

Conformational requirements for inhibition of the pheromone catabolism in *Spodoptera littoralis*

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Abstract

In the present work we report the results of a molecular modeling study aimed at assessing the conformational requirements for binding to the *Spodoptera littoralis* antennal esterase. Comparative conformational analysis of the aliphatic chains of a selected group of trifluoromethyl ketone inhibitors, including good and poor analogs (compounds 1–3 and poor 4–5, respectively) resulted in a unique bioactive conformation, common to all active

analogs. The conformation is open and exhibits its hydrophobic moiety distant from the carbonyl group. The present study also reports the results of an *ab initio* study on the relative strength of the intramolecular hydrogen bond between the hydrate and the function (O, S, SO and SO₂) in β position to the carbonyl of some model compounds. The results correlate well with the relative inhibitory effect displayed by the parent trifluoromethyl ketones.

1 Introduction

Esterases and lipases are hydrolytic enzymes with broad substrate specificity. They play a key role in many biological systems and their inhibition could be of high therapeutic interest [1, 2]. The active site of lipases and esterases is generally formed by a serine, histidine and aspartic acid residues with the catalytic mechanism centered on the serine. The nucleophilic oxygen of the serine forms a tetrahedral hemiacetal intermediate with triacyl glycerides, which is hydrolyzed and the free fatty acid released. If the acid is too tightly bound to the active site the enzyme will be inhibited. A similar mechanism of action has been postulated for the inhibition of esterases by trifluoromethyl ketones (TFMKs), wherein the covalently bound tetrahedral intermediate between the serine hydroxyl group and the carbonyl (or hydrate) group has been established by spectroscopic means [3, 4].

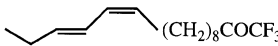
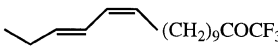

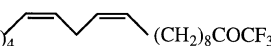
Antennal esterases are the enzymes responsible for the pheromone catabolism in insects. Inhibition of such enzymes might interfere with the pheromone perception

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Key words: esterase; inhibition; modeling; bioactive conformation

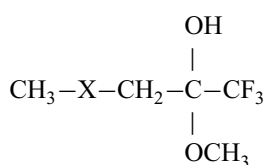
process, opening new approaches to pest control [5, 6]. We have previously reported the synthesis and biological

Table 1. Inhibitory potency of the compounds considered in the present study

Comp. number	Compound	IC ₅₀ /μM
1	 (CH ₂) ₈ COCF ₃	1.4
2	 (CH ₂) ₉ COCF ₃	1.4
3	 (CH ₂) ₁₀ COCF ₃	2.8
4	CH ₃ (CH ₂) ₄  (CH ₂) ₈ COCF ₃	10.7
5	CH ₃ (CH ₂) ₁₄ SCH ₂ COCF ₃	17.6
6	CH ₃ (CH ₂) ₇ SCH ₂ COCF ₃	1.5
7	CH ₃ (CH ₂) ₇ SOCH ₂ COCF ₃	2.2
8	CH ₃ (CH ₂) ₇ SO ₂ CH ₂ COCF ₃	6.1
9	CH ₃ (CH ₂) ₈ COCF ₃	38.2
10	CH ₃ (CH ₂) ₇ SCH ₂ COCH ₃	73.2

evaluation of a series of trifluoromethyl ketones as inhibitors of *Spodoptera littoralis* antennal esterase (7–10). The compounds were designed to probe structural parameters at the active site of the antennal esterase, the best inhibitors being those whose structures closely mimic that of the natural pheromone (Z,E)-9,11-tetradecadienyl acetate [4]. A particularly potent inhibitor was the analogue resulting from replacement of the acetate group by the trifluoroacetyl moiety (IC₅₀ 0.14 μM), but -thiotrifluoromethyl ketones generally exhibited higher inhibitory values than the corresponding parent, devoid of sulfur, compounds. Octylthiotrifluoropropan-2-one (compound **6**, Table 1) is the most active inhibitor found (IC₅₀ 0.08 μM). The results of this study, allowed us to suggest a range of log P values that ligands should exhibit for optimal inhibition [4].

In the present work we report the results of modeling studies addressed, first, to establish the conformational characteristics of the aliphatic chain of the trifluoromethyl ketone for an optimal inhibition and, second, to know the effect of different functional groups, located at position β of the carbonyl group, on the stability of the hypothetical tetrahedral intermediate. Analogs selected include three fairly good inhibitors (compounds **1–3**, Table 1) and two remarkably less active inhibitors (compounds **4–5**, Table 1). These last compounds have been considered in this study as non-binders. The structural determinants of the bioactive form were assessed by assuming that all the active analogs have a common conformation that is not attainable by the non-binders. On the other hand and in order to determine the importance of the intramolecular hydrogen bond between the hydrate, the presumed active species in solution, and the heteroatom, we have calculated the adiabatic torsional barrier of the hydroxy group of the putative hemiacetal intermediate in model molecules of the type



wherein X stands for an ether, thioether, sulfoxide or sulfone group. These model molecules exhibit all relevant structural features of the tetrahedral hemiacetal adduct

serine-hydrate concerning the role of an intramolecular hydrogen bond.

2 Methods

Conformational analysis of the five inhibitors selected for the present study was carried out within the molecular mechanics framework using the AMBER4.0 force field [11] with a dielectric constant of 80 to screen the electrostatic interactions. No cutoff was used to evaluate non-bonded interactions. Molecules were generated using the PREP module of AMBER4.0. All the necessary parameters not included in AMBER4.0 were taken from the literature [12] and ESP charges for each molecule were obtained by fitting the molecular electrostatic potential, computed with a STO-3G basis set, to points located at the nuclei following the Merz-Kollman procedure using the Gaussian94 suite of programs [13].

The conformational space of the molecules was sampled using simulated annealing (SA) in an iterative fashion. The method has been described elsewhere [14]. Extended conformations were used as starting geometries. For every molecule, the starting structure was energy minimized using the conjugate gradient algorithm with the gradient convergence criterium set to 0.001 kcal/mol Å. Subsequently, the structure was heated up to 900 K at a rate of 100 K/ps. Heating is fast in order to force the molecule to jump to a different region of the conformational space. At this point the structure was cooled to 200 K at a rate of 7 K/ps and then minimized. The structure was stored on a file and used as the starting conformation for a new cycle of SA. In this way, a library of low energy conformations was generated. These structures were rank ordered by energy every 100 cycles and checked for uniqueness. The procedure was repeated until no new conformations appeared, excluding those that are local reoptimizations of the side chains, which appeared after a predetermined number of cycles within a 3 kcal/mol energy range with respect to the lowest energy structure already found.

Adiabatic energy profiles of the torsional barrier of the hydroxyl group in the hypothetical tetrahedral intermediate were carried out on model systems as mentioned in the

Table 2. Results of the conformational analysis carried out on the five analogs selected for the present study.

	RCOCF ₃ R	SA cycles	unique structures	structures 0–3 kcal/mol	classes	subclasses
1	Z9E11:C ₁₄ H ₂₅	1800	330	325	29	314
2	Z10E12:C ₁₅ H ₂₇	2300	319	318	29	306
3	Z11:C ₁₄ H ₂₇	2800	691	671	42	639
4	Z9Z12:C ₁₈ H ₃₃	7600	7417	5108	63	4132
5	C15:H ₃₁ SCH ₂	600	424	391	114	387

previous section. Geometries of the four model molecules considered were optimized at the Hartree-Fock level using a 4-31G basis set [15]. Based on these geometries the adiabatic torsional barriers were evaluated with a 6-31G* (16) basis set using the Gaussian94 suite of programs [13].

3 Results

The results of the conformational analysis of compounds 1–5 are summarized in Table 2. The first column lists the number of SA cycles required to achieve convergence for each molecule. The second column shows the number of unique conformations for every compound. The third column lists the number of unique conformations within a 0–3 kcal/mol range with regard to the lowest energy conformation characterized for each molecule.

Comparative conformational analysis was carried out considering all the unique conformations of each inhibitor with a conformational energy up to 3 kcal/mol above the respective global minimum. Conformations were compared for similarity in a two step procedure. First, within each set of conformations, the root-mean square deviation (rmsd) of a subset of atoms, common to all the analogs were computed in a pair-wise manner. Atoms in this subset include: the carbon and the oxygen atoms of the carbonyl group, the carbon of the trifluoromethyl group and eight more carbons of the aliphatic chain preceding the carbonyl group. After visual inspection using molecular graphics, two structures were considered different when the rmsd was larger than 0.88 Å. This procedure allowed us to classify the conformations into classes, so that in every class the lowest energy conformation was chosen as the representative structure of the class. The total number of classes for each of the ligands is listed in column 4 of Table 2. In a further step, the rmsd of the representative structures of the most active inhibitor, 1, were pair-wisely computed with all the representative structures of the different molecules. As

previously chosen, two molecules were considered similar when the rmsd was smaller than 0.88 Å. The presence of analogous conformations between classes of binders (good inhibitors) and non-binders (poor inhibitors) allowed us to discard four classes of conformations of molecule 1 as candidates for being the bioactive conformation. In a second step, two structural parameters were computed for all the unique structures in order to discard other conformations by structural similarity: an angle ϕ , defined by the carbonyl carbon, the carbon C8 of the aliphatic chain, and the carbon of the methyl group at the terminus, and a dihedral angle θ , defined between the preceding atoms plus the oxygen atom of the carbonyl group. With the aid of molecular graphics, the criterium for similarity between two conformations was the following: two conformations belonging to the same class as previously defined, were considered to be analogous when the differences in both parameters and were less than 3°. Following this procedure a large number of subclasses for each molecule was obtained (column 5, Table 2).

In order to assess the bioactive conformation, subclasses of molecule 1 were compared with all subclasses of the different analogs using ϕ and θ as similarity criterium. This procedure generated a set of conformations common to the three inhibitors 1–3. When these conformations were compared with those of the non-binders 4–5, only one conformation remained. Figure 1 shows superimposition of the three common conformations found in 3 binders and not present in those of the non-binder compounds. Table 3 lists the cartesian coordinates of the bioactive conformation of compound 1.

Figure 2 shows the adiabatic profiles of the torsional barrier of the hydroxyl group of the hypothetical tetrahedral intermediate of some model molecules (see above). The minimum of these profiles corresponds to the intramolecular hydrogen bond between the hydroxyl group and the different groups (O, S, SO and SO₂) located in position β . In

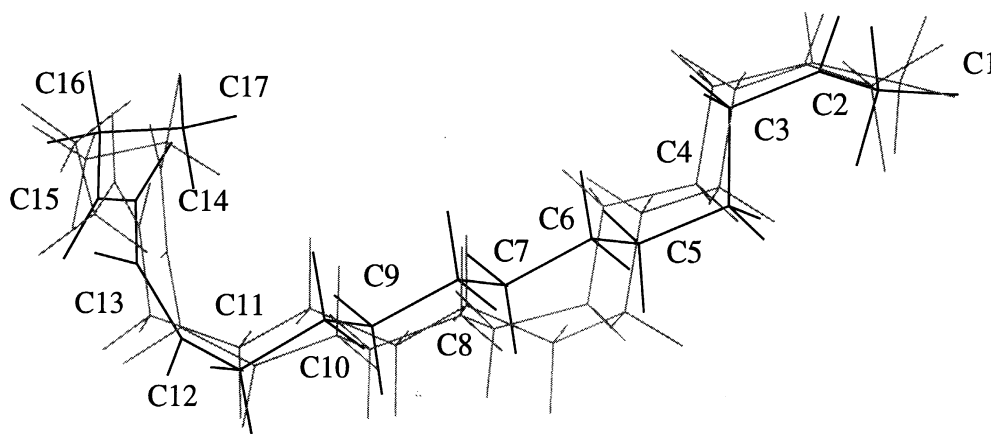


Figure 1. Superimposition of the common bioactive conformations of analogs 1, 2 and 3.

Table 3. Cartesian coordinates of the bioactive form of analog 1 in pdb format.

ATOM	1	C17	ML6	1	2.954	-2.555	2.906
ATOM	2	H171	ML6	1	2.181	-2.199	3.590
ATOM	3	H172	ML6	1	3.030	-1.869	2.062
ATOM	4	H173	ML6	1	3.909	-2.581	3.431
ATOM	5	C16	ML6	1	2.606	-3.961	2.412
ATOM	6	H161	ML6	1	2.565	-4.631	3.272
ATOM	7	H162	ML6	1	3.396	-4.308	1.746
ATOM	8	C15	ML6	1	1.393	-4.021	1.749
ATOM	9	H15	ML6	1	.502	-4.413	2.242
ATOM	10	C14	ML6	1	1.116	-3.631	.485
ATOM	11	H14	ML6	1	1.961	-3.229	-.075
ATOM	12	C13	ML6	1	-.048	-3.654	-.194
ATOM	13	H13	ML6	1	-.132	-4.460	-.925
ATOM	14	C12	ML6	1	-1.119	-2.835	-.107
ATOM	15	H12	ML6	1	-1.897	-3.137	-.809
ATOM	16	C11	ML6	1	-1.298	-1.741	.724
ATOM	17	H111	ML6	1	-1.049	-2.033	1.745
ATOM	18	H112	ML6	1	-2.352	-1.459	.704
ATOM	19	C10	ML6	1	.453	-.518	.345
ATOM	20	H101	ML6	1	.606	-.775	.381
ATOM	21	H102	ML6	1	-.642	.269	1.077
ATOM	22	C9	ML6	1	-.806	.009	-1.051
ATOM	23	H91	ML6	1	-1.869	.249	-1.088
ATOM	24	H92	ML6	1	-.585	-.752	-1.800
ATOM	25	C8	ML6	1	.007	1.268	-1.367
ATOM	26	H81	ML6	1	1.071	1.030	-1.327
ATOM	27	H82	ML6	1	-.213	2.037	-.625
ATOM	28	C7	ML6	1	-.343	1.795	-2.762
ATOM	29	H71	ML6	1	-.120	1.028	-3.504
ATOM	30	H72	ML6	1	-1.407	2.030	-2.801
ATOM	31	C6	ML6	1	.468	3.056	-3.078
ATOM	32	H61	ML6	1	1.529	2.812	-3.037
ATOM	33	H62	ML6	1	.240	3.825	-2.339
ATOM	34	C5	ML6	1	.120	3.576	-4.478
ATOM	35	H51	ML6	1	-.959	3.726	4.535
ATOM	36	H52	ML6	1	.406	2.834	-5.225
ATOM	37	C4	ML6	1	.801	4.912	-4.797
ATOM	38	H41	ML6	1	.537	5.646	-4.034
ATOM	39	H42	ML6	1	.425	5.262	-5.759
ATOM	40	C3	ML6	1	2.327	4.790	-4.886
ATOM	41	H31	ML6	1	2.584	4.003	-5.597
ATOM	42	H32	ML6	1	2.739	4.534	-3.911
ATOM	43	C2	ML6	1	2.957	6.103	-5.340
ATOM	44	O2	ML6	1	3.600	6.165	-6.386
ATOM	45	C1	ML6	1	3.027	7.233	-4.323
ATOM	46	F11	ML6	1	2.008	7.121	-3.463
ATOM	47	F12	ML6	1	2.949	8.409	-4.956
ATOM	48	F13	ML6	1	4.184	7.161	-3.656

addition, all four profiles reveal another point of favourable interaction, that is not a real minimum in these curves since geometries were not optimized at each point. These points of lower energy are associated with the interaction of the hydroxyl group with a fluorine atom of the trifluoromethyl group.

4 Discussion

It is well known that esterases and lipases can accept a broad range of artificial substrates of varying sizes,

suggesting that the protein backbone of these enzymes is flexible and can adopt a variety of conformations to accommodate substrate molecules [17]. X-ray crystallography has shed much light on the structural features of the active site of these types of enzymes although only a few have been determined so far by X-ray studies [18–22]. In these structures the active site is generally situated at the bottom of a deep gorge and consists of a nucleophile-histidine-acidic residue catalytic triad, present in the lipases either as Ser-His-Asp or Ser-His-Glu, that is involved in nucleophilic processes and general acid-base catalysis. In addition, the structures exhibit an extended active site consisting of hydrophobic grooves that are responsible for molecular recognition of hydrophobic moieties. Models based on substrate-receptor interactions with this pattern have been proposed for other esterases, like pig liver esterase [23, 24], cyclohexanone monooxygenase [25, 26], lipase YS [27] and lipase AK [28]. Very recently, we have reported the estimated dimensions of the lipase PS active site, which involves a large hydrophobic pocket to interact with large hydrophobic substituents, a more hydrophilic pocket to bind polar groups, and a tunnel-shape hydrophobic pocket able to accommodate long side chains [29].

At first sight, the chemical simplicity of the esterase inhibitors considered in this work suggests that the trifluoromethyl ketones interact with the enzyme basically by a polar interaction with the carbonyl (or hydrate) group, and by two hydrophobic interactions with the aliphatic chain and the trifluoromethyl group. We have found that the bioactive conformation, shown in Figure 1, is an open conformation with the aliphatic moiety lying distant from the carbonyl group. Furthermore, the curvature of the aliphatic chain in the active form, with regard to the carbonyl moiety, is consistent with a model where a hydrophobic groove runs along the surface of the protein and away from the oxyanion binding site.

With regard to the importance of the intramolecular hydrogen bond between the hydroxyl group of the hypothetical tetrahedral intermediate and the functional group in position β , we considered the difference between the energy of the lowest minimum and the other conformation with a favourable interaction. The results shown in Figure 2 indicate the following order of stability: sulfone > ether ~ sulfoxide ~ thioether. This suggests a correlation with the biological activity of the parent compounds 6–8 (Table 1). The higher energy of the intramolecular hydrogen bond with the sulfone group makes more difficult the acylation of the hydrate, the active form of the TFMK, by the serine residue of the active site. In contrast, the weaker hydrogen bond observed with the sulfoxide and thioether may explain an easier interaction with the serine and, consequently, the higher inhibition potency of compounds 6 and 7. For comparison purposes, compound 9, a very similar aliphatic analog, has also been included in Table 1.

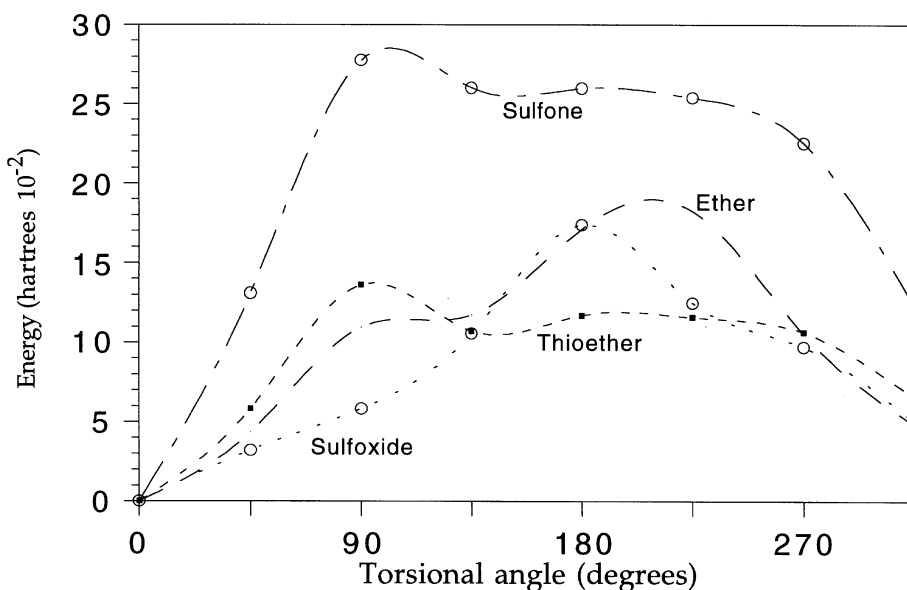


Figure 2. Adiabatic energy profiles of the torsional barrier of the hydroxyl group in model analogs (see text). The origin of the rotational angle corresponds to the lowest energy minimum for each molecule.

In this case, the much lower inhibition effect displayed by the analog is a consequence of the fact that, in aqueous solution, the hydration constant of ketone **9** is much lower than that of the analog containing a sulfur atom in position to the carbonyl, like in compound **6** [30].

Finally, it is worth a comment regarding the role of the trifluoromethyl group in the recognition process. The difference in inhibitory potency of the methyl ketone **10** and that of trifluoromethyl ketone **6** (Table 1) is undoubtedly due to the strong enhancement of the electrophilic character of the carbonyl by the fluorine atoms, increasing the stability of the adducts with the serine.

5 Conclusions

Molecular modeling techniques have been used to assess the stereochemical requirements for *Spodoptera littoralis* antennal esterase enzyme-substrate interaction. Two different aspects were addressed. On the one hand, the conformational characteristics of the aliphatic moiety of the trifluoromethyl ketone and, on the other, if the inhibitory potency of analogs containing different functional groups in position to the carbonyl could be correlated with the strength of an intramolecular hydrogen bond on the hydrate. Conformational analysis of the aliphatic chains of several analogs using molecular mechanics have resulted in a unique bioactive conformation common to all active analogs, which is open and with a hydrophobic pocket lying distant from the carbonyl group. On the other hand, our results show that the strength of the intramolecular hydrogen bond between the hydrate and the function in

position to the carbonyl group of some model compounds correlates well with the relative inhibitory potency of the parent TFMK.

Acknowledgments

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