APPENDIX

HELVETICA CHIMICA ACTA

1994
Volumen 77
Fasciculus
quartus
999–1002

SEPARATUM

VERLAG HELVETICA CHIMICA ACTA
NEUE SCHWEIZERISCHE CHEMISCHE GESELLSCHAFT

93. Mixed Azines of Naloxone with Dihydromorphinone Derivatives

by **Helmut Schmidhammer*** and **Felizia Kaspar**

Institute of Pharmaceutical Chemistry, University of Innsbruck, Innrain 52a, A-6020 Innsbruck

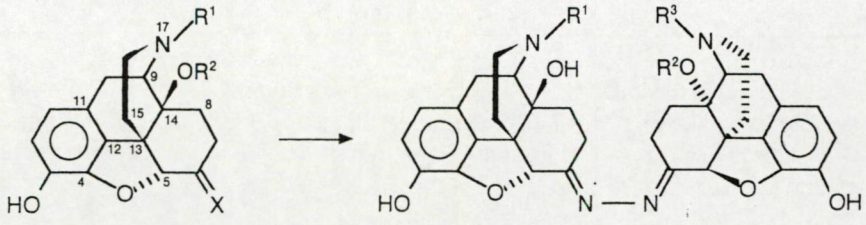
and **Arpad Marki** and **Anna Borsodi**

Institute of Biochemistry, Biological Research Center Szeged, H-6701 Szeged

(15.X.93)

The mixed azines **4** and **5** were prepared by reaction of naloxazone (**2**) with either oxymorphone (**6**) or 14-*O*-methyloxymorphone (**7**) and tested *in vitro* using opioid receptor binding assays and *in vivo* using the AcOH-writhing test in mice. Compound **4** was found to be a partial agonist, while compound **5** was a potent opioid agonist with higher potency than morphine.

Introduction. – Naloxonazine (**1**) is an opioid antagonist which blocks high affinity binding sites (μ_1 receptors) selectively and irreversibly [1–3]. Also some hydrazone derivatives of morphinan-6-ones (*e.g.* naloxazone (**2**) and oxymorphazone (**3**) [4]) block μ_1 receptors selectively and irreversibly. The pharmacological significance of μ_1 sites was evaluated extensively using naloxonazine. Treatment of either rats or mice with **1** eliminated the high affinity binding of a series of radiolabelled opioids. This loss of binding was associated with a dramatic shift of the analgesic dose-response curve to the right, implying that μ_1 sites mediate analgesia. On the other hand, μ_1 blockade did not alter the respiratory depression of morphine or most of the signs associated with morphine dependence [5]. In consideration of the μ_1 selectivity of naloxonazine and its pharmacological action, it was of interest to prepare 'mixed' azines of the opioid antagonist naloxone with opioid agonists of the dihydromorphinone series and to test these compounds biochemically and pharmacologically. We prepared the 'mixed' azine **4** of naloxone and oxymorphone and the 'mixed' azine **5** of naloxone and the highly potent opioid agonist 14-*O*-methyloxymorphone [6].



2 $R^1 = CH_2{=}CHCH_2$, $R^2 = H$, $X = NNH_2$
3 $R^1 = Me$, $R^2 = H$, $X = NNH_2$
6 $R^1 = Me$, $R^2 = H$, $X = O$
7 $R^1 = Me$, $R^2 = Me$, $X = O$

1 $R^1 = R^3 = CH_2{=}CHCH_2$, $R^2 = H$
4 $R^1 = CH_2{=}CHCH_2$, $R^2 = H$, $R^3 = Me$
5 $R^1 = CH_2{=}CHCH_2$, $R^2 = R^3{=}Me$

Chemistry. – Azines **4** and **5** were synthesized by refluxing naloxazone (**2**; prepared essentially as described in [1]) with either oxymorphone (**6**) or 14-*O*-methyloxymorphone (**7**) [6] in MeOH (*Scheme*). The novel azines were not examined regarding their *cis*- and *trans*-isomers. Such azines exist as mixtures of isomers [7] [8].

Biochemical and Pharmacological Evaluation. – Compounds **4** and **5** were evaluated *in vitro* in opioid receptor binding studies [9–11] (*Table 1*). [^{3}H]DAGO (= [D-Ala^2-MePhe^4-Gly^5-ol]enkephaline; μ-selective agonist), [^{3}H]U-69,593 (= (−)-5α,7α,8β-*N*-methyl,*N*-[7-(pyrrolidin-1-yl)cyclohexyl]benzacetamide; κ-selective agonist), and [^{3}H]deltorphin (= Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH$_2$; δ-selective agonist) were used as ligands. For *in vivo* evaluation, the AcOH-writhing test in mice[1]) was performed [17–19] (*Table 2*).

Table 1. *Opioid Receptor Binding Assays*[a])

	[^{3}H]DAGO (μ)	[^{3}H]U-69,593 (κ)	[^{3}H]Deltorphin (δ)
4	1.23 ± 0.43	17.50 ± 3.21	49.70 ± 0.46
5	0.55 ± 0.29	8.83 ± 0.74	8.48 ± 2.74
Cyprodime [12] [13]	1.14 ± 0.23	1070 ± 159	1186 ± 181
Naloxone	3[b])	40[c])	20[d])
Morphine	1[b])	80[c])	70[d])

[a]) The values are K_d in nM. [b]) [^{3}H]DHM (= dihydromorphine) was used; values from [14]. [c]) [^{3}H]EKC (= ethylketocyclazocine) was used; values from [15]. [d]) [^{3}H]DALE (= [D-Ala^2-Leu]enkephaline) was used; values from [16].

Table 2. *AcOH Writhing Test in Mice*

	Agonism test ED_{50} [μg/kg; *s.c.*][a])	Antagonism test	
		Morphine (μ) (1.25 mg/kg; *s.c.*) AD_{50} [mg/kg; *s.c.*][b])	U-50,488 (κ) (2.5 mg/kg; *s.c.*) AD_{50} [mg/kg; *s.c.*][b])
4	WP[c])	1.97	2.51
5	84	–	–
Oxymorphone (**6**)	31	–	–
6·HBr	0.48	–	–
Morphine sulfate	389	–	–
Naloxone	–	0.08	1.12

[a]) The ED_{50} values represent the effective dose at which 50% of the animals showed an analgesic response. [b]) The AD_{50} value is defined as the dose at which the antinociceptive effect of the agonist was antagonized in 50% of the animals. [c]) Weak potency: 30% inhibition of writhing at 5 mg/kg.

In opioid receptor binding, **4** and **5** did not block any of the opioid receptors irreversibly like naloxonazine. The compounds showed preference for μ receptors. The following selectivity ratios were found: compound **4**: δ/μ 40 and κ/μ 14; compound **5**: δ/μ 15 and κ/μ 16.

[1]) The tests were carried out for us at the Lilly Research Laboratories, *Eli Lilly and Company*, Lilly Corporate Center, Indianapolis, IN 46285, USA, through the courtesy of Dr. *J. D. Leander*.

In the AcOH-writhing test, compound **5** possessed considerable antinociceptive potency (*ca.* 5 times more active than morphine), while compound **4** showed only weak antinociceptive potency. Compound **4** exhibited antagonism against both morphine- and U-50,488-induced antinociception, thus representing a partial agonist.

In conclusion, the azine containing the opioid agonist with higher potency (compound **5**) showed considerable opioid agonism, while the azine with the weaker opioid agonist (compound **4**) was a partial agonist.

Experimental Part

General. M.p.: *Kofler* melting-point microscope; uncorrected. IR Spectra: in cm^{-1}; *Beckman-Accu-Lab-2* apparatus. ^{1}H-NMR Spectra: *Jeol-JNM-PMX-60* spectrometer; δ in ppm rel. to $SiMe_4$ as internal reference; J in Hz. EI-MS: *Finnigan-MAT-44S* apparatus. Elemental analyses were performed at the Analytical Department of *F. Hoffmann-La Roche AG*, Basel [^{3}H]DAGO and [^{3}H]U-69,593 were purchased from *Amersham*. [^{3}H]Deltorphin was prepared at the Biological Research Center in Szeged.

Mixed Naloxone-Oxymorphone Azine (= 4,5α-Epoxy-3,14-dihydroxy-17-(prop-2-enyl)morphinan-6-one (= 4,5α-Epoxy-3,14-dihydroxy-17-methylmorphinan-6-ylidene)hydrazone; **4**). A soln. of **2** (460 mg, 1.35 mmol) and **6** (405 mg, 1.34 mmol) in anh. MeOH (5 ml) was refluxed for 3 h. After *ca.* 45 min, **4** began to precipitate. After cooling the mixture to +4°, 732 mg (87%) of **4** were isolated. A small amount was recrystallized from EtOH for analysis. M.p. > 300° (dec.). IR (KBr): 3200 (OH), 1635 (C=N). ^{1}H-NMR ($CDCl_3$): 6.67 (*d*, J = 8, 2 arom. H); 6.52 (*d*, J = 8, 2 arom. H); 5.78 (*m*, 1 olef. H); 5.17 (*m*, 2 olef. H); 4.95 (*s*, H–C(5), H–C(5')); 2.34 (*s*, MeN). EI-MS: 624 (M^+). Anal. calc. for $C_{36}H_{40}N_4O_6$ (624.74): C 69.21, H 6.45, N 8.97; found: C 69.04, H 6.79, N 8.66.

Mixed Naloxone-(14-O-Methyloxymorphone) Azine (= 4,5α-Epoxy-3,14-dihydroxy-17-(prop-2-enyl)morphinan-6-one (4,5α-Epoxy-3-hydroxy-14-methoxy-17-methylmorphinan-6-ylidene)hydrazone; **5**). A soln. of **2** (350 mg, 1.11 mmol) and **7** (379 mg, 1.11 mmol) in anh. MeOH (4 ml) was refluxed for 4.5 h. Compound **5** began to precipitate after *ca.* 1.5 h. After cooling the mixture to +4°, 590 mg (63%) of **5** were collected. An anal. sample was prepared by recrystallization of a small portion from EtOH. M.p. > 210° (dec.). IR (KBr): 3400 (OH), 1630 (C=N). ^{1}H-NMR ($CDCl_3$): 6.56 (*d*, J = 8, 2 arom. H); 6.40 (*d*, J = 8, 2 arom. H); 5.65 (*m*, 1 olef. H); 5.10 (*m*, 2 olef. H); 4.82 (*s*, H–C(5), H–C(5')); 3.21 (*s*, MeO); 2.31 (s, MeN). Anal. calc. for $C_{37}H_{42}N_4O_6 \cdot 2\,H_2O \cdot 0.5$ EtOH (697.83): C 65.41, H 7.08, N 8.03; found: C 65.06, H 6.98, N 7.85.

Pharmacology. For AcOH-writhing tests, see [17–19]. Opioid receptor binding assays were performed using homogenates of rat brain as described in [9–11].

We are greatly indebted to Dr. *J. D. Leander, Eli Lilly & Co.*, for having provided us with the pharmacological data of the AcOH-writhing test. We thank the Analytical Department of *F. Hoffmann-La Roche AG*, Basel, for elemental analyses and Prof. Dr. *K.-H. Ongania* of the Institute of Organic Chemistry, University of Innsbruck, for performing the mass spectra. The authors wish to thank Dr. *G. Toth* for providing tritiated deltorphin and *Zs. Canjavec* for technical assistance (both at the Biological Research Center in Szeged).

REFERENCES

[1] G. W. Pasternak, E. F. Hahn, *J. Med. Chem.* **1980**, *23*, 674.
[2] E. F. Hahn, G. W. Pasternak, *Life Sci.* **1982**, *31*, 1385.
[3] E. F. Hahn, M. Carroll-Buatti, G. W. Pasternak, *J. Neurosci.* **1982**, *2*, 572.
[4] E. Varga, G. Toth, S. Benyhe, S. Hosztafi, A. Borsodi, *Life Sci.* **1987**, *40*, 1579.
[5] Y. Itzhak, in 'The Opiate Receptors', Ed. G. W. Pasternak, The Humana Press, Clifton, New Jersey, 1988, pp. 95–142.
[6] H. Schmidhammer, L. Aeppli, L. Atwell, F. Fritsch, A. E. Jacobson, M. Nebuchla, G. Sperk, *J. Med. Chem.* **1984**, *27*, 1575.
[7] V. M. Kolb, J. R. Gober, *Life Sci. Suppl.1* **1983**, *33*, 419.
[8] V. M. Kolb, D. H. Hua, *J. Org. Chem.* **1984**, *49*, 3824.

[11] B. Buzas, G. Toth, S. Cavagnero, V.J. Hruby, A. Borsodi, *Life Sci.* **1992**, *50*, PL-15.
[12] H. Schmidhammer, W.P. Burkard. L. Eggstein-Aeppli, C.F.C. Smith, *J. Med. Chem.* **1989**, *32*, 418.
[13] H. Schmidhammer, C.F.C. Smith, D. Erlach, M. Koch, R. Krassnig, W. Schwetz, C. Wechner, *J. Med. Chem.* **1990**, *33*, 1200.
[14] G. Toth, M. Kramer, M. Szücs, S. Benyhe, F. Sirokman, *Radiochem. Radioanal.* **1982**, *56*, 209.
[15] S. Benyhe, A. Borsodi, M. Wollemann, *Life Sci.* **1992**, *51*, 1647.
[16] S. Benyhe, G. Toth, J. Kevei, M. Szücs, A. Borsodi, K. Di Gleria, J. Szecsi, E. Varga, K. Medzihradszky, *Neurochem. Res.* **1985**, *10*, 627.
[17] D.M. Zimmerman, J.D. Leander, J.K. Reel, M.D. Hynes, *J. Pharmacol. Exp. Ther.* **1987**, *241*, 374.
[18] J.D. Leander, P.D. Gesellchen, L.G. Mendelsohn, *Pharmacol. Biochem. Behav.* **1988**, *29*, 351.
[19] H. Schmidhammer, J.B. Deeter, N.D. Jones, J.D. Leander, D.D. Schoepp, J.K. Swartzendruber, *Helv. Chim. Acta* **1988**, *71*, 1801.

Regulatory Peptides

AN INTERNATIONAL JOURNAL

VOLUME 54 (1994)

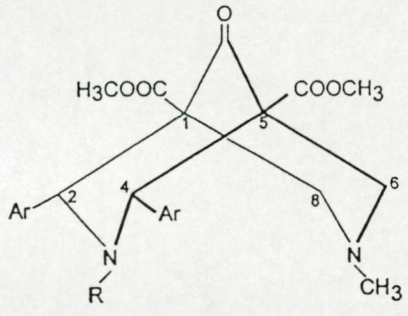
ELSEVIER
AMSTERDAM – LAUSANNE – NEW YORK – OXFORD – SHANNON – TOKYO

STRUCTURALLY NOVEL GROUP OF LIGANDS SELECTIVE FOR KAPPA OPIOID RECEPTORS

Borsodi A, Benyhe S, Holzgrabe* U, Márki Á and Nachtsheim* C, Institute of Biochemistry, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary; *Institute of Pharmacy, University of Bonn, Federal Republic of Germany.

The heterocyclic bicyclo [3.3.1]nonan-9-ones were found to be κ-opioid receptor selective agonists. This novel class of ligands exhibits relatively high affinity for [^{3}H]U-69593 binding sites both in guinea pig cerebellar and in rat brain membranes. It was shown by molecular modelling that heterocyclic bicyclo [3.3.1]nonan-9-ones fit very well with the structure of ketazocine; when compared with the structure of U-69593 a similar geometry was found with a slightly different distribution of the charges. It is postulated, that the essential structure involved in the opioid activity is an aryl-propyl-amine element distributed along the N7-C6-C5-C4-aryl bonds.

A series of compounds with a heterocyclic bicyclo[3.3.1]nonan-9-one skeleton was synthesized in order to examine their potency of labeling CNS opioid receptors. Two of them (designated as compound **a** and **b**; Fig. 1.) were characterized by means of radioligand binding assays and of molecular modelling.



a Ar = 2-pyridyl
R = H

b Ar = 2-chlorophenyl
R = CH_3

a dimethyl-7-methyl-2,4-di-2-pyridyl-3,7-diazabicyclo[3.3.1.]nonan-9-one-1,5-dicarboxylate

b dimethyl-3,7-dimethyl-2,4-di-2-chlorophenyl-3,7-diazabicyclo[3.3.1]nonan-9-one-1,5-dicarboxylate

Fig. 1. Structures and systematic names of compounds **a** and **b**:

Membrane fractions from guinea pig cerebella, rat forebrain, and frog brain were prepared as described previously [1]. The brain tissues were homogenized in Tris-HCl buffer (50 mM, pH 7.4 at 4 °C) and centrifuged (25,000 x g, 20 minutes, 4 °C). The pellets were resuspended in the same buffer containing 0.3 M sucrose. The membranes were frozen in liquid nitrogen and stored at -70 °C until use. Standard radioreceptor binding assays were performed with [^{3}H]U-69593 (47 Ci/mmol; N.E.N) and [^{3}H]EKC (20 Ci/mmol; N.E.N.). Washed membranes, diluted with buffer, (0.4 mg protein in a final volume of 1 ml), were incubated with 1 nM radioligand (30 min, 30 °C, U-69593; or 40 min 24 °C, EKC), in the presence of a wide concentration range of compounds tested for opioid binding. Nonspecific binding was determined with 10 μM naloxone. Samples were filtered on Whatman GF/B filters using a Brandel M24R harvester. Radioactivity was determined by liquid scintillation counting. Data were evaluated by the computer program LIGAND [2].

Molecular modelling calculations were based on the free bases. Starting geometries were generated from X-ray data followed by optimization of the conformations by means of force field (MMX2; PCModel Serena Software, Bloomington, Ind., USA). Semiempirical calculations were carried out using MOPAC (Program no.501, QCPE Bloomington, Ind., USA; on an IBM 3084/3081). Molecular graphics studies were carried out on a PS390/microVAX system (Evans & Sutherland/Digital Equipment Corporation) using SYBYL software (Tripos Associates, St. Louis, Mo., USA). Molecules were fitted using the fit

option as well as the multifit algorithm of SYBYL, which includes an energy minimization of each molecule.

Opioid receptor binding properties of the ligands were tested in particulate membrane fractions. Membranes were labelled either with the agonist-antagonist [^{3}H]EKC, which is considered to be a κ_2 ligand, or with the pure κ_1-agonist [^{3}H]U-69593. The results are shown in Table 1. In guinea pig cerebellar membrane, which is a rich source of κ_1 receptors, compound **a** competes for the [^{3}H]U-69593 and [^{3}H]EKC binding with a K_i of 8.3 and 6.8 nM, whereas it has considerably lower affinity in frog brain. Compound **b** seems to have substantially lower affinities, especially in the case of [^{3}H]EKC binding. However, the selectivity of compound **b** for the κ_1 site is about ten times higher than that of compound **a**. The results also suggest, that compounds **a** and **b** have low affinity at κ_2 receptor sites found predominantly in frog brain [1]. In guinea pig cerebellar membranes, the competition of compound **a** for specific [^{3}H]EKC binding sites was also examined in the presence of NaCl (100 mM) and GppNHp (100 μ M). In both cases, a significant decrease in the affinity was observed, providing biochemical evidence for the opiate agonist property of the ligand.

Table 1. Binding affinity constants for heterocyclic bicyclo[3.3.1]nonan-9-ones in different tissues

	K_i (nM)	
	compound **a**	compound **b**
[^{3}H]U-69593; guinea pig	8.3 ± 0.7	88.3 ± 27
[^{3}H]U-69593; rat	N.D.	410 ± 162
[^{3}H]EKC; guinea pig	6.8 ± 1.7	23,000 ± 2,500
[^{3}H]EKC; rat	N.D.	35,900 ± 2,100
[^{3}H]EKC; frog	196 ± 53	38,000 ± 6,000

Studies of the structure of 2,6-diarylsubstituted 3-oxa-7-aza- and 3,7-diazabicyclo[3.3.1]nonan-9-ones revealed a chair-chair conformation from both crystals and semiempirical calculations [3]. The calculated chair-chair conformation is similar to the geometry found by X-ray analysis [4]. Because the geometries of the heterobicyclic compounds are nearly the same in crystal, solution, and in gaseous state, it is likely, that this conformation is the pharmacologically effective one. The chair-chair conformation of compound **a** showed the best fit with ketazocine when the most favorable conformations were minimized and superimposed with each other. Minimization of compound **a** as well as U-69593 against ketazocine, using the MULTIFIT option of the SYBYL program package (Tripos Assoc.), also shows that compound **a** fits with ketazocine very well and is energetically more favorable than the fit obtained from U-69593 and ketazocine.

From our results it is postulated that the essential structure responsible for the opioid character of these compounds is an aryl-propyl-amine element distributed along the N7-C6-C5-C4-aryl bonds. Comparison of this part of the molecule **a** with the same moiety in ketazocine and U-69593 by means of molecular modelling shows a similar geometry with a slightly different distribution of charges. We conclude, that the discussed group of compounds are of theoretical importance, since it is a completely new chemical structure exhibiting opioid activity. In addition, these unusual compounds, which initially appear to be structurally different from the κ-agonists known until now, should be very promising in developing new highly active κ_1 ligands. Pharmacological analysis of these compounds are also in progress.

REFERENCES

1 S. Benyhe, E. Varga, J. Hepp, A. Magyar, A. Borsodi and M. Wollemann (1990) Neurochem. Res. 15, 899-904.
2 P.J. Munson and D. Rodbard (1981) Anal. Biochem. 107, 220-239.
3 U. Ashauer-Holzgrabe and T. Busch (1988) Z. Naturforsch. 43, 873-77.
4 R. Caujolle, P. Castera, and A. Latters (1984) Z. Kristallographie 166, 81-95.

Pergamon

Bioorganic & Medicinal Chemistry, Vol. 5, No. 2, pp 369–382, 1997

PII: S0968-0896(96)00257-X
0968-0896/97 $17.00+0.00

New Nepenthone and Thevinone Derivatives†‡

János Marton,[a] Csaba Simon,[a] Sándor Hosztafi,[a] Zoltán Szabó,[a] Árpád Márki,[b] Anna Borsodi[b] and Sándor Makleit[c,*]

[a]*Alkaloida Chemical Company Ltd., P.O. Box 1, Tiszavasvári, H-4440 Hungary*
[b]*Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, Post Office Box 521, Szeged, Hungary*
[c]*Department of Organic Chemistry, Lajos Kossuth University, Post Office Box 20, Debrecen, H-4010 Hungary*

Abstract—The diastereoselective reaction of thevinone (**2a**) and nepenthone (**2c**) and their dihydro derivatives (**2b** and **d**) with Grignard reagents afforded new N-substituted (20*S*)- and (20*R*)-phenyl-6,14-ethenomorphinan derivatives (**6a–y**). The Grignard reaction of the N-substituted-*N*-demethyl derivatives **4a–f** and **4m–r** with methylmagnesium iodide resulted in the (20*R*)-phenyl tertiary alcohols **5a–f** and **5m–r**, respectively, but the conversion of **4g–l** and that of the *N*-substituted-dihydrothevinone derivatives with phenylmagnesium bromide afforded the (20*S*)-phenyl derivatives **5g–l** and **5s–y**, respectively. The *N*-cyclopropylmethyl-, *N*-β-phenylethyl-, and *N*-propyl derivatives were prepared by the 3-O-demethylation of compounds **5**. For the synthesis of the *N*-allyl-, *N*-dimethylallyl-, and *N*-propargyl compounds **2a–d** were reacted with the corresponding Grignard reagent, and treatment of the products with cyanogen bromide gave the cyanamides **8a–d**. These latter compounds were transformed into **10a,b,d**, whose alkylation led to the target derivatives **6d–f, j–l, p–r**, and **w–y**. The biochemical investigation of these substances showed that the affinities to the δ-opioid receptors were high, but the selectivity was low. In two cases (**6c** and **11d**) a μ-opioid receptor specificity was observed.

Introduction

Study of 6,14-ethenomorphinans is one of the most promising fields of research of morphine alkaloids, as demonstrated by the discovery and development of the agonist etorphine (**1a**), the antagonist diprenorphine (**1b**), and the mixed agonist–antagonist buprenorphine (**1c**). Most recently, a selective binding of dihydroetorphine to the μ-opioid receptors has also been reported.[1]

The present work was aimed at the synthesis of selective opioid derivatives belonging to the group of the so-called Bentley compounds, carrying a bulky phenyl group at position C-20. The tertiary alcohol, prepared by Bentley et al.[2] from thevinone by treatment with phenylmagnesium bromide and subsequent 3-O-demethylation, was found to be 34 times as active as morphine in *tail pressure* tests on rats. Bentley and his associates[3] supposed that the reactions of thevinone (**2a**) and its analogues with Grignard reagents proceed via six-membered chelate intermediates. Compound **7a** derived as stereochemically homogeneous from the 7α-benzoyl compound **2c** and methyllithium, and the Grignard adduct **7b** available from the 7α-acetyl derivative with phenylmagnesium bromide are in diastereoisomeric relation[4] [(20*R*) and (20*S*), respectively].

By utilizing the advantages in the mechanism of the Grignard reaction we have prepared a series of new (20*S*) and (20*R*)-phenyl-6,14-ethenomorphinan derivatives from thevinone (**2a**) and nepenthone (**2c**). Nepenthone (**2c**) was obtained according to a method published in the literature,[5] and dihydronepenthone was first synthesized by our group.[6]

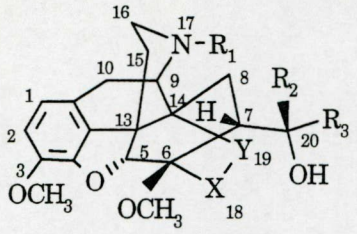
Chemistry

The target compounds were prepared by various independent procedures. N-Demethylation of thevinone (**2a**), dihydrothevinone (**2b**), nepenthone (**2c**), and dihydronepenthone (**2d**) with diethyl azodicarboxylate (DEAD) in benzene gave rise to compounds **3a–d**, whose N-alkylation in *N*,*N*-dimethylformamide in the presence of sodium hydrogen carbonate led to the desired N-substituted-*N*-demethyl derivatives **4a–r** with high yields.[7,8]

Treatment of **4a–f** and **4m–r** with methylmagnesium iodide afforded the N-substituted tertiary alcohols **5a–f** and **5m–r**, respectively, with (20*R*) absolute configuration.

Compounds **4g–l** and the N-substituted derivatives of dihydrothevinone[7,8] were reacted with phenylmagnesium bromide to obtain, in accordance with the mechanism of the Grignard reaction, the (20*S*)-tertiary

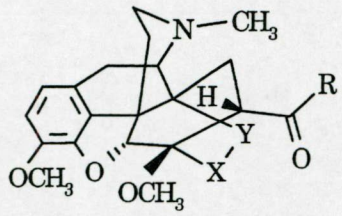
†Morphine alkaloids Part 137. For Part 136 see: Hosztafi, S.; Makleit, S. Synthesis of new apomorphine derivatives containing halogen (Cl, Br) in ring-D (*Synth. Commun.* **1996**, *26*, 3909).
‡This work was a part of the Ph.D. Thesis: Marton, J. Diels–Alder reaction of morphinandienes L. Kossuth University, Debrecen, Hungary, 1995.
Key words: thevinone; nepenthone; 6,14-ethenomorphinans; μ-opioid receptor specificity.



1

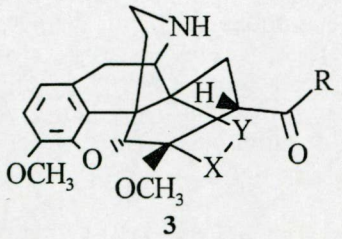
1	R_1	R_2	R_3	X-Y	
a	CH_3	n-Pr	CH_3	CH=CH	Etorphine
b	CPM	CH_3	CH_3	CH_2-CH_2	Diprenorphine
c	CPM	*t*-Bu	CH_3	CH_2-CH_2	Buprenorphine

alcohols **5g–l** and **5s–y**, respectively. The Grignard reactions of the 7α-benzoyl compounds with methylmagnesium iodide, and those of the 7α-acetyl derivatives with phenylmagnesium bromide were carried out with high diastereoselectivity; the diastereoisomeric pair of the product could only be detected or isolated in a very few cases. The major product of the reaction



2

2	R	X-Y
a	CH_3	CH=CH
b	CH_3	CH_2-CH_2
c	Ph	CH=CH
d	Ph	CH_2-CH_2

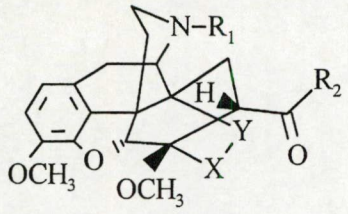


3

3	X-Y	R
a	CH=CH	CH_3
b	CH_2-CH_2	CH_3
c	CH=CH	Ph
d	CH_2-CH_2	Ph

of nepenthone (**2c**) with methylmagnesium iodide was **7a**, but by column chromatography of the mother liquor 5% of **7b** could also be isolated. Based on our experiences in the field of 6,14-ethenomorphinanes, 3-O-demethylation with the KOH/diethylene glycol system was accomplished only in the case of the *N*-cyclopropylmethyl, *N*-(β-phenylethyl), and *N*-(n-propyl) derivatives to afford compounds **6** with 42–63% yield.

The other route employed was the von-Braun reaction leading to N-demethylation, as well. The starting ketones **2a–d** were first reacted with methylmagnesium iodide or phenylmagnesium bromide and the resulting tertiary alcohols (**7a–d**) were treated with cyanogen bromide in chloroform to furnish the cyanoamides **8a–d**. The reaction of these latter compounds with potassium hydroxide gave the *N*-demethyl derivatives **9a–d**, which were then alkylated giving rise to the N-substituted compounds **5a–y** with good yields. A clear advantage of this procedure is that hydrolysis of the cyanoamide and O-demethylation can be carried out as a one-pot procedure, permitting the preparation of **10a,b,d**, which are suitable for converting into derivatives (**6d–f**, **j–l**, **w–y**) containing allyl, 3,3-dimethylallyl and propargyl N-substituents. The above procedure gave **7c**, **8c**, and **9c** but the 3-O-demethylation failed. Treatment of **8c** with KOH in diethylene glycol at 210 °C resulted in an uncontrollable decomposition of the educt. Attempted 3-O-demethylation of **5m–r** with various methods reported for the cleavage of aryl-methyl ethers (i.e. nPrSH/NaH/DMF and diphenyl-phosphine/BuLi) failed, and thus no suitable method for the preparation of the (20*R*) derivatives **6m–r** could be found. 3-O-Demethylation of **5a** and **c** with KOH/diethylene glycol resulted in **12b** and **d** in a longer reaction time (2 h). For structural assignment **5c** was converted with formic acid[9,10] into **12a**. In the ^{1}H NMR spectra of **12b–c** the signal characteristic of the 3-OCH_3 function was missing, but otherwise the spectra were similar to that of **12a**. The presence of the C-6 keto group in these molecules was assigned according to the IR spectra. Thus, the tertiary alcohols **5** undergo rearrangement accompanied by



4

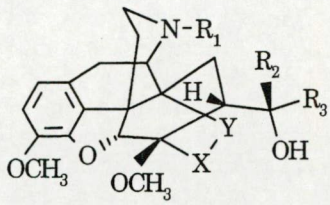
4	X-Y	R_1	R_2
a	CH=CH	CPM	Ph
b	CH=CH	β-Phe	Ph
c	CH=CH	n-Pr	Ph
d	CH=CH	Allyl	Ph
e	CH=CH	diMe-Allyl	Ph
f	CH=CH	Propargyl	Ph
g	CH=CH	CPM	CH_3
h	CH=CH	β-Phe	CH_3
i	CH=CH	n-Pr	CH_3
j	CH=CH	Allyl	CH_3
k	CH=CH	diMe-Allyl	CH_3
l	CH=CH	Propargyl	CH_3
m	CH_2-CH_2	CPM	Ph
n	CH_2-CH_2	β-Phe	Ph
o	CH_2-CH_2	n-Pr	Ph
p	CH_2-CH_2	Allyl	Ph
q	CH_2-CH_2	diMe-Allyl	Ph
r	CH_2-CH_2	Propargyl	Ph

elimination of water under both acidic and alkaline conditions.

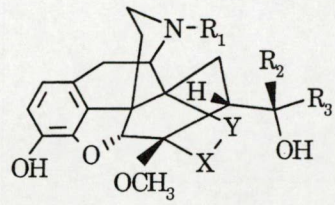
Materials and Methods

Membrane preparation

The rat membrane preparation was according to Pasternak[11] with a small modification (40 000 *g* and Braun Teflon-glass homogenizer instead of 49 000 *g* and Polytron homogenizer, respectively). Rats (Wistar strain) were killed by decapitation. The brains without cerebellum were removed and then homogenized in 20 volumes (wt/vol) of ice-cold buffer (Tris–HCl 50 mM, pH 7.4) with Braun Teflon-glass homogenizer were filtered on four layers of gauze and centrifuged with Sorvall RC5C centrifuge (40 000 *g* 4 °C 20 min). The pellet was resuspended in buffer (50 mM Tris–HCl, pH 7.4) and incubated (37 °C, 30 min). Centrifugation was repeated. The pellet was resuspended in 5 × volumes of buffer (50 mM Tris–HCl, 0.32 M sucrose

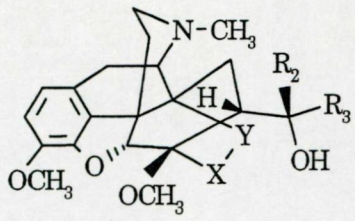


5

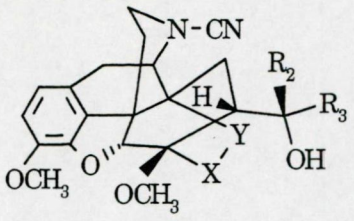


6

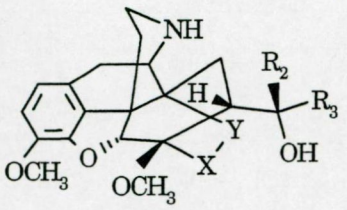
5-6	X-Y	R_1	R_2	R_3
a	CH=CH	CPM	Ph	CH_3
b	CH=CH	β-Phe	Ph	CH_3
c	CH=CH	n-Pr	Ph	CH_3
d	CH=CH	Allyl	Ph	CH_3
e	CH=CH	diMe-Allyl	Ph	CH_3
f	CH=CH	Propargyl	Ph	CH_3
g	CH=CH	CPM	CH_3	Ph
h	CH=CH	β-Phe	CH_3	Ph
i	CH=CH	n-Pr	CH_3	Ph
j	CH=CH	Allyl	CH_3	Ph
k	CH=CH	diMe-Allyl	CH_3	Ph
l	CH=CH	Propargyl	CH_3	Ph
m	CH_2-CH_2	CPM	Ph	CH_3
n	CH_2-CH_2	β-Phe	Ph	CH_3
o	CH_2-CH_2	n-Pr	Ph	CH_3
p	CH_2-CH_2	Allyl	Ph	CH_3
q	CH_2-CH_2	diMe-Allyl	Ph	CH_3
r	CH_2-CH_2	Propargyl	Ph	CH_3
s	CH_2-CH_2	CPM	CH_3	Ph
t	CH_2-CH_2	β-Phe	CH_3	Ph
v	CH_2-CH_2	n-Pr	CH_3	Ph
w	CH_2-CH_2	Allyl	CH_3	Ph
x	CH_2-CH_2	diMe-Allyl	CH_3	Ph
y	CH_2-CH_2	Propargyl	CH_3	Ph



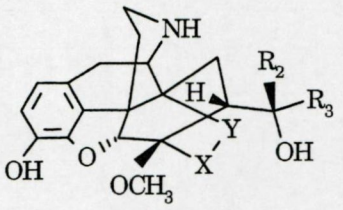
7a-d



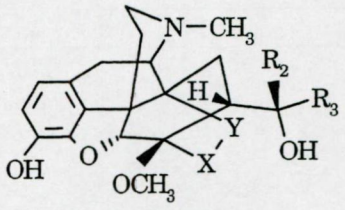
8a-d



9a-d



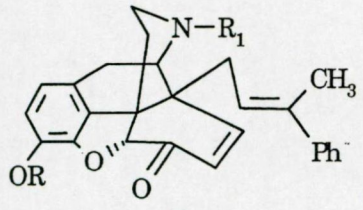
10-a-d



11a-d

7-11	X-Y	R_2	R_3	C-20
a	CH=CH	Ph	CH_3	R
b	CH=CH	CH_3	Ph	S
c	CH_2-CH_2	Ph	CH_3	R
d	CH_2-CH_2	CH_3	Ph	S

pH 7.4). The membranes were stored at −70 °C. Before the using membranes were diluted and centrifuged (40 000 *g*, 4 °C, 20 min) and then the pellet was resuspended in 50 mL buffer (200–300 μg/mL protein). The protein concentration was determined according to Bradford.[12]



12

12	R_1	R
a	n-Pr	CH_3
b	CPM	H
c	n-Pr	H

Receptor binding assays

The frozen membranes were thawed at room temperature and centrifuged in 50 mM Tris–HCl buffer (40 000 *g*, 20 min, 4 °C). Ligand binding experiments were carried out in 50 mM Tris–HCl buffer (pH 7.4) at a final volume of 1 mL containing 100 μL radioligand, 100 μL unlabelled ligand and about 0.3–0.5 mg protein. When [^{3}H]EKC was applied 100 μL DADLE–DAMGO (10–5 M) mixture was used to block μ and δ opioid receptors. In the receptor binding assays the following tritiated ligands were used: [^{3}H]deltorphin II (20 Ci/mmol, Isotope Lab. BRC),[13] [^{3}H]DAMGO (59 Ci/mmol, Amersham),[14] and [^{3}H]EKC (15 Ci/mmol, New England Nuclear) (Table 1). Incubations were started by addition of membrane suspension and continued in a shaking water bath until steady-state was achieved. The reaction was terminated by rapid filtration on Brandel M24 R cell harvester through Whatmann GF/C or GF/B filters and washed with 3 × 5 mL of ice-cold Tris–HCl (pH 7.4) buffer. The filters were dried at 37 °C in the heating room, and the bound radioactivity was determined in a toluene based scintillation cocktail in Beckman 5000 TD spectrophotometer.

Table 1. Experimental procedures of receptor binding assay

Labelled compound	Specific radioactivity (Ci/mmol)	Temperature (°C)	Incubation time (min)	Concentration (nM)	Filter type
[^{3}H]DAMGO[a]	60	35	45	0.5	C
[^{3}H]EKC[b]	15	24	40	1.5	B
[^{3}H]DT[c]	20	35	45	2.0	C

[a]DAMGO: (D-Ala^2-(Me)Phe^4-Gly^5-ol)enkephalin.
[b]EKC: Ethylketocyclazocine.
[c]DT: Tyr-D-Ala-Phe-Glu-Val-Gly-NH_2.

The total binding was defined as that measured in the absence of a competing agent. Nonspecific binding was determined in the presence of 10 μL unlabelled naloxone. All assays were carried out at least three times in duplicate. Competition data were analyzed by the LIGAND 3.1.4.[15] program utilizing a nonlinear least squares fitting algorithm.

Results

Biochemistry

First, the delta receptor affinity of compounds were examined with labelled deltorphin II. In that case when K_i values were less then 100 nM further competition studies were carried out with [^{3}H]DAMGO (μ opioid receptor agonist) and [^{3}H]EKC (κ opioid receptor agonist). Only four compounds (**6h**, **6b**, **5o**, and **5p**) showed K_i values greater than 100 nM. The remaining six ligands possess high affinity (K_i: 5–60 nM) toward the delta, mu, and kappa opioid receptors as well (Table 2). These ligands did not show any opioid receptor type selectivity except **6c** (δ/μ 15.0, κ/μ 5.0) and **11d** (δ/μ 17.7, κ/μ 17.0), which are somewhat mu selective ligands.

Experimental

Melting points (uncorrected) were measured with a Büchi-apparatus in open capillary tubes. The purity of the synthesized compounds were checked by TLC. For column chromatography Kieselgel 60 (particle size 0.063–0.2 mm) was employed with an eluent system $CHCl_3$: MeOH (9:1). The optical rotation values were measured with a Polmat-A (Zeiss, Jena) polarimeter.

^{1}H NMR spectra were recorded with a Varian-Gemini-200 instrument at 20 °C in $CDCl_3$ (or in the case of **10** in DMSO-d_6). For the ^{1}H NMR examinations TMS (δ = 0.00 ppm) was used as the internal standard. In the cases of **12a–c** the proton and carbon assingnments are based on COSY-45 and HETCOR experiments. Abbreviations: cProp: protons of the cyclopropyl ring; Ar: aromatic protons; Ph: protons of the C-20-phenyl group; All: protons of the allyl or 3,3-dimethyallyl groups; Prop: protons of the propargyl group; [a]: deuterable. Mass spectra were obtained with a VG Trio-2 instrument by using the EI method (70 eV), or occasionally by means of the 'thermospray technique' (TSP).

The elemental analyses were carried out at the Department of Organic Chemistry, Lajos Kossuth University with a Carlo Erba automatic analyser.

Table 2. Opioid receptor binding results

Unlabelled compounds	K_i (nM)		
	[^{3}H]DT II	[^{3}H]DAMGO	[^{3}H]EKC
6h	262.8	—	—
6b	106.4	—	—
6v	14.8	1.0	1.7
6c	61.4	4.1	20.7
11d	5.3	0.3	5.1
11c	7.2	2.8	10.7
6i	38.9	7.2	8.3
5o	280.5	—	—
5p	194.5	—	—
5m	32.5	27.5	35.5

Typical procedure for the preparation of compounds 3a–c

To a soln of the *N*-methyl derivatives **3a–c** (17.3 mmol) in abs benzene (89 mL) diethyl azodicarboxylate (5.1 mL) was added, the mixture was heated under reflux for 7 h and then evapd. The residue was dissolved in EtOH (56 mL), pyridinium chloride (1.8 g) added and the reaction mixture stirred for 8 h at room temperature. The hydrochloride that precipitated was filtered off. It was washed with cold EtOH and air dried.

3a [*N*-demethylthevinone]. Yield 68%, mp 347–348 °C [HCl] [EtOH] (Lit.[2]: 350 °C). ^{1}H NMR ($CDCl_3$): δ 1.40 (dd, 1H, 8α-H), 3.62 (s, 1H, 6-OCH_3), 3.78 (s, 3H, 3-OCH_3), 4.57 (d, 1H, 5β-H), 5.56 (d, 1H, 19-H), 5.92 (dd, 1H, 18-H), 6.54 (d, 1H, 1-H), 6.67 (d, 1H, 2-H). MS (EI 70 eV) *m/z* 367 (100) [M^+]. $[\alpha]_D^{25}$ [HCl] −217.0° (H_2O, *c* 0.5). $C_{22}H_{25}NO_4$ (367.4).

3b [*N*-demethylnepenthone]. Yield 71%, mp 238–240 °C [EtOH]. ^{1}H NMR ($CDCl_3$): δ 1.50 (dd, 1H, 8α-H), 3.45 (s, 1H, 6-OCH_3), 3.83 (s, 3H, 3-OCH_3), 4.64 (d, 1H, 5β-H), 5.54 (d, 1H, 19-H), 6.12 (dd, 1H, 18-H), 6.53 (d, 1H, 1-H), 6.65 (d, 1H, 2-H), 7.40–8.02 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 429 (58), 148 (100). $[\alpha]_D^{25}$ [HCl] −206.0° (H_2O, *c* 0.5). $C_{27}H_{27}NO_4$ (429.5) calcd C 75.50; H 6.34; N 3.26; Found C 75.58; H 6.30; N 3.17.

3c [*N*-demethyldihydronepenthone]. Yield 82% [HCl] mp 151–152 °C [hexane]. ^{1}H NMR ($CDCl_3$): δ 0.80 (m, 1H, 8α-H), 3.25 (s, 3H, 6-OCH_3), 3.88 (s, 3H, 3-OCH_3), 4.54 (d, 1H, 5β-H), 6.63 (d, 1H, 1-H), 6.76 (d, 1H, 2-H), 7.40–8.05 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 431 (5) [M^+], 104 (100). $[\alpha]_D^{25}$ −111.0° ($CHCl_3$, *c* 0.1). $C_{27}H_{29}NO_4$ (431.5) calcd C 75.15; H 6.77; N 3.25; Found C 75.20; H 6.85; N 3.18.

General method for the preparation of compounds 4a–r

The hydrochloride salts of the *N*-demethyl derivatives (*N*-demethyl-dihydrothevinone[7] and **3a–c**; 7.4 mmol) were converted into the syrupy free bases and dissolved in abs DMF (20 mL). To this soln $NaHCO_3$ (1.9 g), and 11.1 mmol of the *N*-alkylating agent (cyclopropylmethyl bromide, β-phenylethyl bromide, *n*-propyl bromide, allyl bromide, 3,3-dimethylallyl bromide and propargyl bromide) were added and the reaction mixture stirred at 90 °C (oil-bath) for 20 h. After filtration of the inorganic salts the filtrate was concd, the residue suspended in water, alkalized with 25% aq NH_4OH and extracted with $CHCl_3$. The combined organic layer was washed with water, dried over

Na_2SO_4 and concd. The residual crude product was crystallized from the appropriate solvent.

(5*R*,6*R*,7*S*,9*R*,13*S*,14*R*)-7-Benzoyl-4,5-epoxy-3,6-dimethoxy-17-substituted-6,14-ethenomorphinan derivatives (4a–f) [*N*-demethyl-N-substituted-nepenthone derivatives]

4a: Yield 74%, mp 136–137 °C [EtOH]. ^{1}H NMR ($CDCl_3$): δ 0.13–0.83 (m, 5H, cProp), 1.50 (dd, 1H, 8α-H), 3.48 (s, 3H, 6-OCH_3), 3.85 (s, 3H, 3-OCH_3), 4.70 (d, 1H, 5β-H), 5.72 (d, 1H, 19-H), 6.13 (dd, 1H, 18-H), 6.54 (d, 1H, 1-H), 6.67 (d, 1H, 2-H), 7.43–8.04 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 483 (14) [M^+], 105 (100). $[\alpha]_D^{25}$ −247.0° ($CHCl_3$, *c* 1). $C_{31}H_{33}NO_4$ (483.6) calcd C 76.99; H 6.88; N 2.90; Found C 76.91; H 6.93; N 2.83.

4b: Yield 83%. The residual crude product was dissolved in EtOH and the pH adjusted to 2 with satd HCl/EtOH. The solvent was evapd and the residue treated with Et_2O. The produced hydrochloride salt was filtered off, mp 221–223 °C [HCl] [diethylether]. ^{1}H NMR ($CDCl_3$): δ 1.30 (dd, 1H, 8α-H), 3.47 (s, 3H, 6-OCH_3), 3.82 (s, 3H, 3-OCH_3), 4.65 (d, 1H, 5β-H), 5.53 (d, 1H, 19-H), 6.10 (dd, 1H, 18-H), 6.53 (d, 1H, 1-H), 6.65 (d, 1H, 2-H), 7.05–7.35 (m, 5H, Ar), 7.40–8.00 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 442 (40) [M-91]$^+$, 105 (100). $[\alpha]_D^{25}$ −151.5° ($CHCl_3$, *c* 1). $C_{35}H_{35}NO_4$ (533.6) calcd C 78.77; H 6.61; N 2.62; Found C 78.68; H 6.70; N 2.68.

4c: Yield 55%, mp 129–130 °C [EtOH]. ^{1}H NMR ($CDCl_3$): δ 0.90 (t, 3H, $CH_3CH_2CH_2$), 1.45 (m, 1H, 8α-H), 3.47 (s, 3H, 6-OCH_3), 3.83 (s, 3H, 3-OCH_3), 4.68 (d, 1H, 5β-H), 5.57 (d, 1H, 19-H), 6.10 (dd, 1H, 18-H), 6.53 (d, 1H, 1-H), 6.64 (d, 1H, 2-H), 7.40–8.02 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 471 (13) [M^+], 105 (100). $[\alpha]_D^{25}$ −230.8° ($CHCl_3$, *c* 1). $C_{30}H_{33}NO_4$ (471.6) calcd C 76.41; H 7.05; N 2.97; Found C 76.36; H 7.12; N 3.05.

4d: Yield 69%, mp 104–105 °C [EtOH]. ^{1}H NMR ($CDCl_3$): δ 1.45 (dd, 1H, 8α-H), 3.48 (s, 3H, 6-OCH_3), 3.83 (s, 3H, 3-OCH_3), 4.68 (d, 1H, 5β-H), 5.05–5.28 (m, 2H, All), 5.56 (d, 1H, 19-H), 5.67–5.90 (m, 1H, All), 6.10 (dd, 1H, 18-H), 6.54 (d, 1H, 1-H), 6.66 (d, 1H, 2-H), 7.40–8.02 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 469 (15) [M^+], 105 (100). $[\alpha]_D^{25}$ −246.9° ($CHCl_3$, *c* 1). $C_{30}H_{31}NO_4$ (469.5) calcd C 76.73; H 6.65; N 2.98; Found C 76.65; H 6.70; N 3.05.

4e: Yield 86%, oil. ^{1}H NMR ($CDCl_3$): δ 1.44 (dd, 1H, 8α-H), 1.67 (s, 3H, CH_3), 1.74 (s, 3H, CH_3), 3.47 (s, 3H, 6-OCH_3), 3.83 (s, 3H, 3-OCH_3), 4.68 (d, 1H, 5β-H), 5.15 (t, 1H, All), 5.57 (d, 1H, 19-H), 6.09 (dd, 1H, 18-H), 6.55 (d, 1H, 1-H), 6.65 (d, 1H, 2-H), 7.40–8.04 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 497 (12) [M^+], 105 (100). $[\alpha]_D^{25}$ −221.0° ($CHCl_3$, *c* 1). $C_{32}H_{35}NO_4$ (497.6).

4f: Yield 56%, mp 126–127 °C [EtOH]. ^{1}H NMR ($CDCl_3$): δ 1.48 (dd, 1H, 8α-H), 2.22 (t, 1H, Prop), 3.34 (d, 2H, Prop), 3.47 (s, 3H, 6-OCH_3), 3.83 (s, 3H, 3-OCH_3), 4.67 (d, 1H, 5β-H), 5.57 (d, 1H, 19-H), 6.12 (dd, 1H, 18-H), 6.54 (d, 1H, 1-H), 6.65 (d, 1H, 2-H), 7.40–7.98 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 467 (18) [M^+], 105 (100). $[\alpha]_D^{25}$ −236.9° ($CHCl_3$, *c* 1). $C_{30}H_{29}NO_4$ (467.5) calcd C 77.07; H 6.25; N 3.00; Found C 76.98; H 6.31; N 3.09.

(5*R*,6*R*,7*S*,9*R*,13*S*,14*R*)-7-Acetyl-4,5-epoxy-3,6-dimethoxy-17-substituted-6,14-ethenomorphinan derivatives (4g–l) [*N*-demethyl-N-substituted-thevinone derivatives]

4g: Yield 58%, mp 95–96 °C [EtOH] (Lit.[16]: oil). ^{1}H NMR ($CDCl_3$): δ 0.10–0.92 (m, 5H, cProp), 1.34 (dd, 1H, 8α-H), 2.14 (s, 3H, 7α-Ac), 3.61 (s, 3H, 6-OCH_3), 3.82 (s, 3H, 3-OCH_3), 4.58 (d, 1H, 5β-H), 5.59 (d, 1H, 19-H), 5.90 (dd, 1H, 18-H), 6.52 (d, 1H, 1-H), 6.63 (d, 1H, 2-H). MS (EI 70 eV) *m/z* 421 (80) [M^+], 246 (100). $[\alpha]_D^{25}$ −238.3° ($CHCl_3$, *c* 1); (Lit.[16]: $[\alpha]_D^{20}$ −145° (CH_2Cl_2, *c* 0.67)). $C_{26}H_{31}NO_4$ (421.5).

4h: Yield 76%, mp 136–137 °C [EtOH] (Lit.[2]: 137 °C). ^{1}H NMR ($CDCl_3$): δ 1.25 (dd, 1H, 8α-H), 2.13 (s, 3H, 7α-Ac), 3.58 (s, 3H, 6-OCH_3), 3.82 (s, 3H, 3-OCH_3), 4.56 (d, 1H, 5β-H), 5.55 (d, 1H, 19-H), 5.88 (dd, 1H, 18-H), 6.53 (d, 1H, 1-H), 6.88 (d, 1H, 2-H), 7.14–7.33 (m, 5H, Ar). MS (EI 70 eV) *m/z* 471 (4) [M]$^+$, 380 (100). $[\alpha]_D^{25}$ −195. 1° ($CHCl_3$, *c* 1). $C_{30}H_{33}NO_4$ (471.6).

4i: Yield 51%, mp 94–95 °C [EtOH] (Lit.[2]: oil). ^{1}H NMR ($CDCl_3$): δ 0.92 (t, 3H, $CH_3CH_2CH_2$), 1.35 (m, 1H, 8α-H), 2.14 (s, 3H, 7α-Ac), 3.59 (s, 3H, 6-OCH_3), 3.82 (s, 3H, 3-OCH_3), 4.58 (d, 1H, 5β-H), 5.58 (d, 1H, 19-H), 5.90 (dd, 1H, 18-H), 6.52 (d, 1H, 1-H), 6.63 (d, 1H, 2-H). MS (EI 70 eV) *m/z* 409 (80) [M^+], 380 (100). $[\alpha]_D^{25}$ −236.0° ($CHCl_3$, *c* 1). $C_{25}H_{31}NO_4$ (409.5).

4j: Yield 53%, mp 92–94 °C [EtOH] (Lit.[2]: oil). ^{1}H NMR ($CDCl_3$): δ 1.34 (dd, 1H, 8α-H), 2.14 (s, 3H, 7α-Ac), 3.60 (s, 3H, 6-OCH_3), 3.82 (s, 3H, 3-OCH_3), 4.57 (d, 1H, 5β-H), 5.07–5.27 (m, 2H, All), 5.56 (d, 1H, 19-H), 5.67–5.84 (m, 1H, All), 5.90 (dd, 1H, 18-H), 6.52 (d, 1H, 1-H), 6.64 (d, 1H, 2-H). MS (EI 70 eV) *m/z* 407 (100) [M^+], 232 (90). $[\alpha]_D^{25}$ −237.5° ($CHCl_3$, *c* 1). $C_{25}H_{29}NO_4$ (407.5).

4k: Yield 87%, oil (Lit.[2]: oil). ^{1}H NMR ($CDCl_3$): δ 1.30 (dd, 1H, 8α-H), 1.65 (s, 3H, CH_3), 1.73 (s, 3H, CH_3), 2.13 (s, 3H, 7α-Ac), 3.58 (s, 3H, 6-OCH_3), 3.80 (s, 3H, 3-OCH_3), 4.56 (d, 1H, 5β-H), 5.15 (t, 1H, All), 5.54 (d, 1H, 19-H), 5.87 (dd, 1H, 18-H), 6.51 (d, 1H, 1-H), 6.62 (d, 1H, 2-H). MS (EI 70 eV) *m/z* 435 (48) [M^+], 177 (100). $[\alpha]_D^{25}$ −193.0° ($CHCl_3$, *c* 1). $C_{27}H_{33}NO_4$ (435.5).

4l: Yield 56%, mp 119–120 °C [EtOH] (Lit.[2]: oil). ^{1}H NMR ($CDCl_3$): δ 1.37 (dd, 1H, 8α-H), 2.13 (s, 3H, 7α-Ac), 2.24 (t, 1H, Prop), 3.34 (d, 2H, Prop), 3.59 (s, 3H, 6-OCH_3), 3.82 (s, 3H, 3-OCH_3), 4.58 (d, 1H, 5β-H), 5.58 (d, 1H, 19-H), 5.92 (dd, 1H, 18-H), 6.53 (d, 1H, 1-H), 6.64 (d, 1H, 2-H). MS (EI 70 eV) *m/z* 405

(40) [M⁺], 230 (100). $[\alpha]_D^{25}$ −239.1° ($CHCl_3$, *c* 1). $C_{25}H_{27}NO_4$ (405.5).

(5*R*,6*R*,7*S*,9*R*,13*S*,14*S*)-7-Benzoyl-4,5-epoxy-18,19-dihydro-3,6-dimethoxy-17-substituted-6,14-ethenomorphinan derivatives (4m–r) [*N*-demethyl-N-substituted-dihydronepenthone derivatives]

4m: Yield 72% mp 104–105 °C [hexane]. ^{1}H NMR ($CDCl_3$): δ 0.10–0.80 (m, 5H, cProp), 0.85 (m, 1H, 8α-H), 3.21 (s, 3H, 6-OCH_3), 3.88 (s, 3H, 3-OCH_3), 4.58 (d, 1H, 5β-H), 6.58 (d, 1H, 1-H), 6.73 (d, 1H, 2-H), 7.40–8.05 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 485 (25) [M⁺], 105 (100). $[\alpha]_D^{25}$ −173.5° ($CHCl_3$, *c* 1). $C_{31}H_{35}NO_4$ (485.6) calcd C 76.67; H 7.26; N 2.88; Found C 76.58; H 7.30; N 2.95.

4n: Yield 84%, oil. ^{1}H NMR ($CDCl_3$): δ 0.79 (m, 1H, 8α-H), 3.20 (s, 3H, 6-OCH_3), 3.87 (s, 3H, 3-OCH_3), 4.57 (d, 1H, 5β-H), 6.57 (d, 1H, 1-H), 6.73 (d, 1H, 2-H), 7.08–7.34 (m, 5H, Ar), 7.42–8.04 (m, 5H, 20-Ph). MS (TSP) *m/z* 536 (100) $[M+1]^+$. $[\alpha]_D^{25}$ −125.0° ($CHCl_3$, *c* 0.5). $C_{35}H_{37}NO_4$ (535.6).

4o: Yield 54%, mp 103–104 °C [hexane]. ^{1}H NMR ($CDCl_3$): δ 0.79 (m, 1H, 8α-H), 0.87 (t, 3H, $CH_3CH_2CH_2$), 3.22 (s, 3H, 6-OCH_3), 3.88 (s, 3H, 3-OCH_3), 4.57 (d, 1H, 5β-H), 6.59 (d, 1H, 1-H), 6.73 (d, 1H, 2-H), 7.40–8.04 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 473 (8) [M⁺], 444 (100). $[\alpha]_D^{25}$ −220.0° ($CHCl_3$, *c* 0.1). $C_{30}H_{35}NO_4$ (473.6) calcd C 76.08; H 7.45; N 2.96; Found C 75.97; H 7.50; N 3.02.

4p: Yield 81%, mp 112–113 °C [EtOH]. ^{1}H NMR ($CDCl_3$): δ 0.78 (m, 1H, 8α-H), 3.20 (s, 3H, 6-OCH_3), 3.87 (s, 3H, 3-OCH_3), 4.57 (d, 1H, 5β-H), 5.03–5.23 (m, 2H, All), 5.63–5.84 (m, 1H, All), 6.60 (d, 1H, 1-H), 6.73 (d, 1H, 2-H), 7.40–8.03 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 471 (22) [M⁺], 105 (100). $[\alpha]_D^{25}$ −174.2° ($CHCl_3$, *c* 1). $C_{30}H_{33}NO_4$ (471.6) calcd C 76.41; H 7.05; N 2.97; Found C 76.50; H 7.12; N 2.88.

4q: Yield 90%, mp 160–163 °C [HCl] [EtOH]. ^{1}H NMR ($CDCl_3$): δ 0.78 (m, 1H, 8α-H), 1.63 (s, 3H, CH_3), 1.71 (s, 3H, CH_3), 3.20 (s, 3H, 6-OCH_3), 3.87 (s, 3H, 3-OCH_3), 4.57 (d, 1H, 5β-H), 5.10 (t, 1H, All), 6.59 (d, 1H, 1-H), 6.72 (d, 1H, 2-H), 7.38–8.03 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 499 (8) [M⁺], 105 (100). $[\alpha]_D^{25}$ −145.0° (EtOH, *c* 0.5). $C_{32}H_{37}NO_4$ (499.6) calcd C 76.92; H 7.46; N 2.80; Found C 76.85; H 7.52; N 2.87.

4r: Yield 52%, mp 143–146 °C [EtOH]. ^{1}H NMR ($CDCl_3$): δ 0.82 (m, 1H, 8α-H), 2.22 (t, 1H, Prop), 3.21 (s, 3H, 6-OCH_3), 3.30 (d, 2H, Prop), 3.88 (s, 3H, 3-OCH_3), 4.58 (d, 1H, 5β-H), 6.62 (d, 1H, 1-H), 6.75 (d, 1H, 2-H), 7.40–8.03 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 469 (55) [M⁺], 105 (100). $[\alpha]_D^{25}$ −196.0° ($CHCl_3$, *c* 1). $C_{30}H_{31}NO_4$ (469.5) calcd C 76.73; H 6.65; N 2.98; Found C 76.80; H 6.58; N 2.94.

Typical procedure for the preparation of (5*R*,6*R*,7*R*,9*R*,13*S*,14*R*,20*R*)-4,5-epoxy-α-phenyl-3,6-dimethoxy-α-methyl-17-substituted-6,14-ethenomorphinan-7-methanol derivatives (5a–f) and (5*R*,6*R*,7*R*,9*R*,13*S*,14*S*,20*R*)-4,5-epoxy-α-phenyl-18,19-dihydro-3,6-dimethoxy-α-methyl-17-substituted-6,14-ethenomorphinan-7-methanol derivatives (5m–r) [reaction of *N*-demethyl-N-substituted-nepenthone- and dihydronepenthone derivatives with methylmagnesium iodide]

To a suspension of the Grignard reagent (prepared from 0.8 g of Mg-shavings and 2 mL of MeI in a mixture of 5 mL of abs toluene and 11 mL of abs tetrahydrofuran) a solution of the *N*-demethyl-N-substituted derivative (**4a–f** and **4m–r**; 5.3 mmol) in abs toluene (18 mL) was added over a period of 1 h. Then the reaction mixture is stirred under reflux for 1 h, cooled to ambient temperature, and poured into 120 mL of satd aq NH_4Cl soln. Following extraction with toluene (3 × 40 mL) the combined organic layer was washed with satd aq NaCl, dried (Na_2SO_4), and concd. The residual product was crystallized from the appropriate solvent.

(5*R*,6*R*,7*R*,9*R*,13*S*,14*R*,20*R*)-4,5-Epoxy-α-phenyl-3,6-dimethoxy-α-metyl-17-substituted-6, 14-ethenomorphinan-7-methanol derivatives (5a–f)

5a: Yield 85%, mp 140–141 °C [EtOH]. ^{1}H NMR ($CDCl_3$): δ 0.13–0.95 (m, 5H, cProp), 1.22 (dd, 1H, 8α-H), 1.60 (s, 3H, 20-CH_3), 3.70 (s, 3H, 6-OCH_3), 3.78 (s, 3H, 3-OCH_3), 4.50 (d, 1H, 5β-H), 4.87 (d, 1H, 19-H), 5.02 (dd, 1H, 18-H), 5.90 (s[a], 1H, 20-OH), 6.40 (d, 1H, 1-H), 6.53 (d, 1H, 2-H), 7.08–7.38 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 499 (17) [M⁺], 378 (100). $[\alpha]_D^{25}$ −220.9° ($CHCl_3$, *c* 1). $C_{32}H_{37}NO_4$ (499.6) calcd C 76.92; H 7.46; N 2.80; Found C 76.87; H 7.39; N 2.75.

5b: Yield 83%, oil. ^{1}H NMR ($CDCl_3$): δ 1.02 (dd, 1H, 8α-H), 1.48 (s, 3H, 20-CH_3), 3.58 (s, 3H, 6-OCH_3), 3.76 (s, 3H, 3-OCH_3), 4.47 (d, 1H, 5β-H), 4.82 (d, 1H, 19-H), 4.96 (dd, 1H, 18-H), 5.88 (s[a], 1H, 20-OH), 6.42 (d, 1H, 1-H), 6.53 (d, 1H, 2-H), 7.12–7.40 (m, 10H, 20-Ph, Ar). MS (EI 70 eV) *m/z* 458 (100) $[M-91]^+$ $[\alpha]_D^{25}$ −175.0° ($CHCl_3$, *c* 1). $C_{36}H_{39}NO_4$ (549.7).

5c: Yield 74%, mp 120–121 °C [EtOH]. ^{1}H NMR ($CDCl_3$): δ 0.96 (t, 3H, $CH_3CH_2CH_2$), 1.20 (dd, 1H, 8α-H), 1.52 (s, 3H, 20-CH_3), 3.68 (s, 3H, 6-OCH_3), 3.77 (s, 3H, 3-OCH_3), 4.49 (d, 1H, 5β-H), 4.86 (d, 1H, 19-H), 5.00 (dd, 1H, 18-H), 5.82 (s[a], 1H, 20-OH), 6.40 (d, 1H, 1-H), 6.54 (d, 1H, 2-H), 7.10–7.37 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 487 (20) [M⁺], 366 (100). $[\alpha]_D^{25}$ −252.8° ($CHCl_3$, *c* 1). $C_{31}H_{37}NO_4$ (487.6) calcd C 76.36; H 7.65; N 2.87; Found C 76.40; H 7.74; N 2.94.

5d: Yield 67%, mp 139–140 °C [EtOH]. ^{1}H NMR ($CDCl_3$): δ 1.20 (dd, 1H, 8α-H), 1.53 (s, 3H, 20-CH_3), 3.70 (s, 3H, 6-OCH_3), 3.78 (s, 3H, 3-OCH_3), 4.50 (d, 1H, 5β-H), 4.85 (d, 1H, 19-H), 5.00 (dd, 1H, 18-H), 5.12–5.32 (m, 2H, All), 5.75–5.98 (m, 1H, All), 5.94 (s[a], 1H, 20-OH), 6.42 (d, 1H, 1-H), 6.55 (d, 1H, 2-H), 7.08–7.37 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 485 (15)

[M⁺], 364 (100). $[\alpha]_D^{25}$ −244.6° ($CHCl_3$, *c* 1). $C_{31}H_{35}NO_4$ (485.6) calcd C 76.67; H 7.26; N 2.88; Found C 76.65; H 7.32; N 2.93.

5e: Yield 68%, mp 169–170 °C [EtOH]. ^1H NMR ($CDCl_3$): δ 1.17 (dd, 1H, 8α-H), 1.52 (s, 3H, 20-CH_3), 1.68 (s, 3H, CH_3), 1.78 (s, 3H, CH_3), 3.70 (s, 3H, 6-OCH_3), 3.76 (s, 3H, 3-OCH_3), 4.48 (d, 1H, 5β-H), 4.85 (d, 1H, 19-H), 4.98 (dd, 1H, 18-H), 5.24 (t, 1H, All), 5.84 (s[a], 1H, 20-OH), 6.42 (d, 1H, 1-H), 6.54 (d, 1H, 2-H), 7.12–7.36 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 513 (30) [M⁺], 392 (100). $[\alpha]_D^{25}$ −265.0° ($CHCl_3$, *c* 1). $C_{33}H_{39}NO_4$ (513.6) calcd C 77.16; H 7.65; N 2.73; Found C 77.21; H 7.58; N 2.81.

5f: Yield 71%, mp 155–156 °C [EtOH]. ^1H NMR ($CDCl_3$): δ 1.24 (dd, 1H, 8α-H), 1.52 (s, 3H, 20-CH_3), 2.30 (t, 1H, Prop), 3.34 (d, 2H, Prop), 3.70 (s, 3H, 6-OCH_3), 3.77 (s, 3H, 3-OCH_3), 4.51 (d, 1H, 5β-H), 4.87 (d, 1H, 19-H), 4.98 (dd, 1H, 18-H), 5.96 (s[a], 1H, 20-OH), 6.42 (d, 1H, 1-H), 6.55 (d, 1H, 2-H), 7.08–7.37 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 483 (40) [M⁺], 362 (100). $[\alpha]_D^{25}$ −239.8° ($CHCl_3$, *c* 1). $C_{31}H_{33}NO_4$ (483.6) calcd C 76.99; H 6.88; N 2.90; Found C 76.87; H 6.91; N 2.95.

(5*R*,6*R*,7*R*,9*R*,13*S*,14*S*,20*R*)-4,5-Epoxy-α-phenyl-18,19-dihydro-3,6-dimethoxy-α-methyl-17-substituted-6,14-ethenomorphinan-7-methanol derivatives (5m–r)

5m: Yield 70%, mp 143–145 °C [EtOH]. ^1H NMR ($CDCl_3$): δ 0.10–0.85 (m, 5H, cProp), 1.60 (s, 3H, 20-CH_3), 3.44 (s, 3H, 6-OCH_3), 3.83 (s, 3H, 3-OCH_3), 4.34 (d, 1H, 5β-H), 6.13 (s[a], 1H, 20-OH), 6.47 (d, 1H, 1-H), 6.65 (d, 1H, 2-H), 7.16–7.62 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 501 (45) [M⁺], 380 (100). $[\alpha]_D^{25}$ −156.8° ($CHCl_3$, *c* 1). $C_{32}H_{39}NO_4$ (501.6) calcd C 76.62; H 7.84; N 2.79; Found C 76.54; H 7.80; N 2.82.

5n: Yield 80%, oil. ^1H NMR ($CDCl_3$): δ 1.52 (s, 3H, 20-CH_3), 3.40 (s, 3H, 6-OCH_3), 3.80 (s, 3H, 3-OCH_3), 4.30 (d, 1H, 5β-H), 6.12 (s[a], 1H, 20-OH), 6.45 (d, 1H, 1-H), 6.63 (d, 1H, 2-H), 7.13–7.57 (m, 10H, 20-Ph, Ar). MS (EI 70 eV) *m/z* 460 (18) [M-91]⁺, 206 (100). $[\alpha]_D^{25}$ −113.4° ($CHCl_3$, *c* 1). $C_{36}H_{41}NO_4$ (551.7).

5o: Yield 73%, mp 110–111 °C [EtOH]. ^1H NMR ($CDCl_3$): δ 0.97 (t, 3H, $CH_3CH_2CH_2$), 1.63 (s, 3H, 20-CH_3), 3.45 (s, 3H, 6-OCH_3), 3.85 (s, 3H, 3-OCH_3), 4.36 (d, 1H, 5β-H), 6.14 (s[a], 1H, 20-OH), 6.52 (d, 1H, 1-H), 6.67 (d, 1H, 2-H), 7.18–7.62 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 489 (10) [M⁺], 460 (100). $[\alpha]_D^{25}$ −166.0° ($CHCl_3$, *c* 1). $C_{31}H_{39}NO_4$ (489.6) calcd C 76.04; H 8.03; N 2.86; Found C 75.98; H 8.12; N 2.92.

5p: Yield 67%, mp 152–153 °C [EtOH]. ^1H NMR ($CDCl_3$): δ 1.60 (s, 3H, 20-CH_3), 3.43 (s, 3H, 6-OCH_3), 3.82 (s, 3H, 3-OCH_3), 4.34 (d, 1H, 5β-H), 5.10–5.28 (m, 2H, All), 5.73–5.96 (m, 1H, All), 6.13 (s[a], 1H, 20-OH), 6.50 (d, 1H, 1-H), 6.66 (d, 1H, 2-H), 7.16–7.62 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 487 (42) [M⁺], 366 (100). $[\alpha]_D^{25}$ −145.2° ($CHCl_3$, *c* 0.5). $C_{31}H_{37}NO_4$ (487.6) calcd C 76.36; H 7.65; N 2.87; Found C 76.28; H 7.73; N 2.95.

5q: Yield 61%, mp 126–128 °C [HCl] [EtOH]. ^1H NMR ($CDCl_3$): δ 1.60 (s, 3H, 20-CH_3), 1.68 (s, 3H, CH_3), 1.78 (s, 3H, CH_3), 3.43 (s, 3H, 6-OCH_3), 3.83 (s, 3H, 3-OCH_3), 4.34 (d, 1H, 5β-H), 5.22 (t, 1H, All), 6.13 (s[a], 1H, 20-OH), 6.50 (d, 1H, 1-H), 6.65 (d, 1H, 2-H), 7.14–7.60 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 515 (33) [M⁺], 394 (100). $[\alpha]_D^{25}$ −99.9° (EtOH, *c* 0.5). $C_{33}H_{41}NO_4$ (515.6) calcd C 76.86; H 8.01; N 2.72; Found C 76.78; H 7.96; N 2.76.

5r: Yield 65%, mp 170–171 °C [EtOH]. ^1H NMR ($CDCl_3$): δ 1.60 (s, 3H, 20-CH_3), 2.27 (t, 1H, Prop), 3.30 (d, 2H, Prop), 3.43 (s, 3H, 6-OCH_3), 3.83 (s, 3H, 3-OCH_3), 4.36 (d, 1H, 5β-H), 6.11 (s[a], 1H, 20-OH), 6.49 (d, 1H, 1-H), 6.67 (d, 1H, 2-H), 7.20–7.60 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 499 (25) [M⁺], 452 (100). $[\alpha]_D^{25}$ −147.5° ($CHCl_3$, *c* 1). $C_{32}H_{37}NO_4$ (499.6) calcd C 76.92; H 7.46; N 2.80; Found C 76.85; H 7.37; N 2.75.

General method for the preparation of (5*R*,6*R*,7*R*,9*R*,13*S*,14*R*,20*S*)-4,5-epoxy-α-phenyl-3,6-dimethoxy-α-methyl-17-substituted-6,14-ethenomorphinan-7-methanol derivatives (5g–l) and (5*R*,6*R*,7*R*,9*R*,13*S*,14*S*,20*S*)-4,5-epoxy-α-phenyl-18,19-dihydro-3,6-dimethoxy-α-methyl-17-substituted-6,14-ethenomorphinan-7-methanol derivatives (5s–y) [reaction of *N*-demethyl-N-substituted-thevinone- and dihydrothevinone derivatives with phenylmagnesium bromide]

To a suspension of the Grignard reagent (prepared from 0.52 g of Mg-shavings, and 2.3 mL of freshly distilled bromobenzene in a mixture of 5 mL of abs toluene and 10 mL of abs ether) a soln of the *N*-demethyl-N-substituted derivative (4g–l or *N*-demethyl-N-substituted-dihydrothevinone;[7] 3.5 mmol) in abs toluene (18 mL) was added and the reaction mixture stirred under reflux for 1 h. After cooling, it was poured onto 65 mL satd aq NH_4Cl, extracted with toluene (3 × 20 mL), the combined organic layer was washed with satd aq NaCl, and dried (Na_2SO_4). Evapn of the solvent gave a residue, which was crystallized from the appropriate solvent.

(5*R*,6*R*,7*R*,9*R*,13*S*,14*R*,20*S*)-4,5-Epoxy-α-phenyl-3,6-dimethoxy-α-methyl-17-substituted-6,14-ethenomorphinan-7-methanol derivatives (5g–l)

5g: Yield 67%, mp 159–160 °C [EtOH]. ^1H NMR ($CDCl_3$): δ 0.35–0.75 (m, 5H, cProp), 1.44 (s, 3H, 20-CH_3), 3.80 (s, 3H, 6-OCH_3), 3.85 (s, 3H, 3-OCH_3), 4.58 (d, 1H, 5β-H), 5.35 (s[a], 1H, 20-OH), 5.45 (d, 1H, 19-H), 6.03 (dd, 1H, 18-H), 6.47 (d, 1H, 1-H), 6.62 (d, 1H, 2-H), 7.18–7.48 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 499 (32) [M⁺], 378 (100). $[\alpha]_D^{25}$ −138.1° ($CHCl_3$, *c* 1). $C_{32}H_{37}NO_4$ (499.6).

5h: Yield 74%, mp 162–164 °C [EtOH]. ^1H NMR ($CDCl_3$): δ 0.57 (dd, 1H, 8α-H), 1.43 (s, 3H, 20-CH_3), 3.82 (s, 3H, 6-OCH_3), 3.86 (s, 3H, 3-OCH_3), 4.59 (d, 1H, 5β-H), 5.32 (s[a], 1H, 20-OH), 5.43 (d, 1H, 19-H), 6.03 (dd, 1H, 18-H), 6.47 (d, 1H, 1-H), 6.62 (d, 1H, 2-H), 6.98–7.16 (m, 5H, Ar), 7.27–7.50 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 458 (100) [M-91]⁺, TSP: 550 (100)

$[M+1]^+$. $[\alpha]_D^{25}$ −94.4° ($CHCl_3$, *c* 1). $C_{36}H_{39}NO_4$ (549.7).

5i: Yield 68%, mp 187–188 °C [EtOH]. ^1H NMR ($CDCl_3$): δ 0.57 (dd, 1H, 8α-H), 0.69 (t, 3H, $C\underline{H}_3CH_2CH_2$), 1.43 (s, 3H, 20-CH_3), 3.82 (s, 3H, 6-OCH_3), 3.86 (s, 3H, 3-OCH_3), 4.58 (d, 1H, 5β-H), 5.28 (s[a], 1H, 20-OH), 5.43 (d, 1H, 19-H), 6.03 (dd, 1H, 18-H), 6.46 (d, 1H, 1-H), 6.62 (d, 1H, 2-H), 7.15–7.48 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 487 (33) [M^+], 366 (100). $[\alpha]_D^{25}$ −132.7° ($CHCl_3$, *c* 1). $C_{31}H_{37}NO_4$ (487.6).

5j: Yield 69%, mp 202–203 °C [EtOH]. ^1H NMR ($CDCl_3$): δ 0.58 (dd, 1H, 8α-H), 1.43 (s, 3H, 20-CH_3), 3.82 (s, 3H, 6-OCH_3), 3.85 (s, 3H, 3-OCH_3), 4.58 (d, 1H, 5β-H), 4.97–5.14 (m, 2H, All), 5.30 (s[a], 1H, 20-OH), 5.44 (d, 1H, 19-H), 5.52–5.60 (m, 1H, All), 6.04 (dd, 1H, 18-H), 6.49 (d, 1H, 1-H), 6.63 (d, 1H, 2-H), 7.16–7.50 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 485 (22) [M^+], 70 (100). $[\alpha]_D^{25}$ −126.9° ($CHCl_3$, *c* 1). $C_{31}H_{35}NO_4$ (485.6).

5k: Yield 80%, oil. ^1H NMR ($CDCl_3$): δ 0.56 (dd, 1H, 8α-H), 1.43 (s, 3H, 20-CH_3), 1.54 (s, 3H, CH_3), 1.67 (s, 3H, CH_3), 3.80 (s, 3H, 6-OCH_3), 3.84 (s, 3H, 3-OCH_3), 4.58 (d, 1H, 5β-H), 5.00 (t, 1H, All), 5.32 (s[a], 1H, 20-OH), 5.43 (d, 1H, 19-H), 6.02 (dd, 1H, 18-H), 6.47 (d, 1H, 1-H), 6.62 (d, 1H, 2-H), 7.07–7.63 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 513 (3) [M^+], 344 (100). $[\alpha]_D^{25}$ −88.2° ($CHCl_3$, *c* 1). $C_{33}H_{39}NO_4$ (513.6).

5l: Yield 73%, mp 260–261 °C [EtOH]. ^1H NMR ($CDCl_3$): δ 0.63 (dd, 1H, 8α-H), 1.44 (s, 3H, 20-CH_3), 2.17 (t, 1H, Prop), 3.25 (d, 2H, Prop), 3.82 (s, 3H, 6-OCH_3), 3.86 (s, 3H, 3-OCH_3), 4.60 (d, 1H, 5β-H), 5.32 (s[a], 1H, 20-OH), 5.47 (d, 1H, 19-H), 6.04 (dd, 1H, 18-H), 6.49 (d, 1H, 1-H), 6.63 (d, 1H, 2-H), 7.18–7.48 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 483 (15) [M^+], 68 (100). $[\alpha]_D^{25}$ −119.8° ($CHCl_3$, *c* 1). $C_{31}H_{33}NO_4$ (483.6).

(5*R*,6*R*,7*R*,9*R*,13*S*,14*S*,20*S*)-4,5-epoxy-α-phenyl-18,19-dihydro-3,6-dimethoxy-α-methyl-17-substituted-6,14-ethenomorphinan-7-methanol derivatives (5s–y)

5s: Yield 72%, mp 159–160 °C [EtOH]. ^1H NMR ($CDCl_3$): δ 0.35–0.75 (m, 5H, cProp), 1.80 (s, 3H, 20-CH_3), 3.62 (s, 3H, 6-OCH_3), 3.91 (s, 3H, 3-OCH_3), 4.44 (d, 1H, 5β-H), 5.54 (s[a], 1H, 20-OH), 6.53 (d, 1H, 1-H), 6.72 (d, 1H, 2-H), 7.22–7.58 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 501 (48) [M^+], 380 (100). $[\alpha]_D^{25}$ −71.1° ($CHCl_3$, *c* 0.5). $C_{32}H_{39}NO_4$ (501.6).

5t: Yield 74%, mp 185–187 °C [EtOH]. ^1H NMR ($CDCl_3$): δ 1.82 (s, 3H, 20-CH_3), 3.63 (s, 3H, 6-OCH_3), 3.90 (s, 3H, 3-OCH_3), 4.46 (d, 1H, 5β-H), 5.57 (s[a], 1H, 20-OH), 6.55 (d, 1H, 1-H), 6.73 (d, 1H, 2-H), 6.98–7.22 (m, 5H, Ar), 7.28–7.60 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 551 (2) [M^+], 460 (100). $[\alpha]_D^{25}$ −57.4° ($CHCl_3$, *c* 0.5). $C_{36}H_{41}NO_4$ (551.7).

5v: Yield 68%, mp 132–133 °C [EtOH]. ^1H NMR ($CDCl_3$): δ 0.63 (t, 3H, $C\underline{H}_3CH_2CH_2$), 1.80 (s, 3H, 20-CH_3), 3.61 (s, 3H, 6-OCH_3), 3.88 (s, 3H, 3-OCH_3), 4.43 (d, 1H, 5β-H), 5.52 (s[a], 1H, 20-OH), 6.53 (d, 1H, 1-H), 6.72 (d, 1H, 2-H), 7.16–7.55 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 489 (10) [M^+], 460 (100). $[\alpha]_D^{25}$ −63.4° ($CHCl_3$, *c* 0.5). $C_{31}H_{39}NO_4$ (489.6).

5w: Yield 70%, mp 170–171 °C [EtOH]. ^1H NMR ($CDCl_3$): δ 1.80 (s, 3H, 20-CH_3), 3.60 (s, 3H, 6-OCH_3), 3.88 (s, 3H, 3-OCH_3), 4.43 (d, 1H, 5β-H), 4.93–5.08 (m, 2H, All), 5.43–5.67 (m, 1H, All), 5.52 (s[a], 1H, 20-OH), 6.53 (d, 1H, 1-H), 6.72 (d, 1H, 2-H), 7.20–7.55 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 487 (33) [M^+], 121 (100). $[\alpha]_D^{25}$ −36.0° ($CHCl_3$, *c* 0.1). $C_{31}H_{37}NO_4$ (487.6).

5x: Yield 82%, oil. ^1H NMR ($CDCl_3$): δ 1.47 (s, 3H, CH_3), 1.66 (s, 3H, CH_3), 1.80 (s, 3H, 20-CH_3), 3.60 (s, 3H, 6-OCH_3), 3.88 (s, 3H, 3-OCH_3), 4.42 (d, 1H, 5β-H), 4.94 (t, 1H, All), 5.52 (s[a], 1H, 20-OH), 6.54 (d, 1H, 1-H), 6.73 (d, 1H, 2-H), 7.23–7.65 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 515 (40) [M^+], 393 (100). $[\alpha]_D^{25}$ −56.5° ($CHCl_3$, *c* 0.1). $C_{33}H_{41}NO_4$ (515.6).

5y: Yield 65%, mp 205–206 °C [EtOH]. ^1H NMR ($CDCl_3$): δ 1.78 (s, 3H, 20-CH_3), 2.14 (t, 1H, Prop), 3.16 (d, 2H, Prop), 3.60 (s, 3H, 6-OCH_3), 3.88 (s, 3H, 3-OCH_3), 4.44 (d, 1H, 5β-H), 5.52 (s[a], 1H, 20-OH), 6.53 (d, 1H, 1-H), 6.73 (d, 1H, 2-H), 7.22–7.55 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 485 (80) [M^+], 452 (100). $[\alpha]_D^{25}$ −119.0° ($CHCl_3$, *c* 0.1). $C_{31}H_{35}NO_4$ (485.6).

Preparation of compounds **7a–d** from the corresponding ketones (**3a–d**) with phenylmagnesium bromide or methylmagnesium iodide was accomplished as described above in the general procedures.

7a: Yield 78%, mp 147–148 °C [EtOH] (Lit.[3] mp: 152 °C). ^1H NMR ($CDCl_3$): δ 1.25 (dd, 1H, 8α-H), 1.53 (s, 3H, 20-CH_3), 2.37 (s, 3H, NCH_3), 3.72 (s, 3H, 6-OCH_3), 3.78 (s, 3H, 3-OCH_3), 4.52 (d, 1H, 5β-H), 4.87 (d, 1H, 19-H), 5.02 (dd, 1H, 18-H), 5.86 (s[a], 1H, 20-OH), 6.43 (d, 1H, 1-H), 6.56 (d, 1H, 2-H), 7.08–7.37 (m, 5H, 20Ph). MS (EI 70 eV) *m/z* 459 (25) [M^+], 164 (100). $[\alpha]_D^{25}$ −235.3° ($CHCl_3$, *c* 1). $C_{29}H_{33}NO_4$ (459.6). By column chromatography of the mother liquor 5% of **7b** could be isolated.

7b: Yield 76%, mp 213–214 °C [EtOH] (Lit.[3] mp: 208 °C). ^1H NMR ($CDCl_3$): δ 0.62 (dd, 1H, 8α-H), 1.44 (s, 3H, 20-CH_3), 2.22 (s, 3H, NCH_3), 3.83 (s, 3H, 6-OCH_3), 3.87 (s, 3H, 3-OCH_3), 4.60 (d, 1H, 5β-H), 5.34 (s[a], 1H, 20-OH), 5.47 (d, 1H, 19-H), 6.05 (dd, 1H, 18-H), 6.52 (d, 1H, 1-H), 6.64 (d, 1H, 2-H), 7.20–7.50 (m, 5H, 20Ph). MS (EI 70 eV) *m/z* 459 (40) [M^+], 164 (100). $[\alpha]_D^{25}$ −134.5° ($CHCl_3$, *c* 1). $C_{29}H_{33}NO_4$ (459.6).

7c: Yield 72%, mp 173–174 °C [EtOH]. ^1H NMR ($CDCl_3$): δ 1.62 (s, 3H, 20-CH_3), 2.34 (s, 3H, NCH_3), 3.44 (s, 3H, 6-OCH_3), 3.83 (s, 3H, 3-OCH_3), 4.35 (d, 1H, 5β-H), 6.14 (s[a], 1H, 20-OH), 6.50 (d, 1H, 1-H), 6.65 (d, 1H, 2-H), 7.18–7.58 (m, 5H, 20Ph). MS (EI 70 eV) *m/z* 461 (44) [M^+], 340 (100). $[\alpha]_D^{25}$ −121.1° ($CHCl_3$, *c* 1). $C_{29}H_{35}NO_4$ (461.6) calcd C 75.46; H 7.64; N 3.03; Found C 75.38; H 7.70; N 2.95.

7d: Yield 79%, mp 203–204 °C [EtOH] (Lit.[3] mp: 202 °C). ^{1}H NMR ($CDCl_3$): δ 1.80 (s, 3H, 20-CH_3), 2.14 (s, 3H, NCH_3), 3.60 (s, 3H, 6-OCH_3), 3.88 (s, 3H, 3-OCH_3), 4.44 (d, 1H, 5β-H), 5.54 (s[a], 1H, 20-OH), 6.55 (d, 1H, 1-H), 6.72 (d, 1H, 2-H), 7.20–7.56 (m, 5H, 20Ph). MS (EI 70 eV) *m/z* 461 (71) [M$^+$], 340 (100). $[\alpha]_D^{25}$ −72.6° ($CHCl_3$, *c* 1). $C_{29}H_{35}NO_4$ (461.6).

General method for the reaction of compounds 7a–d with cyanogen bromide

Compounds **7a–d** (50 mmol) were dissolved in 100 mL of $CHCl_3$ (previously dried over $CaCl_2$), 10 g cyanogen bromide was added and the reaction mixture heated under reflux for 10 h. The excess of the reagent and the solvent were removed by evapn under diminished pressure, the residue was taken up with EtOH (150 mL) and the resulting solution evapd to its half-volume. The product (**8a–d**) crystallized from the solution upon cooling was filtered off and dried.

8a: Yield 90%, mp 205–206 °C [EtOH]. ^{1}H NMR ($CDCl_3$): δ 1.38 (dd, 1H, 8α-H), 1.54 (s, 3H, 20-CH_3), 3.70 (s, 3H, 6-OCH_3), 3.78 (s, 3H, 3-OCH_3), 4.49 (d, 1H, 5β-H), 4.80 (d, 1H, 19-H), 5.11 (dd, 1H, 18-H), 5.67 (s[a], 1H, 20-OH), 6.47 (d, 1H, 1-H), 6.60 (d, 1H, 2-H), 7.10–7.35 (m, 5H, 20Ph). MS (EI 70 eV) *m/z* 470 (12) [M$^+$], 121 (100). $[\alpha]_D^{25}$ −295.0° ($CHCl_3$, *c* 1). $C_{29}H_{30}N_2O_4$ (470.5).

8b: Yield 93%, mp 258–259 °C [EtOH]. ^{1}H NMR ($CDCl_3$): δ 0.74 (dd, 1H, 8α-H), 1.43 (s, 3H, 20-CH_3), 3.83 (s, 3H, 6-OCH_3), 3.87 (s, 3H, 3-OCH_3), 4.60 (d, 1H, 5β-H), 5.17 (s[a], 1H, 20-OH), 5.40 (d, 1H, 19-H), 6.13 (dd, 1H, 18-H), 6.54 (d, 1H, 1-H), 6.68 (d, 1H, 2-H), 7.23–7.48 (m, 5H, 20Ph). MS (EI 70 eV) *m/z* 470 (23) [M$^+$], 121 (100). $[\alpha]_D^{25}$ −119.8° ($CHCl_3$, *c* 0.5). $C_{29}H_{30}N_2O_4$ (470.5).

8c: Yield 92%, mp 218–219 °C [EtOH]. ^{1}H NMR ($CDCl_3$): δ 1.64 (s, 3H, 20-CH_3), 3.48 (s, 3H, 6-OCH_3), 3.87 (s, 3H, 3-OCH_3), 4.37 (d, 1H, 5β-H), 5.95 (s[a], 1H, 20-OH), 6.58 (d, 1H, 1-H), 6.74 (d, 1H, 2-H), 7.20–7.60 (m, 5H, 20Ph). MS (EI 70 eV) *m/z* 472 (8) [M$^+$], 121 (100). $[\alpha]_D^{25}$ −130.6° ($CHCl_3$, *c* 1). $C_{29}H_{32}N_2O_4$ (472.5).

8d: Yield 94%, mp 222–224 °C [EtOH]. ^{1}H NMR ($CDCl_3$): δ 1.80 (s, 3H, 20-CH_3), 3.42 (s, 3H, 6-OCH_3), 3.90 (s, 3H, 3-OCH_3), 4.43 (d, 1H, 5β-H), 5.37 (s[a], 1H, 20-OH), 6.62 (d, 1H, 1-H), 6.76 (d, 1H, 2-H), 7.23–7.53 (m, 5H, 20Ph). MS (EI 70 eV) *m/z* 472 (12) [M$^+$], 121 (100). $[\alpha]_D^{25}$ −73.7° ($CHCl_3$, *c* 1). $C_{29}H_{32}N_2O_4$ (472.5).

General method for the hydrolysis of compounds 8a–d

Potassium hydroxide (4.6 g) was dissolved in diethylene glycol (32 mL) at 105 °C, the solution cooled to 70 °C, and a suspension of the cyanoamide **8a–d** (10.6 mmol) in diethylene glycol (32 mL) added. The reaction mixture was stirred vigorously at 170 °C for 75 min, cooled, poured into 300 mL of ice-water and stirred for 30 min. The crystalline precipitate (**9a–d**) was filtered off, dried in a vacuum desiccator over $CaCl_2$, and purified by precipitating from a $CHCl_3$ solution with ethanol.

9a: Yield 90%, mp 208–210 °C [$CHCl_3$–EtOH]. ^{1}H NMR ($CDCl_3$): δ 1.22 (dd, 1H, 8α-H), 1.57 (s, 3H, 20-CH_3), 3.68 (s, 3H, 6-OCH_3), 3.79 (s, 3H, 3-OCH_3), 4.00 (s[a], 1H, NH), 4.56 (d, 1H, 5β-H), 4.83 (d, 1H, 19-H), 5.13 (dd, 1H, 18-H), 5.73 (s[a], 1H, 20-OH), 6.50 (d, 1H, 1-H), 6.63 (d, 1H, 2-H), 7.13–7.40 (m, 5H, 20Ph). MS (EI 70 eV) *m/z* 445 (26) [M$^+$], 121 (100). $[\alpha]_D^{25}$ −187.3° (EtOH, *c* 0.1). $C_{28}H_{31}NO_4$ (445.5).

9b: Yield 90%, mp 254–255 °C [$CHCl_3$–EtOH]. ^{1}H NMR ($CDCl_3$): δ 0.63 (dd, 1H, 8α-H), 1.43 (s, 3H, 20-CH_3), 3.82 (s, 3H, 6-OCH_3), 3.87 (s, 3H, 3-OCH_3), 4.57 (d, 1H, 5β-H), 5.29 (s[a], 1H, 20-OH), 5.42 (d, 1H, 19-H), 6.08 (dd, 1H, 18-H), 6.50 (d, 1H, 1-H), 6.64 (d, 1H, 2-H), 7.18–7.48 (m, 5H, 20Ph). MS (EI 70 eV) *m/z* 445 (15) [M$^+$], 121 (100). $[\alpha]_D^{25}$ −114.9° ($CHCl_3$, *c* 1). $C_{28}H_{31}NO_4$ (445.5).

9c: Yield 92%, mp 128–130 °C [$CHCl_3$–EtOH]. ^{1}H NMR ($CDCl_3$): δ 1.60 (s, 3H, 20-CH_3), 3.42 (s, 3H, 6-OCH_3), 3.82 (s, 3H, 3-OCH_3), 4.32 (d, 1H, 5β-H), 6.07 (s[a], 1H, 20-OH), 6.52 (d, 1H, 1-H), 6.67 (d, 1H, 2-H), 7.15–7.58 (m, 5H, 20Ph). MS (EI 70 eV) *m/z* 447 (45) [M$^+$], 414 (100). $[\alpha]_D^{25}$ −89.2° ($CHCl_3$, *c* 0.5). $C_{28}H_{33}NO_4$ (447.5).

9d: Yield 94%, mp 229–230 °C [$CHCl_3$–EtOH]. ^{1}H NMR ($CDCl_3$): δ 1.80 (s, 3H, 20-CH_3), 3.60 (s, 3H, 6-OCH_3), 3.88 (s, 3H, 3-OCH_3), 4.39 (d, 1H, 5β-H), 5.53 (s[a], 1H, 20-OH), 6.56 (d, 1H, 1-H), 6.73 (d, 1H, 2-H), 7.20–7.57 (m, 5H, 20Ph). MS (EI 70 eV) *m/z* 447 (32) [M$^+$], 121 (100). $[\alpha]_D^{25}$ −46.0° ($CHCl_3$, *c* 1). $C_{28}H_{33}NO_4$ (447.5).

For the preparation of **10a–d** the 3-O-demethylation procedure described for **6a–c** (vide infra) was applied, and the progress of the reaction was monitored by TLC. O-Demethylation proceeded following the hydrolysis of the cyanoamide, and a complete conversion was observed in ca 70–75 min reaction time.

10a: Yield 64%, mp 294–295 °C [$CHCl_3$–EtOH]. ^{1}H NMR (DMSO-d_6): δ 1.03 (dd, 1H, 8α-H), 1.44 (s, 3H, 20-CH_3), 3.10 (s, 3H, 6-OCH_3), 4.44 (d, 1H, 5β-H), 4.70 (s[a], 1H, 20-OH), 5.10 (d, 1H, 19-H), 5.38 (dd, 1H, 18-H), 6.28 (d, 1H, 1-H), 6.40 (d, 1H, 2-H), 7.10–7.48 (m, 5H, 20Ph), 8.74 (s[a], 1H, 3-OH). MS (EI 70 eV) *m/z* 431 (18) [M$^+$], 121 (100). $[\alpha]_D^{25}$ −213.9° (EtOH, *c* 0.1). $C_{27}H_{29}NO_4$ (431.5).

10b: Yield 66%, mp 295–297 °C [$CHCl_3$–EtOH]. ^{1}H NMR ($CDCl_3$): δ 0.62 (dd, 1H, 8α-H), 1.43 (s, 3H, 20-CH_3), 3.80 (s, 3H, 6-OCH_3), 4.56 (d, 1H, 5β-H), 5.22 (s[a], 1H, 20-OH), 5.40 (d, 1H, 19-H), 6.03 (dd, 1H, 18-H), 6.45 (d, 1H, 1-H), 6.60 (d, 1H, 2-H), 7.27–7.46 (m, 5H, 20Ph). MS (EI 70 eV) *m/z* 431 (30) [M$^+$], 121 (100). $[\alpha]_D^{25}$ −258.0° (EtOH, *c* 0.1). $C_{27}H_{29}NO_4$ (431.5).

10d: Yield 62%, mp 308–310 °C [$CHCl_3$–EtOH]. ^{1}H NMR (DMSO-d_6): δ 1.62 (s, 3H, 20-CH_3), 3.05 (s, 3H, 6-OCH_3), 4.28 (d, 1H, 5β-H), 5.04 (s[a], 1H, 20-OH), 6.38 (d, 1H, 1-H), 6.56 (d, 1H, 2-H), 7.12–7.54 (m, 5H, 20Ph), 8.93 (s[a], 1H, 3-OH). MS (EI 70 eV) *m/z* 433 (12) [M^+], 105 (100). $[\alpha]_D^{25}$ −12.6° (EtOH, *c* 0.1). $C_{27}H_{31}NO_4$ (433.5).

For the preparation of **11a–d** the O-demethylation procedure described for **6a–c** (vide infra) was employed and the progress of the reaction monitored by TLC.

11a: Yield 50%, mp 104–105 °C [EtOH]. ^{1}H NMR ($CDCl_3$): δ 1.25 (dd, 1H, 8α-H), 1.52 (s, 3H, 20-CH_3), 2.37 (s, 3H, NCH_3), 3.70 (s, 3H, 6-OCH_3), 4.52 (d, 1H, 5β-H), 4.70 (s[a], 1H, 3-OH), 4.85 (d, 1H, 19-H), 4.97 (dd, 1H, 18-H), 5.80 (s[a], 1H, 20-OH), 6.38 (d, 1H, 1-H), 6.53 (d, 1H, 2-H), 7.14–7.45 (m, 5H, 20Ph). MS (EI 70 eV) *m/z* 445 (100) [M^+]. $[\alpha]_D^{25}$ −218.0° (EtOH, *c* 0.1). $C_{28}H_{31}NO_4$ (445.5).

11b: Yield 56%, mp 260–261 °C [EtOH]. (Lit.[2] mp: 252 °C). ^{1}H NMR ($CDCl_3$): δ 0.63 (dd, 1H, 8α-H), 1.44 (s, 3H, 20-CH_3), 2.23 (s, 3H, NCH_3), 3.83 (s, 3H, 6-OCH_3), 4.62 (d, 1H, 5β-H), 4.73 (s[a], 1H, 3-OH), 5.30 (s[a], 1H, 20-OH), 5.46 (d, 1H, 19-H), 6.02 (dd, 1H, 18-H), 6.47 (d, 1H, 1-H), 6.60 (d, 1H, 2-H), 7.18–7.47 (m, 5H, 20Ph). MS (EI 70 eV) *m/z* 445 (33) [M^+], 121 (100). $[\alpha]_D^{25}$ −111.8° ($CHCl_3$, *c* 1). $C_{28}H_{31}NO_4$ (445.5).

11c: Yield 25%, mp 238–240 °C [EtOH]. ^{1}H NMR ($CDCl_3$): δ 1.58 (s, 3H, 20-CH_3), 2.32 (s, 3H, NCH_3), 3.43 (s, 3H, 6-OCH_3), 4.36 (d, 1H, 5β-H), 4.66 (s[a], 1H, 3-OH), 6.07 (s[a], 1H, 20-OH), 6.45 (d, 1H, 1-H), 6.64 (d, 1H, 2-H), 7.17–7.58 (m, 5H, 20Ph). MS (EI 70 eV) *m/z* 447 (32) [M^+], 105 (100). $[\alpha]_D^{25}$ −136.4° (EtOH, *c* 0.1). $C_{28}H_{33}NO_4$ (447.5).

11d: Yield 58%, mp 243–245 °C [EtOH]. ^{1}H NMR ($CDCl_3$): δ 1.78 (s, 3H, 20-CH_3), 2.14 (s, 3H, NCH_3), 3.57 (s, 3H, 6-OCH_3), 4.45 (d, 1H, 5β-H), 4.68 (s[a], 1H, 3-OH), 5.45 (s[a], 1H, 20-OH), 6.52 (d, 1H, 1-H), 6.69 (d, 1H, 2-H), 7.21–7.55 (m, 5H, 20Ph). MS (EI 70 eV) *m/z* 447 (25) [M^+], 121 (100). $[\alpha]_D^{25}$ −73.9° (EtOH, *c* 0.1). $C_{28}H_{33}NO_4$ (447.5).

General method for the preparation of compounds 6a–c, g–i, and s–v

Potassium hydroxide (3.9 g) was dissolved at 105 °C in 32 mL diethylene glycol under a nitrogen atmosphere. The solution was cooled to 70 °C and a suspension of the aryl-methyl ether (**5a–c**, **g–i**, and **s–v**; 2.6 mmol) in diethylene glycol (4 mL) added. The temperature of the reaction mixture was raised to 210–220 °C and stirred for 90 min. After cooling it was poured into 150 mL satd aq NH_4Cl, extracted with ether (3 × 50 mL), dried (Na_2SO_4) and concd. The residue was dissolved in hot EtOH, the solution decolorized with charcoal, filtered and the pH adjusted to 2 with satd HCl/EtOH. The produced hydrochloride salt was filtered off.

General method for the preparation of compounds 6d–f, j–l, and w–y

To a soln of the N-demethyl derivative **10a**, **b**, or **d** (3.5 mmol) in abs. DMF (9 mL) $NaHCO_3$ (1.10 g) and 3.85 mmol of the alkylating agent (allyl bromide, 3,3-dimethylallyl bromide and propargyl bromide) were added and the reaction mixture stirred at 90 °C (oil-bath) for 20 h. After filtration of the inorganic salts the solvent was distilled off under reduced pressure, the residue suspended in water, alkalized with 25% aq NH_4OH and extracted with $CHCl_3$. The combined organic layer was washed with water, dried (Na_2SO_4) and concd. The residue was crystallized from the appropriate solvent, or isolated in form of the hydrochloride salt.

(5*R*,6*R*,7*R*,9*R*,13*S*,14*R*,20*R*)-4,5-Epoxy-α-phenyl-3-hydroxy-6-methoxy-α-methyl-17-substituted-6,14-etheno-morphinan-7-methanol derivatives

6a: Yield 52%, mp 238–240 °C [HCl] [EtOH]. ^{1}H NMR ($CDCl_3$): δ 0.10–0.95 (m, 5H, cProp), 1.22 (dd, 1H, 8α-H), 1.53 (s, 3H, 20-CH_3), 3.68 (s, 3H, 6-OCH_3), 4.53 (d, 1H, 5β-H), 4.87 (d, 1H, 19-H), 4.94 (dd, 1H, 18-H), 4.97 (s[a], 1H, 3-OH), 5.86 (s[a], 1H, 20-OH), 6.35 (d, 1H, 1-H), 6.52 (d, 1H, 2-H), 7.08–7.36 (m, 5H, 20-Ph). MS (TSP) *m/z* 486 (100) $[M+1]^+$. $[\alpha]_D^{25}$ −289.0° ($CHCl_3$, *c* 0.1). $C_{31}H_{35}NO_4$ (485.6) calcd [HCl] C 71.32; H 6.95; N 2.68; Found C 71.25; H 6.88; N 2.61.

6b: Yield 60%, mp 254–255 °C [HCl] [EtOH]. ^{1}H NMR ($CDCl_3$): δ 1.02 (dd, 1H, 8α-H), 1.47 (s, 3H, 20-CH_3), 3.65 (s, 3H, 6-OCH_3), 4.48 (d, 1H, 5β-H), 4.67 (s[a], 1H, 3-OH), 4.81 (d, 1H, 19-H), 4.93 (dd, 1H, 18-H), 5.82 (s[a], 1H, 20-OH), 6.36 (d, 1H, 1-H), 6.52 (d, 1H, 2-H), 7.10–7.40 (m, 10H, 20-Ph, Ar). MS (EI 70 eV) *m/z* 535 (4) [M^+], 444 (100). $[\alpha]_D^{25}$ −155.1° (EtOH, *c* 0.1). $C_{35}H_{37}NO_4$ (535.6) calcd [HCl]: C 73.48; H 6.69; N 2.45; Found C 73.35; H 6.61; N 2.50.

6c: Yield 58%, mp 266–267 °C [HCl] [EtOH]. ^{1}H NMR (DMSO-d_6): δ 0,85 (t, 3H, $CH_3CH_2CH_2$), 1,42 (s, 3H, 20-CH_3), 3.34 (s, 3H, 6-OCH_3), 4.48 (d, 1H, 5β-H), 4.73 (s[a], 1H, 20-OH), 5.17 (d, 1H, 19-H), 5.38 (dd, 1H, 18-H), 6.30 (d, 1H, 1-H), 6.41 (d, 1H, 2-H), 7.08–7.50 (m, 5H, 20-Ph), 8.73 (s[a], 1H, 3-OH). MS (EI 70 eV) *m/z* 473 (68) [M^+], 352 (100). $[\alpha]_D^{25}$ −249.6° ($CHCl_3$, *c* 0.5). $C_{30}H_{35}NO_4$ (473.6) calcd [HCl] C 70.64; H 7.11; N 2.75; Found C 70.50; H 7.13; N 2.78.

6d: Yield 65%, mp 243–245 °C [HCl] [EtOH]. ^{1}H NMR ($CDCl_3$): δ 1.20 (dd, 1H, 8α-H), 1.53 (s, 3H, 20-CH_3), 3.65 (s, 3H, 6-OCH_3), 4.51 (d, 1H, 5β-H), 4.67 (s[a], 1H, 3-OH), 4.84 (d, 1H, 19-H), 4.96 (dd, 1H, 18-H), 5.12–5.30 (m, 2H, All), 5.75–6.00 (m, 1H, All), 5.88 (s[a], 1H, 20-OH), 6.38 (d, 1H, 1-H), 6.52 (d, 1H, 2-H), 7.10–7.40 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 471 (10) [M^+], 241 (100). $[\alpha]_D^{25}$ −36.7° (EtOH, *c* 0.1). $C_{30}H_{33}NO_4$ (471.6) calcd [HCl] C 70.92; H 6.75; N 2.76; Found C 70.95; H 6.82; N 2.70.

6e: Yield 68%, mp 192–194 °C [HCl] [EtOH]. ^{1}H NMR ($CDCl_3$): δ 1.20 (dd, 1H, 8α-H), 1.25 (s, 3H, CH_3), 1.52 (s, 3H, 20-CH_3), 1.80 (s, 3H, CH_3), 3.68 (s, 3H, 6-OCH_3), 4.53 (d, 1H, 5β-H), 4.70 (s[a], 1H, 3-OH), 4.85 (d, 1H, 19-H), 4.97 (dd, 1H, 18-H), 5.23 (t, 1H, All), 5.80 (s[a], 1H, 20-OH), 6.38 (d, 1H, 1-H), 6.53 (d, 1H, 2-H), 7.12–7.40 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 499 (80) [M^+], 378 (100). $[\alpha]_D^{25}$ −50.5° (EtOH, *c* 0.1). $C_{32}H_{37}NO_4$ (499.6) calcd [HCl] C 71.69; H 7.14; N 2.61; Found C 71.57; H 7.20; N 2.64.

6f: Yield 74%, mp 148–149 °C [EtOH]. ^{1}H NMR ($CDCl_3$): δ 1.20 (dd, 1H, 8α-H), 1.50 (s, 3H, 20-CH_3), 2.30 (t, 1H, Prop), 3.34 (d, 2H, Prop), 3.67 (s, 3H, 6-OCH_3), 4.52 (d, 1H, 5β-H), 4.60 (s[a], 1H, 3-OH), 4.88 (d, 1H, 19-H), 4.98 (dd, 1H, 18-H), 5.80 (s[a], 1H, 20-OH), 6.38 (d, 1H, 1-H), 6.53 (d, 1H, 2-H), 7.12–7.40 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 469 (25) [M^+], 89 (100). $[\alpha]_D^{25}$ −265.0° ($CHCl_3$, *c* 0.1). $C_{30}H_{31}NO_4$ (469.5) calcd C 76.73; H 6.65; N 2.98; Found C 76.68; H 6.70; N 3.03.

(5*R*,6*R*,7*R*,9*R*,13*S*,14*R*,20*S*)-17-Substituted-4,5-epoxy-α-phenyl-3-hydroxy-6-methoxy-α-methyl-6,14-ethenomorphinan-7-methanol derivatives

6g: Yield 63%, mp 257–258 °C [HCl] [EtOH]. ^{1}H NMR ($CDCl_3$): δ 0.40–0.95 (m, 5H, cProp), 0.65 (dd, 1H, 8α-H), 1.44 (s, 3H, 20-CH_3), 3.82 (s, 3H, 6-OCH_3), 4.50 (s[a], 1H, 3-OH), 4.62 (d, 1H, 5β-H), 5.26 (s[a], 1H, 20-OH), 5.45 (d, 1H, 19-H), 6.02 (dd, 1H, 18-H), 6.43 (d, 1H, 1-H), 6.58 (d, 1H, 2-H), 7.20–7.47 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 485 (46) [M^+], 364 (100). $[\alpha]_D^{25}$ −73.0° (EtOH, *c* 0.1). $C_{31}H_{35}NO_4$ (485.6) calcd [HCl] C 71.32; H 6.95; N 2.68; Found C 71.35; H 6.87; N 2.73.

6h: Yield 62%, mp 252–253 °C [HCl] [EtOH]. ^{1}H NMR ($CDCl_3$): δ 0.55 (dd, 1H, 8α-H), 1.44 (s, 3H, 20-CH_3), 3.80 (s, 3H, 6-OCH_3), 4.60 (d, 1H, 5β-H), 5.20 (s[a], 1H, 3-OH), 5.37 (s[a], 1H, 20-OH), 5.42 (d, 1H, 19-H), 5.98 (dd, 1H, 18-H), 6.42 (d, 1H, 1-H), 6.54 (d, 1H, 2-H), 6.95–7.15 (m, 5H, Ar), 7.20–7.50 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 444 (100) [M-91]$^+$ $[\alpha]_D^{25}$ −92.2° (EtOH, *c* 0.1). $C_{35}H_{37}NO_4$ (535.6) calcd [HCl] C 73.48; H 6.69; N 2.45; Found C 73.45; H 6.75; N 2.53.

6i: Yield 54%, mp 255–256 °C [HCl] [EtOH]. ^{1}H NMR (DMSO-d_6): δ 0.86 (t, 3H, $CH_3CH_2CH_2$), 1.38 (s, 3H, 20-CH_3), 3.55 (s, 3H, 6-OCH_3), 4.76 (d, 1H, 5β-H), 5.33 (d, 1H, 19-H), 5.84 (dd, 1H, 18-H), 6.42 (d, 1H, 1-H), 6.56 (d, 1H, 2-H), 7.15–7.48 (m, 5H, 20-Ph), 8.45 (s[a], 1H, 20-OH), 9.10 (s[a], 1H, 3-OH). MS (EI 70 eV) *m/z* 473 (90) [M^+], 352 (100). $[\alpha]_D^{25}$ −103.3° (EtOH, *c* 0.1). $C_{30}H_{35}NO_4$ (473.6) calcd [HCl] C 70.64; H 7.11; N 2.75; Found C 70.57; H 7.20; N 2.84.

6j: Yield 52%, mp 230–232 °C [EtOH]. ^{1}H NMR ($CDCl_3$): δ 0.58 (dd, 1H, 8α-H), 1.58 (s, 3H, 20-CH_3), 3.82 (s, 3H, 6-OCH_3), 4.50 (s[a], 1H, 3-OH), 4.62 (d, 1H, 5β-H), 5.00–5.15 (m, 2H, All), 5.25 (s[a], 1H, 20-OH), 5.42 (d, 1H, 19-H), 5.50–5.70 (m, 1H, All), 6.00 (dd, 1H, 18-H), 6.44 (d, 1H, 1-H), 6.61 (d, 1H, 2-H), 7.20–7.45 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 471 (58) [M^+], 241 (100). $[\alpha]_D^{25}$ −100.0° (EtOH, *c* 0.1). $C_{30}H_{33}NO_4$ (471.6) calcd C 76.41; H 7.05; N 2.97; Found C 76.48; H 7.13; N 2.89.

6k: Yield 64%, mp 219–220 °C [EtOH]. ^{1}H NMR ($CDCl_3$): δ 0.55 (dd, 1H, 8α-H), 1.42 (s, 3H, 20-CH_3), 1.53 (s, 3H, CH_3), 1.67 (s, 3H, CH_3), 3.80 (s, 3H, 6-OCH_3), 4.55 (s[a], 1H, 3-OH), 4.60 (d, 1H, 5β-H), 4.98 (t, 1H, All), 5.24 (s[a], 1H, 20-OH), 5.43 (d, 1H, 19-H), 5.98 (dd, 1H, 18-H), 6.44 (d, 1H, 1-H), 6.58 (d, 1H, 2-H), 7.18–7.47 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 449 (100) [M^+]. $[\alpha]_D^{25}$ −117.2° ($CHCl_3$, *c* 1). $C_{32}H_{37}NO_4$ (499.6) calcd C 76.92; H 7.46; N 2.80; Found C 76.95; H 7.53; N 2.89.

6l: Yield 62%, mp 250–251 °C [EtOH]. ^{1}H NMR ($CDCl_3$): δ 0.62 (dd, 1H, 8α-H), 1.42 (s, 3H, 20-CH_3), 2.16 (t, 1H, Prop), 3.24 (d, 2H, Prop), 3.82 (s, 3H, 6-OCH_3), 4.61 (d, 1H, 5β-H), 4.70 (s[a], 1H, 3-OH), 5.25 (s[a], 1H, 20-OH), 5.44 (d, 1H, 19-H), 6.02 (dd, 1H, 18-H), 6.44 (d, 1H, 1-H), 6.60 (d, 1H, 2-H), 7.22–7.47 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 469 (100) [M^+]. $[\alpha]_D^{25}$ −99.6° (EtOH, *c* 0.1). $C_{30}H_{31}NO_4$ (469.5) calcd C 76.73; H 6.65; N 2.98; Found C 76.65; H 6.71; N 3.07.

(5*R*,6*R*,7*R*,9*R*,13*S*,14*S*,20*S*)-4,5-Epoxy-α-phenyl-18,19-dihydro-3-hydroxy-6-methoxy-α-methyl-17-substituted-6,14-ethenomorphinan-7-methanol derivatives

6s: Yield 58%, mp 262–263 °C [HCl] [EtOH]. ^{1}H NMR (DMSO-d_6) [HCl]: δ 0.32–0.84 (m, 5H, cProp), 1.52 (s, 3H, 20-CH_3), 3.45 (s, 3H, 6-OCH_3), 4.46 (d, 1H, 5β-H), 4.54 (s[a], 1H, 3-OH), 5.03 (s[a], 1H, 20-OH), 6.51 (d, 1H, 1-H), 6.68 (d, 1H, 2-H), 7.08–7.58 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 487 (61) [M^+], 366 (100). $[\alpha]_D^{25}$ −76.5° (EtOH, *c* 0.1). $C_{31}H_{37}NO_4$ (487.6) calcd [HCl] C 71.04; H 7.31; N 2.67; Found C 71.17; H 7.34; N 2.75.

6t: Yield 61%, mp 264–265 °C [HCl] [EtOH]. ^{1}H NMR ($CDCl_3$): δ 1.80 (s, 3H, 20-CH_3), 3.58 (s, 3H, 6-OCH_3), 4.47 (d, 1H, 5β-H), 4.83 (s[a], 1H, 3-OH), 5.48 (s[a], 1H, 20-OH), 6.49 (d, 1H, 1-H), 6.68 (d, 1H, 2-H), 6.95–7.20 (m, 5H, Ar), 7.25–7.58 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 537 (3) [M^+], 446 (100). $[\alpha]_D^{25}$ −67.1° (EtOH, *c* 0.1). $C_{35}H_{39}NO_4$ (537.7) calcd [HCl] C 73.22; H 7.02; N 2.44; Found C 73.15; H 7.10; N 2.51.

6v: Yield 42%, mp 253–255 °C [HCl] [EtOH]. ^{1}H NMR ($CDCl_3$): δ 0.63 (t, 3H, $CH_3CH_2CH_2$), 1.80 (s, 3H, 20-CH_3), 3.57 (s, 3H, 6-OCH_3), 4.46 (d, 1H, 5β-H), 4.64 (s[a], 1H, 3-OH), 5.42 (s[a], 1H, 20-OH), 6.50 (d, 1H, 1-H), 6.68 (d, 1H, 2-H), 7.18–7.54 (m, 5H, 20-Ph). MS (TSP) *m/z* 476 (100) [M+1]$^+$ $[\alpha]_D^{25}$ −31.5° (EtOH, *c* 0.1). $C_{30}H_{37}NO_4$ (475.6) calcd [HCl] C 70.36; H 7.48; N 2.74; Found C 70.25; H 7.39; N 2.80.

6w: Yield 58%, mp 259–260 °C [HCl] [EtOH]. ^{1}H NMR ($CDCl_3$): δ 1.80 (s, 3H, 20-CH_3), 3.58 (s, 3H, 6-OCH_3), 4.45 (d, 1H, 5β-H), 4.82 (s[a], 1H, 3-OH),

4.93–5.10 (m, 2H, All), 5.45 (s[a], 1H, 20-OH), 5.50–5.65 (m, 1H, All), 6.50 (d, 1H, 1-H), 6.68 (d, 1H, 2-H), 7.18–7.55 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 473 (50) [M$^+$], 352 (100). $[\alpha]_D^{25}$ −74.4° (EtOH, *c* 0.1). $C_{30}H_{35}NO_4$ (473.6) calcd [HCl] C 70.64; H 7.11; N 2.75; Found C 70.57; H 7.15; N 2.81.

6x: Yield 61%, mp 239–240 °C [HCl] [EtOH]. ^{1}H NMR ($CDCl_3$): δ 1.50 (s, 3H, CH_3), 1.67 (s, 3H, CH_3), 1.78 (s, 3H, 20-CH_3), 3.55 (s, 3H, 6-OCH_3), 4.45 (d, 1H, 5β-H), 4.90 (s[a], 1H, 3-OH), 5.02 (t, 1H, All), 5.45 (s[a], 1H, 20-OH), 6.51 (d, 1H, 1-H), 6.71 (d, 1H, 2-H), 7.23–7.56 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 501 (60) [M$^+$], 380 (100). $[\alpha]_D^{25}$ −45.0° ($CHCl_3$, *c* 0.1). $C_{32}H_{39}NO_4$ (501.6) calcd [HCl] C 71.42; H 7.49; N 2.60; Found C 71.46; H 7.54; N 2.69.

6y: Yield 75%, mp 104–105 °C [HCl] [EtOH]. ^{1}H NMR ($CDCl_3$): δ 1.79 (s, 3H, 20-CH_3), 2.13 (t, 1H, Prop), 3.14 (d, 2H, Prop), 3.55 (s, 3H, 6-OCH_3), 4.44 (d, 1H, 5β-H), 4.80 (s[a], 1H, 3-OH), 5.48 (s[a], 1H, 20-OH), 6.48 (d, 1H, 1-H), 6.67 (d, 1H, 2-H), 7.22–7.45 (m, 5H, 20-Ph). MS (EI 70 eV) *m/z* 471 (45) [M$^+$], 459 (100). $[\alpha]_D^{25}$ −70.0° (EtOH, *c* 0.1). $C_{30}H_{33}NO_4$ (471.6) calcd [HCl] C 70.92; H 6.75; N 2.76; Found C 70.90; H 6.71; N 2.65.

12a: Compound **5c** (0.2 g, 0.4 mmol) was heated under reflux with 2 mL of 98% formic acid[9] for 3 h. The reaction mixture was cooled to room temperature and then alkalized with 25% aq NH_4OH with external cooling in an ice-water bath. Yield 0.15 g (82%), mp 78–80 °C [EtOH]. ^{1}H NMR ($CDCl_3$): δ 0.98 (t, 3H, $C\underline{H}_3CH_2CH_2$), 1.52 (m, 2H, $CH_3C\underline{H}_2CH_2$), 1.62 (m, 1H, 15-H), 2.01 (s, 3H, CH_3), 2.26 (m, 2H, $CH_3CH_2C\underline{H}_2$), 2.34–2.55 (m, 3H, 15-H, 16-H, 10α-H), 2.82 (m, 0.5H, CH_2C=), 3.12 (d, 1H, 10β-H), 3.18 (d, 1H, 9α-H), 3.42 (m, 0.5H, $C\underline{H}_2$C=), 3.83 (s, 3H, 3-OCH_3), 4.70 (s, 1H, 5β-H), 5.62 (t, 1H, $CH_2C\underline{H}$=C(CH_3)Ph), 6.12 (d, 1H, 8-H), 6.57 (d, 1H, 1-H), 6.65 (d, 1H, 2-H), 6.68 (d, 1H, 7-H), 7.17–7.38 (m, 5H, 20-Ph). ^{13}C NMR 11.73 (CH_3), 16.62 (=C(Ph)C$\underline{H}_3$), 20.69 (CH_2), 22.76 (C-10), 28.71 (C-15), 34.78 ($C\underline{H}_2$-CH=), 44.05 (C-16), 45.26 (C-14), 46.42 (C-13), 56.55 (N-CH_2), 56.93 (OCH_3), 59.92 (C-9), 88.31 (C-5), 114.92 (C-2), 119.67 (C-1), 121.76 (CH), 125.09 (C-11), 131.93 (C-12), 132.66 (C-7), 139.78 (>C=), 143.72 (C-3), 144.44 (C-4), 153.77 (C-8), 194.27 (C-6), aromatic: 125.71; 127.18; 128.47; 144.44. MS (EI 70 eV) *m/z* 455 (75) [M$^+$], 426 (100). $C_{30}H_{33}NO_3$ (455.6).

12b: 3-O-Demethylation of **5a** with KOH/diethylene glycol (2 h) afforded **12b**. Yield 50%, mp 163–164 °C [EtOH]. ^{1}H NMR ($CDCl_3$): δ 0.17 (m, 2H, cPropCH_2syn), 0.56 (m, 2H, cPropCH_2anti), 0.89 (m, 1H, cPropCH), 1.62 (m, 1H, 15-H), 2.02 (s, 3H, NCH_3), 2.24 (m, 2H, NCH_2), 2.28–2.40 (m, 2H, 15-H, 16-H), 2.50 (dd, 1H, 10α-H), 2.85 (m, 1H, 16-H), 2.93 (m, 1H, $C\underline{H}_2$-CH=), 3.08 (d, 1H, 10β-H), 3.32 (m, 1H, $C\underline{H}_2$-CH=), 3.42 (d, 1H, 9α-H), 4.71 (s, 1H, 5β-H), 5.63 (t, 1H, $CH_2C\underline{H}$=C(CH_3)Ph), 6.11 (d, 1H, 8-H), 6.53 (d, 1H, 1-H), 6.64 (d, 1H, 2-H), 6.68 (d, 1H, 7-H), 7.18–7.39 (m, 5H, 20-Ph). ^{13}C NMR 3.37 (cPropCH_2), 3.79 (cPropCH_2), 9.22 (cPropCH), 16.53 (CH_2-CH=C(Ph)C$\underline{H}_3$), 22.37 (C-10), 28.43 (C-15), 34.88 ($C\underline{H}_2$-CH=C(Ph)CH_3), 44.29 (C-16), 45.31 (C-14), 46.55 (C-13), 59.05 (NCH_2), 59.41 (C-9), 88.45 (C-5), 117.17 (C-2), 120.06 (C-1), 121.78 (CH_2-C$\underline{H}$=C(Ph)CH_3), 125.64 (C-11), 131.30 (C-12), 132.24 (C-7), 138.40 (C-3), 139.77 (>C=), 142.76 (C-4), 154.81 (C-8), aromatic: 127.08; 128.37; 143.65. IR (KBr) (cm^{-1}) ν_{CO} 1677; $\nu_{C=C}$ 1622. MS (EI 70 eV) *m/z* 453 (75) [M$^+$], 356 (100). $[\alpha]_D^{25}$ −25.6° (EtOH, *c* 0.1). $C_{30}H_{31}NO_3$ (453.5).

12c: 3-O-Demethylation of **5c** with KOH/diethylene glycol (2 h) furnished **12c**. Yield 53%, mp 263–264 °C [HCl] [EtOH]. ^{1}H NMR ($CDCl_3$): δ 0.97 (t, 3H, $C\underline{H}_3CH_2CH_2$), 1.50 (m, 2H, $CH_3C\underline{H}_2CH_2$), 1.62 (m, 1H, 15-H), 2.00 (s, 3H, CH_3), 2.25 (m, 2H, $CH_3CH_2C\underline{H}_2$), 2.32–2.52 (m, 3H, 15-H, 16-H, 10α-H), 2.68 (m, 1H, 16-H), 2.76 (m, 0.5H, CH_2C=), 3.12 (d, 1H, 10β-H), 3.18 (d, 1H, 9α-H), 3.53 (m, 0.5H, CH_2C=), 4.69 (s, 1H, 5β-H), 5.62 (t, 1H, $CH_2C\underline{H}$=C(CH_3)Ph), 6.10 (d, 1H, 8-H), 6.55 (d, 1H, 1-H), 6.64 (d, 1H, 2-H), 6.68 (d, 1H, 7-H), 7.22–7.40 (m, 5H, 20-Ph). ^{13}C NMR 11.79 (CH_3), 16.71 (=C(Ph)C$\underline{H}_3$), 20.75 (CH_2), 22.88 (C-10), 28.60 (C-15), 34.59 ($C\underline{H}_2$-CH=), 44.12 (C-16), 45.66 (C-14), 46.68 (C-13), 56.55 (N-CH_2), 60.15 (C-9), 88.61 (C-5), 117.19 (C-2), 120.26 (C-1), 121.68 (CH=), 125.88 (C-11), 131.22 (C-12), 132.31 (C-7), 138.34 (C-3), 139.99 (>C=), 142.61 (C-4), 154.37 (C-8), 195.09 (C-6), aromatic: 125.66; 127.20; 128,46; 143.65. IR (KBr) (cm^{-1}) ν_{CO} 1680; $\nu_{C=C}$ 1612. MS (EI 70 eV) *m/z* 441 (46) [M$^+$], 244 (100). $C_{29}H_{31}NO_3$ (441.5).

Acknowledgements

This work was supported in part by grants OMFB 9297–07–0036; OTKA I/3 reg. No.: 1722 and T 0413991. The authors thank Mrs Julianna Nagy and Mrs Julianna Kabai for technical assistance, Mrs Katalin Horváth for measuring the ^{1}H NMR spectra, Mrs Katalin Treszkó and Mrs Mária Orosz for recording mass spectra and Dr Gizella B. Szabó (Analytical Laboratory of L. Kossuth University, Debrecen) for performing the microanalyses.

References

1. Wang, D.-X.; Lu, X.-Q.; Qin, B.-Y. *J. Pharm. Pharmacol.* **1995**, *47*, 669.

2. Bentley, K. W.; Hardy, D. G. *J. Am. Chem. Soc.* **1967**, *69*, 3281.

3. Bentley, K. W.; Hardy, D. G.; Meek, B. *J. Am. Chem. Soc.* **1967**, *89*, 3273.

4. Fulmor, W.; Lancaster, J. E.; Morton, G. O.; Brown, J. J.; Howell, C. F.; Nora, C. T.; Hardy, R. A. J. *J. Am. Chem. Soc.* **1967**, *89*, 3322.

5. Bentley, K. W.; Ball, J. C. *J. Org. Chem.* **1958**, *23*, 1720.

6. Marton, J.; Szabó, Z.; Simon, C.; Hosztafi, S.; Makleit, S. *Liebigs Ann. Chem.* **1996**, 1653.

7. Marton, J.; Szabó, Z.; Hosztafi, S. *Liebigs Ann. Chem.* **1993**, 915.

8. Marton, J.; Hosztafi, S.; Berényi, S.; Simon, C.; Makleit, S. *Monatsh. Chem.* **1994**, *125*, 1229.

9. Lewis, J. W.; Readhead, M. J. *Tetrahedron* **1968**, 24, 1829.

10. Valhari, M. U.; Rahman, A. U.; Memon, M. U.; Nachnani, F. C.; Khan, M. Y. *Sci. Int. (Lahore)* **1992**, *4*, 53.

11. Pasternak, G. W.; Wilson, H. A., Snyder, S. H. *Mol. Pharmacol.* **1975**, *11*, 340.

12. Bradford, M. *Anal. Biochem.* **1976**, *72*, 248.

13. Búzás, B.; Tóth, G.; Cavagnero, S.; Hruby, V. J.; Borsodi, A. *Life Sci.* **1992**, *50*, PL71.

14. Handa, B. K.; Lane, A. C.; Lord, J. A. H.; Morgan, B. A.; Rance, M. J.; Smith, C. F. C. *Eur. J. Pharm.* **1981**, *70*, 531.

15. Munson, P. J.; Rodbard, D. *Anal. Biochem.* **1980**, *107*, 220.

16. Klein, P.; Nelson, W. L.; Yao, Y.-H.; Simon, E. J. *J. Med. Chem.* **1990**, *33*, 2286.

(*Received in U.S.A.* 2 *August* 1996; *accepted* 20 *September* 1996)

Med Chem Res 7:4 (1997) 251–258

MEDICINAL
CHEMISTRY
RESEARCH

SYNTHESIS AND OPIOID BINDING PROPERTIES OF NEW β-FUNALTREXAMINE (β-FNA) ANALOGUES+

Csaba Simon,[1] Sándor Hosztafi,[1] Sándor Makleit,[2]
Árpád Márki,[3] Sándor Benyhe,[3] Anna Borsodi[3]

[1] ICN Alkaloida Company Ltd. Tiszavasvári, H-4440 Hungary

[2]Department of Organic Chemistry, L. Kossuth University, P. O. Box 20, Debrecen, H-4010 Hungary

[3]Biological Research Center of Hungarian Acadamy of Science, P. O. Box 521, Szeged, H-6701 Hungary

SUMMARY: Several 6β-chloroacetamido- and 6β-monomethylfumaramido-4,5α-epoxy-7,8-didehydro-morphinans and their analogues with saturated ring C were prepared and their binding affinities to μ, δ and κ opioid receptors determined. Subtype specifity has also been evaluated by the aid of labeled selective ligands.

During our detailed studies[1] on the applicability of the Mitsunobu reaction in the field of morphine alkaloids an efficient method was elaborated for the diastereoselective synthesis of the 6-deoxy-6β-amino and 6-deoxy-6β-amino-14-hydroxy derivatives of codeine and morphine, as well as for the preparation[2-4] of the corresponding dihydro analogues. It is to be emphasized that, up to the present time, this procedure represents the only way of obtaining the 6β-amino derivatives (**1, 2, 3** and **6**) of these alkaloids carrying a $\Delta^{7,8}$ double bond.

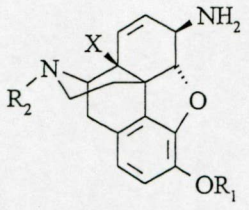
Most recently Hutchinson et al. described[5] the preparation and biological investigation of a few chloroacrylamido derivatives of 4,5α-epoxy-morphinans, and this paper contains previous literature data of a related field of a research, as well.

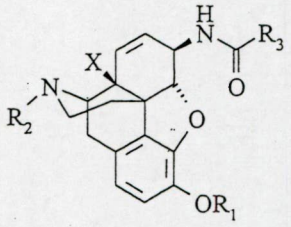
CHEMISTRY

As a continuation of our work[6] we now report on the synthesis and biological properties of novel acylamido analogues of morphine alkaloids. For the studies the amino derivatives **1-9** were employed, and the acid components were monomethyl fumarate, introduced by Porthoghese et al.,[7] and monochloroacetic acid, which has not been utilized as yet in the alkaloid field. Based on the method known from peptide-chemistry[8] the carboxylic acid was

+ Morphine Alkaloids, Part 141. For Part 140 see: Berényi, S.;.Csutorás, Cs.; Gyulai, S.; Makleit, S.:Org. Prep. Proced. Int. . (accepted for publication)

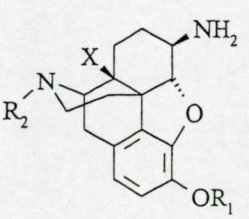
first converted into an "active ester" with N-hydroxysuccinimide, followed by acylation of the amines in dichloromethane solution to obtain the amides **10-23**. This procedure permits the preparation of tritiated compounds as well. The amides **13**, **18** and **19**, and four labeled derivatives have also been described in our previous communication[6]. The physical data and spectral properties of the new compounds are summarized in Table 1 and Table 2, respectively.

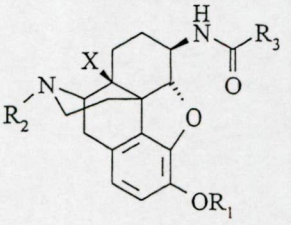




	R_1	R_2	X
1	Me	Me	H
2	Me	Me	OH
3	H	Me	H
4	H	n-Pr	H
5	H	allyl	H
6	H	Me	OH

	R_1	R_2	R_3	X
10	Me	Me	$ClCH_2$	H
11	Me	Me	fum	H
12	Me	Me	$ClCH_2$	OH
13	H	Me	$ClCH_2$	H
14	H	n-Pr	$ClCH_2$	H
15	H	n-Pr	fum	H
16	H	allyl	$ClCH_2$	H
17	H	allyl	fum	H
18	H	Me	$ClCH_2$	OH
19	H	Me	fum	OH





7	Me	Me	OH
8	H	allyl	OH
9	H	CPM	OH

20	Me	Me	fum	OH
21	H	allyl	$ClCH_2$	OH
22	H	allyl	fum	OH
23	H	CPM	fum	OH

Me = methyl; Pr = propyl; fum = trans-CH=CHCOOMe; CPM = cyclopropylmethyl

Table 1.

Physical data of compounds prepared[#]

	Formula	m.p. (°C)	Recystallization solvent	yield (%)	
10	$C_{20}H_{23}N_2O_3Cl$	>250*	abs. ethanol	87	tartaric acid salt
11	$C_{23}H_{26}N_2O_5$	oil		57	
12	$C_{20}H_{23}N_2O_4Cl$	oil		45	
14	$C_{21}H_{25}N_2O_3Cl$	>200*	abs. ethanol	62	tartaric acid salt
15	$C_{24}H_{28}N_2O_5$	>200*	abs. ethanol	58	tartaric acid salt
16	$C_{21}H_{23}N_2O_3Cl$	92-95	abs. ethanol	80	
17	$C_{24}H_{26}N_2O_5$	199-201*	abs. ethanol	51	tartaric acid salt
20	$C_{23}H_{28}N_2O_6$	240-242	abs. ethanol	65	
21	$C_{21}H_{25}N_2O_4Cl$	>200*	abs. ethanol	70	tartaric acid salt
22	$C_{24}H_{28}N_2O_6$	133-135	ether-hexane	58	280[9] HCl
23	$C_{25}H_{30}N_2O_6$	124-126	ether-hexane	48	101-103[7]

All new compounds were analyzed for C, H, N; the results agreed to within ± 0.4 % of the theoretical values.

[#]For the physical and spectral data of compounds **13, 18** and **19** see Ref. 6.

* decomposition

RECEPTOR BINDING STUDIES

The novel compounds were further characterised in radioligand binding assays using neuronal membrane fractions prepared from rat brain. Five substances (**13-16** and **18**) with satisfactory affinity were selected for detailed biochemical assays. Firstly, opioid binding affinities of the compounds were determined by heterologous competition experiments using the general opioid antagonist, [^{3}H]naloxone. These studies also included the estimation of the so-called 'sodium index'. In the presence of 100 mM NaCl the affinity of agonists, but not antagonists, is significantly decreased.[10] The sodium (Na^+) index, i. e. the ratio of K_i or IC_{50} values of a compound measured in

Table 2.

Spectral data of the compounds prepared

	^{1}H-NMR δ (ppm)								MS
	C-5βH	C-6H	C-7H	C-8H	N-R	O-R	others	NH	%
10	4.8: s	4.5; m	5.8; m	5.7; m	2.4; s; 3H; Me	3.8; s; 3H; Me	4.1; m; CH_2Cl	6.4; b	374[M^+](10); 281(30)
11	4.8: s	4.5; m	5.8; m	5.6; dd	2.4; s; 3H; Me	3.7-3.9: 6H;	6.9*; dd; 2H; t-CH=CH	6.9*; b	410[M^+](15); 281(70)
12	4.6; s	4.8; m	6.1; m	5.8; dd	2.4; s; 3H; Me	3.8; s; 3H; Me	4.1; m; 2H; CH_2Cl	7.8; b	390[M^+](15)
14	4.7; s	4.5; m	5.7;m*	5.7;m*	0.9; t; 3H; n-Pr		4.1; m; CH_2Cl	6.5*; b	388[M^+](10); 359(35)
15	4.7; s	4.5; m	5.8; m	5.7; m	0.9; t; 3H; n-Pr	3.8; s; 3H; COOMe	6.9; dd; 2H; t-CH=CH	7.1; b	424[M^+](10); 395(50)
16#	4.6; s	4.2; m	5.8;m*	5.8;m*	5.3; m; 2H* and 6.0*; m; 1H allyl H		4.1; s; 2H; CH_2Cl	8.7; b	386[M^+](10)
17#	4.5; s	4.3; m	5.8;m*	5.8;m*	5.3; m; 2H* and 6.0*; m; 1H allyl H	3.4; s; 3H; COOMe		7.4; b	422[M^+](10)
20	4.5; d	4.0; m			2.4; s; 3H; Me	3.7-3.9; 6H	6.9; dd; 2H; t-CH=CH	7.2; b	428[M^++H]
21	4.4; d	4.2; m			5.3; m; 2H and 6.0; m; 1H allyl H		4.1; m; 2H; CH_2Cl	7.1; b	404[M^+](100); 370(25)
22	4.5; d	4.2; m			5.2; m; 2H and 5.8; m; 1H allyl H	3.8; s; 3H; COOMe	6.9; dd; 2H; t-CH=CH	7.2; b	441[M^++H]$^{\&}$
23	4.4; d	4.2; m			0.1-0.9; m; 5H; c-Pr-H	3.8; 3H; COOMe		7.2; b	454[M+](10)

* overlapping signals

$^{\&}$ measured with the thermospray technique

DMSO-d_6

the presence and absence of sodium ions, correlates well with the pharmacological property of the given compound. Thus, pure opioid agonists have sodium ratio >>1, pure antagonists show sodium indices <1, and intermediate values are characteristic for mixed agonist/antagonist ligands. Data obtained in [^{3}H]naloxone displacement studies are shown in Table 3. Compounds have high affinities in competing for [^{3}H]naloxone binding, although their concentrations producing 50% inhibition of specific binding (IC50 values) are about one order of magnitude higher than those measured for naltrexone and levorphanol. N-methyl derivatives **13** and **14** are agonists on the basis of their sodium indices, whereas the N-allyl (**16**) and N-propyl (**14** and **15**) compounds show antagonist properties. This is in agreement with previous literature data on several N-substituted epoxymorphinans.[11] In that study, ligands with small substituents, such as H- or methyl-, were found to be agonists, however, compounds having bulky substituents (e. g., allyl- or cyclopropylmethyl-) behaved as pure antagonists.

Table 3.

Inhibition of [^{3}H]naloxone binding to rat brain membranes by unlabeled ligands

Compound	IC50 values (nM)		Na^+-index*	Suggested agonist/antagonist character
	$-Na^+$	$+Na^+$		
13	2.1±0.5	54.5±10	26	agonist
14	2.1±0.4	2.0±0.4	0.9	antagonist
15	5.4±1.1	4.7±0.6	0.9	antagonist
16	1.4±0.5	2.7±0.4	1.9	antagonist
18	2.8±0.1	71±14	25	agonist
Naltrexone	0.2±0.05	0.1±0.02	0.5	antagonist
Levorphanol	0.5±0.05	25±8	50	agonist

* $IC50_{+Na} / IC50_{-Na}$; Sodium ions were added to the samples as 100 mM NaCl.

Data are expressed as mean values ±S.E.M.

Opioid receptors are known to be heterogeneous structures, consisting of three major types, mu (μ), delta (δ) and kappa (κ).[12] Neither endogenous opioids nor synthetic opiate ligands possess absolute specificity for a given receptor type, but can interact with more than one type of receptors. Therefore binding properties of the new compounds were further examined using receptor-type selective radioligands. Morphine (μ) receptor was labeled with its selective agonist ligand, [^{3}H]D-Ala^2-(Me)Phe^4-Gly^5-ol-enkephalin (DAMGO). Binding to δ-opiate receptor was

measured with [^{3}H]D-Ser^2-Leu^5-Thr^6-enkephalin (DSLET), while κ-receptor affinities were determined with the κ-specific, non-peptide agonist ligand, [^{3}H]U-69,593. Data from heterologous competition experiments are listed in Table 4. Relative affinity values, introduced by Kosterlitz and co-workers,[13] as a measure of receptor-type selectivity are also presented. The highest affinities were obtained in [^{3}H]DAMGO binding assays indicating that all ligands bind preferably to μ-opioid receptors. At the δ-receptor, labeled with [^{3}H]DSLET, significantly lower affinities were found. The good μ/δ selectivity of the compounds is demonstrated by their relative affinity values (Table 4.). Most of the ligands showed only moderate IC50 values in [^{3}H]U-69,593 binding studies, although compound **15** and **16** exhibited quite good affinity, so substantial interaction with the κ-receptors occurred. This is consistent with the data reported by Ward and co-workers,[14] who described considerable effects mediated by κ-receptors in the case of β-funaltrexamine. Interestingly, the affinity of the antagonist ligand **14**, carrying N-propyl group, is higher than that of the agonist ligands **13** and **18** substituted with a methyl group at the piperidine nitrogen atom. These compounds are potential affinity-reagents for the opioid receptors, although the irreversible nature of their binding has not yet been demonstrated.

Table 4.

Inhibition of the binding of receptor-type selective radioligands to rat brain membranes by unlabeled compounds

Compound	IC50 values, (nM)			Relative affinity, * (%)		
	[^{3}H]DAMGO (μ)	[^{3}H]DSLET (δ)	[^{3}H]U-69,593 (κ)	μ	δ	κ
13	0.77±0.1	56±11	49±8	97	1	2
14	0.99±0.3	53±17	20±5	94	2	4
15	1.60±0.3	96±24	7.9±3	81	2	17
16	0.89±0.3	35±12	3.3±1	76	2	22
18	1.48±0.06	170±48	101±25	98	1	1
Morphine	2.8±0.5	48±16	n.d.			
DSLET	33±8	5.0±1.9	n.d.			
U-69,593	n.d.	n.d.	2.7±0.8			

* Relative affinity constant (ref. 14.) = $IC50_x^{-1}$ / ($IC50_\mu^{-1}$ + $IC50_\delta^{-1}$ + $IC50_\kappa^{-1}$) x 100]

Data are expressed as mean values ±S.E.M. n.d.: not determined.

Summarising our results, the newly synthesised morphinan analogues have high affinities in opioid receptor binding assays. Each of the ligands possess quiet good affinities toward the μ binding sites, whereas some of them also cross-react with the κ-opioid receptors. Interactions with the δ-binding sites (enkephalin receptors) are less pronounced. On the basis of their sodium indices the agonist or antagonist nature of the compounds may be prognosticated, but further *in vivo* or *in vitro* pharmacological experiments are necessary to confirm these biochemical predictions. Experiments are in progress to elucidate whether any of the compounds will bind irreversibly to the opioid receptors.

EXPERIMENTAL

Preparation of the acylamino-derivatives was carried out as reported in our previous paper.[6] Receptor binding experiments were performed with rat brain membranes. Particulate membrane fractions were prepared from rat brain (Wistar strain, both sexes) by differential centrifugation according to the procedure of Pasternak and Snyder.[15] Ligand binding assays were conducted in 50 mM Tris/HCl buffer (pH 7.4). Membranes were incubated with 1 nM radioligand in the presence or absence of various concentrations (ranging 10^{-11} - 10^{-5} M) of the unlabeled compounds. Sample volume was 1 ml and contained 0.3-0.4 mg membrane protein. Opioid receptors were labeled by the radioligands listed below. [^{3}H]Naloxone (70 Ci/mmol) was prepared in the Isotope Laboratory of BRC, Szeged, Hungary as described earlier.[16] [^{3}H]D-Ala^2-(Me)Phe^4-Gly^5-ol-enkephalin (DAMGO; 55 Ci/mmol), [^{3}H]D-Ser^2-Leu^5-Thr^6-enkephalin (DSLET, 41.5 Ci/mmol) and [^{3}H]U-69,593 (5α,7α,8β-(−)-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro(4,5)-dec-8-yl)]-benzacetamide; 47.5 Ci/mmol) were purchased from New England Nuclear. Incubation conditions were selected according to the equilibrium binding properties of the radioligand (Naloxone: 0 °C, 60 min; DAMGO: 35 °C, 45 min; DSLET: 25 °C, 45 min; U-69,593: 25 °C, 45 min). Incubations were terminated by rapid filtration under vacuum followed by washing with 2 x 7 ml ice-cold Tris/HCl buffer through Whatman glass fibre filters using an automated Brandel M24R cell harvester. This method allows to separate bound and free radioligand. The radioactivity was measured by liquid scintillation counting using a Beckman LS 5000TD spectrophotometer. Non-specific binding was determined in the presence of 10 μM unlabeled naloxone. All experiments were performed in duplicates and repeated several times. Equilibrium binding data were evaluated by the LIGAND program as described by Munson and Rodbard.[17]

ACKNOWLEDGEMENT:

The authors thank to Prof. K. Medzihradszky (Budapest) for a fruitful discussion on the method of acylation of amines. This work was financially supported in part, by a grant (OTKA reg. No.: 013991) obtained from the National Science Foundation (Budapest, Hungary)

REFERENCES:

1. Simon, Cs. Ph.D. Thesis, Lajos Kossuth University, Debrecen, 1993
2. Simon, Cs.; Hosztafi, S.; Makleit, S., Synth. Commun., **1992**, *22*, 913-921.
3. Simon, Cs.; Hosztafi, S.; Makleit, S., Heterocycles, **1994**, *38*, 1347-1354.
4. Simon, Cs.; Hosztafi, S.; Makleit, S., Tetrahedron, **1994**, *50*, 9757-9768.
5. Hutchinson, I.; Archer, S.; Hill, K. P.; Bidlack, J. M., BioMed. Chem. Lett., **1996**, *6*, 1563-1566.
6. Ötvös, F.; Tóth, G.; Lovas, S.; Simon, Cs.; Hosztafi, S., Helv. Chim. Acta, **1996**, *79*, 133-136.
7. Portoghese, P. S.; Larson, D. L.; Sayre, L. M.; Fries, D. S.; Takemori, A. E., J. Med. Chem., **1980**, *23*, 233-234.
8. Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M., J. Am. Chem. Soc., **1964**, *86*, 1839.
9. Sayre, L. M.; Larson, D. L.; Takemori, A. E.; Porthogese, P. S., J. Med. Chem., **1984**, *27*, 1325-1335.
10. Pert, C.B.; Snyder, S.H. Mol. Pharmacol., **1974**, *10*, 868-879.
11. Gero, A. Life Sci., **1985**, *36*, 1021-1024.
12. Kieffer, B. Cell. Mol. Neurobiol., **1995**, *15*, 615-635.
13. Kosterlitz, H.W.; Paterson, S.J. Proc. Royal Soc. London, **1980**, *210*, 113-122.
14. Ward, S.J.; Portoghese, P.S.; Takemori, A.E. J. Pharmacol. Exp. Ther., **1982**, *220*, 494-498.
15. Pasternak, G.W.; Snyder, S.H. Mol. Pharmacol., **1974**, *10*, 183-193.
16. Tóth, G.; Krámer, M.; Sirokmán, F.; Borsodi, A.; Rónai, A.Z. J. Label. Comp. Radiopharm., **1982**, *19*, 1021-1030.
17. Munson, P.J.; Rodbard, D. Anal. Biochem., **1980** *107*, 220-239.

Received: 3-10-97 *Accepted: 9-11-97*

Reprinted from

European Journal of Pharmacology

European Journal of Pharmacology 383 (1999) 209–214

μ-Opioid receptor specific antagonist cyprodime: characterization by in vitro radioligand and [^{35}S]GTPγS binding assays

Árpád Márki [a,b], Krisztina Monory [a], Ferenc Ötvös [a], Géza Tóth [a], Roland Krassnig [c], Helmut Schmidhammer [c], John R. Traynor [d,1], Bernard P. Roques [e], Rafael Maldonado [e,*], Anna Borsodi [a,b]

[a] *Institute of Biochemistry and Isotope Laboratory, Biological Research Center of the Hungarian Academy of Sciences, H-6701 Szeged, POB 521, Hungary*

[b] *Department of Pharmacodynamics, Albert Szent-Györgyi Medical University, H-6721 Szeged, Eötvös U.6, Hungary*

[c] *Institute of Pharmaceutical Chemistry, University of Innsbruck, Innrain 52a, A-6020 Innsbruck, Austria*

[d] *Department of Chemistry, Loughborough University, Leicestershire, LE11 3TU, UK*

[e] *Département de Pharmacochimie Moléculaire et Structurale, INSERM U266, CNRS UMR 8600, UFR des Sciences Pharmaceutiques et Biologiques, 4, avenue de l'Observatoire, 75270 Paris Cedex 06, France*

Received 13 August 1999; accepted 20 August 1999

ELSEVIER

ELSEVIER

European Journal of Pharmacology 383 (1999) 209–214

ejp

www.elsevier.nl/locate/ejpha

μ-Opioid receptor specific antagonist cyprodime: characterization by in vitro radioligand and [^{35}S]GTPγS binding assays

Árpád Márki [a,b], Krisztina Monory [a], Ferenc Ötvös [a], Géza Tóth [a], Roland Krassnig [c], Helmut Schmidhammer [c], John R. Traynor [d,1], Bernard P. Roques [e], Rafael Maldonado [e,*], Anna Borsodi [a,b]

[a] *Institute of Biochemistry and Isotope Laboratory, Biological Research Center of the Hungarian Academy of Sciences, H-6701 Szeged, POB 521, Hungary*

[b] *Department of Pharmacodynamics, Albert Szent-Györgyi Medical University, H-6721 Szeged, Eötvös U.6, Hungary*

[c] *Institute of Pharmaceutical Chemistry, University of Innsbruck, Innrain 52a, A-6020 Innsbruck, Austria*

[d] *Department of Chemistry, Loughborough University, Leicestershire, LE11 3TU, UK*

[e] *Département de Pharmacochimie Moléculaire et Structurale, INSERM U266, CNRS UMR 8600, UFR des Sciences Pharmaceutiques et Biologiques, 4, avenue de l'Observatoire, 75270 Paris Cedex 06, France*

Received 13 August 1999; accepted 20 August 1999

Abstract

The use of compounds with high selectivity for each opioid receptor (μ, δ and κ) is crucial for understanding the mechanisms of opioid actions. Until recently non-peptide μ-opioid receptor selective antagonists were not available. However, *N*-cyclopropylmethyl-4,14-dimethoxy-morphinan-6-one (cyprodime) has shown a very high selectivity for μ-opioid receptor in in vivo bioassays. This compound also exhibited a higher affinity for μ-opioid receptor than for δ- and κ-opioid receptors in binding assays in brain membranes, although the degree of selectivity was lower than in in vitro bioassays. Cyprodime has recently been radiolabelled with tritium resulting in high specific radioactivity (36.1 Ci/mmol). We found in in vitro binding experiments that this radioligand bound with high affinity (K_d 3.8 ± 0.18 nM) to membranes of rat brain affording a B_{max} of 87.1 ± 4.83 fmol/mg. Competition studies using μ, δ and κ tritiated specific ligands confirmed the selective labelling of cyprodime to a μ-opioid receptor population. The μ-opioid receptor selective agonist [D-Ala^2, *N*-MePhe^4,Gly^5-ol]enkephalin (DAMGO) was readily displaced by cyprodime (K_i values in the low nanomolar range) while the competition for δ- ([D-Pen^2,D-Pen^5]enkephalin (DPDPE)) and κ- (5α,7α,8β-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro(4,5)dec-8-yl]-benzene-acetamide (U69,593)) opioid receptor selective compounds was several orders of magnitude less. We also found that cyprodime inhibits morphine-stimulated [^{35}S]GTPγS binding. The EC_{50} value of morphine increased about 500-fold in the presence of 10 μM cyprodime. These findings clearly indicate that cyprodime is a useful selective antagonist for μ-opioid receptor characterization.

Keywords: Cyprodime; μ-Opioid receptor; Radioligand; GTPγS binding; Morphine

1. Introduction

Opioid drugs and opioid peptides produce their pharmacological effects, including antinociception, by interaction with opioid receptors in the central nervous system. Opioid receptors are known to be a heterogeneous population consisting of at least three major types (μ, δ and κ) which exhibit different ligand selectivity profiles (Borsodi and Tóth, 1995). Most endogenous opioids and synthetic ligands do not possess absolute specificity for a given receptor type, but interact with more than one opioid receptor. The situation is further complicated by the fact that multiple receptor types may coexist within a single tissue, or even a single cell (Borsodi, 1991).

The multiplicity of opioid receptors is generally accepted and the primary structure of the δ-opioid receptor

* Corresponding author. Laboratori de Neurofarmacologia, Facultat de Cienciès de la Salut i de la Vida, Universitat Pompeu Fabra, C/Doctor Aiguader 80, 08003 Barcelona, Spain. Tel.: +34-93-542-2845; fax: +34-93-542-2802.

[1] Present address: University of Michigan Medical School, Ann Arbor, MI 48104, USA.

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00610-X

(Kieffer et al., 1992; Evans et al., 1993), μ-opioid receptor (Chen et al., 1993) and κ-opioid receptor (Reisine and Bell, 1993) are known. The further development of highly selective ligands remains a challenge for better characterization for each receptor type and possible subtypes.

Opioid receptor antagonists have been indispensable pharmacological tools for identifying receptor types involved in the actions of endogenous and synthetic opioid receptor agonists. Antagonists are especially useful when the pharmacological endpoints are identical (e.g., antinociception or the inhibition of a smooth muscle contractions), and when it is not easy to distinguish among μ-, δ- and κ-opioid receptor mediated effects. Matthes et al. have recently reported that the μ-opioid receptor gene product is the molecular target of morphine in vivo and that it is a mandatory component for the main pharmacological responses of this opioid (Matthes et al., 1996).

It is known that opioid receptors exert their biological functions by interacting with GTP binding proteins. G_i/G_o proteins to which the opioid receptors are coupled regulate effector molecules such as adenylyl cyclase and/or ion channels (Standifer and Pasternak, 1997). Signal transduction can be monitored in membrane preparations by measuring the binding of the non-hydrolysable GTP analogue, guanosine-5′-*O*-(γ-thio)triphosphate (GTPγS) as a function of the amount of a given ligand (Traynor and Nahorski, 1995).

Cyprodime (*N*-cyclopropylmethyl-4,14-dimethoxymorphinan-6-one) has been shown to be a selective μ-opioid receptor antagonist by using guinea pig ileal longitudinal muscle preparations, rat and mouse vas deferentia and acetic-acid writhing tests (Schmidhammer et al., 1989).

In the present study, we have further defined the in vitro ligand-binding profile of cyprodime and described the biochemical characterization of its tritiated derivative, [^{3}H]cyprodime. We have also evaluated the functional effectiveness of cyprodime to alter [^{35}S]GTPγS binding and to inhibit morphine-stimulated [^{35}S]GTPγS binding.

2. Materials and methods

2.1. Chemicals

Cyprodime was synthesized as previously reported (Schmidhammer et al., 1989). D-Phe-Cys-Tyr-D-Arg-Thr-Pen-Thr-NH_2 (CTAP) and [D-Ser^2, Leu^5, Thr^6]enkephalin (DSLET) were a generous gift from the National Institute of Drug Abuse Drug Supply System (Rockville, MD). (±)Ethylketocyclazocine methanesulfonate was supplied by Sterling Winthrop Research Institute (Rennsealeer, New York). Dihydromorphine, deltorphin II, Tyr-Tic-Phe-Phe-OH (TIPP) and Ile^{5,6}deltorphin II were synthesized as previously reported (Tóth et al., 1982a; Buzas et al., 1992; Nevin et al., 1993, 1994, respectively). 5α,7α,8β-(−)-*N*-Methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro(4,5)dec-8-yl]-benzene-acetamide (U69,593) and *trans*-3,4-dichloro-*N*-methyl-1-*N*-1-pyrrolidinyl-(cyclohexyl)-benznecetamide (U50,488) were obtained from Upjohn (Kalamazoo, MI). [D-Ala^2,*N*-MePhe^4,Gly^5-ol]enkephalin (DAMGO), was purchased from Bachem (Switzerland). All other chemicals were of analytical grade.

[^{3}H]Cyprodime (31.6 Ci/mmol) (Ötvös et al., 1992) and [^{3}H]naloxone (72 Ci/mmol) (Tóth et al., 1982b) were synthesized in our Isotope Laboratory as previously reported. [^{3}H]U69,593 (43 Ci/mmol) was purchased from DuPont-New England Nuclear (Boston, MA, USA) and [^{3}H]DAMGO (59 Ci/mmol) and [^{3}H][D-Pen^2,D-Pen^5]enkephalin (DPDPE) 32 Ci/mmol) from Amersham (Buckinghamshire, England).

2.2. Membrane preparation

Rat brain membranes were prepared according to Pasternak et al. (1975) with a small modification. Rats (PVG/C and Wistar strain) were decapitated. The brains without cerebellum were removed and then homogenized in twenty volumes (w/vol) of ice-cold Tris–HCl buffer (50 mM, pH 7.4) and centrifuged (40,000 × *g*, 4°C, 20 min). The final pellet was resuspended in buffer (50 mM Tris–HCl, pH 7.4) and incubated for 30 min at 37°C. Centrifugation was repeated and the final pellet resuspended in buffer (50 mM Tris–HCl, 0.32 M sucrose, pH 7.4) and stored at −70°C. The guinea pig brain membranes were prepared similarly.

2.3. Radioligand binding assays

Ligand binding experiments were carried out in 50 mM Tris–HCl buffer (pH 7.4) with or without 100 mM NaCl, in a final volume of 1 ml containing approximately 0.3–0.5 mg protein. Incubations were started by addition of membrane suspension and continued in a shaking water bath until steady-state was achieved (40–45 min). The reaction was terminated by rapid filtration on a Brandel M24R cell harvester through Whatmann GF/B or GF/C filters and washed with 3 × 5 ml of ice-cold buffer. The filters were dried and the bound radioactivity was determined in a toluene based scintillation cocktail in Wallac 1409 liquid scintillation counter. Total binding was defined as that measured in the absence of competing agent and non-specific binding as binding remaining in the presence of 10 μM naloxone.

2.4. [^{35}S]GTPγS binding

For [^{35}S]GTPγS binding the same membrane preparation was used. Tubes contained 10 μg of protein, 30 μM GDP, 10^{-10} to 10^{-5} M opioid receptor ligands, and 0.05 nM [^{35}S]GTPγS, all in 50 mM Tris–HCl buffer containing 1 mM EGTA and 3 mM $MgCl_2$ in a final volume of 1 ml. Tubes were incubated for 1 h at 30°C. Total activity was

measured in the absence of tested compounds, and nonspecific binding was measured in the presence of 100 μM non-labelled GTPγS. The incubation was terminated by filtrating the samples through Whatman GF/B glass fiber filters. Filters were washed three times with ice-cold buffer in a Millipore filtration instrument, then dried. Radioactivity was measured in a Wallac 1409 scintillation counter using a toluene based scintillation cocktail. Stimulation is given as percentage of specific binding. Data were calculated from three or four separate experiments done in triplicate.

2.5. Data analysis

All assays were carried out at least three times in duplicate, and values are given as means ± S.E.M. The binding capacity (B_{max}) and K_d of [^{3}H]cyprodime were calculated according to Rosenthal (1967) using GraphPad Prism 2.01 computer program. Competition data were analyzed with the program LIGAND (Munson and Rodbard, 1980), using a non-linear least squares fitting algorithm.

3. Results

3.1. Competition assays

The selectivity of unlabelled cyprodime was tested in rat brain membranes using highly selective radioligands for each receptor (μ, δ and κ) (Table 1). The μ-opioid receptor selective peptide, [^{3}H]DAMGO, was readily displaced by cyprodime (K_i value 5.4 nM). Cyprodime showed much less affinity for δ binding sites, which were labelled with [^{3}H]DPDPE. More than 40-fold difference was observed (244.6 for DPDPE vs. 5.4 nM for DAMGO K_i values) when compared with binding to [^{3}H]DAMGO. Almost a similar low affinity (K_i 213.7 nM) was found when cyprodime competed for the κ binding sites labelled with [^{3}H]U69,593.

Further characteristics of cyprodime in rat brain were investigated when [^{3}H]naloxone, a general opioid receptor antagonist, was displaced in the absence and presence of 100 nM NaCl. Without the salt, a K_i value of 14.95 ± 1.01 nM was estimated, which did not change upon addition of NaCl (K_i value 14.70 ± 1.92 nM). This result clearly demonstrates the antagonistic property of cyprodime.

Table 1
Affinity of cyprodime for opioid receptors labelled with different tritiated ligands
Rat brain membranes were incubated with [^{3}H]DAMGO for 45 min at 35°C, [^{3}H]DPDPE for 150 min at 25°C and [^{3}H]U69,593 for 30 min at 30°C with 11 concentrations of cyprodime (10^{-5} to 10^{-12}). Values represent means ± S.E.M. from three separate experiments.

Tritiated ligands	Specificity	K_i (nM)
[^{3}H]DAMGO	μ	5.4 ± 2.4
[^{3}H]DPDPE	δ	244.6 ± 23.1
[^{3}H]U69,593	κ	2187 ± 42.3

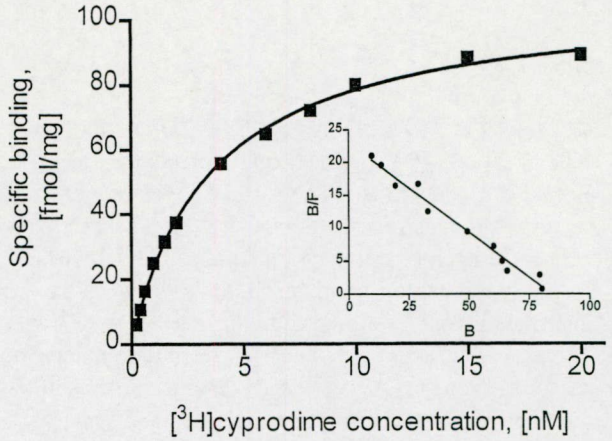


Fig. 1. Equilibrium saturation binding isotherm for [^{3}H]cyprodime binding to rat brain (Wistar strain). Membranes were incubated for 40 min at 25°C. Specific binding was measured at 12 concentrations of radioligand varying from 0.1 to 20 nM using 10 mM naloxone to define nonspecific binding. The insert represents the Rosenthal transformation of the equilibrium binding isotherm. Prism 2.01 computer program was used to fit experimental data and the following parameters were calculated: K_d, 3.8 nM and B_{max}, 87.1 fmol/mg protein.

3.2. Binding of [^{3}H]cyprodime

Binding of [^{3}H]cyprodime to rat brain membranes reached equilibrium at 25°C in 40 min and was stable for at least 90 min. In the saturation experiments, a single class of binding site was detected with a K_d value of 3.83 ± 0.18 nM. The binding capacity was found to be 87.1 ± 4.83 fmol/mg protein in Wistar rats (Fig. 1). The same affinity (3.84 ± 0.12 nM) was observed when another strain (PVG/C) was used. However, it is interesting to note that the B_{max} value was significantly ($p < 0.01$) higher (124 ± 13 fmol/mg protein) in this strain.

Specifically bound [^{3}H]cyprodime was displaced readily from rat brain by cold cyprodime (K_i 8.1 nM) and by the μ-opioid receptor agonists dihydromorphine (K_i 0.4 nM) and DAMGO (K_i 1.1 nM) (Table 2). The μ-opioid receptor selective somatostatin analogue, CTAP, showed less affinity (K_i 43.8 nM). On the other hand, the mixed opioid receptor antagonist, naloxone, exhibited high affinity (K_i 0.9 nM). The weak labelling of δ- and κ-opioid receptors was confirmed by the low affinities of the δ- (deltorphin II and Ile^{5,6}deltorphin II) and κ- (norbinaltorphimine and U50,488) opioid receptor selective ligands. Of the highly δ-opioid receptor specific agonists, deltorphin II and Ile^{5,6}deltorphin II showed very low affinity K_i values, 1186 nM and 1900 nM, respectively. The δ-opioid receptor specific antagonist TIPP was even less potent (K_i 2827 nM). DSLET, the fairly δ-opioid receptor selective ligand which shows cross reactivity with μ-opioid receptor (Gacel

Table 2
Displacement of [^{3}H]cyprodime by opioid ligands in membranes of rat and guinea pig brain
Membranes were incubated with 2 nM [^{3}H]cyprodime for 40 min at 25°C with 11 concentrations (10^{-5} to 10^{-12}) of each competing ligand. Values represent mean ± S.E.M. from three observations. N.T. means not tested.

Competing ligands		K_i (nM)	
		Rat brain (Wistar)	Guinea pig brain
Cyprodime	μ-opioid receptor antagonist	8.1 ± 1.8	26.6 ± 3.9
Dihydromorphine	μ-opioid receptor agonist	0.4 ± 0.1	6.0 ± 2.3
DAMGO	μ-opioid receptor agonist	1.1 ± 2.3	2.6 ± 1.9
CTAP	μ-opioid receptor antagonist	43.8 ± 33.0	48.1 ± 26.9
Naloxone	mixed opioid receptor antagonist	0.9 ± 0.03	1.4 ± 0.5
DSLET	δ-opioid receptor agonist	9.8 ± 2.4	6.3 ± 3.7
TIPP	δ-opioid receptor antagonist	2827 ± 1243	7060 ± 850
Deltorphin II	δ-opioid receptor agonist	1186 ± 104	2878 ± 1242
Ile^{5,6}-deltorphin II	δ-opioid receptor agonist	1900 ± 98	N.T.
U-50,488	κ-opioid receptor agonist	288.1 ± 5.5	303.7 ± 112.8
Norbinaltorphimine	κ-opioid receptor antagonist	171.6 ± 57.0	769.8 ± 321.8

et al., 1988) competes with relatively high affinity (K_i 9.8 nM) for [^{3}H]cyprodime. The affinity of the highly selective κ-opioid receptor agonist U50,488 is much lower (K_i 288.1 nM) and the κ-opioid receptor selective antagonist norbinaltorphimine showed a comparable low affinity (K_i 171.6 nM).

In guinea pig brain, all ligands except DSLET, showed higher K_i values than in rat brain. Cyprodime itself had about 3 × less affinity (K_i 26.6 nM), and DAMGO showed about 2.5 × less affinity (K_i 2.6 nM) than in rat brain. Naloxone still exhibited high affinity (K_i 1.4 nM) whereas dihydromorphine showed a somewhat decreased affinity (K_i 6.0 nM). DSLET exhibited about the same affinity in guinea pig (K_i 6.3 nM) as in rat brain (K_i 9.8 nM). The δ-opioid receptor selective agonist deltorphin II showed a K_i value in the micromolar range (K_i 2878 nM) while the δ-opioid receptor antagonist peptide TIPP was even less potent (K_i 7060 nM). The κ-opioid receptor specific ligands displayed higher affinities than the δ-opioid receptor ligands where K_i values for U50,488 and norbinaltophimine were found to be 303.7 nM and 769.8 nM, respectively.

3.3. [^{35}S]GTPγS binding

We first examined the effects of increasing concentrations of cyprodime on [^{35}S]GTPγS binding. Morphine, a potent μ-opioid receptor agonist, was used as a reference compound. The responses in this assay were detected in the concentration range from 10^{-9} to 10^{-5} M (Fig. 2). Cyprodime caused a slight, but not significant increase of the amount of bound [^{35}S]GTPγS. Thus, the maximal stimulation induced by cyprodime was about 110% above the basal value, while morphine reached a plateau at 155%.

In further experiments, the effects of two different concentrations of cyprodime were studied (Fig. 3). Morphine was incubated either with buffer (negative control), 1 μM cyprodime, 10 μM cyprodime or 1 μM naloxone (positive control). When morphine was incubated with buffer alone an EC_{50} of 244 nM was detected with 155%

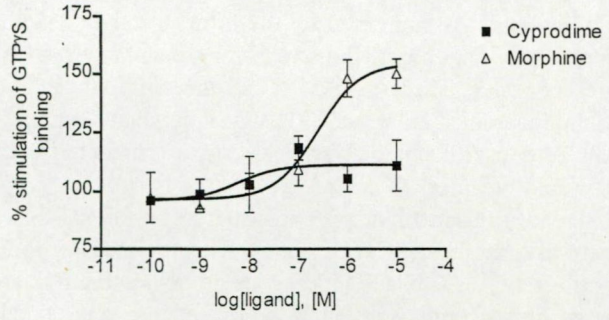


Fig. 2. Effect of different concentrations of morphine [△] and cyprodime [■] on [^{35}S]GTPγS binding to G proteins in crude rat brain membrane preparations. Total binding [without any stimulating agent] is 100%. Data points achieved by the addition of cyprodime do not differ significantly from the basal line. Assay tubes contained 10 mg of protein, 0.05 nmol [^{35}S]GTPγS, 30 mmol GDP, 1 mM EGTA and 3 mM $MgCl_2$ in Tris–HCl buffer, pH 7.4. Incubation was carried out for 60 min at 30°C. Experiments were done three times in triplicate. Data are mean ± S.E.M.

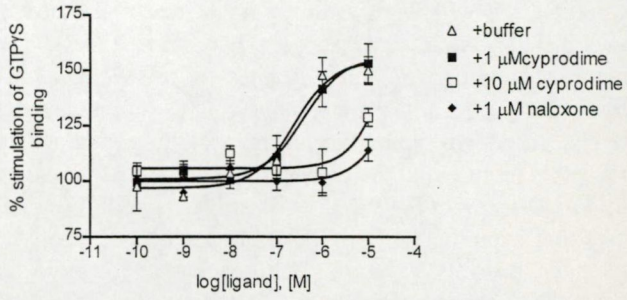


Fig. 3. Stimulation of [^{35}S]GTPγS binding to crude rat brain membranes by various concentrations of morphine was tested in the presence of 0 [△], 1 [■] and 10 [□] mM cyprodime or 1 mM [◆] naloxone. Stimulation of [^{35}S]GTPγS (50 pmol) binding to crude rat brain membranes (10 mg/tube). Assays were performed in the presence of 30 mM GDP for 60 min at 30°C. Points represent means ± S.E.M. from three separate experiments done in triplicate.

maximal stimulation, in agreement with the results of the previous experiment. 1 μM naloxone abolished the stimulating effects of morphine almost completely (EC_{50} ~ 100 μM). 1 μM cyprodime had no significant effect on the morphine dose–response curve when incubated at the lower concentration (1 μg). However, a higher dose of cyprodime (10 μg) dramatically reduced the stimulatory responses of morphine on GTPγS binding. At this concentration of cyprodime, the dose–response curve of morphine shifted to the right, revealing a 500-fold increase of the EC_{50}.

4. Discussion

The present findings with [^{3}H]cyprodime strongly support previous bioassay data which indicated cyprodime to be a highly selective μ-opioid receptor antagonist (Schmidhammer et al., 1989). The μ/κ selectivity ratio in the guinea pig ileum was found to be 37, while in the isolated mouse vas deferens preparation it was 28, which were 2 to 3 × greater values than with naloxone. In the mouse vas deferens preparation the μ/δ selectivity ratio was 15 × greater than with naloxone (Schmidhammer et al., 1989). In the present radioligand binding assays highly selective compounds [^{3}H]DAMGO, [^{3}H]DPDPE and [^{3}H]U69,593 were used in rat brain membranes for labelling μ-, δ- and κ-opioid receptors, respectively. Unlabelled cyprodime displaced [^{3}H]DAMGO with high affinity (K_i 5.4 nM). The affinity of cyprodime for the δ and κ sites was more than 40 × less. It is interesting to note that the same rank order of affinity was seen in guinea pig and frog brain (data not shown) as in rat. The antagonist property of cyprodime was shown in binding assays where [^{3}H]naloxone was displaced by cyprodime (almost identical K_i values were observed when the experiments were performed in the presence or absence of 100 nM NaCl).

Cyprodime was labelled with tritium resulting in a specific radioactivity of 31.6 Ci/mol (Ötvös et al.,1992). The detailed binding properties of this ligand were investigated. The binding was saturable and a single binding site was detected with high affinity: K_d value of 3.8 nM in two different strains of rats (PVG/C and Wistar). The maximal number of binding sites were 87 and 124 fmol/mg protein in Wistar and PVG/C strains, respectively. Although cyprodime labels a single population of receptors in rat brain, the density is less than that measured by [^{3}H]DAMGO (B_{max} 222 ± 5 fmol/mg) and may indicate that cyprodime is only labelling a subset of μ-opioid receptor, possibly supporting the concept of μ-opioid receptor heterogeneity (Varga et al., 1987).

The selectivity of tritiated cyprodime was tested in rat and guinea pig brain. Unlabelled cyprodime, the μ-opioid receptor agonists dihydromorphine and DAMGO, as well as naloxone showed affinities in the low nanomolar range (K_i 0.4–8.1 nM). Somewhat lower potency was detected with CTAP (K_i 43.8 nM) which is a peptide derivative analogue of somatostatin (Pelton et al., 1986). In guinea pig brain all of the above unlabelled ligands showed affinities in the nanomolar range with somewhat higher K_i values (2.6–26.6 nM). These differences might be related to the different ratio of μ- and κ-opioid receptors in the two species (Benyhe et al., 1992). A number of δ-opioid receptor selective ligands (including DSLET, TIPP, deltorphin II and Ile5,6 deltorphin II) were applied to compete for tritiated cyprodime. Low affinities were observed (K_i values in the micromolar range) when using these compounds in rat as well as guinea pig brain. These findings confirm the high selectivity of tritiated cyprodime. Low affinities were also measured using the κ-opioid receptor selective agonist U50,488 (K_i 288 and 303 nM) and the κ-opioid receptor selective antagonist norbinaltorphimine (K_i 171 and 769 nM) for representing κ-opioid receptor specific compounds.

The most currently used non-peptide antagonists, naloxone and naltrexone, do not exhibit high selectivity for any of the opioid receptors. β-funaltrexamine (Takemori et al., 1986) and CTAP (Pelton et al., 1986) were found to be μ-opioid receptor selective antagonists. However, β-funaltrexamine is a non-competitive ligand for μ-opioid receptor and CTAP which does not cross the blood–brain barrier also has high affinity for somatostatin receptors. Such inconveniences limit the application of these compounds. The basic pharmacological properties of cyprodime have been previously described (Schmidhammer et al., 1989). The selective antagonistic properties of this ligand were shown in the guinea pig ileal longitudinal muscle, mouse vas deferentia and rat vas deferentia preparations. High selectivity ratios for cyprodime were shown in these assays (μ/δ: 74 in rat vas deferentia and 100 in mouse vas deferentia; 6 μ/κ: 28 in mouse vas deferentia and 37 in guinea pig ileal longitudinal muscle preparation).

When used as a tritiated ligand, cyprodime labels a population of receptors in rat brain confirmed to be μ-opioid receptor by competition assay. The high selectivity for μ-opioid receptor found with [^{3}H]cyprodime in the binding study is supported by the high selectivity of cold cyprodime also reported in rat brain membranes in the present paper. These results extend the previous findings (Schmidhammer et al., 1989) suggesting that cyprodime can be a useful pharmacological tool to characterize the μ-opioid receptor.

Besides in vitro binding experiments, functional assays were also performed. The effects of cyprodime on agonist stimulated [^{35}S]GTPγS binding in crude rat brain membrane preparations were studied. These functional experiments confirm the finding that cyprodime is an antagonist at μ-opioid binding sites, since it reversed the stimulatory effects of morphine in the [^{35}S]GTPγS binding assay at a concentration of 10 μM. Inhibition could not be detected at lower concentrations of cyprodime showing a weaker antagonist property in comparison with naloxone, which is in agreement with the previous findings in in vivo phar-

macological assays. Indeed, cyprodime exhibited about one-tenth the potency of naloxone to antagonise morphine-induced antinociception in the acetic acid writhing test in mice, and a similar one-tenth ratio was obtained to modify respiratory activity parameters in rabbits and to precipitate withdrawal syndrome in morphine-dependent mice (Schmidhammer et al., 1989). Cyprodime itself produced a negligible and non-significant stimulatory response on [^{35}S]GTPγS binding, which may reflect a very small degree of agonist property of the compound. In binding assays, cyprodime exhibited a lower potency than naltrexone or naloxone to displace [^{3}H]naloxone in the presence of NaCl, but the binding properties of cyprodime were strongly impaired in the absence of NaCl which suggests a pronounced antagonistic activity in this test (Schmidhammer et al., 1989).

In conclusion, the present study provides binding competition and functional results showing the properties of cyprodime as a selective μ-opioid receptor antagonist and indicates that [^{3}H]cyprodime is a selective μ-opioid receptor radioligand with high affinity that has the potential to be a useful tool in probing μ-opioid receptor mechanisms.

Acknowledgements

Helpful discussion of Dr. Sándor Benyhe and excellent technical assistance of Ms. Zsuzsanna Canjavec are greatly acknowledged. This work was supported by COPERNICUS CIPA CT 94 0226, Hungarian National Committee for Technical Development No. 94-97-48-0767, PECO ERB 3530 PL 941123, Biomed II BMH4-95-0510, 0511 and BMH4-98-2267, National Committee for Technical Development OMFB 96-47-48/1205, and National Scientific Research Foundation (OTKA: T022104).

References

Benyhe, S., Szucs, M., Borsodi, A., Wollemann, M., 1992. Species differences in the stereoselectivity of κ-opioid binding sites for [^{3}H]U-69593 and [^{3}H]ethylketocyclazocine. Life Sci. 51, 1647–1651.

Borsodi, A., 1991. In: Bashaum, Besson (Eds.), Towards in New Pharmacotherapy of Pain. Wiley, pp. 241–257.

Borsodi, A., Tóth, G., 1995. Characterization of opioid receptor types and subtypes with new ligands. Ann. N. Y. Acad. Sci. 757, 339–352.

Buzas, B., Toth, G., Cavagnero, S., Hruby, V.J., Borsodi, A., 1992. Synthesis and binding characteristics of the highly delta-specific new tritiated opioid peptide, [^{3}H]deltorphin II. Life Sci. 50, PL75–PL78.

Chen, Y., Mestek, A., Lin, J., Hurley, J.A., Yu, L., 1993. Molecular cloning and functional expression of a μ-opioid receptor from rat brain. Mol. Pharmacol. 44, 8–12.

Evans, C.J., Keith, D.E. Jr., Morrison, H., Magendzo, K., Edwards, R.H., 1993. Cloning of delta opioid receptor by functional expression. Science 258, 1952–1955.

Gacel, G., Daugé, V., Breuze, P., Delay-Goyet, P., Roques, B.P., 1988. Development of conformationally constrained linear peptides exhibiting a high affinity and pronounced selectivity for delta receptors. J. Med. Chem. 31, 1891–1897.

Kieffer, B.L., Befort, K., Gaveriaux-Ruff, C., Hirth, C.G., 1992. The delta opioid receptor: isolation of a cDNA by expression cloning and pharmacological characterization. Proc. Natl. Acad. Sci. U.S.A. 89, 12048–12052.

Matthes, H.W.D., Maldonado, R., Simonin, F., Valverde, O., Slowe, S., Kitchen, I., Befort, K., Dierich, A., Le Meur, M., Dollé, P., Tzavara, E., Hanoune, J., Roques, B.P., Kieffer, B.L., 1996. Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the μ-opioid-receptor gene. Nature 383, 819–823.

Munson, P.J., Rodbard, D., 1980. LIGAND: the versatile computerized approach for characterisation of ligand binding systems. Anal. Biochem. 107, 220–239.

Nevin, S.T., Toth, G., Nguyen, T.M.-D., Schiller, P.W., Borsodi, A., 1993. Synthesis and binding characteristics of the highly specific, tritiated delta opioid antagonist [^{3}H]TIPP. Life Sci. 53, PL57–PL62.

Nevin, S.T., Kabasakal, L., Otvos, F., Toth, G., Borsodi, A., 1994. Binding characteristic of the highly selective delta antagonist, [^{3}H][Ile^{5,6}]deltorphin II. Neuropeptides 26, 261–265.

Ötvös, F., Tóth, G., Schmidhammer, H., 1992. Tritium labelling of cyprodime = (−)-17-cyclopropylmethyl-4,14-dimethoxymorphinan-6-one mu receptor-selective opioid antagonist. Helv. Chem. Acta 75, 1718–1723.

Pasternak, G.W., Wilson, H.A., Synder, S.H., 1975. Differential effect of protein modifying reagents on receptor binding of opiate agonists and antagonists. Mol. Pharmacol. 11, 340–351.

Pelton, J.T., Kazmierski, W., Gulya, K., Yamamura, H.I., Hruby, V.J., 1986. Design and synthesis of conformationally constrained somatostatin analogues with high potency and specificity for mu opioid receptors. J. Med. Chem. 29, 2370–2375.

Reisine, T., Bell, G.I., 1993. Molecular biology of opioid receptors. Trends Pharmacol. Sci. 16, 506–511.

Rosenthal, H.E., 1967. Graphical methods for the determination and presentation of binding parameters in a complex system. Anal. Biochem. 20, 525–532.

Schmidhammer, H., Burkard, W.P., Eggstein-Aeppli, L., Smith, C.F.C., 1989. Synthesis and biological evaluation of 14-alkoxymorphinans: 2. (−)-*N*-Cyclopropylmethyl-4,14-dimethoxymorphinan-6-one, a selective opioid receptor antagonist. J. Med. Chem. 32, 418–421.

Standifer, K.M., Pasternak, G.W., 1997. G proteins and opioid receptor-mediated signalling. Cell Signal 9, 237–248.

Takemori, A.E., Ikeda, M., Portoghese, P.S., 1986. The μ-, κ- and δ-properties of various opioid agonists. Eur. J. Pharmacol. 123, 357–361.

Tóth, G., Krámer, M., Szucs, M., Benyhe, S., Sirokmán, F., 1982a. Preparation of [1,7,8-^{3}H]dihydromorphine of high molar activity and its application in opiate receptor binding experiments. Radiochem. Radioanal. Lett. 56, 209–216.

Tóth, G., Krámer, M., Sirokmán, F., Borsodi, A., Rónai, A., 1982b. Preparation of [7,8,19,20-^{3}H]naloxone of high specific activity. J. Labelled Compd. R. 19, 1021–1026.

Traynor, J.R., Nahorski, S.R., 1995. Modulation by μ-opioid agonists of guanosine-5′-*O*-(3-[^{35}S]thio)trisphosphate binding to membranes from human neuroblastoma SH-SY5Y Cells. Mol. Pharmacol. 47, 848–854.

Varga, E., Toth, G., Benyhe, S., Hosztafi, S., Borsodi, A., 1987. Synthesis and binding of [^{3}H]oxymorphazone to rat brain membranes. Life Sci. 40, 1579–1588.

ELSEVIER

PII S0024-3205(99)00560-3

Life Sciences, Vol. 66, No. 1, pp. 43-49, 2000

0024-3205/00/$–see front matter

TRITIATED KAPPA RECEPTOR ANTAGONIST NORBINALTORPHIMINE: SYNTHESIS AND *IN VITRO* BINDING IN THREE DIFFERENT TISSUES

[#]Á. Márki, F. Ötvös, G. Tóth, [a]S. Hosztafi, [b]A. Borsodi

[b]Institute of Biochemistry and Isotope Laboratory, Biological Research Center, Hungarian Academy of Sciences, Szeged, [b] Department of Pharmacodynamics, Albert Szent-Györgyi Medical University, Szeged, Hungary and [a]Alkaloida Chemical Company Ltd., Tiszavasvári, Hungary.[1]

(Received in final form August 19, 1999)

Summary

Recently a new antagonist with high selectivity for the kappa receptors (norbinaltorphimine) was developed and tested in various systems. This compound was radiolabelled with tritium resulting in high specific radioactivity (47.2 Ci/mmol). [^{3}H]Norbinaltorphimine was characterized by in vitro radioligand binding assays. The radioligand binds to κ-opioid receptors with a high potency and selectivity in guinea pig, frog and rat brain membranes. Our results suggest the κ_1 specificity of the radioligand.

Key Words: **κ-opioid receptors, norbinaltorphimine, tritium labelling, binding studies**

Opioid antagonists are essential tools for studying the opioid system by biochemical and pharmacological means. The most frequently used ligands (naloxone, naltrexone) are potent and universal antagonists; they are capable of antagonizing the agonist effects mediated by multiple opioid receptor types. In order to determine the functional correlates of receptor activation, to study interaction of endogenous opioid peptides with opioid receptor types and subtypes and to determine the receptor selectivity of new opioid agonists, selective antagonists must be used. In the last few years highly selective antagonists for delta (e.g. naltrindole (NTI), 1; naltriben, 2; naltrindole 5'-isothiocyanate, 3; TIPP (Tyr-Tic-Phe-Phe-OH), 4; TIPP[ψ] (Tyr-Tic[Ψ]-Phe-Phe-OH), 5), mu (cyprodime, 6; CTAP (D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2), 7) and kappa (norbinaltorphimine, 8) opioid receptor have been developed. In the mean time some of these compounds became available in radiolabelled form as well NTI (9, 10), TIPP[ψ] (11) and cyprodime (12, 13). The unlabelled norbinaltorphimine (norBNI) behaved as potent and κ-

[#]Present address: Department of Pharmacodynamics, Albert Szent-Györgyi Medical University, Szeged, Hungary
Corresponding author: Dr. Anna Borsodi, Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, Szeged, POB 521, Hungary. Phone/Fax: 36-62-433432, E-mail: borsodi@nucleus.szbk.u-szeged.hu

selective antagonist both in analgesic assays in mice (8) and receptor binding studies in guinea pig brain membrane (14). Here we describe tritium labelling and biochemical characterization of [^{3}H]norBNI in frog, guinea pig and rat brain membrane preparations.

Methods

Chemicals

Unlabelled cyprodime was synthesized and kindly provided by Prof. Schmidhammer (6). (±)Ethylketocyclazocine methanesulfonate (EKC) was supplied by Sterling Winthrop Research Institute (Rennselaer, NY). Naltrindole (NTI) and norbinaltorphimine were provided by Prof. Portoghese (15), [Ile^{5,6}]deltorphin II (Tyr-D-Ala-Phe-Glu-Ile-Ile-Gly-NH_2) (16), TIPP[Ψ] (Tyr-Tic[Ψ]-Phe-Phe-OH) (11), dihydromorphine (17) and MERF (Tyr-Gly-Phe-Phe-Met-Arg-Phe) (18) were synthesized in our laboratory. U50,488 (trans-3,4-dichloro-N-methyl-1-N-1-pyrrolidinyl-(cyclohexyl)-benznecetamide) was from the Upjohn Co. Kalamazoo (MI). CTAP (D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2) and DAMGO ([D-Ala^2-(Me)Phe^4-Gly^5-ol]enkephalin) were generous gifts of National Institute of Drug Abuse Drug Supply System Rockville, MD. Bacitracin, benzamidin, bestatin, ethylenediaminetetraacetic acid (EDTA), ethylenebis-(oxyethylenenitrilo)-tetraacetic acid (EGTA), phenylmethyl-sulphonyl fluoride (PMSF), soybean trypsine inhibitor and thiorphan were purchased from Sigma Chemicals. Gordox® and captopril were from Gedeon Richter Ltd (Hungary, Budapest). All other chemicals were of analytical grade.

Animals

Frogs (*Rana esculenta*) weighing about 50 - 150 g were captured by Tisza Fishing Co. (Szeged, Hungary) and sacrificed immediately. Rats (Wistar strain, 200-250 g, male) were from our Animal House. Guinea pigs (350-400 g, male) were from the Semmelweis Medical University, Budapest. Rats and guinea pigs were housed in groups of six under controlled illumination (lights on from 07-19 h), temperature (22-24 °C) and had free access to food and water.

Membrane preparation

The frog, guinea pig whole brain and rat brain (without cerebellum) membrane fractions were prepared according to Benyhe (19). The brains were removed and then homogenized in twenty volumes (wt/vol) of ice-cold buffer (Tris-HCl 50 mM, pH 7.4) with Braun teflon-glass homogenizer, the suspension was filtered on four layers of gauze and centrifuged with Sorvall RC5C centrifuge (40,000 x g, 4°C, 20 min). The pellet was resuspended in Tris-HCl buffer and incubated at 37°C (30 min). The centrifugation step was repeated. The pellet was resuspended in 5x volumes of buffer (50 mM Tris-HCl, 0.32M sucrose, pH 7.4). The membranes were stored at -70°C. Before using, membranes were diluted and centrifuged (40,000 x g, 4°C, 20 min) and then the pellet was resuspended in 50 ml buffer (Tris-HCl 50 mM, pH 7.4).

Radioreceptor binding assays

The frozen membranes were thawed at room temperature and centrifuged (40,000 x g, 20 min and 4°C) in Tris-HCl buffer. Ligand binding experiments were carried out in buffer D (1000

ml 50 mM Tris-HCl contained 300 mg ethylenediaminetetraacetic acid (EDTA), 380 mg ethylenebis(oxyethylenenitrilo)tetraacetic acid (EGTA), 20 mg bacitracin, 9 mg bestatin, 2 mg thiorphan, 156 mg benzamidin, 4 mg soybean trypsine inhibitor, 2 mg leupeptin, 2.2 mg captopril, 10^{-6} M phenylmethyl-sulphonyl fluoride (PMSF) and 40 KIU/ml aprotinin [Gordox®]; pH:7.4) at the final volume of 1 ml containing 100 mM Na^+-ions and about 0.3 mg protein. The concentration of 0.1 nM tritiated norBNI was used for competition assays and 0.01-0.7 nM for saturation experiments. Incubations were started by addition of membrane suspension and continued in a shaking water bath until steady-state was achieved (25°C, 60 min). The GF/C filters were pretreated with 50 mM Tris-HCl buffer containing 10 μM norBNI one hour prior assay to decrease binding the radioligand to the filter. The reaction was terminated by rapid filtration on Brandel M24R cell harvester through Whatmann GF/C filters and washed with 3x5 ml of ice-cold Tris-HCl (pH 7.4) buffer. The filters were dried at 37°C, and the bound radioactivity was determined in a toluene based scintillation cocktail in Wallac 1409 liquid scintillation counter. The total binding was defined as that measured in the absence of a competing agent. Non-specific binding was determined in the presence of 10 μM unlabelled naloxone and was about 40 percent of total binding.

Data analysis

All assays were carried out at least three times in duplicate, and values are given as means ±SEM. The binding capacity (B_{max}) and the equilibrium dissociation constants (K_d) of [^{3}H]norBNI were calculated according to Rosenthal (20). Data of competition experiments were analyzed using the program LIGAND (21), utilizing a nonlinear least squares fitting algorithm. The kinetic parameters were calculated by GrapPhad Prism 2.01 scientific software.

Results

Synthesis of [^{3}H]norBNI

1,1'-Dibromo-norBNI: 1-bromo-naltrexone·HCl (914 mg, ≈2 mmol) (was prepared according to Tóth et al., 22) and hydrazine·2 HCl (110 mg, ≈1 mmol) were dissolved in glacial acetic acid (30 ml), heated and stirred at 90°C for 6 h under N_2 as described by Nagase et al. (23). After cooling the reaction mixture, 1,1'-dibromo-norBNI·2HCl was precipitated in crystallic form. The crystals were filtered off and washed with cold acetone yielding 850 mg of the crude product. The material was purified by column chromatography on silica using chloroform-methanol-ammonium-hydroxide (9:1:0.5) as an eluent. 350 mg of base was obtained. The hydrochlorid salt was precipitated with HCl in ethanol. 340 mg of 1,1'-dibromo-norBNI x 2 HCl was yielded. Melting point >300°C (decomposition). ^{1}HNMR (base, $CDCl_3$) 6.95S (H-2; 1H) 5.75S (H-5; 1H) 0.9-0.15 m (cyclopropyl protons, 5H).

[^{3}H]NorBNI: 4.54 mg of dibromo-norBNI was dissolved in 1.0 ml N,N-dimethylformamide and 17 mg Pd/$BaSO_4$ 10% (Merck) and 5 μl of triethylamine was added to the solution. The reaction vessel was connected to the tritium manifold (25), frozen with liquid N_2 and evacuated. After introduction of tritium gas (550 GBq, 15 Ci) the mixture was stirred for 30 min at room temperature. The reaction was terminated by absorption of the unreacted tritium gas by pyrophoric uranium. The catalyst was removed by filtration through Whatman GF/C filter and washed several times with ethanol. Labile tritium was removed by repeated evaporation of ethanol-water (1:1)

solution from the reaction mixture. The radioactivity of the crude material was 11.1 GBq (300 mCi). The crude product was purified by TLC using Fertigplatten Kieselgel $60F_{254}$ (Merck) with the solvent of n-butanol-acetic acid-water (2:1:1) resulting tritiated norBNI with a total activity of 5.56 GBq (153 mCi). The purity of the final product was determined by TLC and radiochromatogram scanner (Berthold) in three different solvent systems [A: butanol-acetic acid-water (2:1:1); B: chloroform-methanol (80:24); C: chloroform-methanol-ammonium-hydroxide (9:1:0.5); R_f(A): 0.42; R_f(B): 0.18; R_f(C): 0.26] and found to be at least 95%. The amount of the compound was determined by UV spectrometry using unlabelled norBNI as standard. Specific radioactivity was 1.75 TBq/mmol (47.24 Ci/mmol).

Biochemical characterization

Here we describe detailed kinetic studies on *guinea pig* whole brain membrane preparation which carries high concentration of κ-opioid receptors. The association (k_{+1}) and dissociation (k_{-1}) rate constants of [^{3}H]norBNI (0.1 nM) were determined at 25°C. The binding reached steady state in 60 min. The value of 3.28±0.43 sec^{-1} for k_{-1} and $1.09\pm0.30\cdot10^{10}$ $sec^{-1}M^{-1}$ for k_{+1} was calculated. The K_d was found to be 0.30 nM in this assay. The association binding studies were also carried out on rat and frog brain membranes and steady state was reached in 60 min in both preparations.

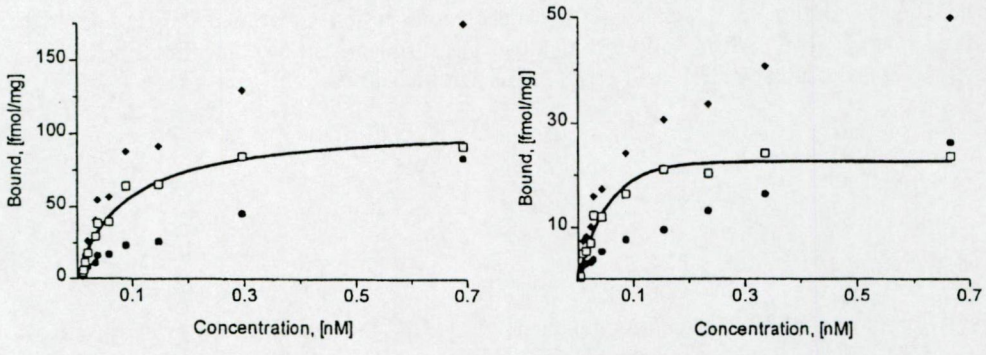


Fig. 1

Representative saturation binding isotherm for [^{3}H]norBNI bound to guinea pig and rat brain membranes, respectively. The figure shows the total (◆), nonspecific (●) and specific (□) binding. Specific binding was measured at 11 concentrations (0.01-0.7 nM) using 10 μM naloxone to define nonspecific binding.

The results of the saturation experiments on *guinea pig* and *rat brain* membrane preparations are shown on **Figure 1**. The concentration of radioligand varied between 0.01 and 0.7 nM. The binding was saturable and the Scatchard plot of data suggested single binding site in both tissues. [^{3}H]norBNI labels kappa receptors with high potency in guinea pig (K_d: 0.10±0.015 nM) as well as in rat (K_d: 0.10±0.013 nM) brain membranes. This value is comparable to K_d value obtained from binding kinetic studies. The maximal number of binding sites are 115.7±6.66 fmol/mg protein in guinea pig and 36.3±0.81 fmol/mg protein in rat brain membranes.

In competition experiments the selectivity of tritiated norBNI was examined with mu, delta and kappa selective unlabelled ligands in frog, guinea pig and rat brain membrane preparations (**Table 1.**). All tested kappa ligands (EKC, dynorphin (1-13), U50,488) proved to be good competitors (K_i: 3-10 nM) of [^{3}H]norBNI in guinea pig brain membrane except MERF, which inhibited labelled norBNI binding at high nanomolar concentration (K_i: 742 nM) while mu and delta specific ligands possessed very low affinities (K_i: ≥ 10,000 nM). The norBNI and U50,488 recognized two receptor types with high (K_i: 0.31 and 4.7 nM) and low (K_i: 17.1 and 476 nM) in rat brain. The K_i value of dynorphin (1-13) were higher in rat than in guinea pig brain, while MERF, cyprodime, CTAP and naltrindole were better competitors of tritiated norBNI in rat brain compared to guinea pig brain. The kappa ligands, except MERF, displayed lower affinity to norBNI-sensitive kappa receptors in frog brain than in guinea pig brain. The K_i value of norBNI from homologous displacement studies was found to be lower by two order of magnitude in frog brain compared to rat and guinea pig brain. Most of the delta and mu ligands proved to be better competitors of labelled norBNI in frog than in guinea pig or in rat brain.

Table I

[^{3}H]norBNI type specificity in rat, guinea pig and frog brain membrane preparations

Unlabelled ligands	K_i [nM] Rat	Guinea pig	Frog
norBNI	0.19 ± 0.12 17.1 ± 2.7	0.22 ± 0.09	13..5 ± 2.9
EKC	2.4 ± 2.3	2.9 ± 2.6	41.6 ± 29.7
dynorphin (1-13)	50.1 ± 30.5	9.4 ± 5.2	11.0 ± 2.5
U50,488	4.7 ± 2.3 476 ± 9.3	5.4 ± 0.4	246 ± 114
MERF	282 ± 77	742 ± 369	499 ± 186
cyprodime	307 ± 207	4107 ± 6	248 ± 18
CTAP	1781 ± 992	≈ 10000	1521 ± 614
DAMGO	>10000	>10000	167 ± 40
naltrindole	61.1 ± 1.7	257 ± 32	13.9 ± 1.2
Ile^{5,6}-deltorphin II	>10000	≈ 10000	386 ± 53
TIPP[Ψ]	>10000	>10000	>10000

All assays were carried out at least three times in duplicate, the given values are means ±S.E.M.

Discussion

Takemori et al. (14) have previously reported that unlabelled norBNI proved to be highly selective (mu/kappa: 169 delta/kappa: 153) antagonist and it possessed very good affinity (K_i: 0,47 nM) to κ-opioid receptors in guinea pig brain membrane. In our experiments we have examined the binding properties of tritiated norBNI in three different tissues. We found that the labelled norbinaltorphimine is even more selective in our conditions (mu/kappa: >18600 delta/kappa: >1170) than in that reported by Takemori et al (14). The lower the density of kappa$_1$ receptor (guinea pig > rat > frog) (25) the lower the selectivity and affinity of the labelled norBNI. The K_i values and Hill coefficients of kappa ligands indicated that the tritiated norBNI labeled only one receptor type (kappa$_1$) in guinea pig brain (n_H : 0.98 - 1.03) and (kappa$_2$) in frog brain (n_H

: 0.80 - 1.11). In contrast two sites were recognized in rat brain (n_H: 0.50 - 0.57). In each case there is about two orders of magnitude difference for the favor of kappa$_1$ site. The calculated B_{max} values with tritiated norBNI are very similar to those obtained by others for the kappa$_1$ receptor subtype in rat (κ_1: 16 fmol/mg, κ_2: 111 fmol/mg) and guinea pig brain (κ_1: 80 fmol/mg) measured with [^{3}H]EKC (26), while the maximal number of binding sites of [^{3}H](d)-N-allylnormetazocine and [^{3}H]bremazocine (non-selective kappa ligands) were much higher: 260 fmol/mg (27) and 378 fmol/mg (28) in rat brain, respectively. On the basis of these observation we suggest that [^{3}H]norBNI has a preference for the κ_1 site.

It appears from Table 1 that non-peptide mu and delta selective ligands are better competitors of [^{3}H]norBNI than peptides. The reason for this is probably that buffer D contained 100 mM Na^+ which decreases the affinity of opioid agonist ligands. However, this is inconsistent with the K_i values of CTAP which has a weaker affinity to norBNI sensitive binding sites than cyprodime. The real explanation is probably that the κ-opioid receptors have different binding domains for peptide and non-peptide ligands (29). It is conspicuous that naltrindole is the best non-kappa competitor of [^{3}H]norBNI (Table I) to such a degree that the K_i value of NTI is equal to the same value of norBNI in frog brain. These two ligands have a very common message part but the address is different; the 14'-N is missing in NTI which is a responsible part for kappa selectivity (30).

In summary, the opioid antagonist [^{3}H]norBNI exhibits high affinity and selectivity to kappa$_1$ opioid receptors. On the basis of these results, this new radioligand is an excellent tool for further characterization of kappa$_1$ opioid receptors at the molecular level.

Acknowledgments

The authors wish to acknowledge Dr. Mária Wollemann and Dr. Sándor Benyhe for helpfull discussion, Dr. Ian Kitchen for reading the manuscript and to Zsuzsanna Canjavec for the excellent technical assistance. This work was supported by COPERNICUS CIPA CT 94 0226, Hungarian National Committee for Technical Development No. 94-97-48-0767, OTKA No. 22104 and Minisitry of Welfare No 132/96grant.

References

1. P.S. PORTOGHESE, H. NAGASE, A.W. LIPKOWSKI, D.L. LARSON, and A.E. TAKEMORI, J. Med. Chem. **31** 836-841 (1988)
2. P.S. PORTOGHESE, J. Med. Chem. **34** 1757-1762 (1991)
3. P.S. PORTOGHESE, M. SULTANA, and A.E. TAKEMORI, J. Med. Chem. **33** 1547-1548 (1990)
4. P.W. SCHILLER, T.M-D. NGUYEN, G. WELTROWSKA, B.C. WILKES, B.J. MARSDEN, C. LEMIEUX, and N.N. CHUNG, Proc. Natl. Acad. Sci. USA, **89** 11871-11875 (1992)
5. P.W. SCHILLER, G. WELTROWSKA, T.M-D. NGUYEN, B.C. WILKES, N.N. CHUNG, and C. LEMIEUX, J. Med. Chem. **36** 3182-3187 (1993)
6. H. SCHMIDHAMMER, P.W. BURKARD, L. EGGSTEIN-AEPPLI, and F.C. SMITH, J. Med. Chem. **32** 418-421 (1989)
7. J.T. PELTON, W. KAZMIERSKI, K. GULYA, H.I. YAMAMURA, and V.J. HRUBY, J. Med. Chem. **29** 2370-2375 (1986)

8. P.S. PORTOGHESE, A.W. LIPKOWSKI, and A.E. TAKEMORI, Life Sci. **40** 1287-1292 (1987)
9. A. BORSODI, G. OZDEMIRLER, S.T. NEVIN, L. KABASAKAL, F. OTVOS, G. TOTH, Br. J. Pharmacol. **109** 17 (1993)
10. M.S. YAMAMURA, R. HORVATH, G. TOTH, F. OTVOS, E MALATYNSKA, R.J. KNAPP, V.J. HRUBY, H.I. YAMAMURA, Life Sci. **50** PL119-124 (1992)
11. S.T. NEVIN, L. KABASAKAL, F. ÖTVÖS, G. TÓTH, and A. BORSODI, Neuropeptides, **26** 261-265 (1994)
12. F. ÖTVÖS, G. TÓTH, H. SCHMIDHAMMER, Helv. Chem. Acta **75** 1718-1720 (1992)
13. Á. MÁRKI, K. MONORY, F. ÖTVÖS, G. TÓTH, H. SCHMIDHAMMER, J.R. TRAYNOR, R. MALDONADO, B.P. ROQUES and A. BORSODI, Eur. J. Pharmacol *in press*
14. A.E. TAKEMORI, Y. BEGONIA, S. NAESETH, and P.S. PORTOGHESE, J. Pharm. Exp. Ther. **246** 255-258 (1988)
15. P.S. PORTOGHESE, M. SULTANA, and A.E. TAKEMORI, J. Med. Chem. **33** 1714-1720 (1990)
16. S.T. NEVIN, G. TÓTH, G. WELTROWSKA, P. SCHILLER, and A. BORSODI, Life Sci. **56** PL225-PL230 (1994)
17. G. TÓTH, M. KRÁMER, M. SZUCS, S. BENYHE, and F. SIROKMÁN, Radiochem. Radioanal. Letters, **56** 209-212 (1982)
18. M. WOLLEMAN, J. FARKAS, G. TÓTH, and S. BENYHE, J. Neurochem. **63** 1460-1465 (1994)
19. S. BENYHE, M. SZUCS, A. BORSODI and M. WOLLEMANN, Life Sci. **51** 1647-1655 (1992)
20. H.E. ROSENTHAL, Anal. Biochem. **20** 525-532 (1967)
21. P.J. MUNSON, and D. RODBARD, Anal. Biochem. **107** 220-239 (1980)
22. G. TÓTH, F. ÖTVÖS, and S. HOSZTAFI, Helvetica Chimica Acta **76** 2274-2278 (1993)
23. H. NAGASE, Y. KATSURA, Chem. Abstr. **116** 6800w Japan Kokai Tokyo Koho JP 03, 193, 782 (1992)
24. G. TÓTH, S. LOVAS, and F. ÖTVÖS, *Methods in Molecular Biology, Neuropeptide Protocols*, G.B. Irvine, C.H. Williams (Eds.), **73** 219-230 Humana Press Inc. Totowa, N.J. (1997)
25. M. WOLLEMANN, S. BENYHE, and J. SIMON, Life Sci. **52** 599-611 (1993)
26. R.S. ZUKIN, M. EGHBALI, D. OLIVE, E.M. UNTERWALD, and A. TEMPEL, Proc. Natl. Acad. Sci. USA, **85** 1061-1065 (1988)
27. Y. ITZHAK, J.M. HILLER, and E.J. SIMON, Mol. Pharmacol. **27** 46-52 (1985)
28. R.B. ROTHMAN, V. BYKOV, J.A. DANKS, A.E. JACOBSON, T.R. BURKE JR., K.C. RICE, and M. HERKENHAM, Neuropeptides **6** 503-516 (1985)
29. J-C. XUE, C. CHEN, J. ZHU, S. KUNAPULI, K. DERIEL, L. YU, and L-Y. LIU-CHEN, J. Biol. Chem. **269** 30195-30199 (1994)
30. C.E. LIN, A.E. TAKEMORI and P.S. PORTOGHESE, J. Med. Chem. **36** 2412-2415