Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 3855–3860

BIOORGANIC & MEDICINAL CHEMISTRY

# Synthesis of Novel Morphiceptin Analogues Modified in Position 3 and Their Binding to μ-Opioid Receptors in Experimental Mammary Adenocarcinoma

A. Janecka,[a,*] J. Fichna,[a] R. Wiercioch[b] and M. Mirowski[b]

[a]*Department of Medicinal Chemistry, Medical University of Lodz, Mazowiecka 6/8, Lodz, Poland*
[b]*Department of Pharmaceutical Biochemistry, Medical University, Lodz, Poland*

Received 11 February 2003; accepted 30 June 2003

**Abstract**—Binding of the $^{125}$I-labeled μ-opioid receptor selective ligands, morphiceptin (Tyr-Pro-Phe-Pro-$NH_2$) and [D-Phe$^3$]morphiceptin, to membranes isolated from experimental mouse mammary adenocarcinoma was examined in vitro using a cross-linking assay followed by a Western blot technique. The radioactive complex had a molecular weight of about 65 kDa and was detectable by anti-μ-opioid receptor antibody, indicating the presence of μ-opioid receptors in tumor membranes. A series of new morphiceptin analogues, modified at the pharmacophoric position 3, was synthesized in order to find the correlation between the lipophilicity, electronic and steric properties of the amino acid in this position and the in vitro affinity of new analogues for μ-opioid receptors on mouse brain and tumor membranes. In in vivo studies the uptake of $^{131}$I-labeled analogues by experimental mammary adenocarcinoma was estimated. The highest affinity for μ-opioid receptors in both, in vitro and in vivo experiments was observed for [D-Phe$^3$]morphiceptin and [D-ClPhe$^3$]-morphiceptin.


## Introduction

In the last decade, a significant progress has been made in the development of target specific radiopharmaceuticals. Since the discovery of peptide receptors it has been recognized that small biologically active peptides can provide new approaches for radiopharmaceutical development.[1–3] In many cancers, an over-expression of receptors is observed which makes such receptors attractive targets for tumor imaging.[4,5] The majority of radiolabeled peptides that has now found a routine application in nuclear medicine are these that target somatostatin receptors.[6–8] The opioid system, like somatostatinergic, is inhibitory and has also been implicated in the control of tumor growth in different organs, including breast.[9,10] For example morphine, the prototype μ-ligand, was found to inhibit proliferation of the T47D human breast cancer cells.[11] Over-expression of opioid receptors on different tumors makes opioid peptides another interesting group of potential targeting agents.

An opioid peptide, morphiceptin (Tyr-Pro-Phe-Pro-$NH_2$), is a tetrapeptide amide,[12,13] structurally related to β-casomorphin, originally isolated from bovine β-casein.[14] Morphiceptin is a highly selective opioid peptide agonist for μ-receptor.[15] The amino acid composition and receptor selectivity of morphiceptin are very similar to those of endomorphin-1 and -2, two recently identified endogenous μ-receptor ligands.[16] The [D-Phe$^3$]morphiceptin was found to have even higher affinity than morphiceptin in the binding assay[17,18] and can be used as a parent compound in structure–activity studies of morphiceptin. From systematic studies of chemical modifications of morphiceptin and other μ-opioid ligands the requirement for the amino and phenolic groups of the Tyr residue and aromatic group of the Phe residue have been well explored.[17,19]

In the present study, we have synthesized several new [D-Phe$^3$]morphiceptin analogues which were designed to probe the steric, electronic and lipophilic requirements of the residue 3 side-chain aromatic function. The replacement of the D-Phe$^3$ side-chain with substituted phenyl rings, with larger aromatic groups, or with heteroatomic aromatic side chains may provide interesting insights into binding requirements of this series

*Corresponding author. Tel./fax: +48-42-678-4277; e-mail: ajanecka@zdn.am.lodz.pl

0968-0896/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0968-0896(03)00448-6

to μ-opioid receptors in in vitro studies and in in vivo uptake of new analogues by experimental mouse mammary adenocarcinoma.

## Results

Binding of $^{125}$I-morphiceptin and $^{125}$I-[D-Phe$^3$]morphiceptin to membrane proteins of mouse mammary adenocarcinoma was determined in vitro. The membranes were incubated with the labeled peptide in the presence of a cross-linking agent, ethylene glycol-bis(succinimidyl succinate) (EGS), followed by denaturing polyacrylamide gel electrophoresis. Autoradiographic analysis has shown $^{125}$I-morphiceptin and $^{125}$I-[D-Phe$^3$]morphiceptin migrating as a single band and an additional radioactive complex with molecular weight of about 65 kDa. This complex analyzed by Western blot technique reacted with polyclonal anti-μ-opioid receptor antibody (Fig. 1), which indicated that μ-opioid receptors were present in the tumor membrane preparations.

A series of [D-Phe$^3$]morphiceptin analogues with different unnatural aromatic amino acids in the third position has been synthesized. The following amino acids were used: 3-(4-chlorophenyl)-D-alanine (D-ClPhe), 3-(3,4-dichlorophenyl)-D-alanine (D-Cl$_2$Phe), 3-(2-naphthyl)-D-alanine (D-2-Nal), 3-(3-quinolyl)-D-alanine (D-Qal), and 3-(3-pyridyl)-D-alanine (D-Pal). Each of these modifications was designed to test the influence of varying steric and electronic character and lipophilicity in this region of the peptide molecule which appears to play an important role in the interaction with the opioid binding sites. Physicochemical data for new analogues are presented in Table 1.

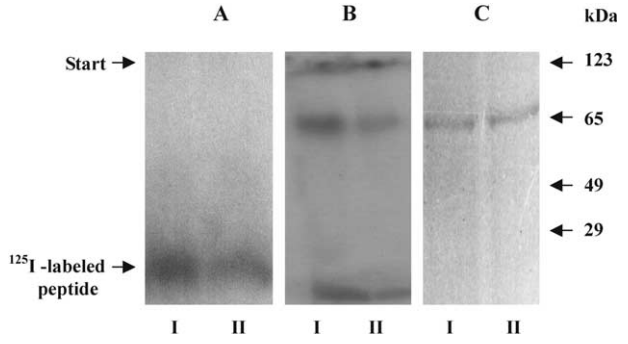


**Figure 1.** Autoradiographic analysis of $^{125}$I-morphiceptin (I) and $^{125}$I-[D-Phe$^3$]morphiceptin (II) before (A) and after incubation with the mouse mammary adenocarcinoma membrane proteins in the presence of cross-linking reagent (B) and Western blot analysis (C).

The binding of $^{125}$I-labeled [D-Phe$^3$]morphiceptin **1** analogues to the membranes from mouse brain and mouse mammary adenocarcinoma was assayed in the saturation experiments. The obtained $K_d$ (dissociation equilibrium constant) and $B_{max}$ (concentration of binding sites) values are summarized in Table 2. The $K_d$ values obtained for brain preparations were slightly lower but the same order of magnitude as the ones obtained for tumor preparations. All $K_d$ values were in a nanomolar range. The lowest $K_d$ values in the brain preparations were found for [D-Phe$^3$]morphiceptin **1**, [D-ClPhe$^3$]morphiceptin **2** and [D-Cl$_2$Phe$^3$]morphiceptin **3** (0.29, 0.15 and 0.13 nM, respectively). The affinity of other analogues was 2 orders of magnitude lower. Similar results were observed for tumor preparations. $B_{max}$ values in the tumor were significantly higher than in the brain for all tested analogues. The highest $B_{max}$ value (1112 fmol/mg protein) was obtained for [D-Phe$^3$] morphiceptin **1** in the tumor.

In vivo binding experiments with $^{131}$I-labeled analogues administered intraperitoneally to tumor bearing mice were performed. Accumulation of the analogues in the tumor (% of dose/g of tissue) was determined 0.5, 2 and 3 and 6 h after injection (Table 3). The highest accumulation

**Table 1.** Structure and physicochemical data of morphiceptin analogues

| Analogue no. | Sequence | FAB-MS (mol. wt) | HPLC retention time[a] (min) | LogP |
|---|---|---|---|---|
| **1** | Tyr-Pro-D-Phe-Pro-NH$_2$ | 522 | 8.064 | 0.53 |
| **2** | Tyr-Pro-D-ClPhe-Pro-NH$_2$ | 556 | 9.972 | 1.05 |
| **3** | Tyr-Pro-D-Cl$_2$Phe-Pro-NH$_2$ | 590 | 11.458 | 1.57 |
| **4** | Tyr-Pro-D-2-Nal-Pro-NH$_2$ | 572 | 11.368 | 1.53 |
| **5** | Tyr-Pro-D-Qal-Pro-NH$_2$ | 573 | 10.028 | 1.09 |
| **6** | Tyr-Pro-D-Pal-Pro-NH$_2$ | 522 | 3.337 | 0.15 |

[a]HPLC elution on a Vydac C-18 column (0.46 × 25 cm) using a linear gradient of 20–90% B in 30 min at a flow rate of 1 mL/min. Solvent system was 0.1% TFA in water/80% acetonitrile in water containing 0.1% TFA.

**Table 2.** In vitro binding studies of morphiceptin analogues

| Analogue no. | Sequence | Brain | | | Tumor | | |
|---|---|---|---|---|---|---|---|
| | | $K_d$ (nM) | Relative affinity | $B_{max}$ (fmol/mg protein) | $K_d$ (nM) | Relative affinity | $B_{max}$ (fmol/mg protein) |
| **1** | Tyr-Pro-D-Phe-Pro-NH$_2$ | 0.29 | 1.00 | 105 | 0.39 | 1.00 | 1112 |
| **2** | Tyr-Pro-D-ClPhe-Pro-NH$_2$ | 0.15 | 1.93 | 209 | 1.80 | 0.22 | 720 |
| **3** | Tyr-Pro-D-Cl$_2$Phe-Pro-NH$_2$ | 0.13 | 2.23 | 164 | 1.12 | 0.35 | 783 |
| **4** | Tyr-Pro-D-2-Nal-Pro-NH$_2$ | 12.81 | 0.02 | 162 | 18.53 | 0.02 | 156 |
| **5** | Tyr-Pro-D-Qal-Pro-NH$_2$ | 14.15 | 0.02 | 120 | 23.82 | 0.02 | 105 |
| **6** | Tyr-Pro-D-Pal-Pro-NH$_2$ | 21.34 | 0.01 | 110 | 32.57 | 0.01 | 167 |

$K_d$ values ± SEM were determined from saturation studies using nonlinear regression analysis. Results are the means ± SEM of three independent experiments.

**Table 3.** In vivo binding studies of morphiceptin analogues

| Analogue no. | Sequence | Accumulation of $^{131}$I-labeled analogues in mouse mammary adenocarcinoma (% dose/g tissue) | | | |
|---|---|---|---|---|---|
| | | 0.5 h | 2 h | 3 h | 6 h |
| **1** | Tyr-Pro-D-Phe-Pro-$NH_2$ | 2.4±0.1 | 3.0±0.5 | 4.5±0.3 | 2.5±0.1 |
| **2** | Tyr-Pro-D-ClPhe-Pro-$NH_2$ | 5.5±0.1 | 3.4±0.1 | 5.8±0.2 | 3.9±0.1 |
| **3** | Tyr-Pro-D-$Cl_2$Phe-Pro-$NH_2$ | 5.2±0.3 | 2.7±0.2 | 1.4±0.1 | 1.0±0.2 |
| **4** | Tyr-Pro-D-2-Nal-Pro-$NH_2$ | 1.3±0.1 | 1.6±0.2 | 1.6±0.2 | 0.8±0.1 |
| **5** | Tyr-Pro-D-Qal-Pro-$NH_2$ | 1.9±0.2 | 1.8±0.2 | 1.5±0.2 | 0.9±0.1 |
| **6** | Tyr-Pro-D-Pal-Pro-$NH_2$ | 0.9±0.1 | 0.9±0.1 | 0.9±0.1 | 0.45±0.1 |

(5.8% after 3 h) was found for [D-ClPhe$^3$]morphiceptin **2**. A slightly lower result (4.5%) was observed for analogue **1**. [D-$Cl_2$Phe$^3$]morphiceptin **3** showed a very high value (5.2%) after 0.5 h, but was quickly eliminated from the tumor to reach only 1.4% after 3 h and 1% after 6 h. The values obtained for the remaining analogues **4**–**6** were rather low (0.9–1.6% after 3 h).

## Discussion

Opioid peptides and their receptors produce a large spectrum of physiological effects, ranging from inducing pain relief to preventing diarrhea.[20] More recent evidence points to their possible involvement in pathological states. The exact mechanisms involved in the action of opioids in relation to oncogenic events are unknown. There has been some evidence to suggest a regulatory role of opioids in the growth and development of different types of cancer,[21–24] including breast cancer.[25–27]

In the present study, the binding of μ-opioid ligand morphiceptin to membrane proteins from experimental mouse mammary adenocarcinoma was demonstrated. A method based on chemical cross-linking between the receptor and the peptide ligand, followed by the separation of the formed complex by SDS-PAGE autoradiography, was adopted. Such cross-linking technique was previously successfully used for detection of somatostatin receptors in human breast cancer[28] and α-fetoprotein receptors in experimental mammary adenocarcinoma and adenoma.[29] The results obtained in this study demonstrate the existence of μ-opioid receptors in the experimental breast tumor. The 65 kDa radioactive complex corresponded with the molecular weight of the μ-receptor cloned by Chen et al.[30]

Compounds **2**–**5** were designed to assess the effects of altered size, lipophilicity and electronic character of the residue 3 side-chain, while maintaining aromaticity. The more lipophilic character of the peptides was reflected by higher values of logP, which were in agreement with longer RP-HPLC elution times (Table 1). Earlier studies of Ambo et al.[31] revealed that analogues of dermorphin and deltorphins with increased lipophilic character at the third residue showed higher receptor affinities. In our experiments the same tendency was observed for [D-Phe$^3$]-, [D-ClPhe$^3$]-, and [D-$Cl_2$Phe$^3$]morphiceptin (**1**–**3**) in in vitro and in vivo studies. Higher lipophilicity of chloro- and dichloro-derivatives resulted in their better binding to the membrane proteins. Replacement of the Phe ring in **4** with a naphthyl moiety increased the lipophilicity, but also the steric bulk of this crucial residue, which resulted in the reduction of μ-binding affinity. In analogues **5** and **6** the electronic and lipophilic character of the residue 3 side-chain was modified considerably by the incorporation of heteroatomic moieties. Replacement of D-2-Nal by the isosteric but hydrophilic D-Qal caused a great loss of affinity. The greatly increased hydrophilicity of analogue **6**, reflected both in the logP value and HPLC retention time, resulted in a 100-fold reduction in μ-affinity. D-Pal$^3$ modification was found to be the most detrimental for μ-affinity. The results observed here are consistent with previous observations in cyclic μ-receptor selective tetrapeptides.[32] Analogues **4**–**6** containing naphthyl-, quinolyl- and pyridyl-rings in the third position showed about 2 orders of magnitude lower $K_d$ values and greatly decreased accumulation in the tumor though logP values for analogues **2** and **3** were about the same as for analogues **5** and **4**, respectively. These data suggest that other than lipophilicity factors, like electronic or steric properties, can influence receptor binding.

The in vitro experiments were further confirmed by in vivo binding of morphiceptin analogues to experimental mouse mammary adenocarcinoma. Analogues with lower $K_d$ values were found to better accumulate in the tumor after intraperitoneal administration. The highest peptide concentration in mouse mammary adenocarcinoma was observed for [D-Phe$^3$]morphiceptin and [D-ClPhe$^3$]morphiceptin 3 h after administration, whereas for [D-$Cl_2$Phe$^3$]morphiceptin after 0.5 h, which indicates at the fastest elimination of this analogue. Minimum energy calculations were performed for all analogues, assuming the *cis* form about the Tyr-Pro amide bond.[33,34] The obtained structures were superimposed to compare their conformation with the minimal energy conformation of [D-Phe$^3$]morphiceptin. The similarity between the minimum energy conformations of [D-Phe$^3$]morphiceptin with analogues **2** and **3** were found (Fig. 2a and b). The differences between [D-Phe$^3$]morphiceptin and analogues **4**–**6** were observed for the relative spatial arrangement of the aromatic side chains in position 3 (Fig. 2c–e). These results are in good agreement with the binding data presented above.

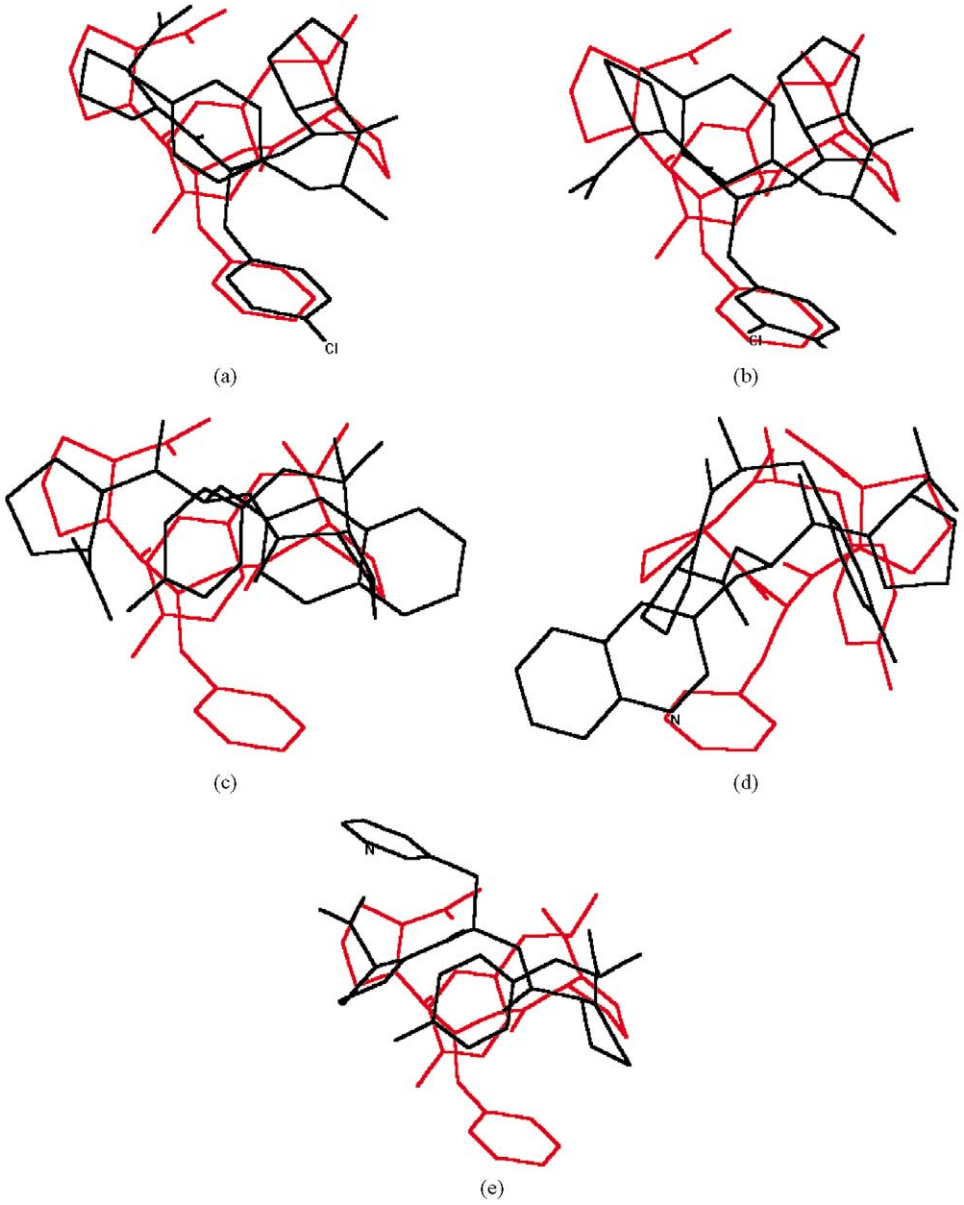


**Figure 2.** Superposition of minimum energy conformations of [D-ClPhe$^3$]morphiceptin (a), [D-$Cl_2$Phe$^3$]morphiceptin (b), [D-2-Nal$^3$]morphiceptin (c), [D-Qal$^3$]morphiceptin (d) and [D-Pal$^3$]morphiceptin (e) (black) upon [D-Phe$^3$]morphiceptin (grey) with the *cis* configuration about the Tyr-Pro amide linkage.

## Conclusions

The results obtained in this study indicate the presence of μ-opioid receptors in the experimental mouse mammary adenocarcinoma. Analogues of morphiceptin with substitutions at the pharmacophoric position 3 were found to bind in vitro and in vivo to tumor membranes and their affinity depended on the character of the aromatic side-chain in position 3. High lipophilicity of the analogues was not a sufficient factor to assure high binding affinity. Electronic and steric properties were found to be equally important. Analogues with chloro- and dichloro-substituted D-Phe in position 3 displayed the highest μ-opioid receptor affinity in both, in vitro and in vivo experiments. In contrast, substituents in position 3 with bigger aromatic moieties or heteroatoms containing rings were not advantages for affinity. Minimum energy calculations performed for chloro- and dichloro-analogues revealed good superimposing of the aromatic rings in position 3 with [D-Phe]morphiceptin, while for analogues with lower affinity to the μ-opioid receptor the differences in relative spatial arrangement were observed.

## Experimental

### Chemistry

Protected unnatural amino acids were purchased from Bachem, Torrance, CA with the exception of Boc-D-$Cl_2$Phe, which was synthesized in our laboratory according to the known procedures.[35] All peptides were synthesized by a standard solid-phase method using Boc chemistry on a methylbenzhydryl amine resin. Trifluoroacetic acid (TFA) was used for deprotection and 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoro-

borate (TBTU) was employed to facilitate coupling. The peptides were cleaved from the resin and deprotected in HF for 1 h at 0°C. Crude peptides were purified by RP HPLC on a Vydac $C_{18}$ column (1.0 × 25 cm) using the solvent system of 0.1% TFA in water (A)/80% acetonitrile in water containing 0.1% TFA (B) with a linear gradient elution of 20–90% B over 40 min. Calculated values for protonated molecular ions were in agreement with those obtained using FAB mass spectrometry.

Labeling of peptides with $^{125}I$ and $^{131}I$ was carried out by chloramine T method.[36]

### Animals

In the experiments, female C3H/Bi mice with transplantable mammary tumors were used. The animals were purchased from the Institute of Oncology, Warsaw, Poland and kept under standard conditions: temperature 22 °C, a 12 h light–dark cycle, and allowed tap water (containing 0.1% KI to minimize free radioiodide uptake by the thyroid) and rodent chow ad libitum. Mice with tumors over 0.5 cm in diameter were used for in vivo studies. Over 95% of the investigated tumors were histologically classified as adenocarcinoma.[29] All experiments on living animals were carried out according to protocol approved by the local ethical committee.

### Preparation of membranes

Brains without cerebelli from male C3H-Bi mice or tumours without necrotic fragments were weighed and homogenized in 0.32 M sucrose at 4 °C. The homogenates were centrifuged at 2000 rpm for 10 min. The supernatants were centrifuged at 19,000 rpm for 20 min. The pellets from two such centrufugations were suspended in 10 volumes of 50 mM Tris–HCl, pH 7.5 and incubated for 1 h at room temperature to remove endogenous opioid ligands. The membrane pellets from final centrifugations at 19,000 rpm for 20 min were suspended in 50 mM Tris–HCl and stored at −80 °C for up to 6 weeks. Protein concentration was determined using the Lowry method with BSA as a standard.

### Cross-linking assay

Fifty microliters of membrane proteins (1 μg/μL) were incubated at room temperature for 90 min with 10 μL of $^{125}I$-morphiceptin (about 100,000 cpm). The cross-linking agent, ethylene glycol bis(succinimidyl succinate) was added up to $10^{-4}$ M and the samples were incubated at 4 °C for 15 min. The cross-linking step was stopped by addition of the sample buffer (0.0625 M Tris–HCl, pH 6.8; 4% SDS; 50% glycerol: 20% β-mercaptoethanol and 0.25% bromophenol blue).

### Electrophoresis

The samples obtained from the cross-linking assay (20 μL) were mixed with the same volume of the sample buffer (as above), incubated for 15 min at room temperature, heated for 1 min at 95 °C and electrophoresed in 12.5% SDS-PAG in a Miniprotean II apparatus (Bio-Rad, Hercules, CA) for 40 min at 100 V. Gels were stained with Coomassie brilliant blue or electroblotted onto polyvinylidene difluoride (PVDF) membranes (Perkin-Elmer Life Sciences, Boston, MA) for Western blot and autoradiography analysis.

### Western blot analysis

Proteins transferred to PVDF membranes were blocked by incubation with 5% milk solution in TTBS (0.1 M Tris–HCl, pH 7.4 containing 0.9% NaCl and 0.1% Tween 20) for 2 h at room temperature. Then, the rabbit polyclonal anti-μ-opioid receptor antibody (Biosource Int., Camarillo, CA) at 1:100 dilution in 5% milk solution in TTBS was added and incubated with PVDF for 1 h at room temperature with constant shaking. After three 15-min washes in TTBS, the PVDF membranes were incubated for 1 h at room temperature with secondary antibody (goat anti-rabbit biotynylated IgG) at 1:300 dilution. After being washed three times in TTBS, PVDF membranes were incubated in ABC reagent (DAKO) for 30 min in the dark and developed with 0.06% diaminobenzidine and 0.009% $H_2O_2$ in 0.05 M Tris–HCl buffer, pH 7.5. When the color developed, the membranes were washed extensively with distilled water.

### Receptor binding assay

The binding of the $^{125}I$-labeled peptides was performed in 50 mM Tris–HCl buffer, pH 7.5 (0.5 mL) at a protein concentration of 0.5–1.0 mg protein. Specific binding was determined in either presence or absence of the corresponding unlabeled peptide. The entire mixture was then incubated at 25 °C for 2 h, centrifuged and the supernatants and the pellets collected. The radioactivity was counted in a Wallac gamma counter, Pharmacia-LKB, Turku, Finland.

### Accumulation of $^{131}I$-labeled peptides in the tumor

Tumor bearing mice were given intraperitoneal injections (pi) of approximately 0.3 μCi (11 kBq) of $^{131}I$-analogue (specific activity ca. 3.0 MBq/μg of peptide). The radioactivity in the tumors was determined at intervals 0.5, 2, 3, and 6 h after the injection. For each interval, four mice were used. Animals were sacrificed. Tumors were removed, weighed and radioactivity was measured in a gamma counter. The results were calculated and expressed as the mean ± SD of percentage of the injected dose per gram of tissue (%ID/g).

### Calculation of lipophilicity (logP)

The values of *n*-octanol/water partition coefficient (logP), characterizing the lipophilicity of the synthesized peptides were calculated with HyperChem 6.01 Molecular Modelling Software (Hypercube, Inc., Gainesville, FL).

### Conformational analysis

Conformational analysis was performed with the use of HyperChem 6.01 Molecular Modelling System

(Hypercube, Inc.). Initial structures were created by using standard bond lengths and bond angles. The energy convergence criterion of 0.01 kcal/mol or 1095 maximum cycles were used in minimization performed in the study. Default settings were used for all other variable parameters.

The conformational search to find low energy conformations was performed by varying specified dihedral angles.

## Acknowledgements

The work was supported by grants 502-11-812 and 502-313-2 from Medical University of Lodz, Poland.

## References and Notes

1. Thakur, M. L. *Nucl. Med. Commun.* **1995**, *16*, 724.
2. Liu, S.; Edwards, D. S.; Barrett, J. A. *Bioconjugate Chem.* **1997**, *8*, 621.
3. Fichna, J.; Janecka, A. *Bioconjugate Chem.* **2003**, *14*, 3.
4. McAfee, J. G.; Neumann, R. D. *Nucl. Med. Biol.* **1996**, *23*, 673.
5. Reubi, J. C. *Q. J. Nucl. Med.* **1997**, *41*, 63.
6. Lamberts, S. W.; Krenning, E. P.; Reubi, J. C. *Endocr. Rev.* **1991**, *12*, 450.
7. Schally, A. V. *Cancer Res.* **1988**, *48*, 6977.
8. De Jong, M.; Bakker, W. H.; Krenning, E. P.; Breeman, W. A.; van der Pluijm, M. E.; Bernard, B. F.; Visser, T. J.; Jermann, E.; Behe, M.; Powell, P.; Macke, H. *Eur. J. Nucl. Med.* **1997**, *24*, 368.
9. Zagon, I. S.; McLaughlin, E. J.; Goodman, S. R.; Rhodes, R. E. *J. Nucl. Cancer Inst.* **1987**, *79*, 1059.
10. Scholar, E. M.; Violi, L.; Hexum, T. D. *Cancer Lett.* **1987**, *35*, 133.
11. Hatzoglou, A.; Bakogeorgou, E.; Hatzoglou, C.; Martin, P. M.; Castanas, E. *Eur. J. Pharmacol.* **1996**, *310*, 217.
12. Chang, K.-J.; Killian, A.; Hazun, E.; Cuatrecasas, P.; Chang, J.-K. *Science* **1981**, *121*, 75.
13. Chang, K.-J.; Su, I. F.; Brent, D. A.; Chang, J.-K. *J. Biol. Chem.* **1985**, *250*, 9706.
14. Brantl, V.; Teschemacher, H.; Blasig, J.; Henschen, A.; Lottspeich, F. *Hoppe-Seyler's Z. Physiol. Chem.* **1979**, *360*, 1211.
15. Janecka, A.; Fichna, J.; Mirowski, M.; Janecki, T. *Mini Rev. Med. Chem.* **2002**, *2*, 565.
16. Zadina, J. E.; Hackler, L.; Ge, L. J.; Kastin, A. J. *Nature* **1997**, *386*, 499.
17. Yamazaki, T.; Ro, S.; Goodman, M.; Chung, N. N.; Schiller, P. *J. Med. Chem.* **1993**, *36*, 708.
18. Keller, M.; Boissard, C.; Patiny, L.; Chung, N. N.; Lemieux, C.; Mutter, M.; Schiller, P. *J. Med. Chem.* **2001**, *44*, 3896.
19. Yang, Y. R.; Chiu, T. H.; Chen, C. L. *Eur. J. Pharmacol.* **1999**, *372*, 229.
20. Rapaka, R. S. *Life Sci.* **1986**, *39*, 1825.
21. Maneckjee, R.; Biswas, R.; Vonderhaar, B. K. *Cancer Res.* **1990**, *50*, 2234.
22. Maneckjee, R.; Minna, J. D. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 3294.
23. Ard, M. D.; Goldstein, M. N.; Nash, D. R.; Gottlieb, D. L. *Biochem. Biophys. Res. Commun.* **1985**, *133*, 60.
24. Kampa, M.; Bakogeorgu, E.; Hatzoglou, A.; Damianaki, A.; Martin, P. M.; Castanas, E. *Eur. J. Pharmacol.* **1997**, *335*, 255.
25. Scopsi, L.; Balslev, E.; Brunner, N.; Poulsen, H. S.; Andersen, J.; Rank, F.; Larsson, L. I. *Am. J. Pathol.* **1989**, *134*, 473.
26. Maneckjee, R.; Biswas, R.; Vonderhaar, B. K. *Cancer Res.* **1990**, *50*, 2234.
27. Aylsworth, C. F.; Hodson, C. A.; Meites, J. *Proc. Soc. Exp. Biol. Med.* **1979**, *161*, 18.
28. Prevost, G.; Provost, P.; Salle, V.; Lanson, M.; Thomas, F. *Eur. J. Cancer* **1993**, *29*, 1589.
29. Mirowski, M.; Świtalska, J.; Wiercioch, R.; Byszewska, E.; Niewiadomski, H.; Michalska, M. *Nucl. Med. Commun.* **2003**, *24*, 297.
30. Chen, Y.; Mestek, A.; Liu, J.; Hurley, J. A.; Yu, L. *Mol. Pharmacol.* **1993**, *44*, 8.
31. Ambo, A.; Murase, H.; Niizuma, H.; Ouchi, H.; Yamamoto, Y.; Sasaki, Y. *Bioorg. Med. Chem. Lett.* **2002**, *25*, 879.
32. Heyl, D. L.; Mosberg, H. I. *Int. Pep. Protein Res.* **1992**, *39*, 450.
33. Mierke, D. F.; Nossner, G.; Schiller, P. W.; Goodman, M. *Int. J. Pept. Protein Res.* **1990**, *35*, 35.
34. Yamazaki, T.; Probsti, A.; Schiller, P. W.; Goodman, M. *Int. J. Pept. Protein Res.* **1991**, *37*, 364.
35. Solladie-Cavallo, A.; Schwarz, J. *Tetrahedron: Asymmetry* **1994**, *5*, 1621.
36. Greenwood, F. C.; Hunter, W. M.; Glower, J. S. *Biochem. J.* **1963**, *89*, 114.