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Structural Approach of the Mechanism of Inhibition of α-Chymotrypsin by Coumarins

Johan Wouters,[a,†] Marjorie Huygens,[a] Lionel Pochet,[b] Bernard Pirotte,[c] François Durant[a] and Bernard Masereel[b,*]

[a]*Laboratoire de Chimie Moléculaire Structurale, Facultés Universitaires N.-D. de la Paix, 61 rue de Bruxelles, B-5000 Namur, Belgium*
[b]*Department of Pharmacy, University of Namur, FUNDP, 61 rue de Bruxelles, B-5000 Namur, Belgium*
[c]*Service de Chimie Pharmaceutique, 1 avenue de l'Hôpital, B-4000 Liège, Belgium*

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Abstract—A pharmacophore associated to the inhibiton of α-chymotrypsin has been built based on the structural and electronic characterization of a series of coumarin derivatives.

As part of our interest for inhibitors of serine proteases, a series of coumarins has been designed, synthesized and their inhibitory potency measured.[1] Among those compounds, we were interested in rationalizing the activities of compounds **1**–**5** towards α-chymotrypsin (Table 1).

As a result of this study, a pharmacophore model associated to the inhibition of α-chymotrypsin by coumarins is proposed.

Conformational Studies

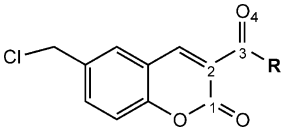
Crystal structures of compounds **1** and **3** have been obtained by X-ray crystallography (see Experimental; Fig. 1). Compound **1** is characterized by a *syn* conformation of the *exo*-carbonyl function versus the lactone [T1 [1-2-3-4] = −16.2(4)° close to 0°]. In the benzyl-keto analogue **3**, this torsion angle is close to 120° [144.7(3)°], probably due to an intra-molecular H bond between one methylene H atom of the benzyl substituent and the lactone C=O oxygen atom. Theoretical conformational scans (ab initio 3-21G*) around the T1 torsion angle indicate that the *syn* conformation of **3** is also energetically accessible (ΔE 4.2 kcal/mol).

No suitable crystals of the thioester isoster **2**, **4** and the amide isoster **5** have been obtained so far. Therefore, statistical analysis (within the Cambridge Structural Database, CSD[2]) of fragments representative of those compounds have been carried out in order to further approach the conformation of those molecules (see Experimental).

Combination of single crystal structures of **1** and **3**, and data contained in the CSD allowed the modeling of 13 conformations representative of the geometry of **1**–**5**: 2 for **1**, 3 for **2**, 5 for **3**, 2 for **4**, and 1 for **5** (Fig. 2). The experimental conformations are in good agreement with theoretical predictions obtained from conformation scans around T1.

Amide analogue **5**, the less active compound in the series, always adopts an *anti* conformation (T1 = 180°), favored by the presence of an intra-molecular H bond between the NH and C=O (lactone) groups, in contrast with (thio)ester **1**, **2** or carbonyl **3**, **4** substituted coumarins for which a *syn* conformation is allowed. Com-

Table 1. Inhibitory potency towards α-chymotrypsin[1c]

	R =	k_{inact}/K_M [a]
	O–Ph (**1**)	100,000
	S–Ph (**2**)	48,000
	CH_2–Ph (**3**)	10,000
	Ph (**4**)	2400
	NH–Ph (**5**)	200

[a]In M^{-1} s^{-1}. Torsion angle T1 is defined by atoms [1-2-3-4].

*Corresponding author. Tel./fax: +32-8172-4338; e-mail: bernard.masereel@fundp.ac.be
†Present address: Institut de Recherches Microbiologiques Wiame, Av E Gryzon, B-1070 Brussels, Belgium.

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pound **4**, which is also a poor inhibitor, adopts *gauche* conformations (T1 torsion angles close to + or −120°).

We therefore suggest that the *syn* conformation corresponds to the bioactive conformation of those inhibitors. This geometrical constraint will be incorporated into the pharmacophore model.

Electrostatic Potential

A conserved pattern of attractive (−40 kcal/mol) molecular electrostatic potential generated by the carbonyls (Fig. 3) characterizes the active compounds when they adopt a *syn* conformation (T1 [1–2-3–4] close to 0°). Both carbonyls of the inhibitors thus represent characteristic anchoring points to the active site of the protein.

This characteristic pattern, common to active analogues **1**–**3**, is still present (but attenuated) for compound **4**. It is not retained by the inactive amide analogue **5**.

Binding Site of α-Chymotrypsin

Identification of potential binding sites in the crystal structures of serine proteases has been carried out using the GRID program[3] (see Experimental). In the particular case of α-chymotrypsin, a series of probes were used to identify functional groups (H bond donor/acceptors, ionic interactions, hydrophobic pockets) potentially involved in stabilization of inhibitors. Beside the catalytic residues (Asp102-His57-Ser195), amino acids from the specificity pocket (e.g., Ser190, Ser217, Tyr228, Trp215) could further interact with ligands.

Pharmacophoric Model

Based on the stereo chemical characterization of coumarins **1**–**5**, a model of pharmacophore (Fig. 4) has been proposed. It is complementary to functional groups defining the binding site of the protein.

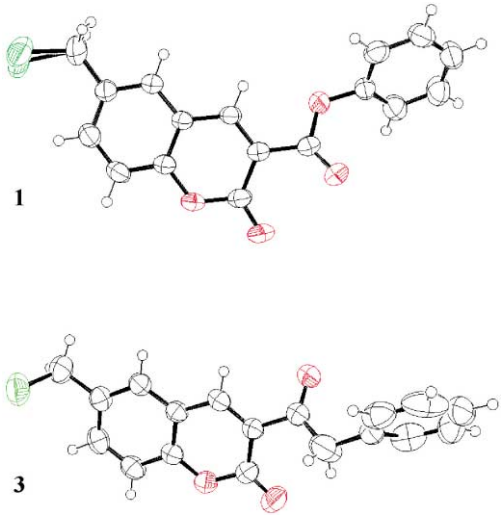

Figure 1. Crystal structure conformations of compounds **1** and **3**.

Relative position of the hydrophobic substituent (lateral phenyl ring in **1**–**5**) has also been established. From the superposition of representative conformations of **1**–**5** (Fig. 2) geometrical descriptors were measured: the distances between centroids of rings A and B is about 8.2 Å and the position of ring B versus the essential lactone function (defining the characteristic attractive MEP pattern) is about 6.4 Å.

The distances derived in the pharmacophore model (Fig. 4) are compatible with the geometry of the binding site of α-chymotrypsin.

Therefore, we suggest that:

- the carbonyls (*C*) orient in such a way that they interact with Ser190 and the catalytic serine 195 potentially leading to the acyl-enzyme.[1]

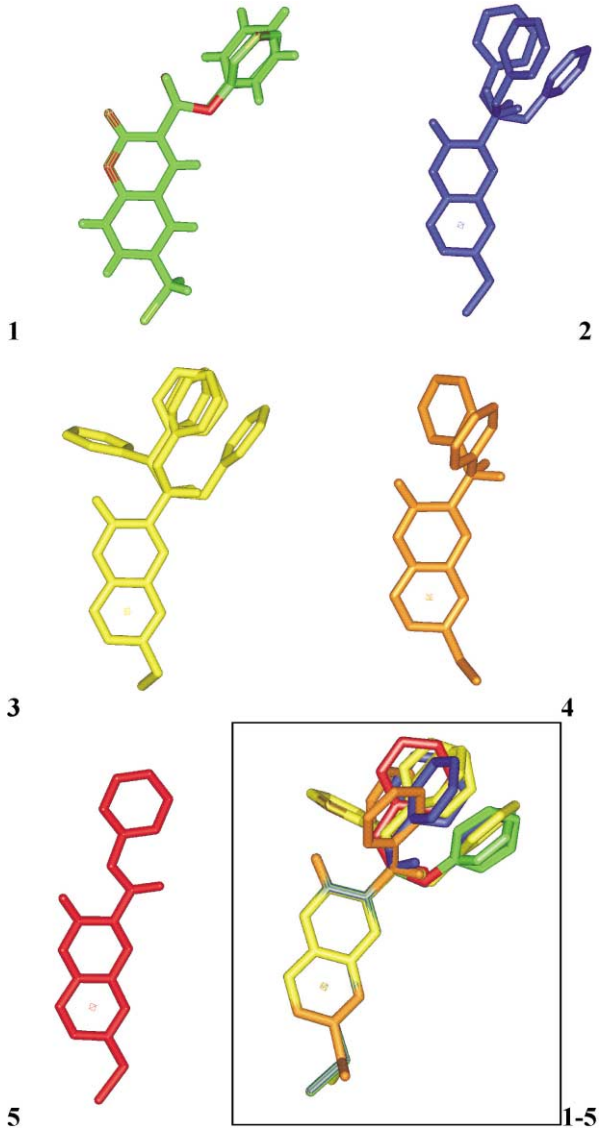

Figure 2. Representative conformations of compounds **1**–**5** (2 for **1**, 3 for **2**, 5 for **3**, 2 for **4**, and 1 for **5**) modeled on the basis of small molecule crystallographic data.

- the coumarin ring (*A*) binds into the active site in order to fit the chloro methyl function close to His57, the nucleophile potentially implied in formation of the alkyl-enzyme.[1]
- the phenyl substituent (*B*) could fit into the hydrophobic specificity pocket of the protein.

Docking Simulation

Docking of **1** in the active site of α-chymotrypsin has been performed incorporating the structural features derived form the model of pharmacophore and the functional groups defining the binding site of the protein.

The *syn* conformation of **1** allows optimal interaction in the carbonyls (*C*) with Ser190 and the catalytic serine 195 (Fig. 5). The coumarin ring (*A*) is stabilized by van der Waals forces that involve, among other residues, Trp215. The phenyl substituent (*B*) fits the hydrophobic specificity pocket of the protein. From Figure 5, it is clear that for less active compounds (e.g., **5**), probably adopting an *anti* conformation, the *B* lateral aromatic ring cannot fit into the hydrophobic specificity pocket and clashes with the enzyme.

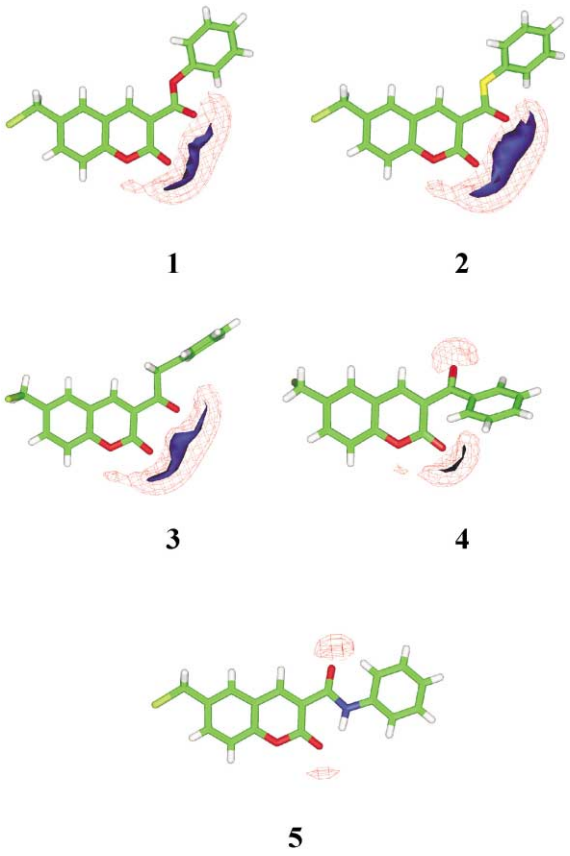

Figure 3. Molecular electrostatic potential (MEP) calculated (HF 3-21G*) on the representative geometries of molecules **1**–**5**.

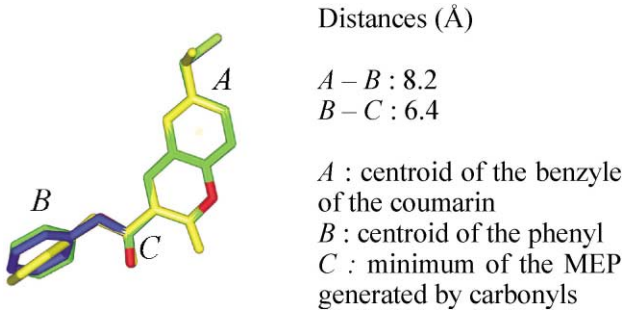

Figure 4. Superimposition of conformations of compounds **1**–**3** allowing measurement of characteristic geomtries between pharmacophoric elements (A, B, C) defining the pharmacophore. Those elements find their counter part in the binding site of the enzyme.

Experimental

Crystallography

Crystals of compounds **1** and **3** were obtained by slow evaporation of concentrated solutions in toluene/*i*ProOH and *i*BuOH/acetone, respectively.

1. $C_{17}H_{11}O_4Cl$, orthorhombic, $P2_12_12_1$, $a=4.989(1)$ $b=12.220(3)$, $c=21.160(4)$ Å, $V=1395(1)$ Å^3, $Z=4$, $\mu=2.57$ mm^{-1}, $D_x=1.498$ g cm^{-3}, λ (Cu $K\alpha$) $=1.54178$ Å, $F(000)=648$, 2287 unique reflections ($R_{int}=0.012$), $R_1=0.0351$ for 2070 $F_o^2>2\sigma(F_o^2)$ and $wR_2=0.100$, $GooF=S=1.040$, $T_{min}=0.759$, $T_{max}=0.967$, $\Delta\rho_{min}=-0.26$, $\Delta\rho_{max}=0.17$ e Å^{-3}.

3. $C_{18}H_{13}O_3Cl$, monoclinic, $P2_1/c$, $a=5.889(1)$ $b=15.797(1)$, $c=16.017(1)$ Å, $\beta=95.62(2)°$, $V=1482(1)$ Å^3, $Z=4$, $\mu=2.37$ mm^{-1}, $D_x=1.401$ g cm^{-3}, λ (Cu K_α) $=1.54178$ Å, $F(000)=648$, 2911 unique reflections ($R_{int}=0.048$), $R_1=0.0526$ for 1297 $F_o^2>2\sigma(F_o^2)$ and $wR_2=0.1288$, $GooF=S=0.975$, $T_{min}=0.517$, $T_{max}=0.871$, $\Delta\rho_{min}=-0.25$, $\Delta\rho_{max}=0.21$ e Å^{-3}.

Refinement by full matrix least-squares on F^2 using the program SHELXL97.[4] Data have been corrected for absorption effects. Lists of atomic coordinates,

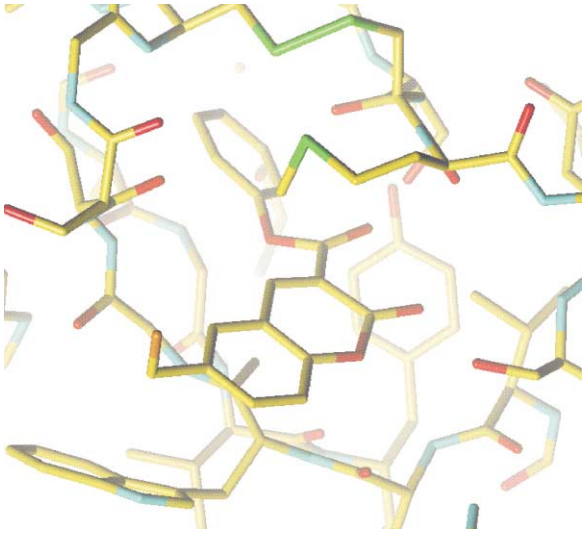

Figure 5. Docking of **1** in the active site of α-chymotrypsin.

displacement parameters, and complete geometry have been deposited with the IUCr.

Programs. Data collection: Enraf-Nonius CAD-4. Cell refinement: Enraf-Nonius CAD-4. Data reduction: *HELENA*.[5] Solution: *SIR97*.[6] Refinement: *SHELXL97*.[4] Molecular graphics: *PLATON*.[7]

Statistical search in the CSD

Molecules containing fragments characteristic of compounds **1**–**5** have been retrieved (*Quest* program) from the Cambridge Structure Database[2] and geometrical descriptors (T1, T2, T3) statistically analyzed (*Vista* program).

Ab initio calculations

Conformational scans and molecular electrostatic potentials (MEP) have been calculated ab initio (HF 3-21G*) using the program Gaussian94.[8] The input geometry of molecules **1**–**5** was deduced in Conformational Studies (above).

GRID analysis

The GRID[3] program was used to probe the active site of α-chymotrypsin. A series of probes (carbonyl, amine, hydrophilic, hydrophobic, aromatic) have been used to identify potential anchoring points within the enzyme.

Docking simulation

After manual docking of **1** in the active site of α-chymotrypsin, the structure of the complex has been submitted to energy minimization (cff91 force field; steepest descent and conjugate gradient algorithms; final energy derivatives less than 0.01 kcal/mol) using the Discover program (MSI simulation). Residues within 6 A of the inhibitor were allowed to move.

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