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Synthesis, molecular modeling and biological evaluation of two new chicoric acid analogs

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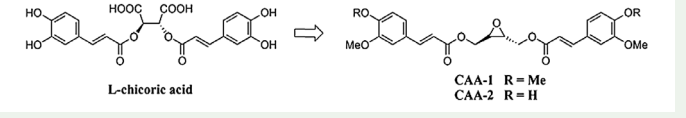
ABSTRACT

Two conformationally constrained compounds similar to chicoric acid but lacking the catechol and carboxyl groups were prepared. In these analogues, the single bond between the two caffeoyl fragments has been replaced with a chiral oxirane ring and both aromatic residues modified protecting completely or partially the catechol moiety as methyl ether. Preliminary molecular modeling studies carried out on the two analogs showed interactions near the active site of HIV integrase; however, in comparison with raltegravir, the biological evaluation confirmed that CAA-1 and CAA-2 were unable to inhibit infection at lower concentration.



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1. Introduction

Among the three enzymes encoded by HIV-1 *pol* gene and translated as apolyprotein, protease, reverse transcriptase and integrase (IN), the latter was an 'orphan' in terms of approved antiretroviral drugs, until the FDA approval of raltegravir (Steigbigel et al. 2008; McColl & Chen 2010) (Figure 1) in late 2007 (brand name Isentress).

Currently, other IN inhibitors are in use: Elvitegravir (Figure 1), trade name Vitekta, approved by the FDA on August 2012, a low molecular weight that shares the core structure of quinolone antibiotics (Shimura et al. 2007) and dolutegravir (Figure 1), brand name Tivicay, approved by the FDA in 2013 and recently gained European approval in January 2014 (Eron et al. 2013).

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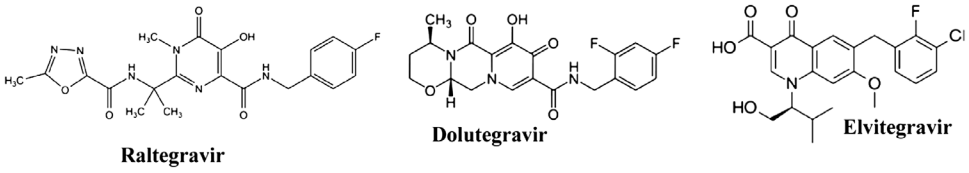


Figure 1. IN inhibitors currently in use.

The HIV-IN, 32 kDa protein containing 288 amino acids, catalyses the virus DNA integration in the cell's genetic code allowing its replication; consequently, this enzyme represents an attractive target for the antiretroviral therapy. Its structure has been separately identified: the N-terminal domain, the central core and the C-terminal domain. In the first two parts, bivalent cations are involved, zinc in the N-terminal domain and magnesium and manganese in the central core, respectively (Brown 1990; Sakai et al. 1993). IN functions in a two-step manner by initially removing a dinucleotide unit from the 3′-ends of the viral DNA (termed '3′-processing'), with the 3′-processed strands then being transferred from the cytoplasm to the nucleus where they are introduced into the host DNA (termed 'strand transfer').

It is known that many compounds, as peptides, oligonucleotides and small polyhydroxylated aromatic compounds inhibit HIV-IN because they are involved in the metal chelation (Reinke et al. 2002; Reinke et al. 2004). This suggests that the formation of coordination complexes with one or two bivalent ions is the key factor in the inhibition (Kawasuji et al. 2006).

Among all reports in the literature, L-chicoric acid, a compound extracted from a variety of plant species (Chkhikvishvili & Kharebava 2001; Hammami et al. 2013; Elansary & Mahmoud 2015), is one of the most potent HIV-IN inhibitors with moderate anti-HIV activity (Lee et al. 2007; Dayam et al. 2008), exhibiting IC_{50} values of 0.15 μM for the 3′-P and 0.13 μM for the ST, an ED_{50} concentration of 1–2 μM and a CT_5 value of 264 μM.

Numerous SAR studies indicate that the presence of bis-catechol and carboxylic groups in this molecule is of critical importance for its anti-HIV activity. Nevertheless, these and other structural characteristics make it a weak candidate as a drug: (1) low permeability, (2) liability of the two ester groups, (3) potential toxicity associated with catecholic groups, (4) relatively high number of flexible bonds that could limit oral bioavailability (Veber et al. 2002). These considerations have prompted in the last years the preparation of series of analogues with structural features of L-chicoric acid to develop more active and specific inhibitors. (Hwang et al. 2001; Charvat et al. 2006; Chhipa et al. 2014).

In this regard, we synthesised two conformationally constrained analogues replacing the single bond between the two caffeoyl fragments with a chiral oxirane ring.

An already reported incorporation of cyclohexane ring as central linker (Lin et al. 1999) did not affect IN strand transfer inhibitory potency, even if antiviral activity decreased (Chhipa

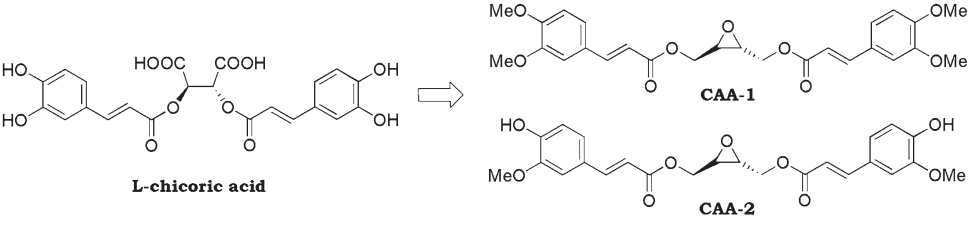


Figure 2. Analogs of L-chicoric acid.

et al. 2014). In our analogues, also the two cinnamic residues have been modified protecting completely or partially the catechol moiety as methyl ether (Figure 2).

2. Results and discussion

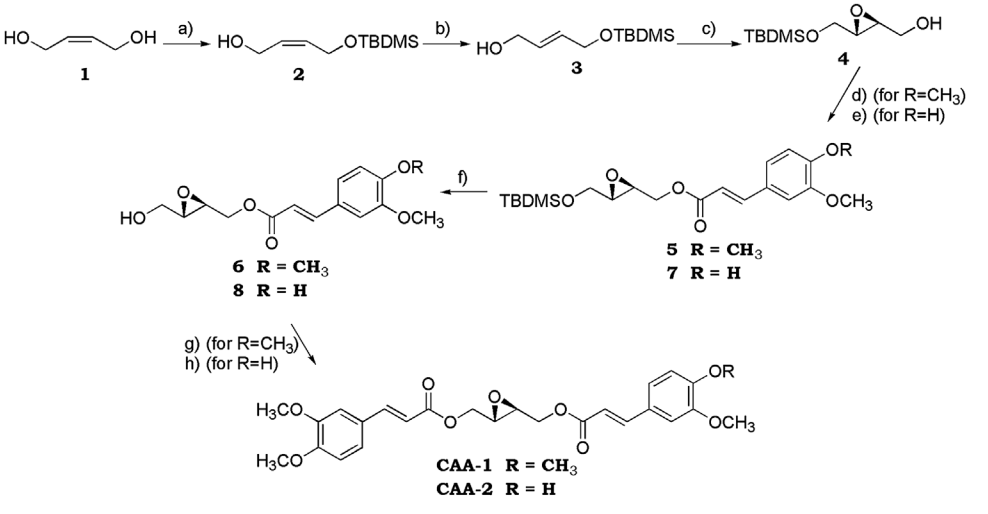
2.1. Chemistry

Retrosynthetic analysis towards the target compounds CAA-1 and CAA-2 involves three crucial steps: two esterification steps with the appropriate cinnamic partners and the Sharpless asymmetric epoxidation.

According to this plan, the preparation of epoxy alcohol **4** has been carried out from the commercially available *cis*-but-2-en-1,4-diol **1** (Scheme 1). The monoprotection of the diol as *tert*butyldimethylsilyl ether, readily provides compound **2** (McDougal et al. 1986). Subsequent *cis*/*trans* isomerisation (Corey & Suggs 1975) afforded the allylic alcohol **3** suitable for the Sharpless epoxidation (Gao et al. 1987), which provided the epoxide **4** in good yield and satisfactory ee%.

The esterification with 3,4-dimethoxycinnamic acid was carried out with DCC/DMAP (*N*,*N*′-dicyclohexylcarbodiimide/(4-(dimethylamino)pyridine) in refluxing DCM (dichloromethane). The cleavage of the silyl ether present in the ester **5**, gave the alcohol **6**, then submitted to esterification with another 3,4-dimethoxycinnamic acid partner to afford the diester CAA-1, the first analogue of L-chicoric acid.

The second analogue of L-chicoric acid CAA-2 was synthesised utilising a pathway similar to CAA-1, in which the esterification conditions were modified (Appendino et al. 2002). In this case, the cinnamic partner was the ferulic, and consequently, we used a method that enabled us to obtain the ester **7** chemoselectively without necessitating protection of



Scheme 1. Reagents and conditions: (a) NaH, TBDMSCl (*tert*-butyldimethylsilyl chloride), THF, 0 °C, 81%; (b) i) PDC (pyridinium chlorochromate), CH_2Cl_2; ii) $NaBH_4$, CH_3OH, 0 °C, 67% (two step); (c) *t*-BuOOH, $Ti(O\text{-}i\text{-}Pr)_4$, (+)-DET (diethyl L-(+)-tartrate), CH_2Cl_2, −20 °C; 86%; (d) $(MeO)_2C_6H_3(CH)_2COOH$, DCC/DMAP (*N*,*N*′-dicyclohexylcarbodiimide/(4-(dimethylamino)pyridine), CH_2Cl_2, reflux, 87%; (e) $(MeO)(HO)C_6H_3(CH)_2COOH$, DIAD (diisopropyl azodicarboxylate), TPP (triphenylphosphine), THF, 57%; (f) TBAF (tetrabutylammonium fluoride), THF, rt, 96%; (g) $(MeO)_2C_6H_3(CH)_2COOH$, DCC, DMAP, CH_2Cl_2, reflux, 68%. (h) $(MeO)(HO)C_6H_3(CH)_2COOH$, DIAD, TPP, THF, 26%.

phenolic group (Swamy et al. 2009). After deprotection of the alcoholic group of **7**, the latter esterification was carried out under the same conditions, thereby producing CAA-2, although in low yield.

2.2. Molecular modeling: a preliminary study

Docking studies were performed in order to investigate the binding of the ligands and metal complexes interactions in the active site of the protein.

According to docking results of CAA-1, this compound interacts with the active site by means of hydrogen bonding to the amino acid residue Asn117 and, in this bound orientation, forms coordinate bonds with Mg^{2+} ion by dimethyl catecholic function (Figure 3(a)). The mean binding energy (MBE) is −2.95 kcal/mol and the estimated free energy of binding (EFEB, ΔG_{bind}) is −3.17 kcal/mol, while the estimated inhibition constant (EIC, K_i) is 4.78 mM. Although these results seemed to suggest favourable interactions and binding with the active site of IN, the docking result was quite low and consistent with the biological evaluation data (Table 1).

A trend quite similar to CAA-1 was observed for CAA-2 and, as shown in Figure 3(b), the two compounds were nearly superimposable.

2.3. Biological evaluation

Compounds CAA-1 and CAA-2 were tested for their cytotoxic activity on monocytoid cell line U937 through an assay assessing the inhibition of mitochondrial metabolic activity. The

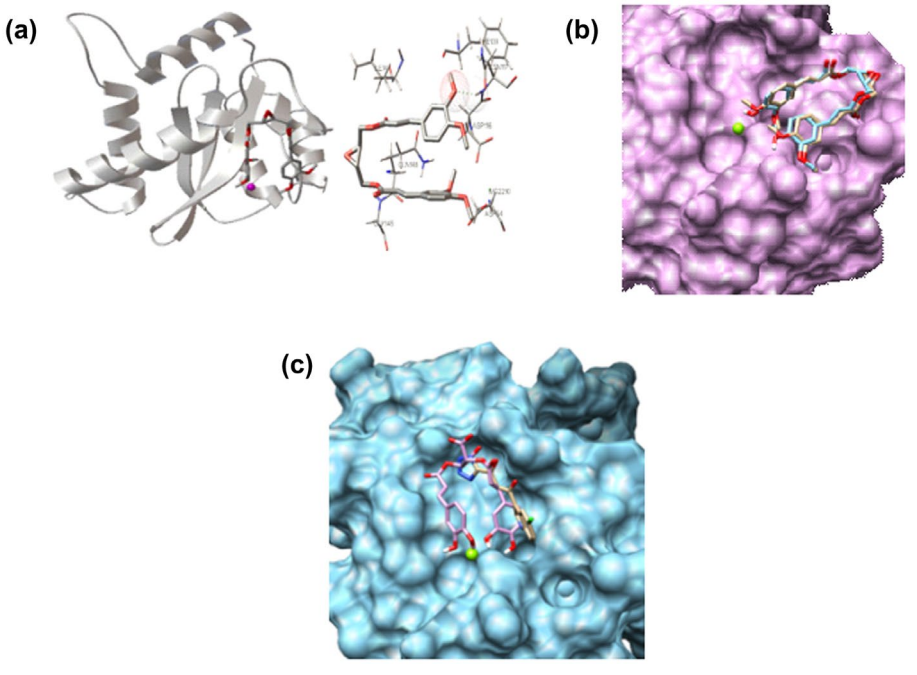


Figure 3. (a) Interaction of CAA-1 with the HIV Integrase active side; (b) Interaction of CAA-1 and CAA-2 with the HIV integrase-active site.

Table 1. Amino acids interactions and hydrogen bonding.

Ligands	%cluster	M.B.E.[a]	E.F.E.B.[b]	E.I.C.,K_i[c]	Interaction aa, H-Bonds
CAA-1	55	−2,95	−3,17	14,78 mM	ASP64 ASP116 **ASN117** PHE139ILE141 GLN148 GLY149 ILE151
CAA-2	89	−2,46	−2,70	10,45 mM	**ASP64** ASP116 **ASN117** ILE141GLN148 GLY149 ILE151 MG2210
L-chicoric acid	12	−7,35	−9,00	253,72 nM	ASP64 CYS65 THR66 **HIS67ASP116** GLN148 ILE151 GLU152 ASN155**LYS-156LYS159** MG2210

[a]M.B.E.= Mean binding energy(kcal/mol).
[b]E.F.E.B. = Estimated free energy of binding(kcal/mol).
[c]E.I.C., K_i = Estimated inhibition constant, K_i.

cytotoxic effect of CAA-1 and CAA-2 were compared to the one of a well-known drug inhibitor of HIV-IN, raltegravir.

The results indicated that CAA-1 was not cytotoxic towards U937 cells exhibiting IC_{50} > 1000 μM, while CAA-2 exhibited a cytotoxic effect overlapping that of raltegravir, about 100 μM. A potential functional activity of CAA-1 and CAA-2 as antiretroviral was assessed by assaying their effect on infection of peripheral blood mononuclear cells with HIV. The results showed that CAA-1 and CAA-2 were unable to inhibit infection at lower concentration in comparison with raltegravir.

Although the functional activity revealed that CAA-1 and CAA-2 were not efficacious in inhibiting HIV infection, their low or equal cytotoxicity in respect to raltegravir, encourages improving their structure – target activity. Actually considering that one of the main drawbacks in HIV infection is the outcome of drugs resistant strains there is urgent need to design and evaluate new antiretroviral molecules.

Therefore, CAA-1 or CAA-2 skeleton could represent a lead structure that could undergo further synthetic changes to increase the specific activity.

3. Conclusion

We reported the synthesis of two conformationally constrained analogues of L-chicoric acid, where the single bond between the two caffeoyl fragments has been replaced with a chiral oxirane ring and both aromatic residues modified protecting completely or partially the catechol moiety as methyl ether.

Even though the preliminary molecular modelling studies carried out on the two analogues seemed to indicate favourable interactions and tight binding with the active site of IN, the biological evaluation showed that CAA-1 and CAA-2 were unable to inhibit infection at lower concentration in comparison with raltegravir. Nevertheless, considering their low or equal cytotoxicity in respect to raltegravir, improvement on their structure – target activity could be valuable.

Supplementary material

Supplementary material regarding the characterisation of all new compounds reported in this article, the molecular modelling and the biological evaluation sections are available online.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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