Topological Exploration of Cyclic Endomorphin-1 Analogues, Structurally Defined Models for Investigating the Bioactive Conformation of MOR Agonists

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Abstract: Although there have been several reports on the conformational analysis of endomorphin-1 (YPWF-NH_2) and related MOR (μ-opioid receptor) agonists, a definitive, convincing model of the biologically active structure is not yet available. We recently reported the synthesis and pharmacological characterization of the atypical endomorphin-analogue agonist *c*[YpwFG]. In this paper we discuss the conformational analysis of *c*[YpwFG] in comparison to its epimers, for investigating the topological features responsible for ligand recognition and receptor activation, and the role of the different pharmacophores.

Keywords: Cyclic endomorphins, bioactive conformation, agonism, MORs, lipophilic peptides.

INTRODUCTION

In the last three decades divergent opinions have been reported on the nature of the interaction between μ-opioid receptor (MOR) and their agonists or antagonists. The structures that have been proposed to represent the biologically active conformations of ligands very often show contrasting features [1,2,3]. Since the X-ray structure of the receptor is not available, structural investigations have been carried out by the conformational analysis of isolated compounds. However, it has been difficult to establish reliable models from endogenous opioid peptides due to their intrinsic flexibility [4], and the flexibility of the receptor cavity, which can modify its inner shape to host different ligand [5].

Ever since the discovery of endomorphin-1, YPWF-NH_2, and endomorphin-2, YPFF-NH_2 [6,7], which are considered to be the endogenous MOR agonists, there has been renewed interest in investigating the bioactive conformation. Nevertheless, some research groups have proposed different structures, depending on the nature of the environment selected for the 2D-NMR analysis and/or computational modeling. These diverse studies could not converge towards a definition of the relative 3D orientation of the pharmacophores or of the cis/trans conformation of the strategic amide bond preceding Pro^2. Based on spectroscopic analysis in DMSO or water, an extended conformation showing a trans Tyr^1-Pro^2 amide bond has been proposed by Podlogar *et al.* [8]. In contrast, studies in membrane-mimetic SDS micelles or AOT reverse micelles have suggested that the Tyr^1-Pro^2 bond adopted a cis disposition [9]. Concerning the pharmacophores, it has been reported that, by varying the environment of endomorphin-1 from water to micelles [10], the spatial disposition of the aromatic side-chains changed from one in which the Tyr^1 and Phe^4 residues are close to each other to one in which the phenolic moiety of Tyr^1 is in close contact with the indole group of Trp^3. Therefore, while there is a general consensus regarding the importance of Tyr^1 as the primary pharmacophore [1,2,6], the role of the other pharmacophores is currently under debate.

The introduction of conformational restrictions [2,10,11] in the sequence of endomorphins has not simplified matters. Indeed, there has been some evidence to support a bioactive conformation with a cis Tyr^1-Pro^2 and, conversely, other evidence to support a trans Tyr^1-Pro^2 amide bond. For instance, analogues of endomorphin-2 containing (4*R*)-thiazolidine-4-carboxylic acid in place of Pro^2 showed a constrained cis Tyr^1-pseudoPro^2 amide bond and maintained a certain activity toward MORs [12]. Furthermore, analogues of endomorphin-2 containing 2',6'-dimethyl-Tyr (Dmt) in place of Tyr^1 showed a 4.6-fold increase in μ-opioid affinity (but also a decreased μ/δ selectivity), and exhibited a 13:7 preference for a cis configuration of the Dmt^1-Pro^2 bond [13]. On the other hand, endomorphin-1 analogues containing a bicyclic scaffold, designed as a type III β-turn mimetic, resulted in a μ-selective agonist [14]. In addition, a μ-selective endomorphin analogue with 1-aminocyclohexane-1- carboxylic acid (Chx) in place of Pro showed a trans-form Tyr-Chx bond and a folded conformation similar to that of D-TIPP and the β-turn of Leu-enkephalin [15]. Finally, a recent paper described an endomorphin-2 analogue showing a N-O turn induced by a α-aminoxyPhe, similar to a turn at the Pro^2-Phe^3 position of the parent peptide [16].

Very recently, we reported a selected library of stereoisomeric cyclic pentapeptides **1**-**14**, Fig. (**1**), based on the sequence of endomorphin-1, containing the amino acids in the L or D configuration [17]. These compounds were designed with the goal of obtaining opioid peptide analogues with improved stability and increased ability to cross biological barriers [18-20]. The cyclic peptide *c*[YpwFG] (**14**) showed good affinity for the MOR and agonist behaviour. Interestingly, this lipophilic peptide, which contains all amide bonds, activated the receptor even without the contribu-

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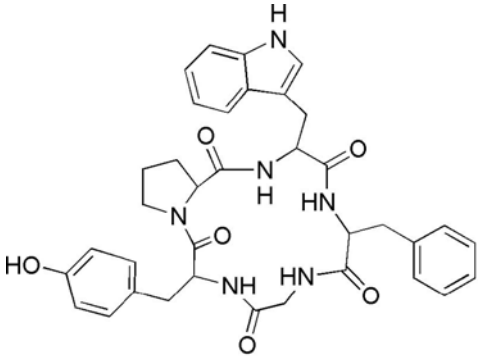
tion of an H-bond involving a protonable primary amine. Generally, the absence or derivatization of the amino group (e.g. acylation) deprived an opioid peptide of biological activity, or converted an agonist into an antagonist [19-22]. For the highly lipophilic peptide **14**, the overall hydrophobic contributions seem to counterbalance the absence of ionic interaction [5,23] Aparently, the ability displayed by **14** to fully activate the receptor means that the ligand and receptor surface are strongly complementary.

In this paper we investigate the correlation between the specific 3D pharmacophore display of selected members of the library. The systematic reversal of the stereochemistry of each amino acid in endomorphins has been advantageously used to explore how 3D changes affect biological activity [24-26]. Therefore, we performed a comparative analysis of the 3D structures of *c*[YpwFG] (**14**) and of the epimers *c*[ypwFG] (**10**), *c*[YpWFG] (**5**), and *c*[YpwfG] (**4**). Within the family of cyclic peptides, cyclic pentamers containing one or two D-amino acids have been widely used as conformationally restricted β- or γ-turn models [27-32] (but with an intrinsic, residual flexibility, see Discussion and References), for arranging the pharmacophores in defined reciprocal orientations [33].



1 c[-Tyr-Pro-Trp-Phe-Gly-]
2 c[-D-Tyr-D-Pro-D-Trp-D-Phe-Gly-]
3 c[-D-Tyr-Pro-Trp-Phe-Gly-]
4 c[-Tyr-D-Pro-D-Trp-D-Phe-Gly-]
5 c[-Tyr-D-Pro-Trp-Phe-Gly-]
6 c[-D-Tyr-Pro-D-Trp-D-Phe-Gly-]
7 c[-Tyr-Pro-D-Trp-Phe-Gly-]
8 c[-D-Tyr-D-Pro-Trp-D-Phe-Gly-]
9 c[-Tyr-Pro-Trp-D-Phe-Gly-]
10 c[-D-Tyr-D-Pro-D-Trp-Phe-Gly-]
11 c[-D-Tyr-Pro-D-Trp-Phe-Gly-]
12 c[-Tyr-D-Pro-Trp-D-Phe-Gly-]
13 c[-D-Tyr-Pro-Trp-D-Phe-Gly-]
14 c[-Tyr-D-Pro-D-Trp-Phe-Gly-]

Figure 1. Structures of stereoisomeric cyclic-endomorphins.

RESULTS

The synthesis of the stereoisomeric cyclic endomorphin analogues, Fig. (**1**), and their MOR affinities has been reported in a previous paper [17].

The conformational analysis of **14** and its epimers **10**, **5**, and **4**, was performed by NMR spectroscopy and Molecular Dynamics (MD) simulations [34]. NMR experiments of the cyclopeptides were conducted using standard techniques at 400 MHz in either DMSO-d_6 or in $CDCl_3$/DMSO-d_6 mixtures. The experiments were performed with a 2 mM peptide concentration, at 293 °K. 2D spectra were acquired in the phase sensitive mode and processed using a 90° shifted, squared sine-bell apodization. Interestingly, for all compounds ^{1}H and ^{13}C-NMR revealed a single set of resonances, suggesting conformational homogeneity or a fast equilibrium [28,33] The unambiguous assignment of the resonances was performed by COSY and HMBC analysis (heteronuclear multiple bond correlation). To deduce the presence of H-bonds, we performed VT-^{1}H-NMR (variable temperature) experiments over the range 291-333 °K [35]. Finally, 2D-ROESY (mixing time 300 ms) allowed to determine the main conformational features of cyclopeptide backbones. For the absence of $H\alpha_i$-$H\alpha_{i+1}$ cross peaks, indicative of a cis peptide bond conformation, the ω bonds of **14**, **5**, and **4** were set at 180°. The observation of strong ROESY cross peaks between TyrHα and ProHδ was also used to infer a trans conformation of Tyr^1-Pro^2 amide bonds. On the contrary, the strong ROESY between D-ProHα and D-TyrHα observed in **10** was used to restrain the ω bond connecting the two residues to 0°. Moreover, the ^{13}C NMR data chemical shifts of Cβ and Cγ of the Pro residue can be utilized for assigning the conformation of the preceding peptide bond; [36] a difference of 4-6 ppm indicates a trans conformation while a difference of 8-10 ppm is expected for a cis conformation. When possible, an analysis of $^3J_{NH\text{-}H\alpha}$ and $^3J_{H\alpha\text{-}H\beta}$ coupling constants was used to estimate the torsion angles [37,38]. In particular, $^3J_{H\alpha\text{-}H\beta}$ coupling constants suggested that side chains are subjected to a fast conformational switch, albeit a certain preference can be deduced. In any case, ^3J were not used for defining angle restraints.

Low-energy conformations of cyclopeptide backbones consistent with spectroscopic analysis were obtained by restrained MD [39], using the distances deduced from ROESY as constraints. Simulations were conducted in vacuo using the AMBER [40] force field, with $\varepsilon = 4 \times r$. No distance cutoffs were used, and chiral centers were constrained to the starting values. A set of 100 random structures was generated by means of a 50 ps unrestrained high-temperature simulation (1200 °K), and each structure was subjected to a 20 ps restrained MD with a 50% scaled force field, followed by 20 ps with full restraints (1200 °K), after which the system was cooled (50 °K) in 5 ps (distance force constant: 7 kcal/mol Å^2; ω bonds were set at 0 or 180° using a force constant of 16 kcal/mol Å^2). The structures were minimized with 2000 cycles of steepest descent and 2000 cycles of conjugated gradient (convergence at 0.01kcal/mol Å). The structures that showed the lowest internal energy and the least number of violations of the experimental data were selected and analyzed, Fig. (**2**).

A representative backbone structure of **14**, c[YpwFG], is reported in Fig. (**2**). This structure is compatible with the presence of a type II β-turn with Tyr-D-Pro in the positions *i+1* and *i+2*, and an inverse γ-turn (γ'-turn) with Phe in the *i+1* position. However, an alternative conformation showing the residues D-Pro-D-Trp occupying the positions *i+1* and *i+2* of an inverse type I β-turn (βI'-turn), and Gly in the position *i+1* of a γ-turn [41], cannot be ruled out. Nevertheless, VT-NMR experiments supported the first hypothesis [35], for the presence of two H-bonds involving GlyNH and

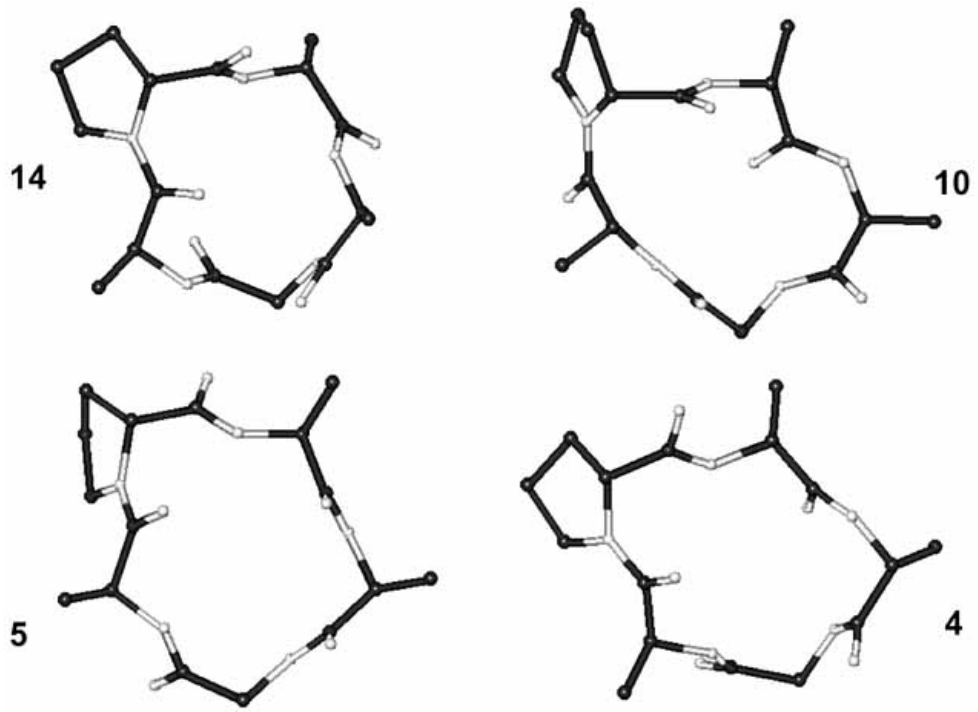


Figure 2. Representative conformations of cyclopeptide backbones calculated by restrained MD with the lowest internal energy and the least number of violations of ROESY data.

D-TrpNH. Indeed, the $\Delta\delta/\Delta t$ values for **14** in DMSO-d_6 over the range of 298-348 °K were (ppb/°K): TyrNH, -4.8; PheNH, -5.3; GlyNH, -1.4; D-TrpNH, -1.5.

In peptide **10**, c[ypwFG], the introduction of D-Tyr^1 induced a cis configuration of the D-Tyr^1-D-Pro^2 bond, Fig. (**2**). This is deduced from the strong ROESY between D-ProHα and D-TyrHα, and also from the large difference between the chemical shifts of Cγ and Cβ of D-Pro^2 in the ^{13}C-NMR spectra, 21.5 and 30.8 ppm, respectively, compared to the small difference between Cγ at 24.3 and Cβ at 26.7 of D-Pro^2 as observed for **14**. It has been reported that the presence in cyclic pentapeptides of a residue (Xaa) preceding proline having the same absolute configuration induced a cis Xaa-Pro ω bond (see for instance the peptide containing the Ala-Pro sequence [42], and the peptide containing the Trp-Pro-Asp sequence, which showed a backbone a structure compatible with a type VIb β-turn [42]). VT-NMR analysis did not furnish any evidence of H-bonds. The cis amide involving D-Tyr^1 ensures that the phenol group remains on the same side of the molecule as in **14**, despite the inversion of the absolute configuration.

Cyclopeptide **5**, c[YpWFG], adopts a conformation compatible with two γ-turns showing D-Pro and Gly in the *i+1* positions [44]. Accordingly, a VT-NMR experiment indicates that TyrNH and TrpNH participate to the formation of H-bonds. The substitution of D-Trp^3 with Trp^3 gives a structure with the indole group on the same side as phenol and phenyl, which maintain the same position as in **14**, therefore the phenol-indole and the phenyl-indole distances are significantly shortened.

The structure of c[YpwfG] (**4**) calculated from NMR data and restrained MD did not furnish a well defined structure, suggesting a conformational freedom. The analysis of the structures showing the smaller violations of the distances indicated by NMR lead to the proposal of a likely average conformation, Fig. (**2**).

In general, the structures calculated from ROESY data failed to reproduce explicit secondary structural elements matching VT-NMR analysis. This observation is not unexpected, for the residual backbone flexibility generally showed by cyclic Pro-containing pentapeptides [28,33, 44-47]. In order to investigate the inherent flexibility of the cyclopeptides, the structures derived from NMR were analyzed by unrestrained MD. Unrestrained MD in explicit water have been performed for 3 ns using the AMBER force field, by placing peptides in a 30x30x30 Å box of standard TIP3P models of equilibrated water, with a minimum solvent-solute distance of 2.3 Å, for a total of 892 H_2O molecules, at constant temperature and pressure (Berendsen scheme, bath relaxation constant 0.2). A 12 Å cutoff was used for non-bonded interactions.

The trajectories confirmed the presumed flexibility of peptide backbones, Fig. (**3**). Nevertheless, most of this freedom seems to be limited to the Gly region [30,44]. Despite of this residual flexibility, the pharmacophoric groups occupy well definite regions around the molecule. Concerning the side chain orientations, for each cyclopeptide the most frequently observed χ angle for Tyr was trans. In **14**, **10**, and **4**, χTrp was predominantly g+, while in **5** χTrp adopted a g- conformation. χPhe was predominantly g- in **14**, **10**, **5**, and g+ in **4**. Interestingly, among the diverse conformations, during the simulations the cyclopeptides clearly adopted well defined β- or γ-turns, in agreement with VT-NMR and/or ROESY data, Fig. (**4**).

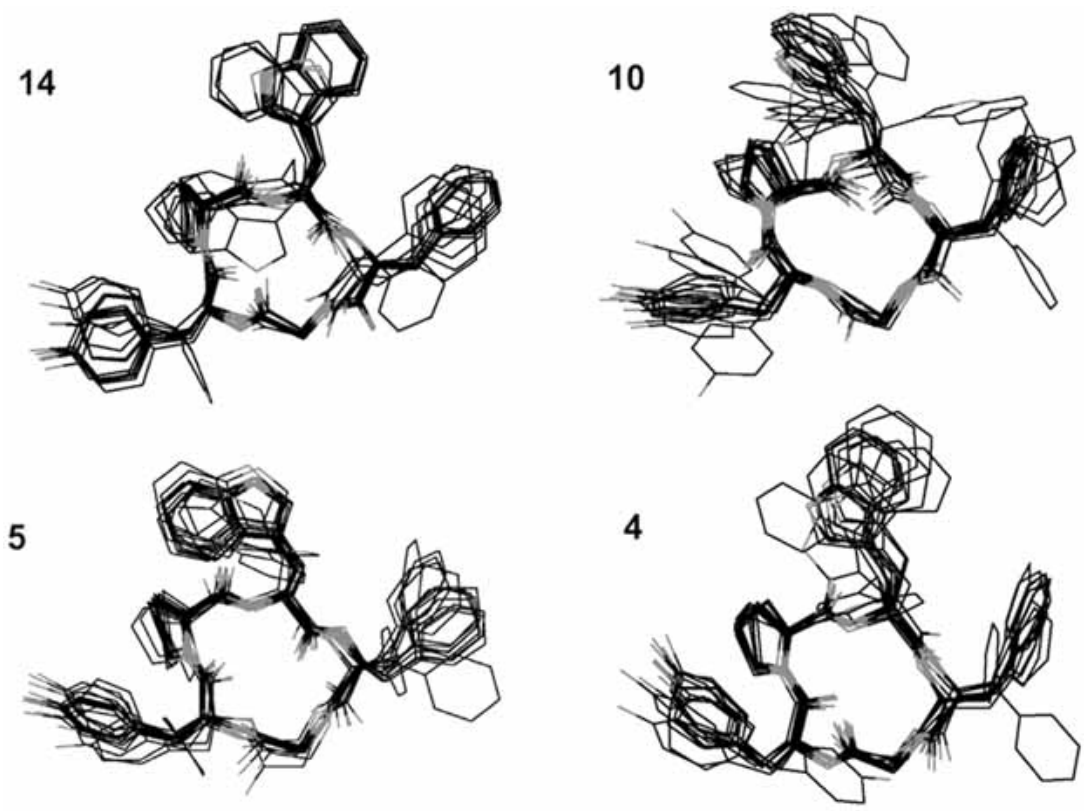


Figure 3. Superimposition of minimized structures of **14**, **10**, **5**, and **4**, sampled every 200 ps from 3 ns unrestrained MD simulations in explicit water.

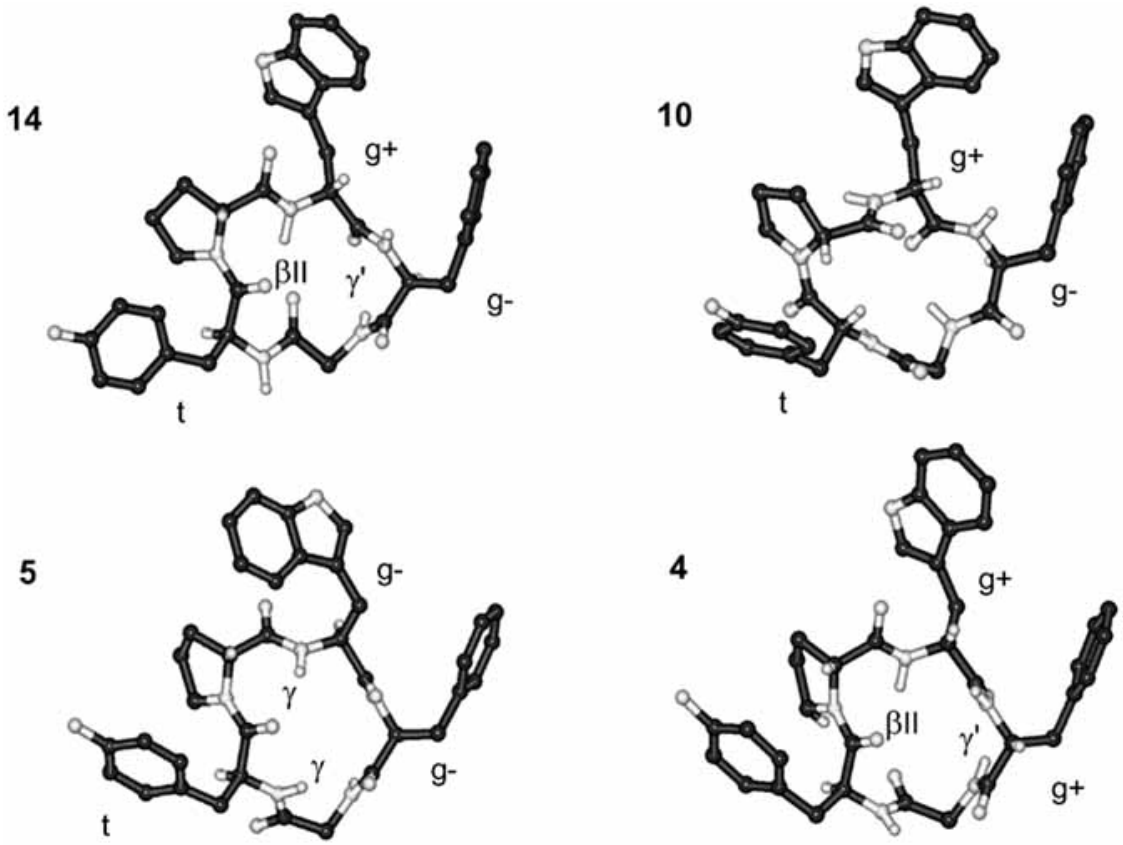


Figure 4. Minimized structures of **14**, **10**, **5**, and **4** obtained from unrestrained MD in agreement with NMR data, along with their secondary conformational features.

DISCUSSION

Since the cyclopeptides confirmed to be suitable scaffolds for positioning the pharmacophores in restricted regions of the space surrounding the molecule, the comparison of the 3D structures deduced from NMR analysis and MD computations could be of value to infer well-defined correlations between structure and activity. One of the most important features to discuss is the definition and relative disposition of the primary pharmacophores. The conformational model obtained for **14**, Fig. (**3**), is in excellent agreement with the literature [3]. Extensive investigations of μ-opioid peptides [2] such as endomorphin-1 [8,24], morphiceptin [48,49], JOM-6, Tyr-c(S-Et-S)[D-Cys-Phe-D-Pen]NH [50], have led to the definition of a trans χTyr^1 angle. Similar studies performed on endomorphin-1 and analogues [24] suggested a g- orientation for χTrp^3. However, the presence of a D-Trp^3 in peptide **14** instead of the L-Trp^3 in endomorphin-1 is consistent with the angle reversal, from g- to g+. The conformation of Phe^4 has been found less well-defined in comparison to the other pharmacophores in both endomorphin-1 [24] and endomorphin-2 [25]. Even an endomorphin-2 derivative that lacks the Phe^4 residue still interacted with the MOR [25]. Nevertheless, more recent data indicated that the introduction of (2S,3S)-β-$MePhe^4$ in endomorphins gave peptides with a preferential g- conformation of the Phe analogue and high receptor affinity, in agreement with **14** [51].

Concerning the reciprocal positions of Tyr^1 and Phe^4, the average distance between the aromatic rings (defined as pseudoatoms) during MD is around 12-13 Å in **14**, 11-12 Å in **4**, around 12 Å in **5**, and around 12-13 Å in **10**. This suggests that even a subtle difference in the distance or in the reciprocal orientation of phenol and phenyl can affect the biological activity. The distance calculated in **14** agrees very well with the requisites described in the literature for MOR agonists [52]. Data obtained from enkephalin-derived cyclic peptides [1,2], and from the investigation of morphiceptin [48] or endomorphin-1[8] and its analogues [25], have established that a relatively large separation of around 12 Å of the two aromatic side chains is required for activity and selectivity.

The role on Trp^3 in endomorphin-1 recognition is somewhat elusive, and the comparison between the structures of **14** and **5** could be of some value. Among the many MOR agonists, only endomorphin-1 has a Trp residue within its sequence, and this could be related to the unusual selectivity respect the other native opiod peptides [1,2,6]. Based on the MOR selectivity models proposed for JOM-6 [50,53], the Trp^3 residue of **14** seems to be an additional recognition motif, since no group in JOM-6 overlaps the indole ring. The absence of protonable nitrogen is certainly responsible for the lower activity of **14**, therefore efficient hydrophobic interactions, plus eventual H-bond(s), are required for compensating in part the lacking electrostatic interaction. As a consequence, the role of Trp^3 as an important contributor to ligand-receptor binding should be considered. According to the general model of MOR [54,55], efficient binding and receptor activation require that **14** was deeply inserted into the binding cavity. The receptor seems to have sufficient room to host Trp^3 in the gap delimited by TM-IV (TM = trans membrane helix), TM-V, and EL-II (EL = extracellular loop). This arrangement also seems to be promising for the possibility of a stabilizing interaction between indole-NH and Glu^{229} on TM-V. The absence of significant activity in **5**, which differed from **14** mainly in the opposite orientation of Trp^3, could be explained on the basis of unfavorable interaction with residues of TM-7, such as Trp^{318} and/or Cys^{321}.

Finally, it is worthwhile to mention that the partial correspondence between the calculated structures of the cyclopeptides could be responsible of the comparatively higher biological activities demonstrated by **10** (K_I = 3.9 μM and IC_{50} = 5.2 μM), **4** (K_I = 2.7 μM and IC_{50} = 3.2 μM), and **5** (K_I = 2.9 μM and IC_{50} = 3.8 μM) [17] respect the other peptides of the library, Fig. (**1**), which in general showed K_I and IC_{50} values in the 10^{-4}-10^{-5} range. Due to the presence of two, three, or four mutated amino acids, it is expected that these peptides should display backbone conformations and side chain orientations quite different from **14**. The only exception is **13**, enantiomer of **14**, which shows affinity (K_I = 6.6 μM and IC_{50} = 8.1 μM) comparable to those of **10**, **4**, and **5** [17]. This result could be ascribed to the mirror disposition of phenol and phenyl respect **14**, and some overlap of the indole group, albeit with an opposite orientation.

CONCLUSIONS

The good activity displayed by c[YpwFG] (**14**) towards MORs can be correlated with the specific 3D arrangement of the pharmacophoric groups. The absence of a protonable nitrogen is responsible for the lower activity respect endomorphin-1; nevertheless, **14** can still activate the receptor. In particular, molecular modeling and spectroscopic analysis revealed that c[YpwFG] displays the aromatic side chains towards opposite, orthogonal directions. The comparison of **14** with its stereoisomers gave interesting clues regarding the role of each pharmacophore. By analogy to the general model, Tyr^1 is the primary pharmacophore (message), while Phe^4 seems to be the secondary pharmacophore responsible for peptide specificity (address) [1,2,56]. The reciprocal distances and relative orientations of the two pharmacophores are in excellent agreement with the requisites generally accepted for MOR agonists. Finally, it could be conjectured that Trp^3 is a supplementary recognition motif, specific for endomorphin-1, and appears to be an important contributor to ligand-receptor binding and selectivity.

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REFERENCES

[1] Hruby, V, J. Agnes, R, S, (**1999**) *Biopolymers, 51*, 391.
[2] Gentilucci, L., Tolomelli, A. (**2004**) *Curr. Top. Med. Chem., 4*, 105.
[3] Malicka, J. Czaplewski, C. Groth, M. Wiczk, W. Oldziej, S. Lankiewicz, L. Ciarkowski, J. Liwo, A. (**2004**) *Curr. Top. Med. Chem., 4*, 123.
[4] Spadaccini, R. Temussi, P. A. (**2001**) *Cell. Mol. Life Sci., 58*, 1572.

[5] Davis, A, M. Teague, S. J. (**1999**) *Angew. Chem. Int. Ed. Engl.*, *38*, 736.
[6] Zadina, J. E. Hackler, L. Ge, L. J. Kastin, A. J. (**1997**) *Nature*, *386*, 499.
[7] Horvath, G. (**2000**) *Pharmacol. Ther.*, *88*, 437.
[8] Podlogar, B. L. Paterlini, M. G. Ferguson, D. M. Leo, G. C. Demeter, D. A. Brown, F. K. Reitz, A. B. (**1998**) *FEBS Lett*., *439*, 13.
[9] Fiori, S. Renner, C. Cramer, J. Pegoraro, S. Moroder, L. (**1999**) *J. Mol. Biol.*, *291*, 163.
[10] Hruby, V. J. Balse, P. M. (**2000**) *Curr. Med. Chem*., *7*, 945.
[11] Janecka, A. Kruszynski, R. (**2005**) *Curr. Med. Chem*., *12*, 471.
[12] Keller, M. Boissard, C. Patiny, L. Chung, N. N. Lemieux, C. Mutter, M. Schiller, P. W. (**2001**) *J. Med. Chem.*, *44*, 3896.
[13] Okada, Y. Fujita, Y. Motoyama, T. Tsuda, Y. Yokoi, T. Li, T. Sasaki, Y. Ambo, A. Jinsmaa, Y. Bryant, S. D. Lazarus, L. H. (**2003**) *Bioorg. Med. Chem., 11*, 1983.
[14] Eguchi, M. Shen, R Y. W. Shea, J. P. Lee, M. S. Kahn, M. (**2002**) *J. Med. Chem*., *45*, 1395.
[15] Doi. M. Asano, A. Komura, E. Ueda, Y. (**2002**) *Biochem. Biophys. Res. Commun*., *297*, 138.
[16] Wei, J. Shao, X. Gong, M. Zhu, B. Cui, Y. Gao, Y. Wang, R. (**2005**) *Bioorg. Med. Chem. Lett*., *15*, 2986.
[17] Cardillo, G. Gentilucci, L. Tolomelli, A. Spinosa, R. Calienni, M. Qasem, A. R. Spampinato, S. (**2004**) *J. Med. Chem*., *47*, 5198.
[18] Lipkowski, A. W. Misicka, A. Carr, D. B. Ronsisvalle, G. Kosson, D. Bonney, I. M. (**2004**) *Pure Appl. Chem*., *76*, 941.
[19] Gentilucci, L. (**2004**) *Curr. Topics Med. Chem., 4*, 19.
[20] Yamada, R. Kera, Y. (**1998**) EXS, 85 (D-Amino Acids in Sequences of Secreted Peptides of Multicellular Organisms), 143.
[21] Ronai, A. Z. Botyanszki, J. Hepp, J. Medzihradszky, K. (**1992**), *Life Sci., 50*, 1371.
[22] Schiller, P. W. Weltrowska, G. Nguyen, T. M. Lemieux, C. Chung, N. N. Lu, Y. (**2003**) *Life Sci*., *73*, 691.
[23] Hansch, C. Hoekman, D. Gao, H. (**1996**) *Chem. Rev*., *96*, 1045.
[24] Paterlini, M. G. Avitabile, F. Ostrowski, B. G. Ferguson, D. M. Portoghese, P. S. (**2000**) *Biophys. J*., *78*, 590.
[25] Okada, Y. Fukumizu, A. Takahashi, M. Shimizu, Y. Tsuda, Y. Yokoi, T. Bryant, S. D. Lazarus, L. H. (**2000**) *Biochem. Biophys. Res. Commun*., *276*, 7.
[26] Fichna, J. do-Rego, J. C. Kosson, P. Costentin, J. Janecka, A. (**2005**) *Biochem Pharmacol*., *69*, 179.
[27] Weisshoff, H. Prasang, C. Henklein, P. Frommel, C. Zschunke, A. Mugge, C. (**1999**) *Eur. J. Biochem*., *259*, 776, and references therein.
[28] Stradley, S. J. Rizo, J. Bruch, M. D. Stroup, A. N. Gierasch, L. M (**1990**) *Biopolymers*, *29*, 263, and references therein.
[29] Kessler, H. Gratias, R. Hessler, G. Gurrath, M. Muller, G. (**1996**) *Pure Appl. Chem.*, 68, 1201.
[30] Dechantsreiter, M. A. Planker, E. Matha, B. Lohof, E. Holzemann, G. Jonczyk, A. Goodman, S. L. Kessler, H. (**1999**) *J. Med. Chem.***,** *42*, 3033.
[31] Wermuth, J. Goodman, S. L. Jonczyk, A. Kessler, H. (**1997**) *J. Am. Chem. Soc*., *119*, 1328, and references therein.
[32] Tamamura, H. Mizumoto, M. Hiramatsu, K. Kusano, S. Terakubo, S. Yamamoto, N. Trent, J.-O. Wang, Z. Peiper, S. C. Nakashima, H. Otaka, H. Fujii, N. (**2004**) *Org. Biomol. Chem., 2*, 1255.
[33] Kessler, H. (**1982**) *Angew. Chem. Int. Ed. Engl., 21*, 512, and references therein.
[34] Cornell, W. D. Cieplak, P. Bayly, C. I. Gould, I. R. Merz, K. M. Ferguson, D. M. Spellmeyer, D. C. Fox, T. Caldwell, J. W. Kollman, P. A. (**1995**) *J. Am. Chem. Soc*., *117*, 5179.
[35] Toniolo, C. (**1980**) *CRC Crit. Rev. Biochem.*, *9*, 1.
[36] Konat, R. K. Mierke, D. F. Kessler, H. Kutscher, B. Bernd, M. Voegeli, R. (**1993**) *Helv. Chim. Acta*, *76*, 1649.
[37] Cung, M. T. Marraud, M. (**1982**) *Biopolymers*, *21*, 953.
[38] Wang, A. C. Bax, A. (**1995**) *J. Am. Chem. Soc*., *117*, 1810.
[39] HyperChem 7.01 Professional, 2002, Hypercube Inc. 1115 NW 4th Street, Gainesville, FL 32601 USA.
[40] Cornell, W. D. Cieplak, P. Bayly, C. I. Gould, I. R. Merz, K. M. Ferguson, D. M. Spellmeyer, D. C. Fox, T. Caldwell, J. W. Kollman, P. A. (**1995**) *J. Am. Chem. Soc*., **117**, 5179.
[41] Paulitz, C. Steglich, W. (**1997**) *J. Org. Chem*., *62*, 8474.
[42] Gierasch, L. M. Karle, I. L. Rockwell, A. L. Yenal, K. (**1985**) *J. Am. Chem. Soc*., *107*, 3321.
[43] Weiβhoff, H. Wieprecht, T. Henklein, P. Frommel, C. Antz, C. Mugge, C. (**1996**) *FEBS Lett., 387*, 201.
[44] Haubner, R. Gratias, R. Diefenbach, B. Goodman, S. L. Joczyk, A. Kessler, H. (**1996**) *J. Am. Chem. Soc., 118*, 7461.
[45] Nikiforovich, G. V. Kover, K. E. Zhang, W.-J. Marshall, G. R. (**2000**) *J. Am. Chem. Soc***.,** *122*, 3262.
[46] Mierke, D. F. Kurz, M. Kessler, H. (**1994**) *J. Am. Chem. Soc., 116*, 1042.
[47] Heller, M. Sukopp, M. Tsomaia, N. John, M. Mierke, D.F. Reif, B. Kessler, H. (**2006**) *J. Am. Chem. Soc., 128*, 13806.
[48] Janecka, A. Fichna, J. Mirowski, M. Janecki, T. (**2002**) *Mini Rev. Med. Chem*., *2*, 565..
[49] Grieco, P. Giusti, L. Carotenuto, A. Campiglia, P. Calderone, V. Lama, T. Gomez-Monterrey, I. Tartaro, G. Mazzoni, M. R. Novellino, E. (**2005**) *J. Med. Chem*., *48*, 3153.
[50] Mosberg, H. I. Fowler, C. B. (**2002**) *J. Pept. Res*., *60*, 329.
[51] Tomboly, C. Kover, K. E. Peter, A. Tourwe, D. Biyashev, D. Benyhe, S. Borsodi, A. Al-Khrasani, M. Ronai, A. Z. Toth, G. (**2004**) *J. Med. Chem.***,** *47*, 735.
[52] Loew, G. Keys, C. Luke, B. Polgar, W. Toll, L. (**1986**) *Mol. Pharm*., *29*, 546.
[53] Fowler, C. B. Pogozheva, I. D. Lomize, A. L. LeVine, H.-III. Mosberg, H. I. (**2004**) *Biochemistry*, *43*, 15796.
[54] Subramanian, G. Paterlini, M. G. Portoghese, P. S. Ferguson, D. M. (**2000**) *J. Med. Chem*., *43*, 381.
[55] Sagara, T. Egashira, H. Okamura, M. Fujii, I. Shimohigashi, Y. Kanematsu, K, (**1996**) *Bioorg. Med. Chem*., *4*, 2151.
[56] Schwyzer, R. (**1980**), *Proc. R. Soc. Lond.. B, 210*, 5.

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