

6-Hydroxy-1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid mimics active conformation of tyrosine in opioid peptides

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Abstract—6-Hydroxy-1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid (6Htc) has been proposed as a rigid mimic of tyrosine conformation in opioid ligand–receptor complex. The significant receptor binding to mu and delta opioid receptors of respective analogues of deltorphin, dermorphin, and endomorphin with D,L-6Htc prove initial prediction.

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1. Introduction

Tyramine moiety is a common element of opioid pharmacophore of both, alkaloids and peptides. In rigid opioid alkaloids, tyramine has a ‘frozen’ conformation. This conformation is not related to any of those that have been found for tyrosine residue in peptides or proteins. It has been postulated that opioid peptides interact with their receptors in a multistep ‘zipper’ mechanism of complementary groups fitting to receptor binding sites.¹ Adaptation of N-terminal tyrosine, especially tyramine moiety, is one of the critical steps of this mechanism. The energetically unfavored transformation of tyrosine conformation probably needs additional supporting groups in the formed peptide–receptor complex. Therefore in opioid peptides, aromatic phenylalanine has been defined as a second determinant of affinity to opioid receptors. The possible common conformation of tyramine moiety of benzomorphan alkaloids and opioid peptides in ligand–receptors complex has been evidenced indirectly through hybridization of rigid benzomorphan alkaloids with peptide C-terminal (‘address’) fragment of various selective opioid peptides that resulted in modulation of receptor selectivity of the final hybrid compounds in the same manner as the parent

peptides.² Trimethyltyrosine (TMT), which substituted Tyr(1) resulted in biologically potent analogues, possessing rotamers that well overlap with alkaloid tyramine.³ Substitution of tyrosine with rigid tyrosine analogue, 7-hydroxy-1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid (Htc) (Fig. 1), resulted in dramatic loss of affinity to mu opioid receptor types but with preservation of affinity to delta opioid receptors.⁴

To get additional data to support hypothesis of conformational similarity of tyramine in peptides and alkaloids, search for constrained amino acid analogues that may simulate benzomorphan tyramine has been performed.

The molecular modeling studies indicated that 6-hydroxy-1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid (6Htc) (Fig. 1) better than Htc simulates tyrosine in

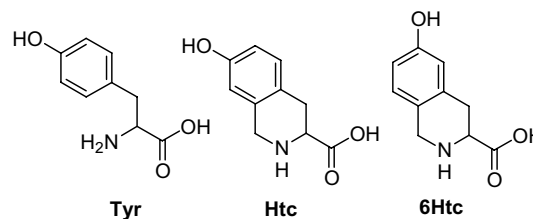


Figure 1. Molecular formulas of Tyr, Htc, and 6Htc.

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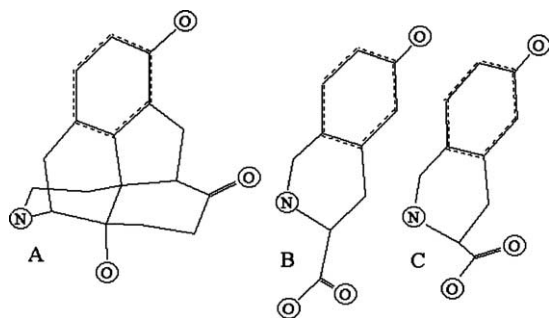


Figure 2. Comparison of overlapped conformations of oxymorphone (A), R-6Htc (B), and S-6Htc (C).

'benzomorphan related conformation' (Fig. 2). Carboxyl group of 6Htc is responsible for link of N-terminal 'tyramine like' element with the other part of opioid peptides. Flexible peptide chains express high degree of structure modification and adaptation on a stage of interactions with receptors. In the molecular simulation level of structure–activity studies of analogs with 6Htc, it was hard to predict, which stereoisomer should be introduced to peptide analogues. Therefore, in the preliminary stage of study, the analogues of opioid selective peptides with racemic 6Htc have been synthesized and their affinities to opioid mu and delta receptors have been tested.

2. Synthesis

The Pictet-Spengler reaction of formaldehyde and D,L-*meta*-tyrosine performed overnight at room temperature in water resulted in precipitation of D,L-6Htc in high (89%) yield and purity, and has been used in next steps without additional purification. The used reaction conditions were much milder than previously used.⁵ D,L-6Htc has been transformed into Boc-D,L-6Htc using standard procedure with commercial (Boc)₂O. Peptide analogues **3** and **6** have been synthesized in solid phase using Fmoc-strategy with Rink-amide resin. 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) in presence of *N*-hydroxybenzotriazole (HOBt) and *N*-diisopropylethyl amine (DIPEA) has been used for coupling reaction. Piperidine has been used for Fmoc-deprotection. Peptides have been liberated from the resin with 95% trifluoroacetic acid. Peptides **8** and **10** have been synthesized in solution step-by-step procedure using commercial Boc-amino acids and phenylalanine amide. For coupling reactions, *N,N'*-dicyclohexylcarbodiimide (DCC) in presence of HOBt and DIPEA has been used. Hydrochloride in acetic acid has been used for Boc-group deprotection. Crude peptide analogues have been purified using gel filtration on Sephadex LH-20 in methanol.

3. Crystal structure determination

The 6Htc has been transformed into hydrochloride salt and crystallized from water. The X-ray experiment was performed using Nonius BV MACH3 diffractometer. Unit cell parameters were obtained by least-squares anal-

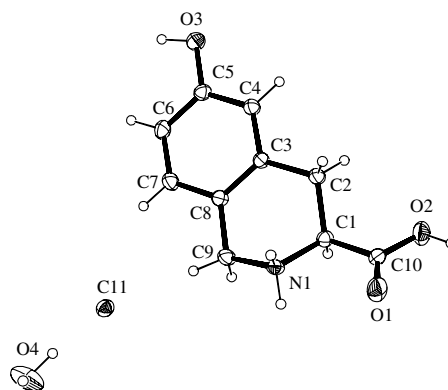


Figure 3. ORTEP diagram of D,L-6Htc hydrochloride monohydrate showing thermal ellipsoids at 30% probability level.

ysis of 15 reflections in θ range 23.1–41.3°: $a = 8.5303(4)$, $b = 6.6333(3)$, $c = 20.0341(9)$ (Å) $\beta = 90.136(3)$ (°); $V = 1133.61(9)$ (Å³) monoclinic space group $P2_1/n$, $Z = 4$, calculated density 1.445 (mg m⁻³). Final R indices for 2075 data and 170 refined parameters with $I > 2\sigma(I)$: $R_1 = 0.0473$, $wR_2 = 0.1344$. The structure (Fig. 3) was solved with SHELXS-97 and refined with SHELXL 97.⁶ Hydrogen atoms were located from $\Delta\rho$ maps (water, N, and O hydrogens) or placed in geometrically idealized positions and constrained to ride on their parent atoms, with $U_{\text{iso}}(\text{H}) = 1.2U_{\text{eq}}(\text{C})$.⁷

4. Receptor affinity measurement

Radioreceptor binding assays have been performed as described previously⁸ after obtaining permission from the Animal Rights Commission of Medical Research Centre of Polish Academy of Sciences. The binding assays were performed on adult male Wistar rats brain homogenates. [³H]Deltorphin II, and [³H]naloxone synthesized by Dr. G. Toth,⁹ Biological Institute, Hungarian Academy of Sciences, Szeged, Hungary have been used as a radioligands of delta and mu receptors, respectively. Naltrexone hydrochloride was used to define non-specific tissue binding. The data were analyzed by a nonlinear least-squares regression analysis computer program Prism Graph Pad. The obtained result are summarized in Table 1.

5. Results and discussion

Molecular modeling search for amino acid that might mimic tyramine moiety of benzomorphan opioid alkaloids resulted in selecting of 6-hydroxy-1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid (6Htc). The X-ray structure of synthetic D,L-6Htc confirmed predicted model structure. Phenol and amino groups of 6Htc and benzomorphan overlapped well. Small, but potentially significant difference in distance between phenol and amino group has been observed. It was not possible to choose between amino acid stereoisomers on the basis of available data. Therefore, for preliminary studies, the synthesis of peptide analogues with racemic 6Htc has been decided. The opioid peptide analogues with

Table 1. Opioid receptor binding of opioid peptide analogs containing D,L-6Htc

Amino acid sequence	K_i (nM)	
	Mu	Delta
1. Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH ₂	2.4	295
2. Htc-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH ₂	1615 ^a	>10,000 ^a
3. D,L-6Htc-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH ₂	253	>10,000
4. Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH ₂	1700	0.73
5. Htc-D-Ala-Phe-Asp-Val-Val-Gly-NH ₂	>10,000 ^a	304 ^a
6. D,L-6Htc-D-Ala-Phe-Asp-Val-Val-Gly-NH ₂	>10,000	10.4
7. Tyr-Pro-Phe-Phe-NH ₂	3.7	2300
8. D,L-6Htc-Pro-Phe-Phe-NH ₂	216.0	>10,000
9. Tyr-D-Ala-Phe-Phe-NH ₂	5.1	1820
10. D,L-6Htc-D-Ala-Phe-Phe-NH ₂	57.8	>10,000

^a Taken from Ref. 4.

tyrosine stereoisomers could express opposite functional properties (agonist vs antagonist). Nevertheless, their potential cross interferences should not be visible in binding assay and their values should be the sum of affinities of both enantiomers. Dermorphin (**1**), deltorphin II (**4**), endomorphin (**7**), and D-Ala²-endomorphin (**9**) have been used as a parent sequences of synthetic analogues. The obtained results of binding to opioid receptors showed that 6Htc indeed is able to simulate active conformation of tyrosine. Substitution of N-terminal tyrosine with 6Htc in delta selective peptide deltorphin II, resulted in delta selective and potent analogue **6**. Substitution of tyrosine in dermorphin, endomorphin, and D-Ala²-endomorphin, three mu selective peptides resulted in **3**, **8**, and **10** analogues that preserved high selectivity to mu receptor types. Nevertheless, the affinity levels are lower than for delta selective analogue. This, with previous data of analogues with Htc,⁴ may strongly indicate the difference in tyramine conformation requirements of delta and mu receptors. The obtained interesting preliminary results rationalize further studies of analogues with individual isomers of 6Htc.

Acknowledgments

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References and notes

- (a) Misicka, A.; Lipkowski, A. W.; Fang, L.; Knapp, R. J.; Davis, P.; Kramer, T.; Burks, T. F.; Yamamura, H. I.; Carr, D. B.; Hruby, V. J. *Biochem. Biophys. Res. Commun.* **1991**, *180*, 1290–1297; (b) Misicka, A.; Lipkowski, A. W.; Horvat, R.; Davis, P.; Kramer, T. H.; Yamamura, H. I.; Hruby, V. J. *Life Sci.* **1992**, *51*, 1025–1032.
- (a) Lipkowski, A. W.; Tam, S. W.; Portoghese, P. S. *J. Med. Chem.* **1986**, *29*, 1222–1223; (b) Lipkowski, A. W.; Misicka, A.; Porreca, F.; Davis, P.; Yamamura, H. I.; Stropova, D.; Hruby, V. J. *Lett. Pept. Sci.* **1995**, *2*, 177–181.
- Shenderovich, M. D.; Liao, S.; Qian, X.; Hruby, V. J. *Biopolymers* **2000**, *53*, 565–580.
- Tourwe, D.; Verschuere, K.; Frycia, A.; Davis, P.; Porreca, F.; Hruby, V. J.; Toth, G.; Jaspers, H.; Verheyden, P.; Van Binst, G. *Biopolymers* **1996**, *38*, 1–12.
- Ornstein, P. L.; Arnold, M. B.; Augenstein, N. K.; Paschal, J. W. *J. Org. Chem.* **1991**, *56*, 4388–4392.
- (a) Sheldrick, G. M. *SHELXS 97, Program for Crystal Structure Solution*; University of Göttingen, Germany; 1990; (b) Sheldrick, G. M. *SHELXS 97, Program for Crystal Structure Refinement*; University of Göttingen, Germany; 1997.
- X-ray parameters for crystal structure of D,L-6Htc have been deposited with the Cambridge Crystallographic Data Base, No. CCDC 232457.
- Olma, A.; Łachwa, M.; Lipkowski, A. W. *J. Pept. Res.* **2003**, *62*, 45–52.
- Buzas, B.; Toth, G.; Cavagnero, S.; Hruby, V. J.; Borsodi, A. *Life Sci.* **1992**, *50*, PL75–PL78.