-Bz:HO-THR282 HO-Bz:NH-GLN281	HO-C3	–	–	–	–	−1.34	0.52
Captopril	N.D.	N.D.	N.D.	N.D.	–	–	–	–	0.37	0.61
Enalapril	N.D.	N.D.	N.D.	N.D.	–	–	–	–	−0.02	0.76

In the case of eugenol, the phenolic and methoxy oxygen atoms that take part in this interaction. With glucosides (**3**) and (**10**) the saccharide hydroxyls participate in the interaction with the catalytic zinc. Except in the case of compound (**5**), glucosylation appears to contribute to an inhibitory activity improvement via both *in silico* and *in vitro* studies. This may be related to the greater number of hydrogen bonds available to be made with the ACE active site. This corroborates the study by Li et al. (2015) that states that these saccharide hydroxyls have a direct influence on the zinc chelation at the active site of the enzyme. In view of the interesting inhibition results, a prediction of the toxicity risks was also made (Table 1). In the case of eugenol (**1**); mutagenicity, tumorigenicity and irritant effects are expected. The predicted toxic effects were basically associated with the overall structure of eugenol in the case of mutagenic potential and the allyl-aryl chain in the case of the two other features. It can be noted that the mutagenic potential can be reduced with the glucosylation strategy. However, safety related to tumorigenic and irritant effects is only achieved when the allyl chain is modified, as predicted for the glucoside (**8**) (Figure 3).

Despite the improvement in the toxicological profile, the presence of a saccharide unit and the changes in aglycone contribute to a considerable reduction in the ClogP values of (**5**), (**8**) and (**10**), which could compromise their oral bioavailability. A good absorption through gastrointestinal membranes is observed for compounds having a ClogP ranging from 1 to 3 (Hetal et al. 2010; Gibson 2016). Since these three compounds have negative ClogP values, indicating low lipophilic features, a low ability to cross lipid membranes can be expected - which compromises its intestinal absorption and its oral bioavailability. Even with this limitation, the contribution of its toxicological and physicochemical features as a whole provides an intermediate Drug Score value which is higher than that of eugenol and its glucosylated derivative (**3**) - indicating better qualities regarding drug evolution. To improve the lipophilicity of compound (**8**), one of the alternatives that could be explored would be employing the synthetic intermediate (**7**), which is the peracetylated precursor of compound (**8**). This could behave as a prodrug to be hydrolysed *in vivo* to the parent active glucoside. Peracetylation does not interfere with the toxicological profile of the glucoside (**8**), as shown in Table 1, but contributes to enhancing its ClogP value bringing it close to 1 - which could already improve its intestinal absorption. Moreover, as this value is less than 2, this would contribute to the lower possibility of the peracetylated derivative accessing the central nervous system, contributing only to the expected peripheral action. Finally, the calculated Drug Score for the peracetylated derivative of (**8**) is higher (0.66) than that of captopril (0.61), indicating better ADMET properties (Absorption, Distribution, Metabolism, Excretion and Toxicity). The use of molecular docking studies, as well as ADMET property prediction findings, would allow for structural modifications aimed at enhancing both the inhibitory profile of these compounds and their pharmacokinetic and toxicological behaviour.

3. Conclusions

The glucosides reported herein are promising candidates for additional structural optimisation as ACEi, especially glycoside compound (**8**). The interchange of the allylic

chain of the eugenol glycoside by a carboxylic group considerably reduces its IC_{50} value. The saccharide unit seems to be the main responsible for interaction with the zinc in the ACE active site, whereas in glycoside (**8**) the carboxylate unit better does this role. Moreover, ADMET property prediction shows that glycoside (**8**) has a good drug score and that its peracetyl derivative may be an alternative for enhancing its lipophilicity.

Supporting information

The experimental section and spectroscopic data associated with this article can be found in the supporting information section.

Disclosure statement

The authors declare that there are no conflicts of interest.

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