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A uniform molecular model of δ opioid agonist and antagonist pharmacophore conformations

Wolfgang Brandt
Institute of Biochemistry, Martin-Luther-University Halle-Wittenberg, Kurt-Mothes-Strasse 3, D-06099 Halle, Germany

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Summary

On the basis of a model of the pharmacophore conformations of agonist of the δ-opioid receptor the corresponding δ-antagonist conformations were determined by means of force field calculations. The results explain the unusual behavior of several cyclic β-casomorphin analogues on the molecular level. Thus, for instance, the model helps to understand why Tyr-c[D-Orn-2-Nal-D-Pro-Gly] is a mixed μ-agonist and δ-antagonist. Furthermore, the model is consistent with low energy conformations of other δ-antagonists such as Tyr-Tic-Phe, Tyr-Tic-Phe-Phe, naltrindole and BNTX. The occupation of a special spatial area by bulky groups close to the protonated N-terminus of opioid peptides is assumed to be highly critical for the switch from agonist to antagonist behavior.

Introduction

The development of potent opioid agonists and antagonists with high affinity for each of the three major receptor types (μ, δ, κ) is an important aim in analgesia research. Opioids with mixed agonist and antagonist action are assumed to be of therapeutic importance to reduce undesired side effects such as drug abuse and tolerance. Such compounds (cyclic β-casomorphin analogues) have been described by Schmidt et al. [1, 2]. Since Brantl and Teschemacher [3–6] have discovered that fragments of the bovine β-casein peptone exhibit analgesic potential, a number of β-casomorphin analogues (β-casomorphin-5= Tyr-Pro-Phe-Pro-Gly) have been synthesized and tested with regard to their μ- and δ-receptor binding affinity [6–9]. In particular, cyclic peptides such as Tyr-c[-D-Xaa-Phe-Pro-Gly-], Tyr-c[-D-Xaa-Phe-D-Pro-Gly] (Xaa = A_2bu, Orn, Lys) were shown to have rather high antinociceptive activity in rats [1, 2, 8]. Interestingly, some of these compounds with 2-Nal in position 3 and D-Pro in position 4 show mixed μ-agonist and δ-antagonist behavior whereas the corresponding 1-Nal^3 or Trp^3 compounds and all L-Pro^4 derivatives behave as δ-agonists (Table 1) [1, 2].

Other highly interesting compounds wherein small modifications lead to the switch from δ-agonists to δ-antagonists have been synthesized and tested by Schiller et al. [10]. For instance, H-Tyr-Phe-Phe-Phe-NH_2 is a weak δ-agonist whereas the substitution of Phe^2 by Tic (1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid) leads to a δ-antagonist. Some relatively rigid compounds have been described by Portoghese et al. [11, 12] displaying high δ opioid receptor affinity and behave as antagonists or by small structural modifications as agonists (see Scheme 1). These authors could show that the relative position of the second aromatic ring with regard to the rest of the compounds is critical for antagonist activity (compare Scheme 1).

Several models have been described for the characterization of molecular structure differences between opioid agonists and antagonists or to explain the antagonist activity of the Tic^2 dermorphin derivatives [13, 14].

This paper is dedicated in honor to the 65th birthday of Prof. Dr. Alfred Barth who initiated and essentially supported molecular modelling research at Halle University.

Table 1. Receptor affinities and in vivo activities (GPI- and MVD-test) of β-casomorphin- and dermorphin derivatives[1]

No.	Compound	[^{3}H]**DAMGO** K_i^μ (nM)	[^{3}H]DSLET K_i^δ(nM)	K_i^δ/K_i^μ	GPI IC_{50}(nM)	MVD IC_{50}(nM)	MVD/GPI	Ref.
1	H-Tyr-c[-D-A_2bu-Phe-D-Pro-Gly-]	0.3	4.78*	15.9	1.06	4.67	4.41	[8]
2	H-Tyr-c[-D-A_2bu-2-Nal-D-Pro-Gly-]	6.41	70.8	11.0	353	1000	–	[1]
3	H-Tyr-c[-D-Orn-Phe-Pro-Gly-]	3.99	1280	321	13.4	69.9	–	[1]
4	H-Tyr-c[-D-Orn-Phe-D-Pro-Gly-]	0.881	13.2	15.0	2.14	4.89	–	[1]
5	H-Tyr-c[-D-Orn-MePhe-D-Pro-Gly-]	123	386*	3.16	41.7	194	4.65	[8]
6	H-Tyr-c[-D-Orn-2-Nal-Pro-Gly-]	81.3	2140	26.3	1220	10000	–	[1]
7	H-Tyr-c[-D-Orn-2-Nal-D-Pro-Gly-]	5.89	17.2	2.92	384	**antagonist**	–	[1]
8	H-Tyr-c[-D-Orn-1-Nal-D-Pro-Gly-]	3.42	23.6	6.90	14.9	29.9	2.01	[1]
9	H-Tyr-c[-D-Lys-Phe-Pro-Gly-]	13.6	43*	3.16	4.65	51.4	11.1	[8]
10	H-Tyr-c[-D-Lys-Phe-D-Pro-Gly-]	0.2	9.6*	48	4.30	16.3	3.79	[8]
11	H-Tyr-c[-D-Lys-2-Nal-D-Pro-Gly-]	17.1	62.6	3.66	609	**antagonist**	–	[1]
12	H-Tyr-c[-D-Orn-Trp-D-Pro-Gly-]	2.09	10.3	4.93	27.5	16.1	0.585	[1]
13	H-Tyr-c[-D-Orn-2-Nal-D-Pro-]	2.74	28.3	10.3	11.8	32.2	2.73	[1]
14	H-Tyr-Tic-Phe-Phe-NH_2	78.8	3.0	0.0381	1700	**antagonist**		[10]
15	H-Tyr-Tic-Phe-Gly-Tyr-Pro-Ser-NH_2	865	1.26	0.0015	6000#	**antagonist**	–	[2]
16	H-Tyr-Tic-Phe-Phe-Tyr-Pro-Ser-NH_2	1250	0.578	0.0005	10000ant.	**antagonist**	–	[2]

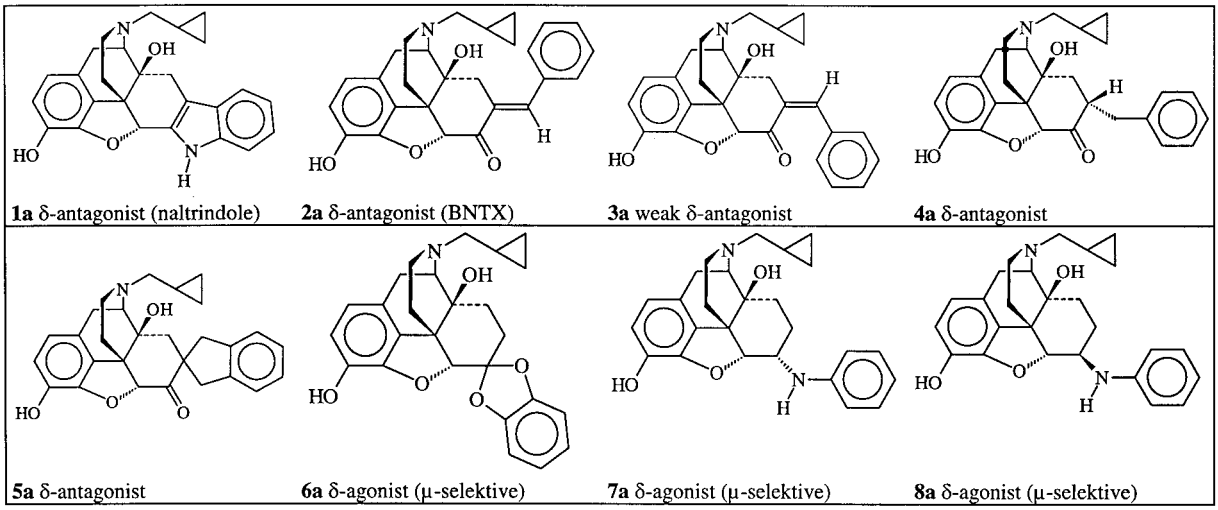
* [^{3}H]DADLE = [^{3}H]-Tyr-D-Ala-Gly-Phe-D-Leu-OH has been used instead of [^{3}H]DSLET = H^3-Tyr-D-Ser-Gly-Phe-Leu-Thr-OH.
\# IC_{30} value.
1 Abbreviations: GPI = guinea pig ileum assay that is μ-receptor-representative and MVD = mouse vas deferens that is δ-receptor-representative, A_2bu = 2,4-diaminobutyric acid, Tic = 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, 1-Nal = 3-(1′-naphthyl)alanine, 2-Nal = 3-(2′-naphthyl)alanine, [^{3}H]DAMGO = H^3-Tyr-D-Ala-Gly-MePhe-Gly-ol.

The structure(conformation)-activity relationships of the ornithine containing β-casomorphin derivatives were already described with respect to their very likely μ- and δ-selective pharmacophore conformations related to agonists [15–19]. The molecular modelling investigations were supported by NMR experiments and could explain some experimental results even in semiquantitative manner in case of the δ-opioid agonist affinity of β-casomorphin analogues [19].

On the basis of the proposed δ-pharmacophore conformations we will show by means of force field calculations what are the structural differences between δ-agonists and antagonists and which molecular feature is mainly responsible for the antagonism.

Computational methods

Conformational analyses were performed with the molecular modelling program SYBYL 6.4 [20] running on SGI Crimson workstations. The used methods for the systematic conformational analysis of cyclic β-casomorphin analogues with Phe in position 3 have already been described extensively [15]. These multitudes of conformation were used to construct the 1-Nal^3, 2-Nal^3 and Trp^3 β-casomorphin derivatives. The phenyl ring of Phe^3 was replaced in each low energy conformation by the corresponding naphthyl ring taking into account two possible orientations ($\chi_2^3 = \chi_2^3 + 180°$). Using the TRIPOS force field [21] and the Powell minimizer contained in the SYBYL/MAXIMIN software package, the conformations were minimized to a convergence of energy gradients less than 0.0005 kcal/mol × Å. The Gasteiger-Hückel method was used to calculate the partial charge distribution of the molecules (PEOE) [22, 23]. Electrostatic interactions were taken into consideration by using a distance dependent dielectric function of $\epsilon = 4r$. It has been shown that this dielectric function together with the TRIPOS force field is appropriate to calculate peptide conformations in agreement with experimental results [15–17]. All molecules were investigated with the N-terminal amino groups in the protonated form. The conformations of Tic^2 dermorphin analogues were taken from [14] but were reoptimized using the aforementioned dielectric constant and function that differ from the one used by Wilkes and Schiller [13, 14] ($\epsilon = 78$, constant).



Scheme 1. Compounds synthesized by Portoghese et al. [12].

Results and discussion

It is not yet clear whether agonists and antagonists bind at the same recognition site to the opioid receptors or not. There are some indications that agonists and antagonists bind in different positions to the opioid receptor. However, the small structural modifications of the compounds considered in this paper do not lead to a change in specificity for one of the three main subtypes of the opioid receptors (μ, δ, κ). If the molecules, e.g., the agonists and antagonists, are so highly similar it seems justified to assume similar recognition areas at the receptor. Based on this assumption we took as the target conformations of the cyclic β-casomorphins the earlier determined δ-pharmacophore conformations [18, 19]. In Table 2 the energetically preferred δ-pharmacophore conformations of selected β-casomorphin derivatives are listed. The comparison and good spatial correlation of cyclic β-casomorphins containing Phe^3 with other δ-selective compounds has already been demonstrated [19]. Interestingly, cyclic β-casomorphins with D- and L-configuration at Pro^4 may adopt comparable conformations (Figure 1).

The modifications in the configuration lead to a *cis-trans* isomerisation of the peptide bond preceding Pro^4 which has already been proved by NMR spectroscopy [15–17].

To analyze the differences between agonists and antagonists two agonists (**6**, **8**) were superimposed with an antagonist (**7**) (Figure 2a) using the phenolic oxygens, the centroids of the Tyr^1 aromatic rings, the nitrogen atoms of the N-termini and all atoms of the peptide bonds of the first three amino acid residues [19]. This match shows that the anellated rings occupy different spaces. Portoghese et al. [12] have already shown that different spatial orientations of aromatic rings are critical for δ-antagonists. The conformation of compound **12** (δ-agonist) with Trp^3 is very similar to the structure of **8** (1-Nal^3) shown in Figure 2a. The indole ring occupies the same area like the anellated ring of **8** but not the one of **7**.

From this first match one could conclude that the spatial area adopted by the anellated ring in **7** is responsible for the δ-antagonism of this compound. To prove this assumption compounds **14**–**15** were taken into consideration. The energetically optimized conformations differ only slightly from those published [14] but the order of energy is completely different because of the modified electrostatic contribution to the total energy. The dihedral angles of conformations relevant for the discussion here are listed in Table 3. The superposition of N-terminal fragments of **14** and **16** with **7** is shown in Figure 2b. Some interesting results may be observed. First, low-energy conformations of **14**, **15** and **16** match very well with the δ-pharmacophore conformations of cyclic β-casomorphins. Particularly surprising is the superposition of the phenyl ring of Phe^3 of the dermorphin analogues with the corresponding ring of 2-Nal^3 or Phe^3 of **3** and **4** (not shown, see [19]). The spatial orientation of these rings with regard to the tyrosine residue in position 1 is widely accepted to be responsible for μ- or δ-selectivity of opioid peptides. Second, and most important for the conclusions to be drawn,

Table 2. The δ pharmacophore conformations of cyclic β-casomorphin analogues

Compound	Xaa	H-Tyr-c[-D-Orn-Xaa-D-Pro-Gly-]				H-Tyr-c[-D-Orn-Xaa-Pro-Gly-]	
		Phe	2-Nal	Trp	1-Nal	Phe	2-Nal
		Dihedral angles					
Tyr[1]	Ψ	159	158	157	159	156	156
	χ_1	−179	−178	−178	−179	174	176
	χ_2	77	79	81	78	76	76
D-Orn[2]	Φ	166	165	165	166	164	166
	Ψ	59	58	57	59	57	57
	χ_1	172	171	172	172	171	170
	χ_2	176	176	177	175	167	168
	χ_3	−68	−78	−77	−79	−72	−78
Xaa[3]	Φ	−140	−138	−143	−139	−169	−170
	Ψ	149	149	149	149	138	149
	ω	178	−179	−174	−175	−3	−4
	χ_1	−51	−45	−51	−47	51	50
	χ_2	108	113	106	112	91	90
D-Pro[4]or	Φ	48	48	49	48	−77	−77
L-Pro[4]	Ψ	36	37	38	36	−17	−17
Gly[5]	Φ	159	159	159	159	−84	−84
	Ψ	−121	−120	−127	−121	−90	−91

The relative energies of all conformations are in a range of 24 kJ/mol. All dihedral angles ω not listed adopt values of about 180°. From two possible orientations (χ_2^3) of the side chain of Xaa the energetically more stable conformation was listed and used.

the aromatic ring of Tic[2] occupies the same spatial area as the anellated ring of 2-Nal in **7** but not the space adopted by compounds **6** or **7** nor **3** and **4**.

Finally, the compounds represented in Scheme 1 were superimposed with the cyclic β-casomorphins. For this purpose, the six carbon atoms of the phenolic rings, the phenolic oxygen atom as well as the protonated nitrogen atom were used as points to be superimposed. The resulting arrangement is shown in Figure 2c. Although the phenolic rings and the protonated nitrogen atom do not match completely it is clearly seen that the aromatic rings modified in the compounds in Scheme 1 occupy in the case of antagonists nearly the same position as the peptide antagonists. As already demonstrated by Portoghese et al. [12] the aromatic rings of the agonists point in another direction. This is in agreement with the results presented here for the cyclic β-casomorphin analogues.

Conclusions

According to our calculations it seems to be justified to conclude that a special spatial area occupied by bulky groups (indicated by the blue arrow in Figures 2a-c) is critical for the switch from δ-opioid agonist to δ-antagonist behavior. This picture seems to be true for peptides as well as non-peptides.

It has been known for a long time, especially in the case of μ-selective compounds, that groups such as allyl or methylcyclopropyl (nalorphine, naloxone) linked to the protonated nitrogen atom of opioids derived from morphine-like compounds cause opioid-antagonism. As demonstrated here not such groups attached to the N-terminal nitrogen but residues occupying a defined position close to the N-terminus seem to be responsible for antagonist action. If this result derived from modeling studies is indeed true, then for instance, a compound similar to naltrindole but with the methylcyclopropyl group replaced by methyl should also be an opioid antagonist.

Exactly this was found by Takemorie et al. [24]. The naltrindole derivative without methyl-cyclopropyl group behaves as a 10-fold more potent δ-antagonist than naltrindole. Up to now, the molecular mechanism of signal transduction initiated by opioid action is not known. Several mechanisms have been discussed. Common to all is that the interaction of opioids (and other ligands of G-protein coupled receptors) with

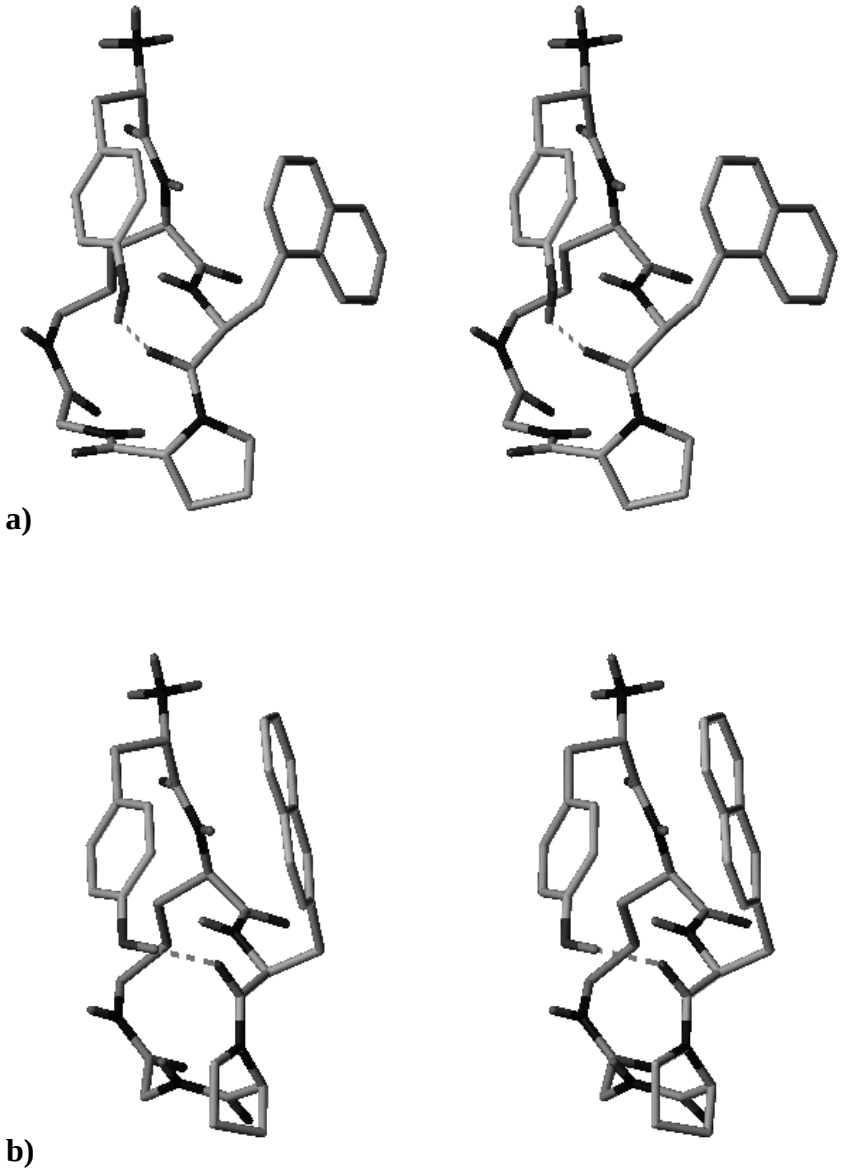


Figure 1. Stereo representation of the proposed δ-pharmacophore conformations of (a) H-Tyr-c[-D-Orn-1-Nal-D-Pro-Gly-] and (b) H-Tyr-c[-D-Orn-2-Nal-Pro-Gly-].

their corresponding receptors cause conformational changes of the receptor inducing a signal transduced from outside into the cell.

There are very probably binding sites within the seven transmembrane helices but also at the extracellular loops [11, 25, 26]. Maybe the special spatial area occupied by the antagonist as shown in this paper prevents a conformational change of the receptors. In agreement with experimental findings and models of binding sites [11, 25, 26, 27], in this way antagonists bind to the receptor by mostly hydrophobic interactions at the extracellular loops but are probably not able to penetrate inside the helices as agonists do.

Altogether, the modeling results give a uniform picture of structure activity relationships for δ-opioid agonists and antagonists. The different action of several cyclic β-casomorphins modified only by small structural changes can now be understood on the mole-

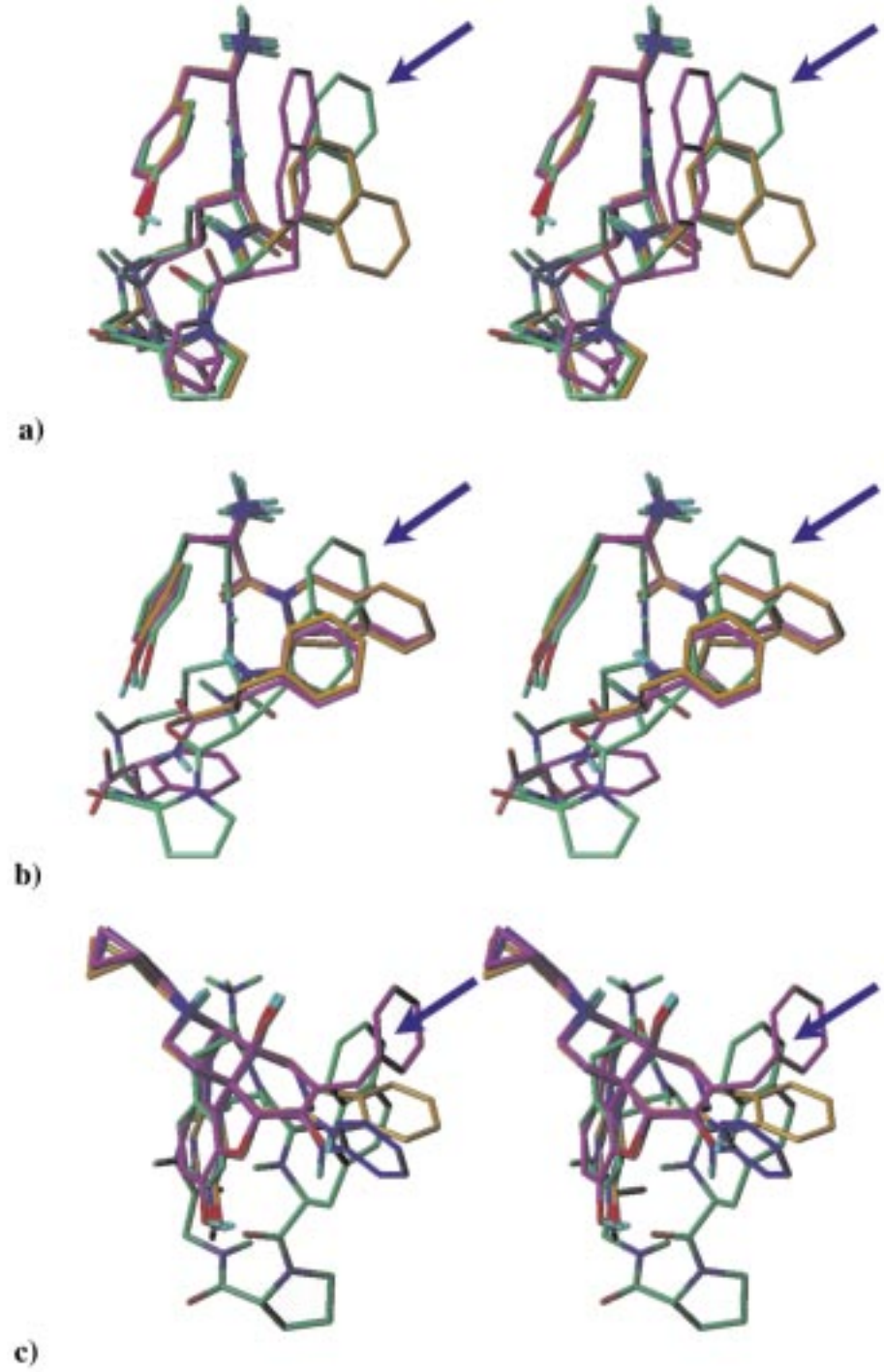


Figure 2. (a) Stereo representation of the superposition of the δ-antagonist H-Tyr-c[-D-Orn-2-Nal-D-Pro-Gly-] (green) with the agonists H-Tyr-c[-D-Orn-1-Nal-D-Pro-Gly-] (orange) and H-Tyr-c[-D-Orn-2-Nal-Pro-Gly-] (magenta). (b) Superposition of the δ-antagonist H-Tyr-c[-D-Orn-2-Nal-D-Pro-Gly-] (green) with the antagonists H-Tyr-Tic-Phe-O^{-} (orange) and H-Tyr-Tic-Phe-Phe-O^{-} (magenta). (c) Superposition of the δ-antagonist H-Tyr-c[-D-Orn-2-Nal-D-Pro-Gly-] (green) with the antagonists naltrindole (**1a**) (orange), BNTX (**2a**) (magenta) and **7a** (blue) of Scheme 1.

Table 3. Low energy conformations of H-Tyr-Tic-Phe-O$^-$

No.	ψ^1	ω^1	χ_1^1	χ_2^1	ϕ^2	ψ^2	χ_1^2	χ_2^2	ϕ^3	χ_1^3	χ_2^3	**E** (kJ/mol)
1	143	−178	175	99	−76	83	54	−47	−67	−61	105	0
	146	−179	178	99	−74	83	53	−47	−71	−60	106	3.5
2	71	−177	−176	71	−78	78	55	−47	−150	−57	99	4.4
	94	**178**	**176**	**74**	**−101**	**59**	**58**	**−38**	**−143**	**−55**	**114**	**0**
3	136	−175	−176	90	−90	163	59	−44	−137	−62	93	4.8
	140	−175	−177	90	−85	162	57	−47	−143	−63	94	4.2
4	113	−168	−177	62	−86	74	57	−46	−151	−63	91	5.1
	120	−170	−177	60	−85	69	56	−47	−152	−62	90	6.9

Each upper row indicates the conformations obtained by Wilkes and Schiller [14] the lower row the results of this work. The conformation represented in Figure 2 is bold highlighted.

cular level. Furthermore, it is obvious that 'structure activity relationships' considering only amino acid sequences but not the three dimensional structures are usually not sufficient to explain the experimental results.

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