Chapter 3

Conformational Study of 1-amino-2-phenylcyclopropanecarboxylic Acid Derivatives and the Search of Novel Leads for Bradykinin Antagonism

3.1 Summary

A thorough conformational search of Hoe 140 has been carried out on Hoe 140 in order to redefine the pharmacophore for the bradykinin (BK) B2 antagonism. Moreover, several conformational searches using the SA technique have been carried out on 1-amino-2-phenylclyclopropanecarboxylic acid (c_3Phe) derivatives designed to be of bradykinin antagonists (BK) inspired on other analogs reported in the literature. On the one hand the 4 diasteroisomers of the peptide of sequence Thi^1-Ser^2-c_3Phe^3-Pro^4-Arg^5 and on the other, the 4 diasteroisomers of peptide sequence Thi^1-Ser^2-Pro^3-c_3Phe^4-Arg^5, where Thi stands for β-(2-thienyl)-alanine. The aim of this work is to predict which one of the analogs better fulfills the BK B_2 pharmacophore structural requirements. The refined pharmacophore has also been used as template to search in different chemical databases using the Catalyst™ software. Following this procedure we have identified novel small molecule candidates for BK B_2 antagonism.

3.2 Introduction

Bradykinin (BK) is a linear nonapeptide hormone of sequence Arg^1-Pro^2-Pro^3-Gly^4-Phe^5-Ser^6-Pro^7-Phe^8-Arg^9, produced from its precursor kininogen by the action of a group of proteases, called kallikreins. The peptide hormone is released in response to inflammation, trauma, burns, shock, allergy and some cardiovascular diseases, influencing vascular tone and permeability and decreasing blood pressure (Regoli, et al., 1980, Farmer et al., 1991, Dray et al., 1993). BK actions are mediated through two G-protein coupled receptors: the B_1 and B_2 kinin receptors. B_1 has a low level of expression in normal tissues, being expressed during trauma or inflammation (Marceau et al., 1998). On the other hand, the B_2 kinin receptor is constitutively expressed by different cell types (Regoli et al., 1980). B_2 receptor agonists are sought for the treatment and prevention of various cardiovascular diseases, such as hypertension, ischemic heart disease, congestive heart failure as well as diabetic disorders. There have been described several potent and selective agonists of B_2 receptor, like RMP-7 (Iobradamil, Cereport; Alkermes), JMV-1116 (Fournier), FR-190997 and FR-191413 (Fujisawa). On the other hand, B_2 antagonists are sought to reduce the effects mediated by BK, like pain and inflammation in several diseases (asthma, rhinitis and septic shock). Different peptide (Altamura et al., 1999) and non-peptide (Heitsch, 2002) antagonists have been disclosed to date, the best known B_2 receptor is the peptide antagonist Hoe 140, with sequence D-Arg^0-Arg^1-Pro^2-Hyp^3-Gly^4-Thi^5-Ser^6-D-Tic^7-Oic^8-Arg^9 (where Hyp stands for

hydroxyproline; Thi, β-(2-thienyl)-alanine; Tic, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; Oic, (2S, 3aS, 7aS)-octahydroindole-2-carboxylic acid) (Hock et al., 1991).

BK in aqueous solution is an average of different conformations in fast exchange. However, Lee et al. (1990) characterized the conformation of BK as a β-turn structure on residues 6-9 in five molar sodium dodecyl sulfate (SDS) micelles. Structures obtained from distance geometry calculations after energy minimization and restrained molecular dynamics yielded different types of β-turns, I, II and VII according to the β-turn classification by Brooks et al. (1988). A previous characterization of the conformational profile of BK carried out in our group (Perez et al., 1995), by means of low and high temperature MD runs in a continuum solvent model and with a dielectric constant of 80 to reproduce the water environment, identified the type I β-turn expanding from residues 6 to 9 as the most frequent conformational motif in the set of low energy conformations. Moreover, the authors identified a bent conformation at the N-terminus that sometimes folded into a β-turn expanding along residues 2 to 5. Analog [Thr^6]-BK is a potent BK agonist present in wasp venom. Its structure has also been determined by NMR spectroscopy in the presence of SDS micelles (Pellegrini et al. 1997). This peptide exhibits a greater tendency than BK to form the β-turn at residues 6 to 9 than BK, based on the presence of a greater larger number of NOEs involving the Thr^6 residue. Distance geometry calculations yielded a structure exhibiting a type I β-turn between residues 6 to 9. Moreover, from ensemble calculations the authors hypothesized that the NMR observables could be explained by the existence in solution of three families of structures containing type I β-turn, type II β-turn and a partially extended conformation.

Analysis of the results obtained from several studies (Kyle et al.1991, Kyle et al, 1992, Chakravarty et al., 1993, Kyle et al., 1993, Liu et al., 1993) led to the hypothesis that the β-turn expanding along residues 6 to 9 was a pre-requisite for activity and that the type of β-turn adopted allowed for discrimination of agonists and antagonists. The structural features of Hoe 140 in SDS micelles, characterized by a combined approach of NMR and molecular dynamics (MD) indicates that the peptide exhibits a type II' and a type II β-turns comprising residues 6-9 and 2-5, respectively (Guba et al., 1994). A few years ago it was carried out a study in order to characterize the conformational profile of Hoe 140 by means of two different computational methods, using high and low temperature MD runs as well as simulated annealing in an iterative fashion (Filizola et al., 1998a). In both studies a continuum solvent model was used with a dielectric constant of 80 to reproduce the aqueous phase. From the results of this study it was deduced that the set of low energy conformations found exhibit a type II' β-turn along residues 6 to 9 and that about 50% of the low-energy conformations characterized exhibited a β-turn at the N-terminus in agreement with the results obtained for Hoe 140 in SDS micelles (Guba et al., 1994).

In the past years a great effort has been devoted to design peptide surrogates and peptidomimetics, based either on the knowledge of the conformation that a peptide exhibits when bound to its receptor, i.e. the bioactive conformation, or the most stable conformation in solution when the former is not known (Saragovi et al., 1991, Marshall et al., 1992). Once the bioactive conformation of a peptide has been partially or totally characterized, a first indirect approach widely used towards the design of more active compounds consists in the synthesis of peptide surrogates containing non-natural amino acids that would restrict the conformational freedom and lock the new molecule into the bioactive conformation, thus reducing the amount of degradation of the molecule and increasing its activity (Hruby et al., 1991, Rizo et al., 1992, Gillespie et al., 1997). α,α-disubstituted amino acids have been generally viewed as conformationally restricted amino acids and therefore as good candidates for peptidomimetic design. An example of this group of molecules is 1-aminocyclopropanecarboxylic acid (Ac_3c) and its derivatives. The conformational profile of Ac_3c has been undertaken and reported by our group (Gomez-Catalan et al., 2000). Two symmetric global minima were identified at $\varphi = \pm 90^o$, $\psi = 0^o$ including when the solvent effects were taken into account. From these minima it could be inferred that Ac_3c could be a good amino acid for the induction of type I and II β-turns when placed as the third residue (*i+2*) of a β-turn. Ac_3c can be substituted at four positions yielding 4 different diasteroisomers. Substitution with phenyl rings yields the 2,3-methanophenylalanine amino acid (c_3Phe) that exhibits a similar conformational behavior (Aleman et al., 2002). The synthesis and the ^{1}H NMR spectroscopy in solution of the 4 dipeptides containing all the possible diasteroisomers of sequence Pro-c_3Phe and the dipeptide of sequence Pro-Ac_3c have been reported (Jimenez et al., 1998). In the absence of the phenyl ring, i.e. in Pro-Ac_3c, the type II β-turn is the most favored structure in CH_2Cl_2. The type II β-turn is the most favored conformation for Pro-(2R,3R)c_3Phe and for the Pro-(2S,3R)c_3Phe, whereas the type I β-turn is significantly populated though not the predominant conformation for Pro-(2R,3S)c_3Phe and becomes the most favored conformation in Pro-(2S,3S)c_3Phe in CH_2Cl_2.

In a previous study carried out within the group a pharmacophore for the antagonism of the BK B_2 receptor was proposed (Filizola, et al. 1998b). The pharmacophore was defined as the distances between the moieties responsible for the interaction with the receptor: an aromatic ring, a hydrophobic group and a positive charged group. In Figure 3.1 these pharmacophoric descriptors are shown on the group of c_3Phe containing molecules studied in the present work. The aim of the present study is to further redefine the pharmacophore calculating the tolerance of the descriptors from the standard deviations obtained from a thorough conformational search of Hoe 140 (see Figure 3.2) using the iterative simulated annealing technique and introducing an exclusion volume in order to reduce the number of hits in the search for new putative BK B_2 antagonists. Concomitantly, based on the tendency of c_3Phe to induce β-turns we want to assess the use of derivatives as BK B_2 agonists or antagonists.

3.3 Methods

All the calculations were carried out within the molecular mechanics framework by means of the AMBER 5 program (Case et al., 1997) using the Cornell all-atom force field (Cornell et al., 1995). All the molecules were studied in its zwitterionic form. No explicit solvent was included in the calculations, although an effective dielectric constant of 80 was used to screen electrostatic interactions and no non-bonded interaction cutoff was considered.

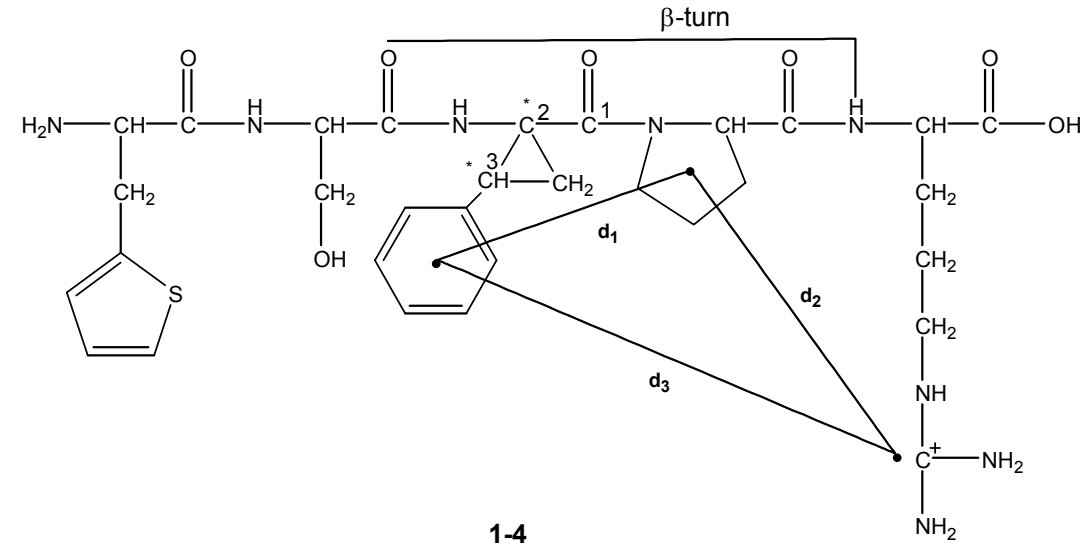
Hoe 140 was built with using the PREP module of AMBER and the charges of the non-natural residues (Hyp, Thi, Tic and Oic) were taken from Filizola et al., 1998. Two different groups of c3Phe containing peptides were studied (see Table 3.1 and Figure 3.2). The first group contained all the 4 possible diasteroisomers of sequence Thi^{1}-Ser^{2}-c_3Phe^{3}-Pro^{4}-Arg^{5} (**1-4**) and the second group contained all the 4 possible diasteroisomers of sequence Thi^{1}-Ser^{2}-Pro^{3}-c_3Phe^{4}-Arg^{5} (**5-8**) (Figure 3.1). RESP charges for the c_3Phe residue were calculated with a 6-31G* basis set with GAUSSIAN 94 (Frisch et al., 1995) by Gomez-Catalan et al. (unpublished data). Specific torsional parameters for the cyclopropane ring were taken from a previous study carried out by the group (Gomez-Catalan et al., 2000).

The conformational spaces of Hoe 140 and the c_3Phe containing peptides were explored using a simulated annealing (SA) protocol in an iterative fashion. The method has been described elsewhere (Filizola et al., 1997). The initial minimized structure is quickly heated up to 900 K at a rate of 100 K/ps, in order to force the molecule to jump to a different region of the conformational space. Subsequently, the 900 K structure is slowly cooled to 200 K at a rate of 7 K/ps and then minimized. The structure obtained at 200 K is stored on a file and used as the starting conformation for a new cycle of SA. In this way an energy rank ordered library of low energy conformations is generated. Low energy conformations are checked for uniqueness by exclusion of those for which at least one of the backbone dihedral angles is different from 60º in respect to the previous conformations already presented in the library. The conformational search is continued until the searching procedure reaches a low level of performance in finding new low energy conformations, i.e. when the efficiency of the procedure, termed λ, is below 0.1 (Corcho et al., 1999).

Table 3.1. Configurations of the peptides investigated in the present study.

NUMBER	SEQUENCE	CONFIGURATION
1	Thi[1]-Ser[2]-c_3Phe[3]-Pro[4]-Arg[5]	(2S,3S)
2		(2S,3R)
3		(2R,3S)
4		(2R,3R)
5	Thi[1]-Ser[2]-Pro[3]-c_3Phe[4]-Arg[5]	(2S,3S)
6		(2S,3R)
7		(2R,3S)
8		(2R,3R)

In order to assess the presence of β-turns in the ensemble of structures obtained by the SA procedure the following criteria were applied: distance *R* between the C^{α} atoms of the residues *i* and *i*+3 and all structures with $R \leq 7$ Å, the dihedral angle formed by the C^{α} atoms of residues *i* to *i*+3 have to be in the range $-90º < \tau < 90º$, the standard torsion angles, described in Table 2, have to be fulfilled at the same time within a range of ± 50º. In some cases this range yields structures fulfilling two different types of β-turns that have been grouped in a different category corresponding to their intersection. Conformations fulfilling the previous criteria and containing ω dihedral angles in cis position were removed from the analysis in case it was not a property required by the type of β-turn. Finally, in order to restrict more the structures having β-turn an additional criterion was enforced: the presence of a hydrogen bond between the O in the carbonyl group of residue *i* and the H in the amide group of residue *i*+3 defined as having a distance ≤ 3.5 Å and a NH···O angle between 120º and 180º.



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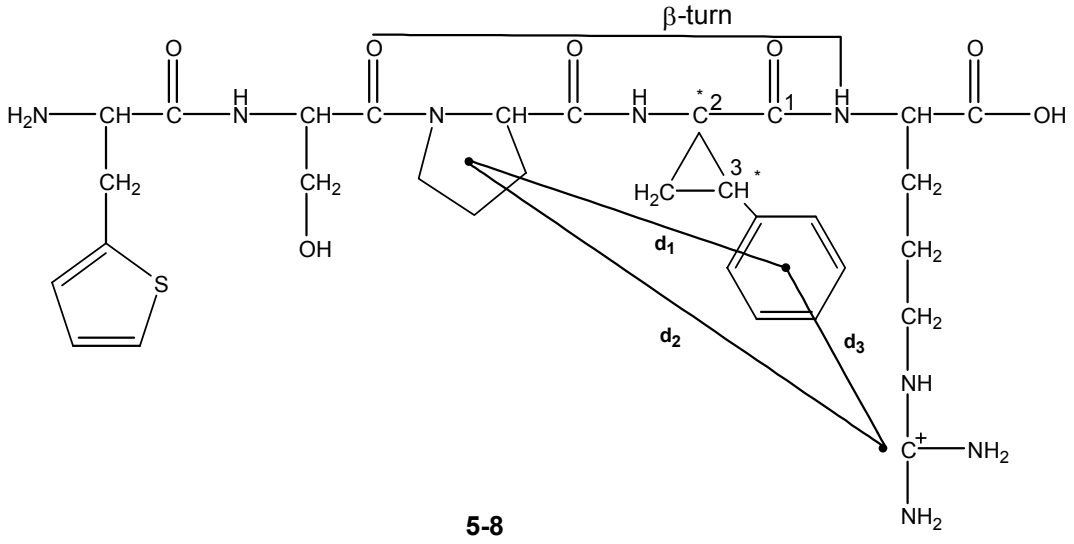

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Figure 3.1. Molecular structures of the peptides investigated in the present study. Distances d_1-d_3 describe the pharmacopore for BK B_2 antagonism defined from the conformational study of Hoe 140. The superindex 1-3 corresponds to the numbering scheme for residue 2,3-methanophenylalanine amino acid (c_3Phe).

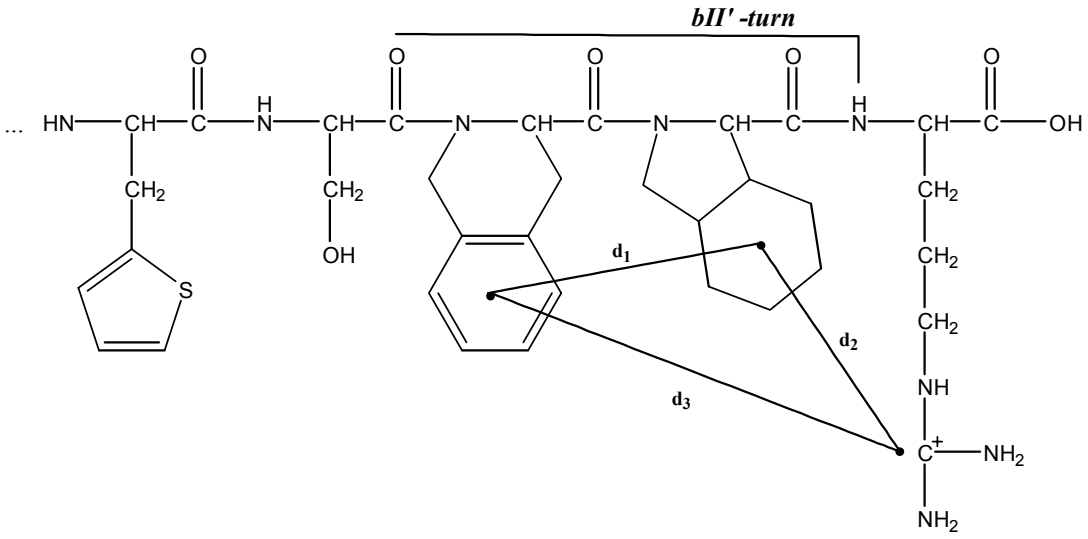

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Figure 3.2. Molecular structure of the last 5 residues of Hoe140 of sequence D-Arg^0-Arg^1-Pro^2-Hyp^3-Gly^4-Thi^5-Ser^6-D-Tic^7-Oic^8-Arg^9 (Hyp, hydroxyproline; Thi, β-(2-thienyl)-alanine; Tic, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; Oic, (2S, 3aS, 7aS)-octahydroindole-2-carboxylic acid). Distances d_1-d_3 describe the pharmacopore for BK B_2 antagonism.

Table 3.2. Standard angles for the types of β-turns used in the present study. Angles were accepted as valid when having a deviation up to ± 50º from the standard value[a,b].

TURN	φ_{i+1}	ψ_{i+1}	φ_{i+2}	ψ_{i+2}
βI	-60	-30	-90	0
βI'	60	30	90	0
βII	-60	120	80	0
βII'	60	-120	-80	0
βIII	-60	-30	-60	-30
βIII'	60	30	60	30
βVIa	-60	120	-90	0
βVIb	-120	120	-60	0
βVIb'	-120	120	-60	150

[a]Angles in degrees
[b]Möhle et al., 1997

3.4 Results and Discussion

3.4.1 Conformational Analysis of Hoe 140

As described in the Methods section, the conformational search of Hoe 140 was performed using a simulated annealing (SA) protocol in an iterative fashion, as sampling technique. The search for new conformations and therefore the efficiency was only assessed on the region expanding residues 5 to 9, as this is the part of the molecule containing the β-turn required for BK agonism and antagonism. The SA procedure was stopped after 6500 cycles, when the efficiency of the sampling (λ) was below the threshold value of 0.1, i.e. 10% of the initial efficiency. The global energy minimum was attained at the 1426 cycle with -5.07 kcal·mol^{-1}.

To the 6500 structures obtained by the SA of Hoe 140 the criteria for the presence of β-turns were applied to residues 6 to 9: distance *R* between the C^{α} atoms of the residues *i* and *i*+3 has to be $\leq$ 7 Å, the dihedral angle formed by the C^{α} atoms of residues *i* to *i*+3 has to be in the range -90º < τ < +90º, the standard torsion angles, described in Table 3.2, have to be fulfilled at the same time with a deviation of ± 50º from the standard value, and conformations containing ω dihedral angles in cis position that were not a required property for the type of β-turn in study were removed. Results are shown in Table 3.3. 1018 fulfilled concomitantly these criteria. The type of β-turn was assigned to each structure from the group of structures fulfilling these criteria and the results are shown in Table 3.4. 941 structures exhibit a type II' β-turn being this type of β-turn the most abundant type (92.4%of all the structures exhibiting a β-turn and 14.5% of the total number of structures). The other type of β-turn present were less abundant: type I (0.4% of total number of structures) and type III (0.7% of the total number of structures).

The histogram depicting relative energy for all structures and the structures fulfilling the type II' β-turn is shown in Figure 3.3. The maxima of the distribution for all the structures and the structures with a type II' β-turn is at 13 and 10 kcal·mol^{-1} respectively, thus indicating that the subgroup of structures exhibiting a type II' β-turn are more stabilized than the average structure. The 3 kcal·mol^{-1} difference could be due to the extra energy provided by the hydrogen bond that stabilizes the structures presenting a type II' β-turn as have been previously suggested (Corcho et al. 2000). When the conformations containing a type II' β-turn are divided by the total number of structures at each interval of 1 kcal·mol^{-1} relative energy (Figure 3.4) it can be observed that the low energy conformations are clearly enriched in conformations containing a type II' β-turn, thus indicating that structures exhibiting this type of β-turn are the most energetically stable

conformations with the conditions used in the present study. Indeed, 47% of structures below 5 kcal mol^{-1} from the global energy minimum exhibit a type II' β-turn. This result is in agreement with the results of Guba et al. (1994) in SDS micelles and by NMR spectroscopy and previous results obtained in our group by using MD runs at high and low temperature and a different iterative simulated annealing protocol from the one used in the present study (Filizola et al. 1998a). Both studies pointed out the existence of a type II' β-turn expanding residues 6 to 9 as the most stable conformation of Hoe 140.

Table 3.3. Fulfillment of the β-turn criteria of the 6500 structures obtained in the SA procedure of Hoe 140.

CRITERIA	NUMBER	PERCENTAGE
Distance ≤ 7Å	3811	58.6
Dihedral angle Cα -90º < τ < +90º	4378	67.4
Standard dihedral angle	1477	22.7
hydrogen bond presence	789	12.1

Table 3.4. Type of β-turn present in the SA procedure of Hoe 140.

TURN	NUMBER	PERCENTAGE
βI	29	0.4
βII'	941	14.5
βIII	48	0.7
βI & βIII	29	0.4
Total	989	15.2

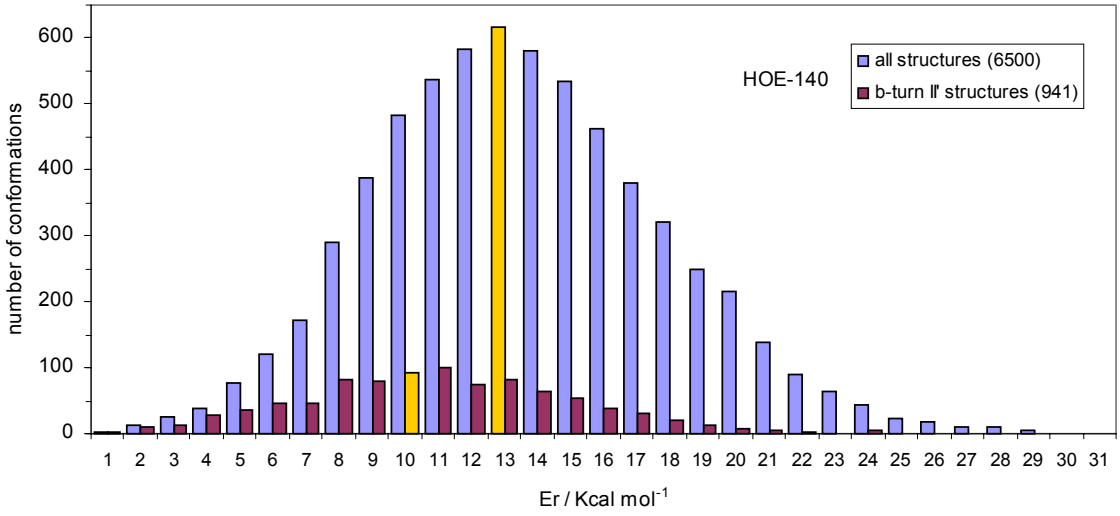


Figure 3.3. Histogram of all structures and of the structures containing the type II' β-turn between residues 6 and 9.

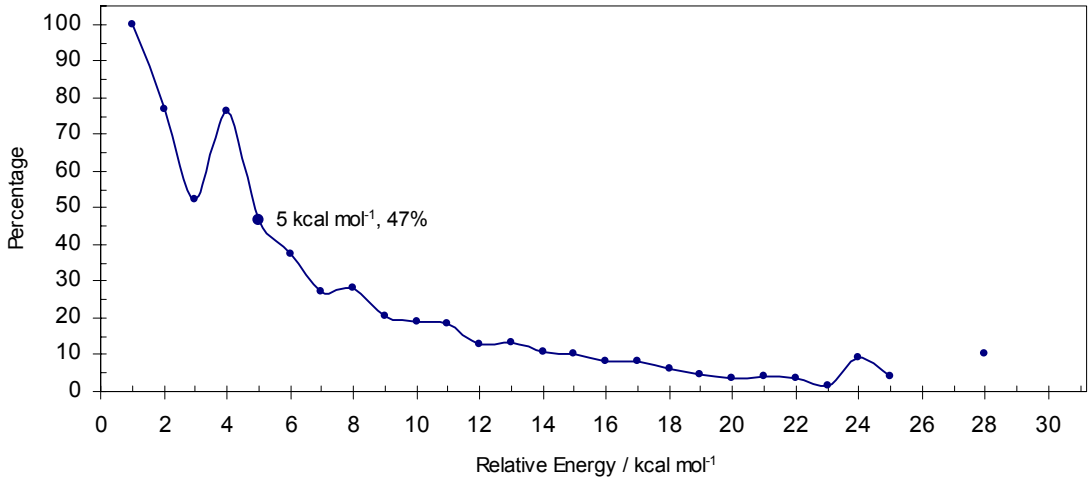


Figure 3.4. Relative Energy versus the percentage of structures containing type II' β-turns for Hoe 140. 47% of structures below 5 kcal mol^{-1} from the global energy minimum exhibit a type II' β-turn.

3.4.2 B_2 Pharmacophore Revisited

In order to have a more restricted group of structures to define the pharmacophore, to all the requirements described above to consider the existence of a β-turn an additional criterion was enforced: the presence of a hydrogen bond between the O in the carbonyl group of residue *i* and the H in the amide group of residue *i*+3 defined as having a distance ≤ 3.5 Å and a NH···O angle between 120º and 180º. Only 789 structures fulfilled this latter criterion, and 679 of these structures were the ones that exhibited a type II' β-turn. This subgroup of structures was used for the pharmacophore definition. The descriptors taken for the definition of the pharmacophore are the ones that have been described by Filizola et al. (1998b) and are depicted in Figure 3.2: d_1 corresponds to the distance between the center of the aromatic ring of Tic[7] and the center of the cyclohexane of Oic[8], d_2 corresponds to the distance between the cyclohexane ring of Oic[8] and the C atom of the guanidinium group of Arg[9] and d_3 corresponds to the distance between the aromatic ring of Tic[7] and the C atom of the guanidinium group of Arg[9]. A comparison of the pharmacophore descriptors for BK B_2 antagonism in the previous study and the ones obtained in the present study are shown in Table 3.5. The average distance, two standard deviations, and the range for the group of structures fulfilling all the criteria for the existence of a type II' β-turn (679) and for all the structures (6500) are shown. Distances found in the present study are similar to the previous study by Filizola et al. (1998b). d_1 corresponding to the distance between the center of the aromatic ring of Tic[7] and the center of the cyclohexane of Oic[8] is the most rigid descriptor of the pharmacophore and the previous and the present value are in total agreement (8.5 Å). For d_2, corresponding to the distance between the cyclohexane ring of Oic[8] and the C atom of the guanidinium group of Arg[9] the previous range is contained within the range obtained in the present study. However, the average value (8.3 Å) is in the higher part of the previous range, thus suggesting that the range should be updated with the new values. d_3, corresponding to the distance between the aromatic ring of Tic[7] and the C atom of the guanidinium group of Arg[9] is the distance with the greatest range due to the flexibility of the Arg[9] side chain. The new average value obtained (10.9 Å) is not contained within the previous range and the new values will be used for database search of compounds fulfilling the properties of the pharmacophore. 92.34% and 55.71% of structures fulfilled the three distances $(d_1\text{-}d_3)$ described by the aromatic ring, the hydrophobic moiety and the positive charge for the subgroup of 679 structures fulfilling the criteria for the presence of a type II' β-turn and for all the 6500 structures obtained in the SA procedure, respectively (Table 3.10). d_3 value, corresponding to the distance between the aromatic ring of Tic[7] and the C atom of the guanidinium group of Arg[9], is the more discriminant descriptor between the 6500

structures obtained in the SA procedure (62.94%) and the subgroup of 679 structures fulfilling the criteria for the presence of a type II' β-turn (97.79%).

The use of the pharmacophore in *Catalyst*^{TM} package (2001) for the search of databases in conjunction with the introduction of an exclusion volume made compulsory the conversion of the pharmacophore from a distance defined one into a volume defined pharmacophore (Table 3.6). The radius of the volumes describing the tolerance was obtained by averaging the two standard deviations for each point. For example the aromatic ring is related to two distancess d_1 and d_3 that have 0.24 and 1.64 as standard deviation values and thus the tolerance is 0.94.

In order to reduce the number of hits obtained in the database search for compounds fulfilling the geometrical properties of the pharmacophore an exclusion volume has been introduced. Given the rigidity that it has been observed for the d_1 distance and the fact that Tic^7 and Oic^8 are conformationally restricted, the position of the receptor in the vicinity of both sidechains can be located. For this purpose the average structure of the 679 structures fulfilling the criteria for the presence of a type II' β-turn has been computed. The structure presenting the lowest RMS distance from the average structure computed taking the backbone atoms of residues 7 to 10 was selected. For this structure an exclusion volume was created by visual inspection. The center of the exclusion volume was positioned at 7.0 Å from the center of the aromatic ring of Tic^7, the C atom of the carbonyl group of Tic^7, the center of the cyclohexane of Oic^8 and the amide N of Oic^8. As a result, the center of the exclusion volume is placed in the plane formed by the β-turn conformation of Hoe 140.

Table 3.5. Comparison of the pharmacophore descriptors for BK B_2 antagonism in a previous study (Filizola et al., 1998b) and the ones obtained in the present study. The average distance, the standard deviation, and the range for the group of structures fulfilling all the criteria for the existence of a type II' β-turn (679) and for all the structures (6500) is shown. d_1 corresponds to the distance between the center of the aromatic ring of Tic[7] and the center of the cyclohexane of Oic[8], d_2 corresponds to the distance between the cyclohexane ring of Oic[8] and the C atom of the guanidinium group of Arg[9] and d_3 corresponds to the distance between the aromatic ring of Tic[7] and the C atom of the guanidinium group of Arg[9].

Distance		Previous Values[a]	Hoe 140 (679) βII'	Hoe 140 (6500)
d_1	Average	8.5	8.5	8.6
	2x Standard Deviation		0.48	0.7
	min/max		7.9/9.3	5.6/9.5
d_2	Average		8.3	8.8
	2x Standard Deviation		4.0	3.5
	min/max	5.5/8.5	4.1/11.9	4.1/12.5
d_3	Average		10.9	8.7
	2x Standard Deviation		3.3	6.1
	min/max	4.5/7.5	3.6/15.2	3.4/15.5

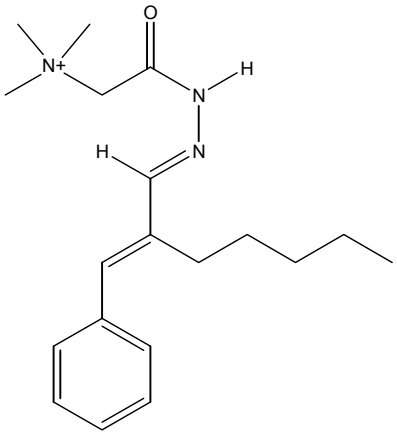
[a]Filizola et al., 1998b.

Table 3.6. Pharmacophore version defined in terms of volumes around each pharmacophoric point. The radius of the volumes describing the tolerance was obtained by averaging the two standard deviations for each point. For example the aromatic ring is related to two distances d_1 and d_3 that have 0.24 and 1.64 as standard deviation values and thus the tolerance is 0.94.

DESCRIPTORS	X	Y	Z	TOLERANCE
Aromatic ring (Tic[7])	3.462	4.246	-0.053	0.94
Hydrophobic (Oic[8])	-0.266	-2.014	3.982	1.10
Positive charge (Arg[9])	-4.229	-2.854	-2.870	1.81
Exclusion volume	4.488	2.776	6.690	3.50

3.4.3 Search of the Pharmacophore in Databases

The pharmacophore defined in terms of volumes (Table 3.6) with the introduction of an exclusion volume in order to reduce the number of hits obtained was built in the *Catalyst*^{TM} package for the search of new leads for BK B_2 antagonism. Proprietary 3D-databases used for the search were Derwent Drug Index-1999, National Cancer Institute-2000, MDL's Available Chemical Directory-2001 and Maybridge-2001. From the 574 hits obtained a shortlist of 11 compounds was obtained that fulfilled the following criteria: the positive charge had to be relatively on the surface of the molecule, the size of the molecule was around 500 of molecular weight, the molecule has to be rigid between the aromatic ring and the hydrophobic moiety and exhibiting some degree of flexibility at the positive charge end. The 11 candidates were ranked in terms of the planarity of the molecule given that the pharmacophore points are disposed on a geometrical plane. Molecules had to be also contained mainly within the triangle defined by the pharmacophore not extending far away from it. From this analysis 6 molecules were finally selected and are shown in Figure 3.5.



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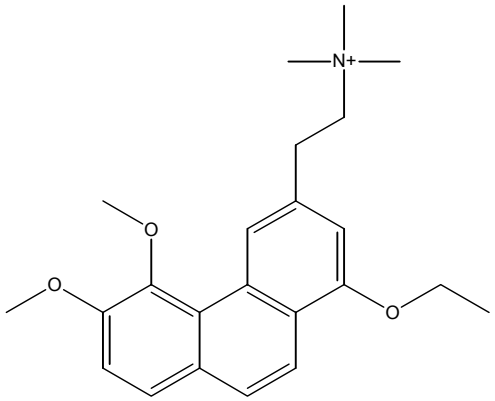

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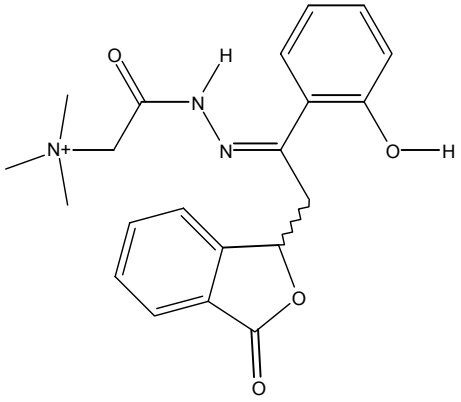

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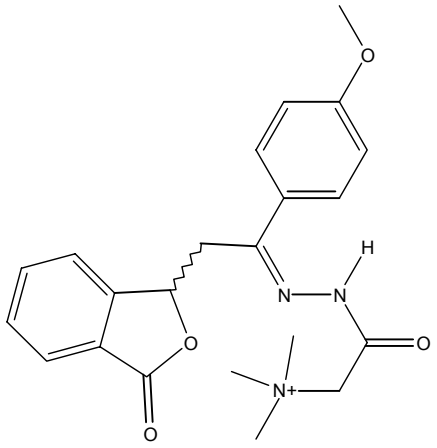
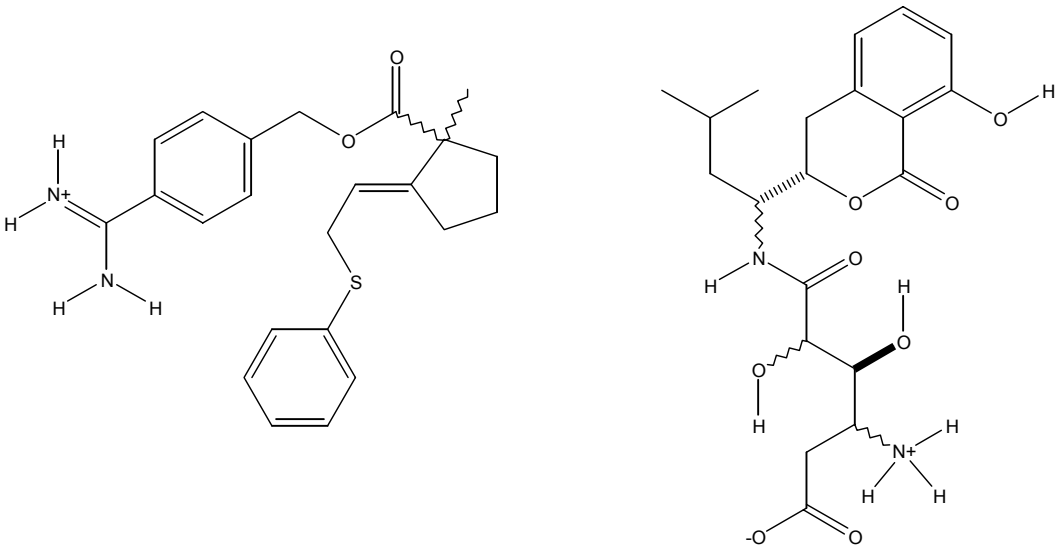


NCI0647605

Figure 3.5. Structure of the best candidates found in databases using the pharmacophore proposed in the present study.



ORG-34092 **AI-77-B**

Figure 3.5. Structure of the best candidates found in databases using the pharmacophore proposed in the present study.

3.4.4 Conformational Analysis of c_3Phe Derivatives of BK

As described in the Methods section, the conformational search of the c_3Phe derivatives of BK (**1-8**) was performed using simulated annealing (SA) in an iterative fashion, as sampling technique. The SA procedure was stopped when the efficiency of the sampling (λ) was below the threshold value of 0.1, i.e. 10% of the initial efficiency. See Table 3.7 for a summary of the results.

Criteria for the presence of β-turns were applied to the structures obtained by the SA of c_3Phe containing peptides. For this purpose we considered residues 2 to 5 and we have measured the different parameters as described in the Methods section. Results of this analysis are shown in Table 3.8. There are no significant differences using the two first criteria: distance *R* between the C^{α} atoms of the residues *i* and *i*+3 has to be $\leq$ 7 Å and the dihedral angle formed by the C^{α} atoms of residues *i* to *i*+3 has to be in the range -90º < τ < +90º. For the last two criteria: the torsion angles with a deviation smaller than $\pm$ 50º from the standard value, and the presence of a hydrogen bond between the O in the carbonyl group

of residue *i* and the H in the amide group of residue *i*+3 defined as having a distance $\leq$ 3.5 Å and a NH···O angle between 120º and 180º, a remarkable difference appears. Thus, compounds **1-4** of sequence Thi[1]-Ser[2]-c_3Phe[3]-Pro[4]-Arg[5] show a smaller proportion of structures fulfilling the more strict criteria for the presence of β-turns than compounds **5-8** of sequence Thi[1]-Ser[2]-Pro[3]-c_3Phe[4]-Arg[5].

The abundance of the different types of β-turns for the c_3Phe containing peptides is summarized in Table 3.9. In general terms, type I and III β-turns are the most predominant for compounds **1-4**. Other less abundant β-turns are types VIb and VIb'. Regarding compounds **5-8**, type I, II and III are the most abundant. Other types of β-turns, like type VIa, VIb and VIb' are present to a lesser extent. Type II' β-turns are only present in compounds **1** and **3** with small percentages, thus suggesting that this type of β-turn is not favored for the family of compounds selected for the present study. By looking at the Ramachandran plots it is clear that the region corresponding to the type II' β-turn at the position *i+1* is abundantly populated in the SA procedure in the case of Hoe 140 and poorly populated for compounds **1-8** (Figures 3.6-3.14). If the presence of a type II' β-turn is a requirement for BK B_2 antagonism, as it has been suggested in the literature, a poor BK B_2 antagonism capacity should be anticipated for the compounds analyzed in the present study.

In the work of Jimenez et al. (1998) the authors found that the most populated β-turn for molecules Pro-(2R,3R)c_3Phe and Pro-(2S,3R)c_3Phe was type II; type I was present to a significant extent for Pro-(2R,3S)c_3Phe and is the most favored conformation for Pro-(2S,3S)c_3Phe. Present results are in agreement with this observation. Thus, for compounds **6** (2S,3R) and **8** (2R,3R), type II β-turn is predominant. For compound **7** (2R,3S) type I and II β-turns are equally favored and for compound **5** (2S,3S) type I β-turn is clearly the most favored conformation. Therefore, the ratio of type I to type II β-turns obtained for compounds **5-8** could be explained solely on the basis of the structural features of the central residues of the β-turn, Pro and c_3Phe.

Table 3.7. Summary of the results for the SA procedure of the c3Phe containing peptides.

COMPOUND	1	2	3	4	5	6	7	8
No. of cycles	6400	8700	7200	9200	3900	4800	6100	4400
Global Energy Minimum	8.3	10.43	10.09	9.59	6.63	6.09	6.81	6.33
Cycle of Minimum	2785	1000	6295	6788	861	1379	2993	3718

Table 3.8. Fulfillment of the β-turn criteria of the structures obtained in the SA procedure of the c_3Phe containing peptides.

COMPOUND		1	2	3	4	5	6	7	8
Distance ≤ 7Å	**No.**	3601	4056	3268	5372	1885	3147	3430	3094
	%	56.3	46.6	45.4	58.4	48.3	65.6	56.2	70.3
Dihedral Angle C_α -90º<t<+90º	**No.**	3832	4552	3553	5610	1957	3295	3640	3390
	%	59.9	52.3	49.3	61	50.2	68.6	59.7	77
Standard Dihedral Angle	**No.**	2064	1869	1457	752	1410	2163	1855	2140
	%	32.3	21.5	20.2	8.2	36.2	45.1	30.4	48.6
Hydrogen Bond Presence	**No.**	261	136	231	261	1518	2269	1995	1871
	%	4.1	1.6	3.2	2.8	38.9	47.3	32.7	42.5

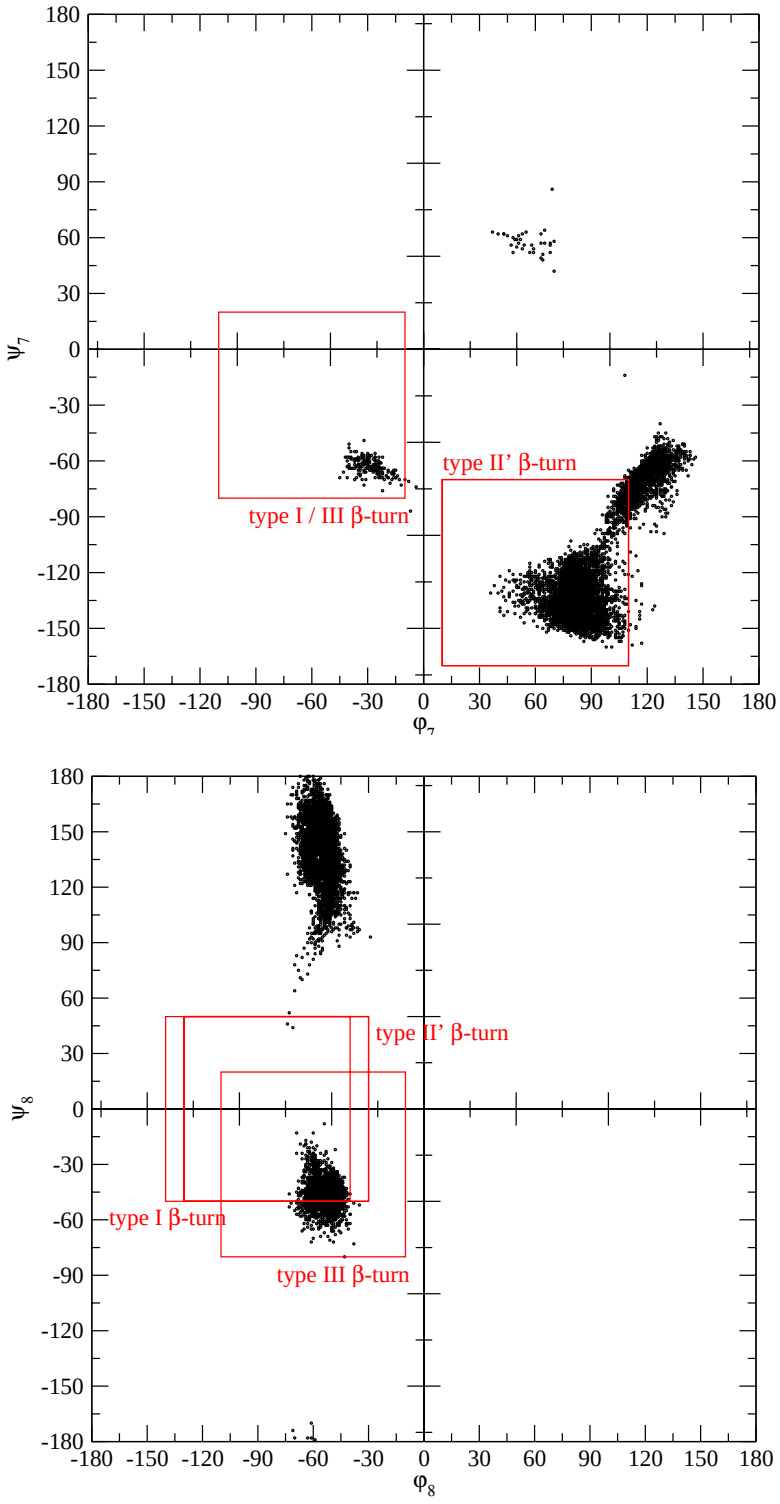


Figure 3.6. Ramachandran plots of residues D-Tic[7] and Oic[8] of Hoe 140 of sequence D-Arg[0]-Arg[1]-Pro[2]-Hyp[3]-Gly[4]-Thi[5]-Ser[6]-D-Tic[7]-Oic[8]-Arg[9].

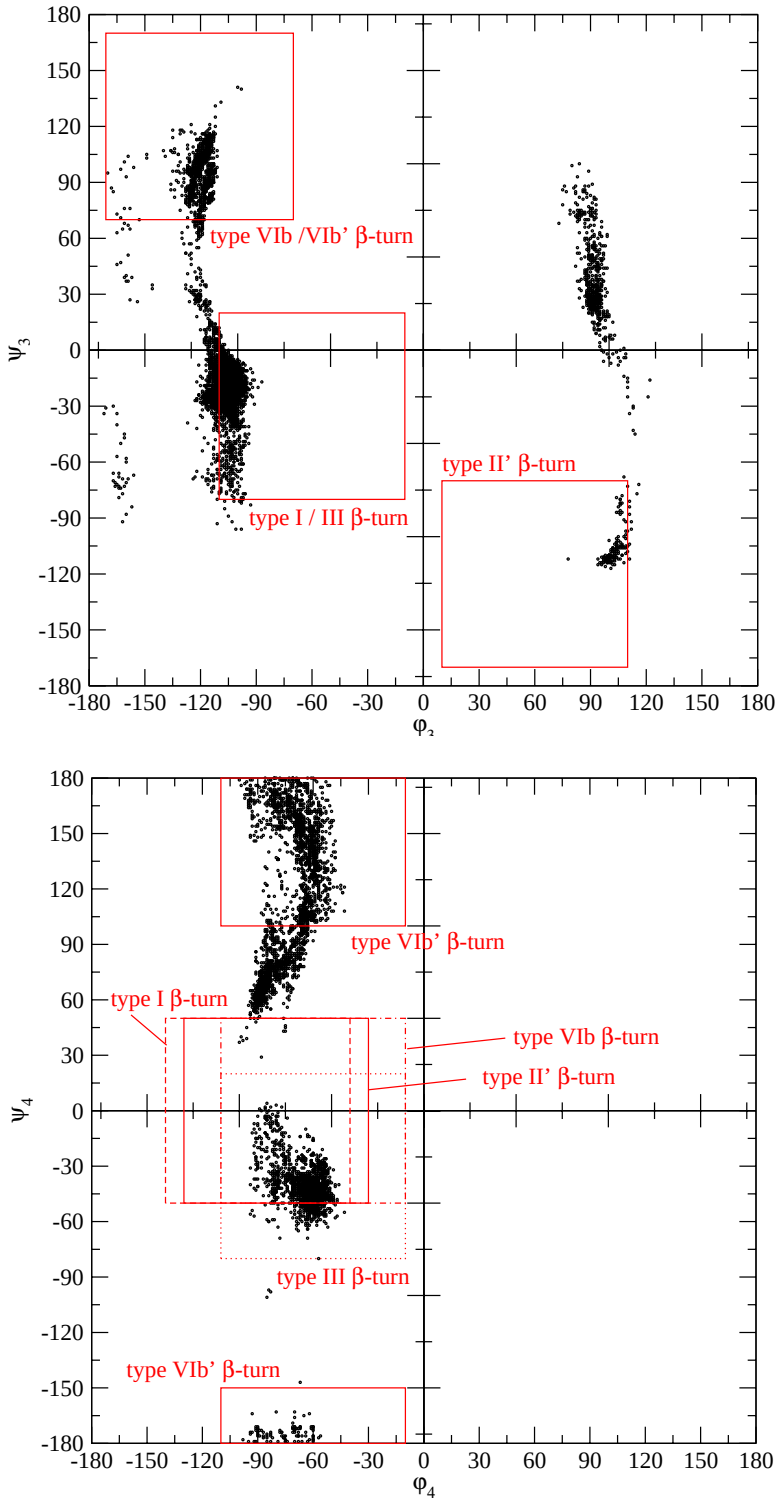


Figure 3.7. Ramachandran plots of residues c_3Phe[3] and Pro[4] of compound **1** of sequence Thi[1]-Ser[2]-(2S,3S)c_3Phe[3]-Pro[4]-Arg[5].

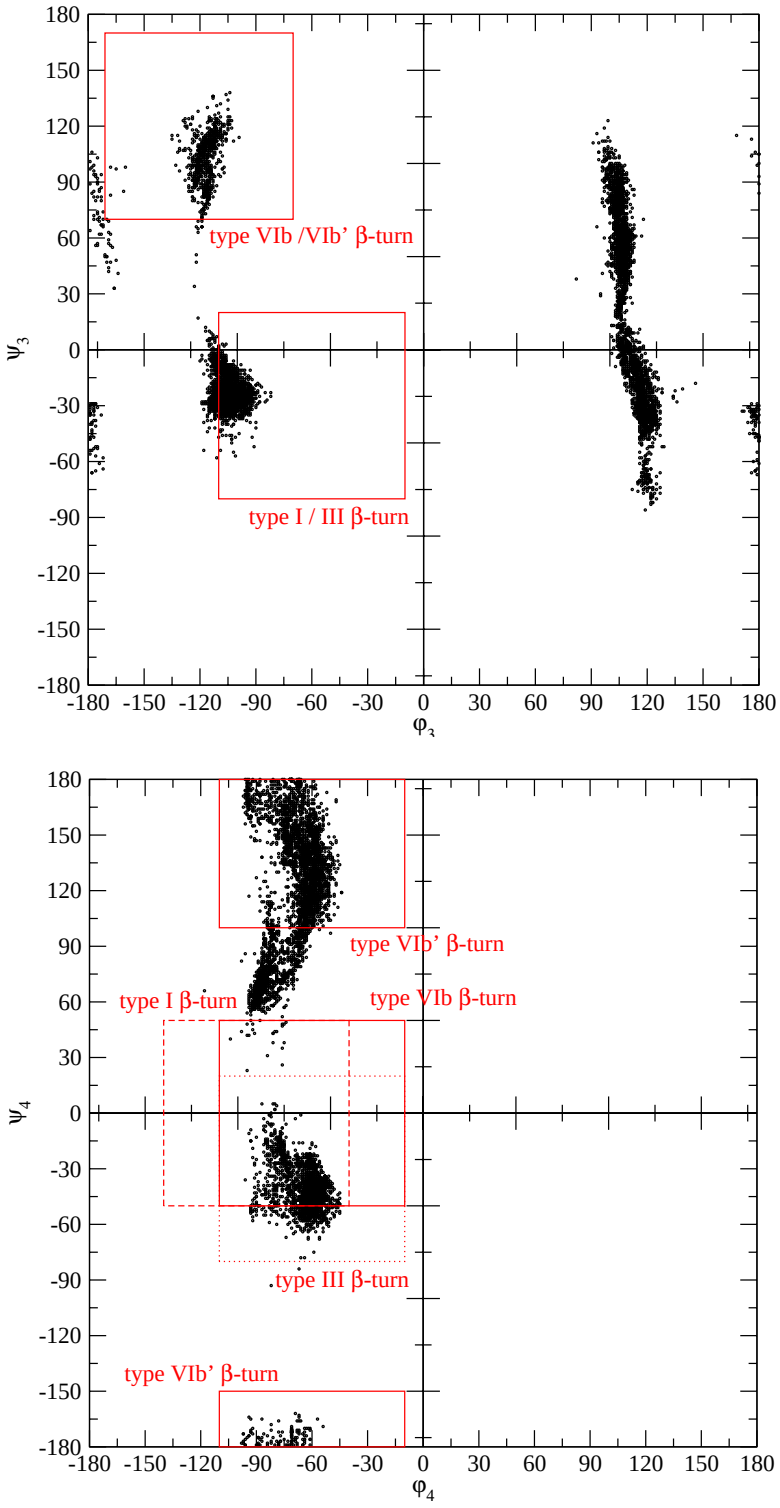


Figure 3.8. Ramachandran plots of residues c_3Phe[3] and Pro[4] of compound **2** of sequence Thi[1]-Ser[2](2S,3R)c_3Phe[3]-Pro[4]-Arg[5].

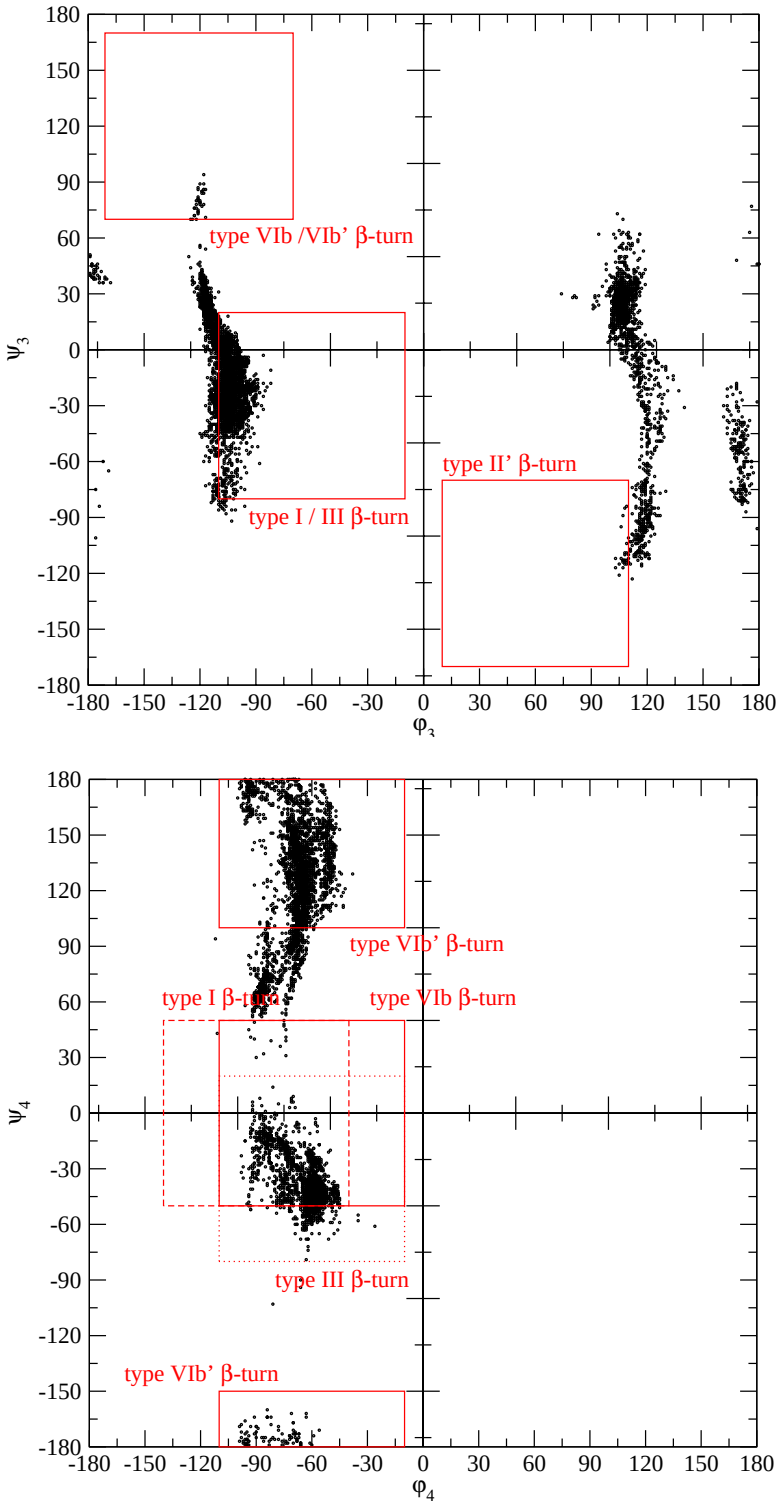


Figure 3.9. Ramachandran plots of residues c_3Phe^3 and Pro^4 of compound **3** of sequence Thi^1-Ser^2(2R,3S)c_3Phe^3-Pro^4-Arg^5.

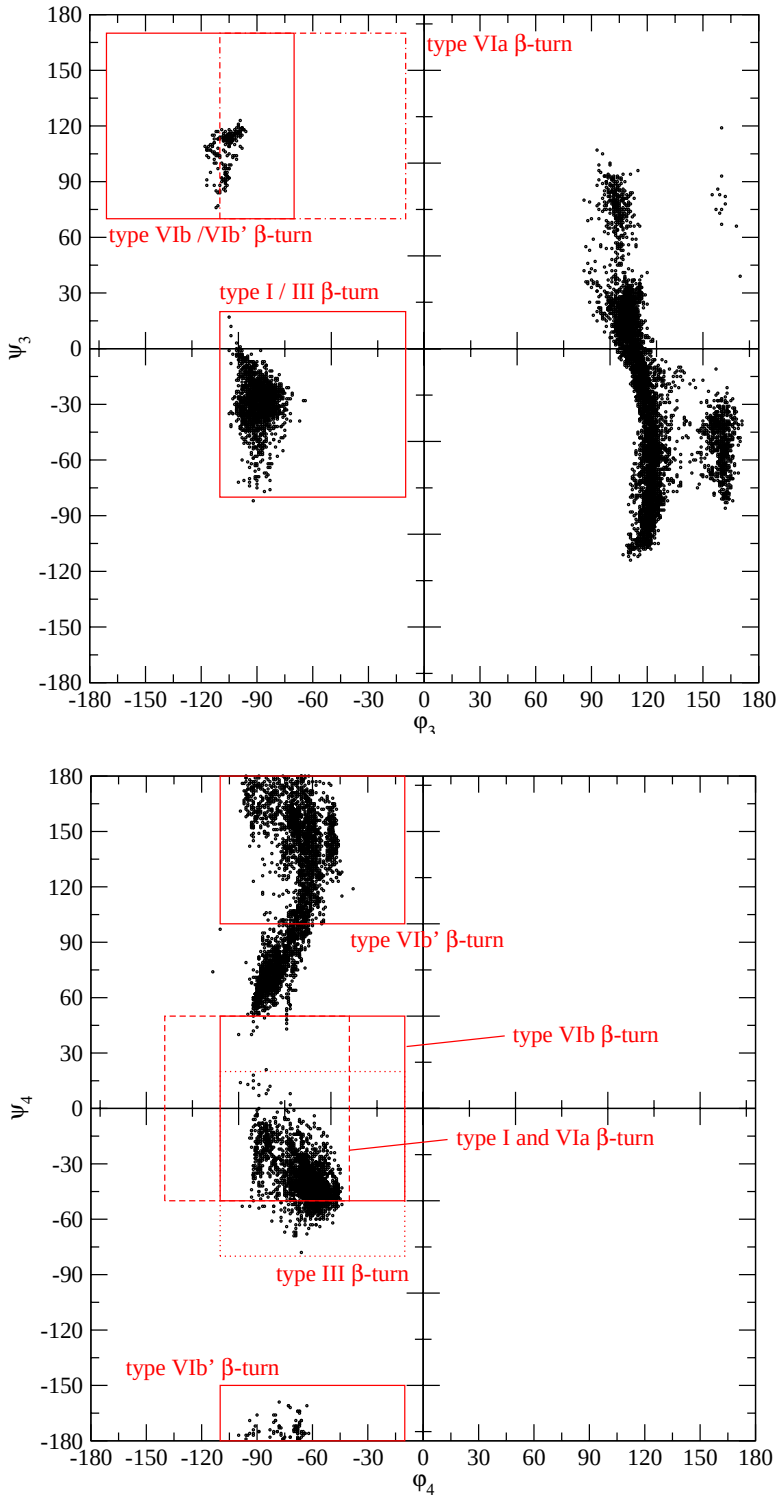


Figure 3.10. Ramachandran plots of residues c_3Phe[3] and Pro[4] of compound **4** of sequence Thi[1]-Ser[2](2R,3R)c_3Phe[3]-Pro[4]-Arg[5].

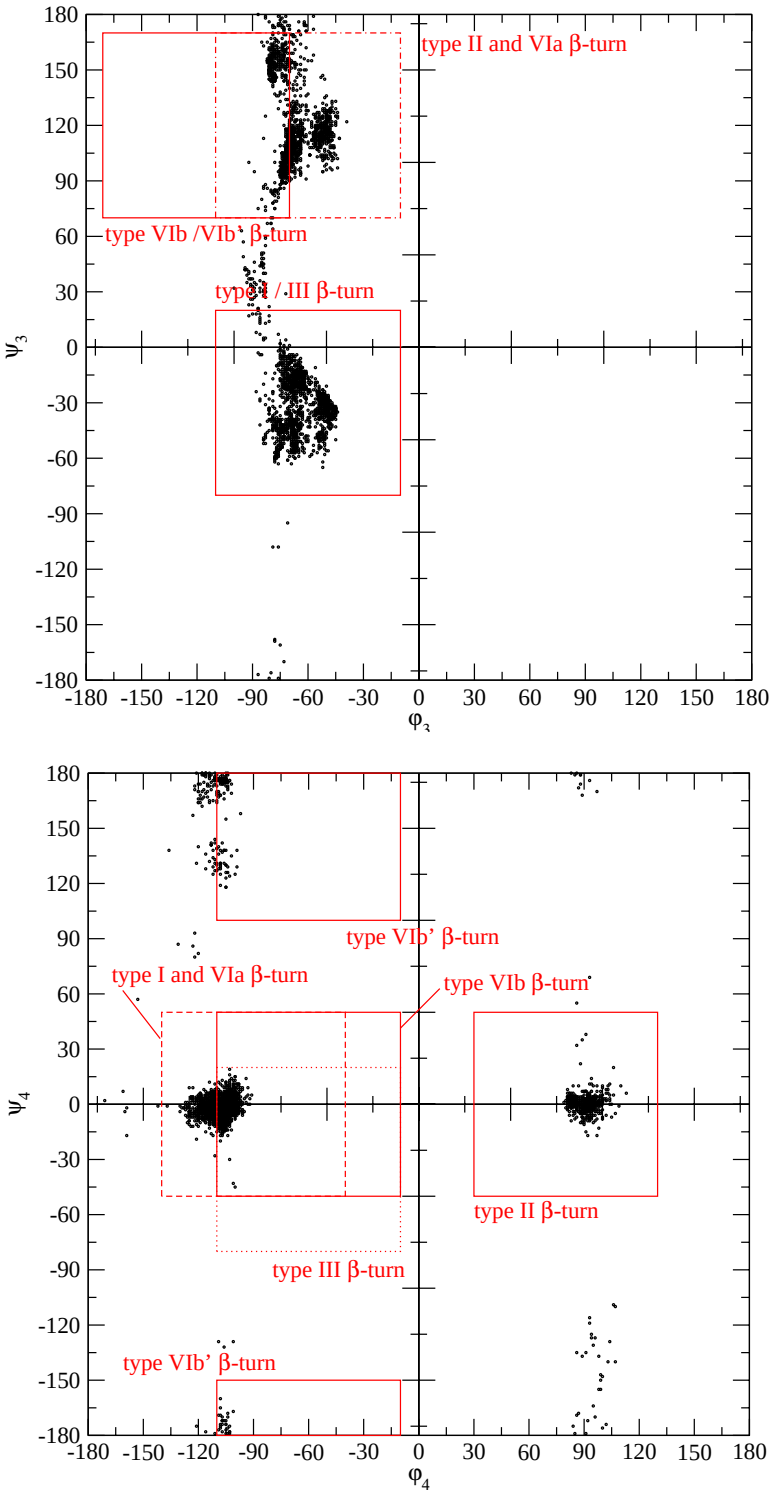


Figure 3.11. Ramachandran plots of residues c_3Phe[3] and Pro[4] of compound **5** of sequence Thi[1]-Ser[2]-Pro[3]-(2S,3S)c_3Phe[4]-Arg[5].

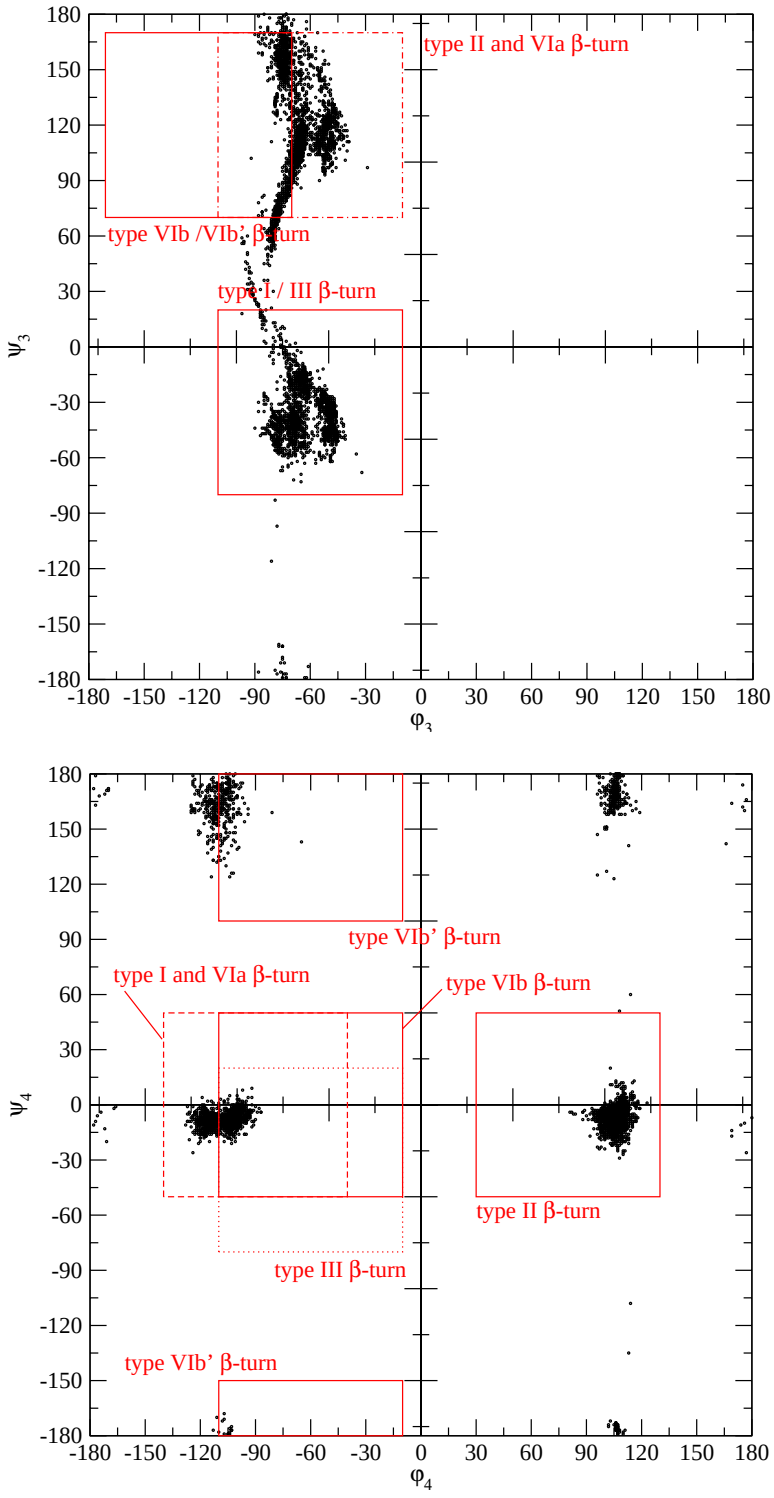


Figure 3.12. Ramachandran plots of residues c_3Phe[3] and Pro[4] of compound **6** of sequence Thi[1]-Ser[2]-Pro[3]-(2S,3R)c_3Phe[4]-Arg[5].

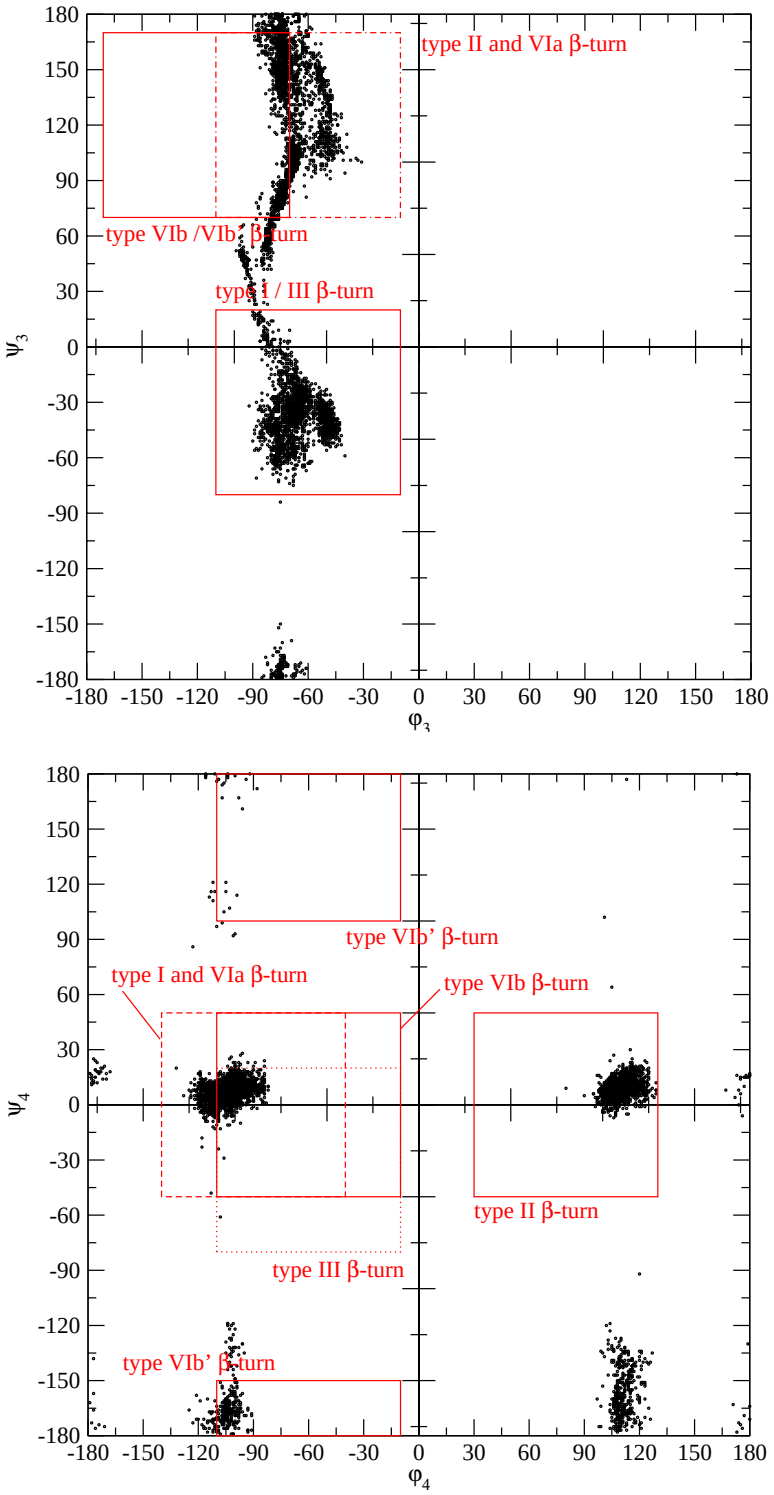


Figure 3.13. Ramachandran plots of residues c_3Phe[3] and Pro[4] of compound **7** of sequence Thi[1]-Ser[2]-Pro[3]-(2R,3S)c_3Phe[4]-Arg[5].

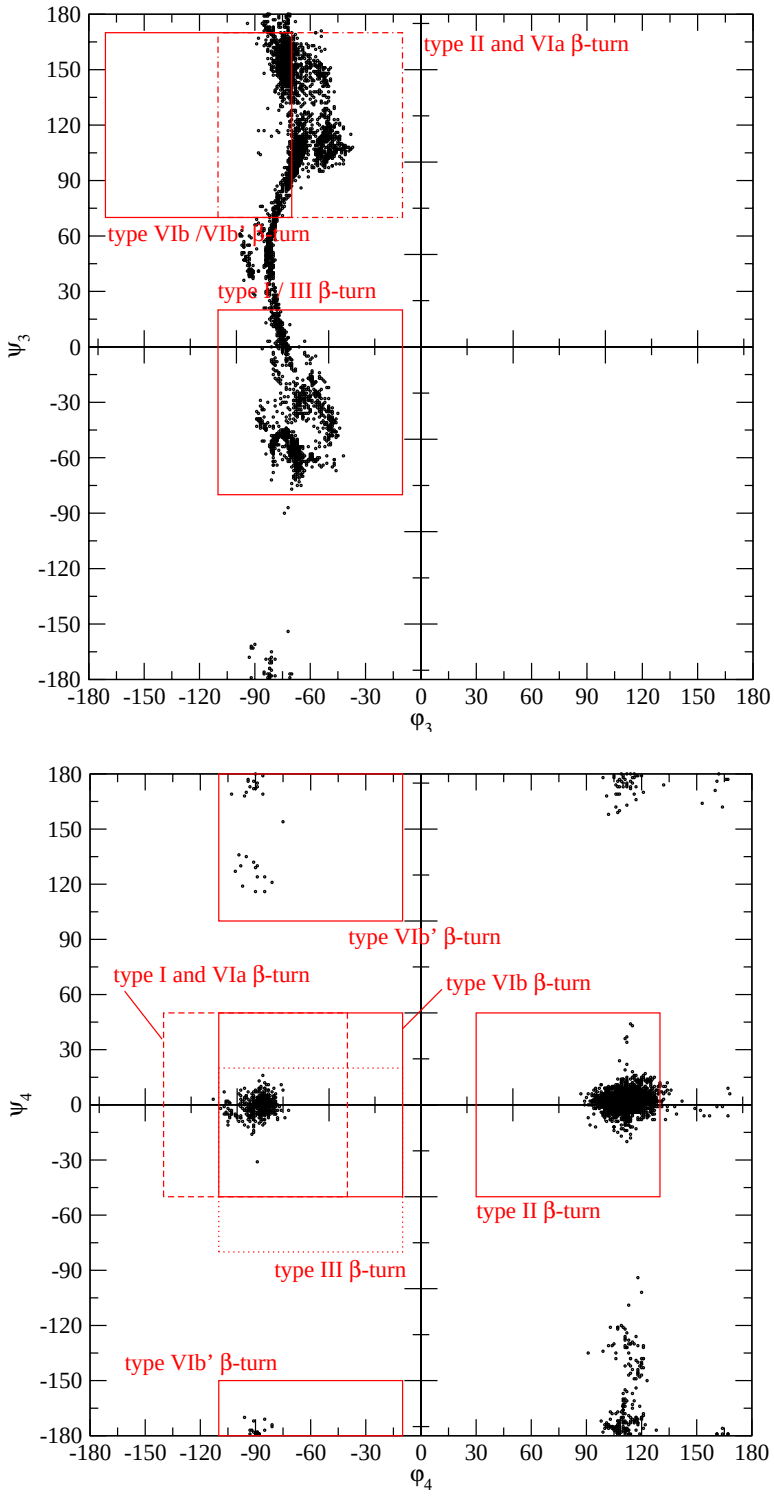


Figure 3.14. Ramachandran plots of residues c_3Phe[3] and Pro[4] of compound **8** of sequence Thi[1]-Ser[2]-Pro[3]-(2R,3R)c_3Phe[4]-Arg[5].

3.4.5 Pharmacophore Fulfillment by c_3Phe Fragments of BK

Distances d_1-d_3 were calculated for all the structures obtained in the SA procedure for compounds **1-8**. Subsequently, the percentages of structures that fulfilled each of the distances and the three distances at the same time with a tolerance of two times the standard deviation were calculated (Table 3.10). Thus, diasteroisomers could be ranked in terms of predicted activity. Compounds **1-5** and **8** did not fulfilled the d_1 distance in any case, thus suggesting that the distance between the aromatic ring in c_3Phe and Pro hydrophobic ring is not large enough to fulfill the pharmacophore requirement. Compounds **1-8** had d_2 values corresponding to the distance between the cyclohexane ring of Oic[8] and the C atom of the guanidinium group of Arg[9] within the range allowed and d_3 values corresponding to the distance between the aromatic ring of Tic[7] and the C atom of the guanidinium group of Arg[9] relatively within the range allowed. Only compounds **6** and **7** had some structures fulfilling the three distances. Indeed, 7.96% and 28.08% of the structures obtained in the SA procedure of **6** and **7** fulfilled the pharmacophore, respectively. Based on the pharmacophore fulfillment we predict that only **6** and **7** exhibit certain activity. Experimental studies for testing the activity should be carried out in order to check the present hypothesis.

Table 3.9. Type of β-turn present in the SA procedure of the c_3Phe containing peptides.

COMPOUND	1		2		3		4	
	No.	%	No.	%	No.	%	No.	%
βI	1639	25.6	1531	17.6	1222	17	636	6.9
βI'	0	0	0	0	0	0	0	0
βII	0	0	0	0	0	0	0	0
βII'	57	0.9	0	0	12	0.2	3	0
βIII	1811	28.3	1755	20.2	1405	19.5	705	7.7
βIII'	0	0	0	0	0	0	0	0
βI&βIII	1633	25.5	1525	17.5	1215	16.9	633	6.9
βI'&βIII'	0	0	0	0	0	0	0	0
βVIa	0	0	1	0	0	0	16	0.2
βVIb	48	0.8	20	0.2	5	0.1	21	0.2
βVIb'	142	2.2	87	1	28	0.4	20	0.2
βVIa&βVIb	0	0	0	0	0	0	16	0.2
TOTAL	2064	32.3	1869	21.5	1457	20.2	752	8.2
COMPOUND	**5**		**6**		**7**		**8**	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
βI	1068	27.4	754	15.7	832	13.6	242	5.5
βI'	0	0	0	0	0	0	0	0
βII	321	8.2	1398	29.1	978	16	1651	37.5
βII'	0	0	0	0	0	0	0	0
βIII	952	24.4	692	14.4	779	12.8	242	5.5
βIII'	0	0	0	0	0	0	0	0
βI&βIII	952	24.4	692	14.4	779	12.8	242	5.5
βI'&βIII'	0	0	0	0	0	0	0	0
βVIa	19	0.5	11	0.2	41	0.7	234	5.3
βVIb	4	0.1	4	0.1	8	0.1	117	2.7
βVIb'	2	0.1	0	0	4	0.1	13	0.3
βVIa&βVIb	4	0.1	4	0.1	8	0.1	117	2.7
TOTAL	1410	36.2	2163	45.1	1855	30.4	2140	48.6

Table 3.10. Fulfillment of the pharmacophore by Hoe 140 and compounds **1-8**. In order to fulfill the pharmacophoric descriptors distances have to be in the range comprised between the average ± two standard deviations.

	% of total fulfillment	% of d_1 fulfillment	% of d_2 fulfillment	% of d_3 fulfillment
Hoe 140 (679) βII'	91.31	94.55	98.53	97.79
Hoe 140 (6500)	52.02	85.38	99.45	60.92
1	0	0	99.50	71.83
2	0	0	99.64	75.30
3	0	0	99.74	48.90
4	0	0	99.60	51.33
5	0	0	96.97	68.77
6	6.12	15.71	95.96	42.75
7	22.49	34.18	96.52	70.93
8	0	0	97.45	86.41

3.5 Conclusions to chapter 3

The SA procedure carried out on Hoe 140 yields a type II' as the most abundant type of β-turn. Moreover, 47% of structures under 5 kcal·mol^{-1} exhibit this type of β-turn, suggesting that this conformation is highly stable and could be the most accessible for the molecule in solution. It had previously been suggested in the literature that a type II' β-turn could be a requisite for BK B_2 antagonism. The results obtained in the conformational study carried out in the present work further reinforce this hypothesis.

Based on the existence of a previous pharmacophore for BK B_2 antagonism we have redefined its distances using the values that were computed for the subgroup of structures that exhibited a type II' β-turn obtained in the SA procedure of Hoe 140. An exclusion volume has been also defined following the hypothesis that the β-turn will orient the side chains of residues at positions *i+1* and *i+2* towards the residues. Thus, as it has been checked by visual inspection, residues Tic[7] and Oic[8] are exposed to the side chains of the receptor responsible for the interaction. Thus, it is possible to safely place an exclusion volume in the vicinity of both side chains. The pharmacophore designed has been built into *Catalyst*^{TM} package and used for the search in chemical databases and 6 promising compounds have been obtained that will be subsequently tested on the receptor for BK B_2 antagonism.

Given the fact, that cyclopropanephenylalanine (c_3Phe) has been postulated as β-turn inducer, the conformational profile of a series of BK derivatives containing c_3Phe has been obtained through iterative simulated annealing. From the exhaustive analysis of the presence of β-turn in this series it can be concluded that peptides with sequence Thi^1-Ser^2-c_3Phe^3–Pro^4-Arg^5 (**1-4**) show a smaller tendency to induce β-turns than peptides of sequence Thi^1-Ser^2- Pro^3-c_3Phe^4-Arg^5 (**5-8**). Moreover, the β-turn present in peptides **5-8** is stabilized by the presence of a hydrogen bond. Greater hydrogen bond presence for compounds **5-8** than for **1-4**. On the other hand, compounds **5-8** have type II and type VIa β-turns that are not present in **1-4**. Thus, c_3Phe is a good inductor of type II β-turns whereas Pro has a tendency to form a type II β-turn when placed in the i+1 position but it does not lead to a type II β-turns when placed in the *i+2* position. A recent analysis of the relative abundance of the 20 amino acids in β-turns in proteins can be of help in order to rationalize differences arising between compounds **1-4** and **5-8**. (Guruprasad et al., 2000). Indeed, the authors showed that Pro is the most abundant residue at position *i+1* for type II β-turn and there are not examples of proteins with type II β-turn containing a Pro at position *i+2*. They also found Pro in the β-turns of the proteins studied at positions *i* and *i+2* but not at position *i+1* for type II' β-turn. This fact could explain why there are only type II' β-turns present for compounds **1-4**, the ones containing Pro at position *i+2* and there are not type II' β-turns for compounds **5-8**. From the Ramachandran plots (Figure 3.6-3.14) it can also be deduced that Pro at position *i+1* induces a restriction of the conformational space on the c_3Phe residue (Figures 3.11-3.14) in respect to the situation where c_3Phe is at position *i+1* and Pro is at position *i+2* (Figures 3.7-3.10).

Only compounds **1** and **3** exhibit type II' β-turns although in low percentages. Thus, if the presence of a type II' β-turn is a strict requirement for BK B_2 antagonism as it has been suggested in the literature, a poor BK B_2 antagonism activity should be predicted for compounds studied in the present study.

Compounds containing the sequence Pro^3-c_3Phe^4 (**5-8**) have different ratios of type I to type II β-turns, going from the greater abundance of type II β-turn for **6** and **8**, the equally abundant type I and type II β-turns for **7** and type I as being the most predominant for compound **5**. This is in agreement with experimental studies containing the sequence Pro-c_3Phe. This suggest that the ratio of type I to type II β-turns could be explained solely on the basis of the structural features of the the Pro-c_3Phe sequence.

By analyzing the pharmacophoric descriptors for compounds **1-8** it has been deduced that the distance between the aromatic ring of c_3Phe and the pyrrolidine ring of Pro is not large enough to fulfill the pharmacophore. Only compounds **6** and **7** partially fulfilled this criterion. The replacement of the Pro residue with Oic could be an appropriate strategy in order to better fulfill the distance requirement between the aromatic ring of c_3Phe and the hydrophobic moiety in the *i+2* position of the β-turn.

3.6 References to chapter 3

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