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# Structure activity relationship studies of cinnamic acid derivatives as inhibitors of human neutrophil elastase revealed by ligand docking calculations

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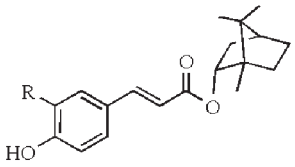
Structure-activity relationship of cinnamic acid derivatives as inhibitors of the human neutrophil elastase is reported. Comparison of the inhibitory concentrations ($IC_{50}$ values) with the results of the ligand docking calculations revealed that the structure element of the aromatic ortho-dihydroxy groups combined with a lipophilic residue seems to be a prerequisite for an optimal binding within the active site.

## 1. Introduction

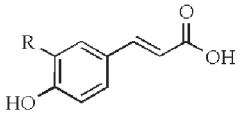
Human neutrophil elastase (HNE) is a major proteinase of human neutrophils located in the azurophilic granules. It is a serine protease with a specificity for small hydrophobic amino acids, occurring in a high concentration in elastases main substrate elastin. Its primary role appears to be in the intracellular degradation of foreign proteins during phagocytosis by neutrophils. A second role is likely to aid the movement of these cells through connective tissue matrices while they are heading towards a target. During these processes, HNE is released and controlled by endogenous proteinase inhibitors. However, intense neutrophil infiltration results in an imbalance between the amount of HNE and endogenous inhibitors. Accumulating HNE can then cause abnormal degradation of healthy tissue resulting in the development of diseases such as pulmonary emphysema, rheumatoid arthritis or cystic fibrosis [1, 2]. Therefore, natural or synthetic inhibitors of HNE could be of considerable interest in the treatment of these inflammatory diseases.

Cinnamic acid esters are a group of natural compounds widespread in higher plants. Esters from cinnamic acid derivatives with borneol isolated from *Verbesina turbacensis* serve special interest. They have been shown to influence inflammatory processes in several ways. Strong antioxidative and radical scavenging properties have been proven. They also influence enzymes playing an important role in the inflammatory process, e.g. 5-lipoxygenase and cyclooxygenase-1 [3]. In *in vivo* test systems like the TPA-induced mouse ear oedema they were also active. Here, the $IC_{50}$ values were similar to that obtained for indometacin [4].

Recently, we have demonstrated that the caffeate, ferulate and *p*-coumarate of borneol, but not the free acids inhibit HNE *in vitro*. Bornyl caffeate was the most active compound with an $IC_{50}$ value of 1.6 µmol/ml [5]. Moreover, the structurally closely related cimicifugic acids and fukinolic acid from *Cimicifuga racemosa* also inhibit HNE in very low concentrations [6].

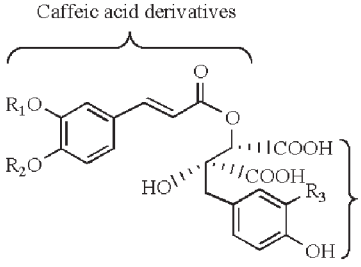


R = OH (-)-bornyl caffeate (**1**)
R = H (-)-bornyl coumarate (**2**)
R = $OCH_3$ (-)-bornyl ferulate (**3**)



R = OH caffeic acid (**4**)
R = H coumaric acid (**5**)
R = $OCH_3$ ferulatic acid (**6**)

Caffeic acid derivatives



Fukiic acid $R_3$=OH
Piscidic acid $R_3$ =H

$R_1$=H $R_2$=H $R_3$=OH Fukinolic acid (**7**)
$R_1$=$CH_3$ $R_2$=H $R_3$=OH Cimicifugic acid A (**8**)
$R_1$=H $R_2$=$CH_3$ $R_3$=OH Cimicifugic acid B (**9**)
$R_1$=$CH_3$ $R_2$=H $R_3$=H Cimicifugic acid F (**10**)

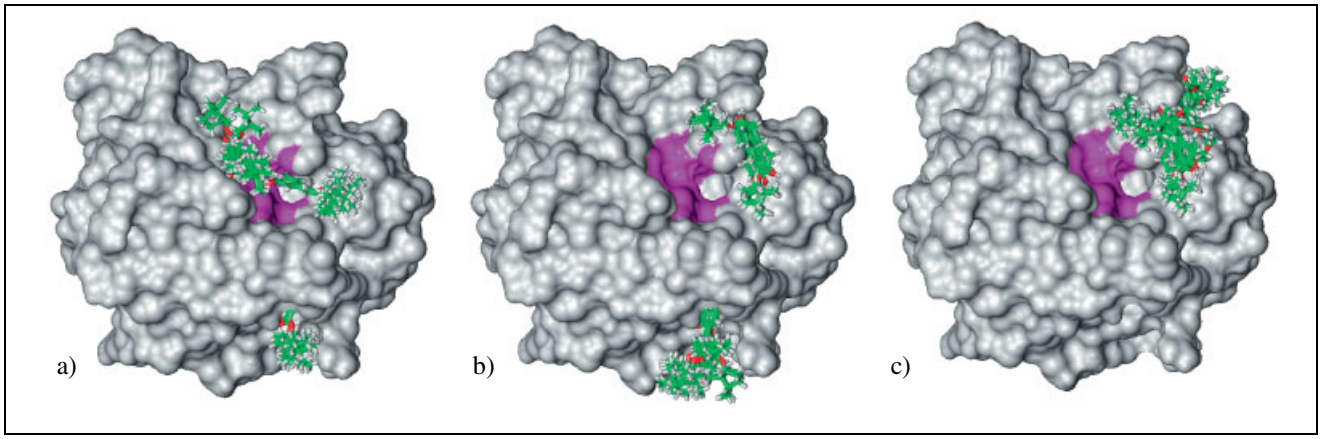



Fig. 1: Positions of the 20 highest ranked docking solutions of (a) bornyl caffeate (**1**), (b) bornyl *p*-coumarate (**2**) and (c) bornyl ferulate (**3**) on the surface of elastase (grey). Only in the case of bornyl caffeate binding to the active site region (magenta) is observed. Carbon atoms oxygens and hydrogens of the ligand are shown in green, red and white, respectively.

Using the X-ray structure of 1HNE [7] and the ligand docking program FlexX [8] we have calculated putative complexes of HNE with the cinnamic acid derivatives. On the basis of the predicted structures the different activities of these substances are discussed.

## 2. Investigations, results and discussion

### *2.1. Ligand docking calculations with the bornyl esters*

Ligand docking calculations with the bornyl ester derivatives **1**, **2** and **3** revealed placements within the active site only in the case of bornyl caffeate (**1**). No complexes with bornyl *p*-coumarate (**2**) and ferulate (**3**) bound to the active site were predicted (Fig. 1). The $IC_{50}$ values of **1**, **2** and **3** correlate well with these findings (Table). For bornyl caffeate a total number of 156 ligand-protein-complexes were calculated, of which 44% showed the inhibitor inside the active site region. As a reasonable subset for a detailed analysis, the 20 highest ranked solutions of each docking calculation were selected. Among these, 15 (75%) showed interactions with the active site. From the calculated complexes follows that the presence of the two *o*-dihydroxy groups in bornyl caffeate is especially favourable for an interaction with the active site of elastase. They enable a simultaneous anchoring of this inhibitor

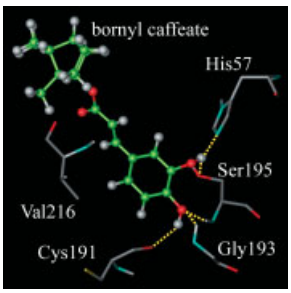



Fig. 2: Highest ranked docking solution of bornyl caffeate with the ligand bound to the active site. The dashed yellow lines indicate the hydrogen bonds between the ligand and HNE. Colour legend: carbon atoms of the protein: grey; carbon atoms of the ligand: green; oxygens: red; nitrogens: blue, hydrogens: white; sulfur: yellow

via hydrogen bonds in the oxyanion hole, consisting of the backbone NH groups of Ser195 and Gly193, at the backbone carbonyl of Cys191, and at the side chains of His57 and Ser195 (see Fig. 2). These two amino acids, together with Asp102, form the catalytic triad. Additionally hydrophobic interactions were calculated for the aromatic ring of the caffeate residue with side chains of amino acids being part of the S1 specificity pocket, especially with Val216 (see Fig. 2). The S1 pocket of

**Table: Inhibitory concentrations and results of the docking calculations for the investigated cinnamic acid derivatives with HNE**

| Compds. | $IC_{50}$ (μM)[b] | $\Delta G^0$ (kJ/mol)[c] | total number of docking solutions | placements in the active site region (%) | placements in the active site region among the 20 best solutions | Number of H-bonds[d] |
|---|---|---|---|---|---|---|
| Bornyl caffeate (**1**) | 1.6 | −18.7 | 156 | 44 | 15 | 5 |
| Bornyl *p*-coumarate (**2**) | 69 | – | 194 | 0 | 0 | 0 |
| Bornyl ferulate (**3**) | 78 | – | 199 | 0 | 0 | 0 |
| Caffeic acid (**4**)[a] | 93 | −21.2 | 115 | 10 | 5 | 5 |
| *p*-Coumaric acid (**5**)[a] | >1000 | – | 115 | 0 | 0 | 0 |
| Ferulic acid (**6**)[a] | >1000 | – | 109 | 0 | 0 | 0 |
| Fukinolic acid (**7**)[e] | 0.23 | −20.9 | 99 | 82 | 16 | 6 |
| Cimicifugic acid A (**8**)[e] | 2.2 | −19.6 | 149 | 75 | 20 | 6 |
| Cimicifugic acid B (**9**)[e] | 11.4 | −20.3 | 70 | 59 | 17 | 6 |
| Cimicifugic acid F (**10**)[e] | 18.0 | −21.5 | 87 | 21 | 0 | 5 |

[a] Docking calculations were carried out with the anionic forms of the acids
[b] Refs. [5, 6]
[c] Calculated binding free energy for the highest ranked docking solution with the ligand bound to the active site
[d] Only hydrogen bonds to the amino acids of the active site are considered
[e] Calculations with all other possible protonation states were performed with **7** leading to comparable results. Therefore, data from the calculations with both protonated aliphatic carboxylic groups are here reported.

elastase has a rather hydrophobic character with no anchoring point and can accommodate medium sized hydrophobic side chains [9].

### 2.2. Ligand docking calculations with the free acids

Previously, it was shown that, compared to the bornyl esters **1**, **2** and **3**, the corresponding free acids **4**, **5** and **6** either need higher concentrations to inhibit HNE, such as caffeic acid (**4**), or do not influence the enzyme up to a concentration of 1000 μM, as it was shown for *p*-coumaric (**5**) and ferulic acid (**6**) [5]. To explain these findings, we performed docking calculations as well. Since the elastase assay was performed at a pH of 7.5, the anionic forms of the acids were used in the calculations. Again, a clear correlation between the number of ligand placements at the active site and the $IC_{50}$ values was obtained (Table). Only for caffeic acid solutions with the ligand bound to the active site were found. They show a similar hydrogen bond network as observed for bornyl caffeate. However, with respect to the calculated binding free energies of bornyl caffeate and caffeic acid no correlation between the $IC_{50}$ values can be stated. An explanation for the relatively high $IC_{50}$ values for **4** may be the fact that caffeate can interact with the positively charged 19 arginine residues which are located on the surface of the protein close to the active site [9].

### 2.3. Ligand docking calculations with Cimicifuga compounds

To extend our studies on structure activity relationships further cinnamic acid derivatives were included in the ligand docking calculations and cimicifugic and fukinolic acids (**7**–**10**) possessing a lipophilic residue were studied. Ligand docking calculations showed a good correlation between the number of placements in the active site and the $IC_{50}$ values of these compounds as well (see Table). Again no correlation between the calculated binding free energies and the $IC_{50}$ values was found. Fukinolic acid (**7**) and the cimicifugic acids A (**8**) and B (**9**) are predicted to bind with their fukiic acid residue in the active site where the aromatic *o*-dihydroxy groups form hydrogen bonds similar to bornyl caffeate. Additional hydrogen bonds between the aliphatic hydroxy group of the fukiic acid residue and the backbone carbonyl of His57 and between the carboxy group of the tertiary carbon and the backbone NH group of Asn61 are observed. Similar to bornyl caffeate one of the aromatic rings interacts with the hydrophobic S1 specificity pocket. The other one contributes to the binding by electrostatic interactions with Val62 and Leu35. The less active compound cimicifugic acid F (**10**) missing a catechol structure only shows very few docking solutions at the active site with relatively high binding energy. Among the 20 best docking solutions no ligand placement within the active site was predicted for this compound, supporting the importance of the *ortho*-dihydroxy groups of the catechol unit for a favourable interaction with the active site of elastase.

### 2.4. Conclusion

No correlation between the $IC_{50}$ values and the calculated binding free energies was found. However, based on geometrical considerations the experimental data can be explained. The structure element of the aromatic *ortho*-dihydroxy groups combined with a lipophilic residue seems to be a prerequisite for a hydrogen bond network within the active site. This is in accordance with an experimental study in which the ortho-dihydroxy groups were also shown to be an important structure element for elastase inhibition [14]. Thus, caffeic acid esters with this structural feature could serve as lead compounds for the development of reversible inhibitors of human leukocyte elastase.

## 3. Experimental

### 3.1. Test compounds

(−)-Bornyl caffeate, (−)-bornyl ferulate and (−)-bornyl *p*-coumarate were isolated from *Verbesina turbacensis*, Asteraceae [3]. Fukinolic acid and the cimicifugic acids A, B and F were isolated form *Cimicifuga racemosa,* Ranunculaceae [10]. Caffeic acid, ferulic acid and *p*-coumaric acid were purchased from ICN Biomedicals (Germany).

### 3.2. Ligand docking calculations

The X-ray structure of Navia et al. [7] (PDB code: 1HNE) was used in our calculations after deletion of the irreversible inhibitor MeO-Suc-Ala-Ala-Pro-Val-$CH_2Cl$ from the PDB file. We chose this structure because of the high resolution (1.8 Å) and because a structure with complexed inhibitor has a lower risk of unduly large structural changes. The region of the protein considered in the calculations was determined with the molecular modelling package WhatIf [11]. All amino acids were included with at least one atom lying within a distance of 15 Å from the Oγ atom of the catalytically active Ser195. Polar hydrogen atoms were placed with the HB2NET command of WhatIf optimizing the hydrogen bond network of the complete protein [12]. Crystallographically determined water molecules were omitted for the docking calculations as they were found not to be located in the active site region. The term active site region is used for the close environment of Ser195 Oγ, i.e. all protein atoms within a radius of 6.5 Å (see Fig. 1). The structures of the test compounds were built with the program Hyperchem™ (Hypercube Inc.) and geometry optimized with the MM+ force field prior to use as input files for the docking calculations.

Docking calculations were carried out with the program FlexX (GMD, Germany) [8], version 1.9.0, on a Silicon Graphics Workstation. FlexX is designed for the docking of small to medium sized organic molecules into protein binding sites. During the docking procedure, the protein is considered as rigid, whereas the ligand conformation is flexible. Directed chemical interactions (predominantly hydrogen bonds and salt bridges) of the ligand with the receptor are used for the calculation of ligand placements applying an incremental construction algorithm. The interaction geometries were deduced from the analysis of crystallographic data. The predicted protein-ligand complexes were optimized and ranked according to the empirical scoring function of FlexX, which estimates the binding free energy, $\Delta G^0$, of the ligand receptor complex [13]. This scoring function was derived using the X-ray structures of a large number of protein-ligand complexes as a basis to fit the free energy function to the experimental data.

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