Stereochemistry and Opioid Receptor Affinity of 2,4-Dipyridine-3,7-diazabicyclo[3.3.1]nonanones – Influence of the Substituent in Position N7

A. Cambareri, D. P. Zlotos, and U. Holzgrabe*

Institut für Pharmazie und Lebensmittelchemie, Universität Würzburg, Am Hubland, D-97074 Würzburg, Germany

W. Englberger and M. Haurand

Abteilung für Pharmakologie, Grünenthal GmbH, Zieglerstr. 6, 52078 Aachen, Germany

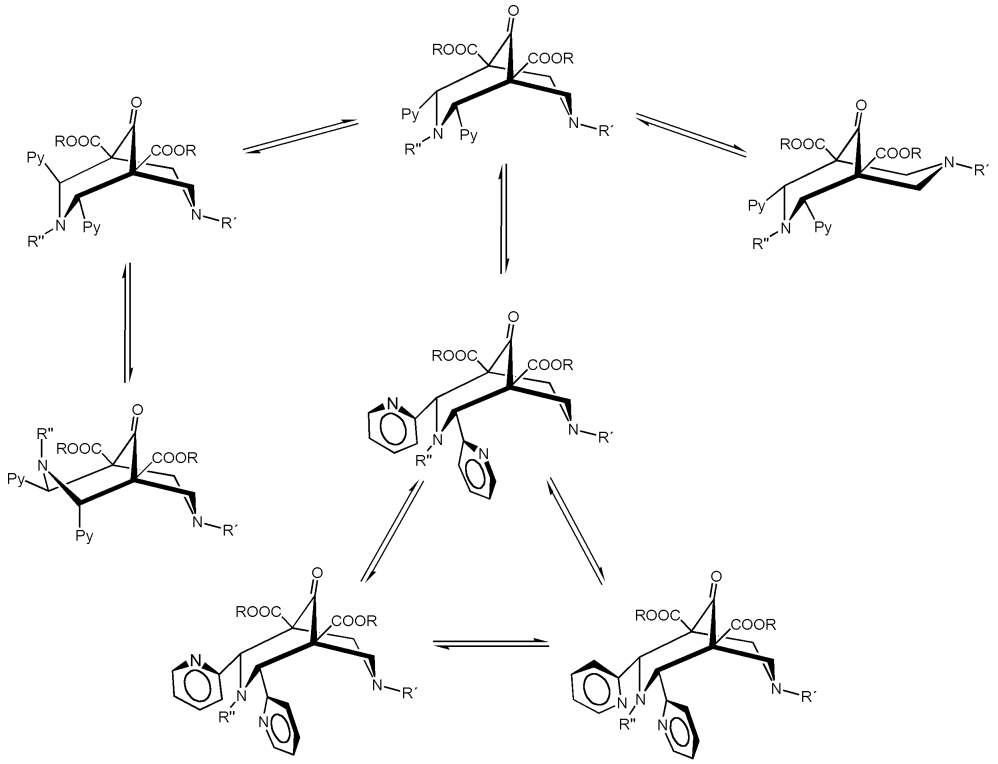
Received January 7, 2002

The stereochemistry the 2,4-di-arene substituted 3,7-diazabicyclo[3.3.1]nonan-9-one 1,5-dicarboxylate skeleton was found to be regulated by the kind of substituents attached to the arene rings as well as to the nitrogens N3 and N7. Conformational isomers, *i.e.*, chair/chair, boat/chair and chair/boat, in addition to *cis*/*trans* configurational isomerism with respect to the arene rings were reported. Since the analgesic potency of the diazabicyclononanones, which is related to their affinity toward the κ-opioid receptor, is governed by the stereochemistry of the molecules, the influence of the substituents at nitrogen N7 was studied herein. The various differently N7 substituted diazabicyclononanones were found to crystallise in a highly symmetrical chair/chair conformation. However, beside HZ2 none of the compounds exhibits high affinity to the κ receptor. In contrast, some compounds with affinity to the μ receptor could be identified. In addition, the N7-(4-carboxybenzyl) substituted compound was found to have affinity to the δ receptor in the submicromolar range of concentration.

J. Heterocyclic Chem., **39**, 789 (2002).

Introduction.

2,4-Di-2-pyridine substituted 3,7-diazabicyclo[3.3.1]-nonan-9-one 1,5-dicarboxylates are attracting pharmacological interest because of their affinity to the κ-opioid receptor and along with this, the analgesic activity [1,2,3,4]. In previous studies, the affinity of the diazabicyclononanes to the three opioid receptor subtypes, μ, κ and δ, was found to depend sensitively on the substitution pattern of the skeleton. Whereas the pyridine rings in 2 and 4 position can be replaced with *p*-methoxy-, *m*-hydroxy- and *m*-fluoro-substituted phenyl rings without any loss of affinity to the κ-receptor [5], the nitrogen N3



Scheme 1: Isomerism of diazabicyclo[3.3.1]nonan-9-one dicarboxylates.

can be substituted with an hydrogen or a methyl group only [6]. Increasing the size of the substituent at this position resulted in a complete loss of affinity. None of the compounds tested till now showed a substantial affinity to the μ or δ opioid receptor.

The aim of this investigation was to study the influence of substituents attached to the nitrogen N7 on the receptor affinity. Since the diazabicyclononanes should specifically bind to a receptor protein, the spatial arrangement of the molecule is pivotal for a high pharmacological activity and has, thus, to be studied. Even though the diazabicyclic skeleton is rather rigid a number of isomers have been described. Depending on the nature of the arene rings in position 2 and 4 and the conditions of the double Mannich reaction *cis* and *trans* isomers can be isolated [1,7,8]. In addition, N3-methyl substituted compounds can form rotational isomers because the methyl group attached to the nitrogen is able to restrict the rotation around the C2-aryl and C4-aryl axes [8,9]. Recently, the influence of substituents of increasing size in position N3 was studied [10]. Interestingly, a boat/chair isomer was isolated which was characterized by a boat conformation in the higher substituted piperidone, a *trans* configuration of the pyridine rings in 2- and 4-position and an axial N3-butyl substituent. In addition, the substitution on the nitrogen at N7 influences sensitively the conformation of the piperidone rings. Caujolle *et al*. [7] reported an equilibrium between a chair/chair and chair/boat conformation with a boat in the less substituted piperidone ring, which depends on the size of the substituents attached to N7. Both ^{1}H and ^{13}C NMR spectroscopic data revealed that an increase in the percentage of the chair/boat conformations correlates with the steric bulk of the N7 substituents. However, due to the lack of coupling constants between the hydrogens attached to the skeleton NMR data were often found to be ambiguous (*cf*. ref. [6] and ref. [10]). Thus, it was aimed to check whether 2,4-di-2-pyridine substituted dimethyl 3-methyl-diazabicyclo[3.3.1]nonan-9-one 1,5-dicarboxylates bearing large substituents such as a *tert*.-butyl group in position 7 take a chair/chair or a chair/boat conformation. In addition, with respect to the receptor recognition it is of interest to find out whether the nitrogens of the pyridine rings point to the C9-keto group or to the bottom of the molecule.

Taken together, the aim of this study was to synthesize diazabicyclononanones with substituents of increasing size in position N7, to study their stereochemistry and to evaluate the affinity to the μ-, κ- and δ-opioid receptor (OR) subtype.

Results and Discussion.

Synthesis.

The 2,4-di-2-pyridine substituted dimethyl 7-alkyl-3-methyl-9-oxo-diazabicyclo[3.3.1]nonane 1,5-dicarboxylates were synthesized by a double Mannich reaction starting with the condensation of 1 mole of methylamine, 2 moles of pyridine-2-aldehyde and 1 mole of dimethyl oxoglutarate to give the intermediate piperidone. These can be converted to the corresponding diazabicyclononanones by refluxing 1 mole of the piperidone with 2 mole of formaldehyde and 1 mole of primary amines in methanol.

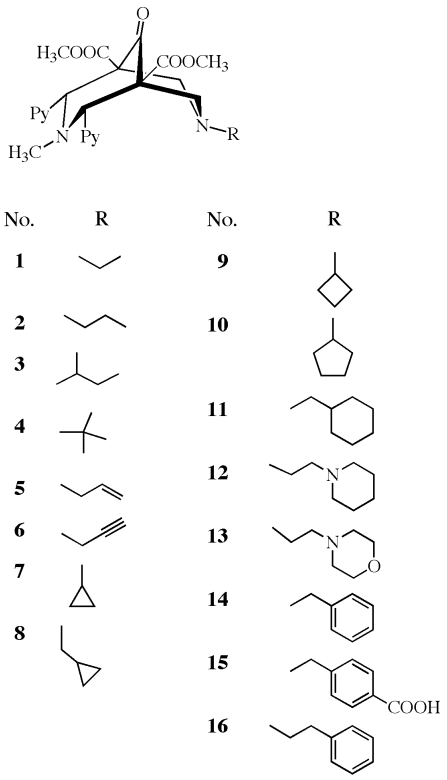


Figure 1. Structural formula of the compounds studied.

Stereochemistry.

The stereochemistry of the 2,4-di-2-pyridine substituted dimethyl 7-*tert*.butyl-3-methyl-9-oxo-diazabicyclo[3.3.1]-nonane 1,5-dicarboxylate **4** will be representatively discussed. The ^{1}H NMR spectrum of the obtained crystals showed half a set of signals indicating a symmetrical structure which is likely to be characterized by a *cis* configuration of the pyridine rings in position 2 and 4. Since only a single isomer was isolated from the Mannich reaction it is impossible to derive the conformation of both piperidone rings of the diazabicyclic skeleton from the chemical shifts of the ^{1}H and ^{13}C NMR spectra.

Crystallisation of the compound from ethanol gave crystals that are suitable for an X-ray analysis. X-ray crystallographic data of **4** are presented in Tables 1 and 2 and an ORTEP view with the numbering used in the crystallographic study is shown in Figure 2.

The diazabicyclic structure is characterized by a chair/chair conformation, the methyl group at N3 and the *tert*-butyl group at N7 as well as the pyridine ring in the 2 and 4 position take equatorial positions. The crystal structure is highly symmetrical (mirror plane) with respect to the orientation of the ester groups and the pyridine rings. Interestingly, the nitrogens of the pyridines point to the keto group at C9. This observation conflicts with findings with **HZ2**, a 3,7-dimethyl-3,7-diazabicyclononanone of a corresponding substitution pattern, whose nitrogens were found to point to the bottom of the molecule by means of a series of NOE difference experiments [3].

Table 1

Crystallographic Data and Structural Refinement for **4**

Empirical formula	$C_{26}H_{32}N_4O_5$
Formula weight	480.56
Crystal size	0.3 x 0.2 x 0.1 mm
Crystal system	orthorhombic
Space group	Pnma
Unit cell dimensions	a = 13.528(2) Å α = 90°
	b= 19.425(2) Å β = 90°
	c = 9.282(1) Å γ = 90°
Unit cell volume	2439.2(5) Å^3
Z	4
Density (calculated)	1.309 g/cm^{-3}
Temperature	193 K
Measuring method	Omega scans
Scan angle increment	0.3°
Measuring time	7 s
θ_{min}	2.10°
θ_{max}	26.37°
Total reflections measured	15269
Unique reflections	2860 (R_{int} = 0.0325)
Computing data collection	Smart 5.622 [18]
Data integration	SaintNT Vers.5/6.0 [18]
R_1, wR_2 [I>2σ(I)]	0.0645, 0.1461
R_1, wR_2 (all data)	0.0696, 0.1494
GOF (all data)	1.116
g1, g2	0.0568, 1.9186
Computing molecular graphics	SHELXTL [19]

Table 2

Atomic Coordinates and Equivalent Isotropic Displacement Parameters (Å^2 x 10^3) for **4**

	x	y	z	U(eq)
C12	0.9251(1)	0.3117(1)	0.1766(2)	24.8(4)
N2	0.7727(1)	0.2500	-0.051(2)	20.8(5)
C11	0.9882(2)	0.2500	-0.0293(3)	24.5(6)
N3	0.8750(2)	0.2500	0.2286(2)	24.7(5)
C6	0.8291(1)	0.3139(1)	-0.0682(2)	20.9(4)
O2	1.0005(1)	0.3802(1)	-0.1777(1)	30.9(4)
N1	0.7959(1)	0.4356(1)	-0.0738(2)	30.2(4)
O3	1.0734(1)	0.2500	-0.0679(2)	36.5(5)
C8	0.9310(1)	0.3140(1)	0.0096(2)	22.2(4)
O1	1.0314(1)	0.4150(1)	0.0476(1)	36.7(4)
C9	0.9922(1)	0.3764(1)	-0.0351(2)	26.1(4)
C2	0.6976(1)	0.3701(1)	0.0903(2)	29.7(4)
C1	0.7698(1)	0.3754(1)	-0.0155(2)	22.6(4)
C4	0.6799(2)	0.4915(1)	0.0819(2)	34.5(5)
C3	0.6524(2)	0.4291(1)	0.1398(2)	34.6(5)

Table 2 (continued)

	x	y	z	U(eq)
C7	0.6927(2)	0.2500	-0.1573(3)	27.3(6)
C10	1.0597(2)	0.4363(1)	-0.2306(3)	41.0(6)
C13	0.8604(2)	0.2500	0.3884(3)	29.4(6)
C5	0.7513(2)	0.4921(1)	-0.0234(2)	34.8(5)
C14	0.8012(3)	0.3127(2)	0.4321(3)	66.9(9)
C15	0.9575(3)	0.2500	0.4725(4)	81.2(2)
C12A	0.9251(1)	0.1883(1)	0.1766(2)	24.8(4)
C6A	0.8291(1)	0.1861(1)	-0.0682(2)	20.9(4)
O2A	1.0006(1)	0.1198(1)	-0.1777(1)	30.9(4)
N1A	0.7959(1)	0.0644(1)	-0.0738(2)	30.2(4)
C8A	0.9310(1)	0.1860(1)	0.0096(2)	22.2(4)
O1A	1.0314(1)	0.0841(1)	0.0476(1)	36.7(4)
C9A	0.9921(1)	0.1236(1)	-0.0351(2)	26.1(4)
C2A	0.6976(1)	0.1300(1)	0.0903(2)	29.7(4)
C1A	0.7698(1)	0.1246(1)	-0.0155(2)	22.6(4)
C4A	0.6799(2)	0.0085(1)	0.0819(2)	34.5(5)
C3A	0.6524(2)	0.0709(1)	0.1398(2)	34.6(5)
C10A	1.0597(2)	0.0637(1)	-0.2306(3)	41.0(6)
C5A	0.7513(2)	0.0079(1)	-0.0234)2)	34.8(5)
C14A	0.8012(3)	0.1873(2)	0.4321(3)	66.9(9)

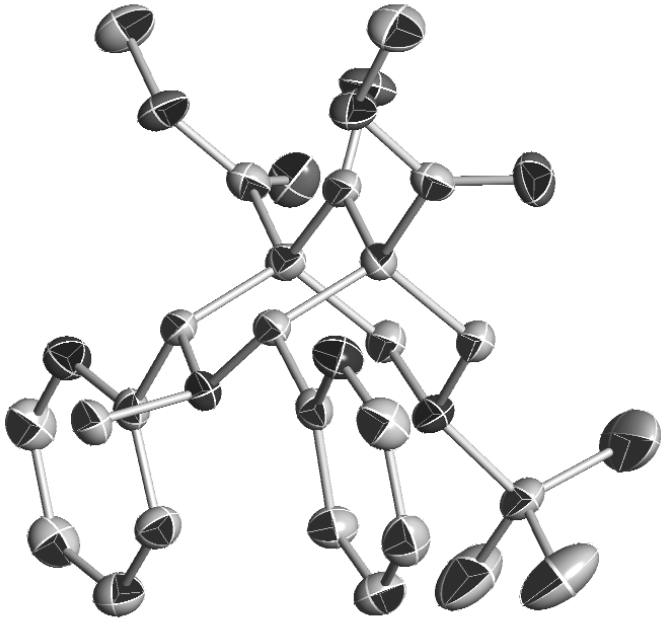


Figure 2. Molecular structure of **4** showing the atomic numbering scheme (thermal ellipsoids at 50% probability). Hydrogen atoms are shown with an arbitrary radius.

The latter finding prompted the elucidation of the structure in solution by means of high-field NMR measurements (see Figure 4), in particular NOESY and NOE difference experiments to achieve the spatial arrangement of the pyridines. The NOESY diagram (see Figure 5) clearly shows cross peaks between the hydrogen Hm´ in the pyridine ring (*cf*. Figure 3) with the hydrogens H2 and H4, respectively, the equatorial H6 and H8 (δ = 3.10 ppm), respectively, N3-CH_3 and N7-$C(CH_3)_3$. These findings

were confirmed by additional NOE difference experiments: On saturation of the signal of Hm´ strong positive NOEs were observed with Hp, H2/4 (δ = 4.65 ppm), N3-CH_3 (δ = 2.00 ppm) and weak NOEs with the equatorial H6/8 and N7-C$(CH_3)_3$ (δ = 0.94 ppm). In turn, on saturation of the H2/4 signal strong positive NOEs were observed with Hm´ and N3-CH_3. These results indicate a close vicinity between Hm´ and H2/4 (27 nm) along with a pyridine nitrogen pointing to the bottom of the molecule. However, on saturation of the signal of the N7-C$(CH_3)_3$ group, clear positive NOEs were found with both H6/8 signals and Hm´. The latter can be observed only when Hm´ is pointing to the bottom and the nitrogen to the keto group of the diazabicyclic moiety.

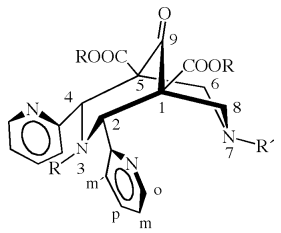


Figure 3. Structural formula and assignment of compound **4**.

These results in solution are in contrast to the crystal structure, and show that the nitrogen is found in both positions. At this stage it cannot be decided whether separate isomers bearing these substituents either both pointing in direction of the keto carbonyl group (Scheme 1) or both to the bottom of the molecule or in different directions are present in solution. Alternatively, the NMR observation can be a product of a rotation around the C2/C4-aryl axis. However, this rotation was previously found to be restricted [9].

Interestingly, in the NMR spectrum of **4** a second set of signals of low intensity (< 20 percent) can be identified which can be assigned to the chair/boat conformation (*cf.* to [11]). In comparison to the observations reported by Caujolle, the percentage of molecules in boat/chair conformation is lower.

Pharmacology.

All compounds synthesized were subjected to radioligand binding assays using [^{3}H]naloxone, [^{3}H]CI-977, or [^{3}H]D-Ala-deltorphine II for human μ, κ and δ_2 receptor subtypes, respectively. The *K*i values and percent inhibition of the

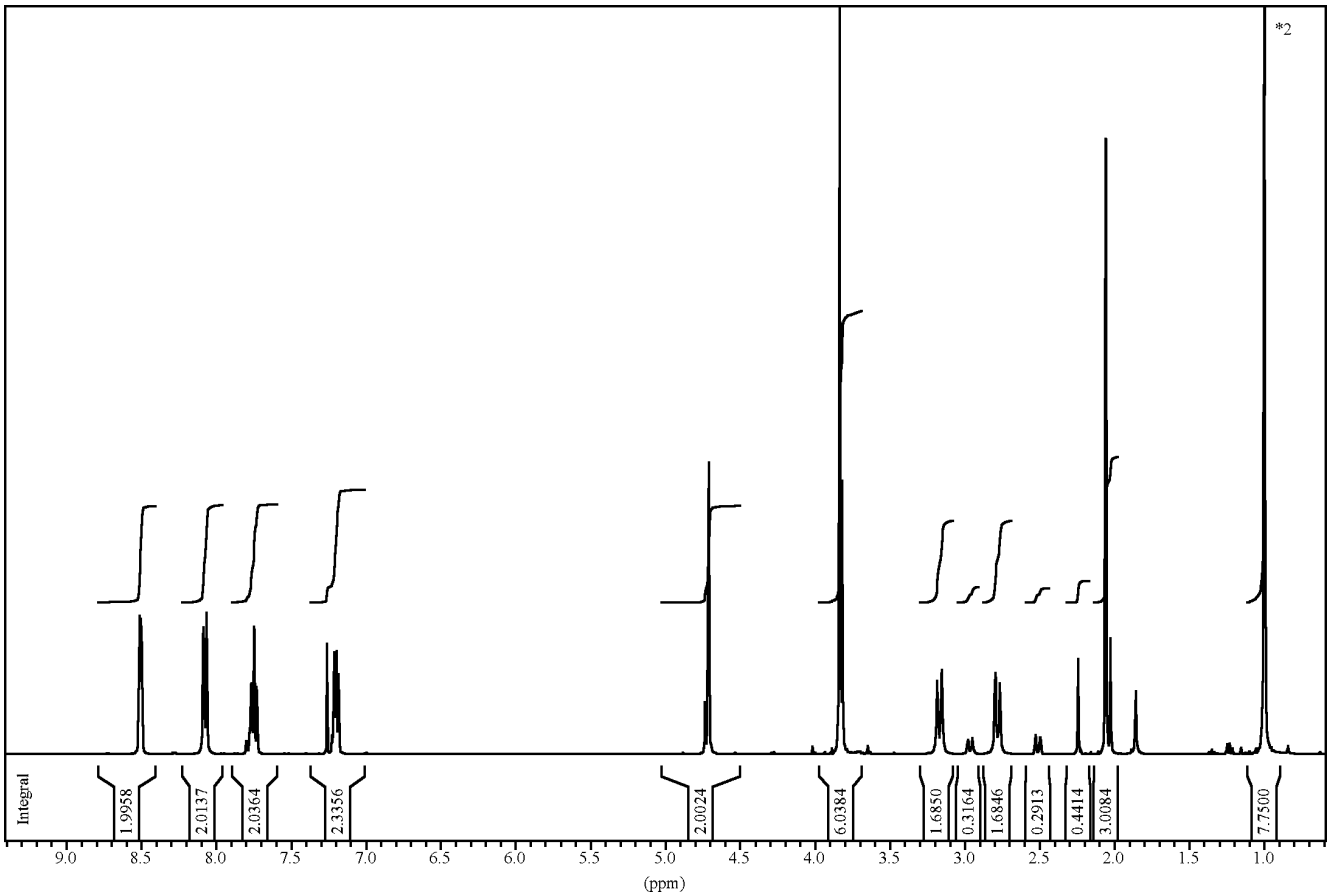


Figure 4. ^{1}H NMR spectrum of the N-*tert*.butyl substituted diazabicyclononanone **4**.

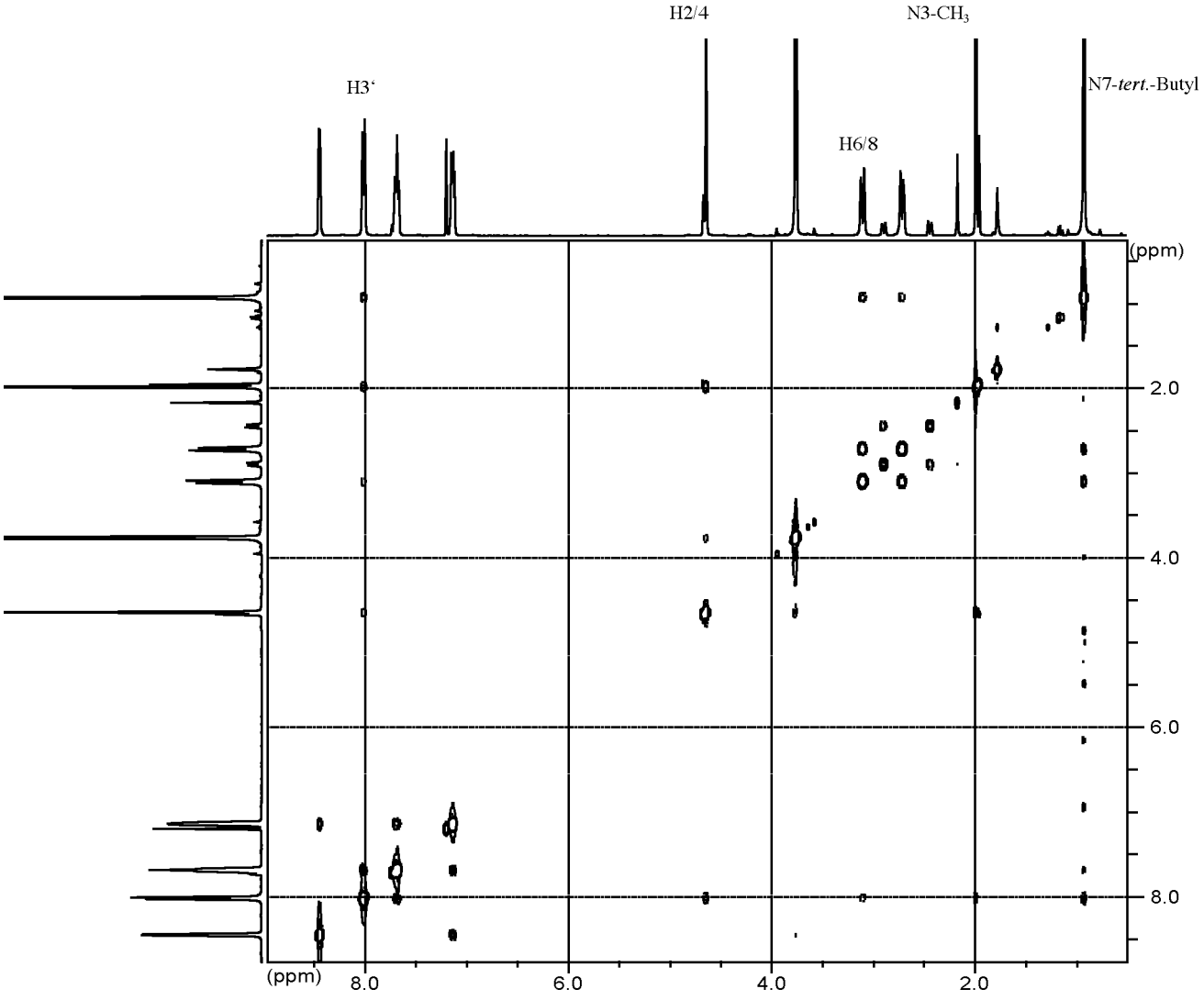


Figure 5. NOESY diagram of the N-*tert*.butyl substituted diazabicyclononanone **4**.

radioligand binding, respectively, are displayed in Table 3. Previously, rat brain homogenates in combination with subtype specific radioligands were used, *e.g.* [^{3}H]naloxone (μ-OR), [^{3}H]Cl-DPDPE (δ-OR) and [^{3}H]CI-977 or [^{3}H]diprenorphine (κ-OR), respectively [4,5,6]. Since the affinities measured in different assays are not directly comparable, for sake of comparison **HZ2**, its oxalate salt, bremazocine and U-50,488 were additionally measured. Interestingly, the affinity of **HZ2** to human κ-OR was found to be lower than the affinity to rat brain receptors (Ki = 15.3 nM (rat brain) versus Ki = 1,2 μM (human)). In comparison to **HZ2**, which is characterized by a methyl group in position 7 and affinity to the κ-receptor, the affinity of compound **1-16** to the κ-OR subtype decreased when increasing the bulk of the N7 substituent. Even an ethyl group seems to be too large for a significant affinity to the κ receptor subtype indicating that the size of the N7 substituent is critical for binding to the receptor pocket. However, the 2-butyl and methylcyclohexyl substituted compounds **3** and **11** exhibit an unexpected high affinity to the κ-OR.

Whereas in previous studies no μ-affinities have been found for any diazabicyclononanone [3,4,5,6] **HZ2** and the corresponding alkyl substituted compounds **1 – 5** show affinity for the human μ-OR in the micromolar range of concentration. Since compounds of mixed affinities to the μ and κ-OR such as buprenorphine and butorphanol are of increasing interest [12], the next step of pharmacological studies will be to work out whether the compounds are agonists or antagonists at either receptors and check the analgesic activity in animals.

In addition, it is worth mentioning that the 4-carboxybenzyl substituted compound **16** exhibit affinity to both μ and

Table 3

Variation of the substituents at the nitrogen N7, radioligand binding data (inhibition constants (K_i)) or % inhibition of the compounds at [^{3}H]radiolabelled opioid receptors (The respective test concentration is indicated in brackets as μM). The radioligands for μ-OR, δ-OR and κ-OR sites were [^{3}H]naloxone, [^{3}H]D-Ala-deltorphine II and [^{3}H]CI977, respectively; the percent inhibition was measured in cell membrane preparations of CHO-K1, HEK293 cells, CHO-K1 cells transfected with human μ, κ, or δ_2 opioid receptor subtype, respectively.

Entry	N7-R	Human μ-OR [^{3}H]naloxone			Human κ-OR [^{3}H]CI-977				Human δ_2-OR [^{3}H]-D-Ala-deltorphine II		
		% (10)	% (1)	Ki (μM)	% (100)	% (10)	% (1)	Ki (μM)	% (10)	% (1)	Ki (μM)
HZ2	methyl	62	49	1.4	90	62	24	1.2	-3	1	
HZ2-oxalate	methyl	74	34	1.8	88	60	41	0.91			
1	ethyl	42	8	6.9	30	10	-2		1	-3	
2	propyl	50	9	2.2	46	18	3	38.6	-4	-8	
3	2-butyl	56	8	4.9	72	34	11	8.3	11	2	
4	*tert.*-butyl	49	16	6.0		23	-1		7	0	
5	allyl	47	12	6.0	29	0	0		4	-2	
6	propargyl		8*							-17*	
7	cyclopropyl		15*				-6*			-2.5*	
8	methyl-cyclopropyl-	34	7		41	12	0		-8	-8	
9	cyclobutyl	35	2		60	19	4	22.1	-6	-13	
10	cyclopentyl	35	6		47	19	7	37.6	1	-7	
11	methyl-cyclohexyl	81	41	1.2	91	37	5	4.9	19	-3	
12	ethylpiperidine	18	8		34	18	1		1	-4	
13	ethylmorpholine	13	6		22	5	1		-3	-5	
14	benzyl		10*				28*				
15	4-carboxy-benzyl	71	16	2.3	-	12	-5		98	34	0.67
16	ethylphenyl	58	24	3.3	45	17	4	39.0	10	-4	
Bremazocine				0.0011			99	0.000025			0.0036
U-50,488		61	44	0.78			100	0.0024	37	12	

* The radioligands for μ-OR, δ-OR and κ-OR sites were [^{3}H]naloxone, [^{3}H]Cl-DPDPE and [^{3}H]CI977, respectively; the percent inhibition was measured in rat brain.

δ-receptors. Since the δ-affinity was found to be in a submicromolar concentration this compound may be a new lead compound for the development of ligands for the δ-OR.

Conclusion.

Whereas substituents in the higher substituted piperidones ring of the 2,4-di-2-pyridyl-3,7-diazabicyclononanones are able to influence the configuration and the conformation of skeleton strongly, substituents in the less substituted piperidone ring in position N7 do not change the conformation and configuration. All compounds studied were isolated in a chair/chair conformation. In no case do the molecules crystallise in a chair/boat conformation. Even though the molecules are able to adopt the pharmacophoric chair/boat conformation, none of the diazabicyclononanones showed as high an affinity to the κ receptor as **HZ2**. This indicates that the binding pocket of the κ-OR seems to be too small to be visited by molecules with large substituents attached to N7 and N3 [10]. However, compounds **3** and **11** carrying bulky 2-butyl and methylcyclohexyl substituents, respectively, are characterized by considerable affinities for the κ-OR. Some of the compounds **1-5**, **11** and **15**, **16** are active at the μ-OR and **15** additionally at the δ-OR giving the idea to develop analgesics with mixed affinities to opioid receptors which may have a high analgesic potency with less side effects.

EXPERIMENTAL

Melting points were determined with Dr. Tottoli melting point apparatus (Büchi, Switzerland) and were not corrected. IR spectra, recorded as KBr discs, were obtained using a Perkin-Elmer 298 spectrometer. TLC was performed using Merck silica gel F_{254} plates (Merck 5554), 0.2 mm; eluent mixture: cyclohexane/ethyl acetate/methanol (10/4/1). Chemicals were of analytical grade and were purchased from Aldrich, Steinheim, or Merck, Darmstadt, FRG. Dry solvents were used throughout. Elemental analysis was performed by means of a Leco 932 CHNS and the results were found to be within ±0.4% of the theoretical value.

Synthesis.

The dimethyl N-methyl-4-oxo-2,6-di(2-pyridyl)-4-piperidine-3,5-dicarboxylate was synthesized according to reference [6].

General Procedure for Synthesis of Dimethyl 7-Alkyl-3-methyl-9-oxo-2,4-di-2-pyridine-3,7-diazabicyclo[3.3.1]nonane 1,5-dicarboxylates.

The piperidone (0.005 mol) was dissolved in acetone (I) or in methanol (II) and 1.2 ml of 35 % formaldehyde and 0.005 mol of

Table 4

Analytical Data of the Compounds **1-16**

No.	R	Molecular formula MW	Yield % Method	Mp °C (dec.)	Combustion analysis	C	H	N	IR (cm^{-1}) (KBr)
1	ethyl	$C_{26}H_{28}N_4O_5$ 480.57	51.2 II B/E	162	Found	63.58	6.34	12.42	3470, 1690, 1260, 1180,
					Cal.	63.70	6.24	12.38	720
2	propyl	$C_{25}H_{30}N_4O_5$ 466.54	50.6 II B/E	178	Found	63.91	6.44	11.89	2940, 1740, 2800, 1230,
					•$^1/_2$ EtOH				1140, 740
					Cal.	63.81	6.78	11.40	
3	2-butyl	$C_{26}H_{32}N_4O_5$ 480.57	35.4 II B/E	209	Found	64.92:	6.77	11.69	2970, 2790, 1720, 1410,
					Cal:	64.89,	6.71	11.66	1250, 1070, 730
4	t.butyl	$C_{26}H_{32}N_4O_5$ 480.57	23.75 II A/E	210	Found	64.64	6.85	11.72	2980, 2800, 1720, 1420,
					Cal.	64.98	6.71	11.66	1260, 740
5	allyl	$C_{25}H_{28}N_4O_5$ 464.53	78.37 I A/M	176	Found	64.60	6.01	12.03	2980, 1710, 1380, 1250,
					Cal.	64.64	6.08	12.06	750
6	propargyl	$C_{25}H_{26}N_4O_5$ 462.51	17.24 I A/M	190	Found	64.86	5.99	11.97	3240, 2950, 2780, 1720,
					Cal	64.92	5.67	12.11	1240, 750
7	cyclopropyl	$C_{25}H_{28}N_4O_5$ 464.53	40.26 II B/M	199	Found	63.54	6.24	11.64	2930, 2800 1760, 1430,
					•$^1/_2$ MeOH				1280, 710
					Cal.	63.76	6.29	11.67	
8	methyl-cyclopropyl	$C_{26}H_{30}N_4O_5$ 478.55	53.56 II B/E	199	Found	65.30	6.46	11.70	3100, 2800, 1730, 1430,
					Cal.	65.26	6.32	11.71	1270, 750
9	cyclobutyl	$C_{26}H_{30}N_4O_5$ 478.55	59.66 II B/M	202	Found	65.34	6.42	11.71	2970, 2800, 1760, 1430,
					Cal.	65.26	6.32	11.71	1270, 760
10	cyclopentyl	$C_{27}H_{32}N_4O_5$ 482.58	70.61 II A/M	226	Found	65.70	6.88	11.36	2980, 2790, 1770, 1380,
					Cal.	65.84	6.55	11.37	1210, 700
11	methyl cyclohexyl	$C_{29}H_{36}N_4O_5$ 520.63	61.51 II B/E	199	Found	66.78	7.43	10.78	2950, 1760, 1430, 1250,
					Cal.	66.90	6.97	10.76	760
12	ethyl-piperidine	$C_{29}H_{37}N_5O_5$ 535.65	II B/E	166	Found	65.00	6.92	12.95	2930, 2790, 1730, 1430,
					Cal.	65.03	6.96	13.07	1270, 750
13	ethyl-morpholine	$C_{28}H_{35}N_5O_6$ 537.62	34.23 II B/E	182	Found	62.46	6.43	12.95	2970, 2810, 1720, 1420,
					Cal.	62.56	6.56	13.03	1270, 740
14	benzyl	$C_{29}H_{30}N_4O_5$ 514.49	48.40 II B/M	209	Found	67.31	5.90	11.02	2960, 2790, 1710, 1410,
					Cal.	67.69	5.88	10.89	1250, 720
15	4-carboxy-benzyl	$C_{30}H_{30}N_4O_7$ 558.80	II B/M	194	Found	62.07	5.68	9.52	3000-2700, 2970, 1730,
					•1 H_20	62.49	5.59	9.72	1420, 1230, 730
					Cal.				
16	ethylphenyl	$C_{30}H_{32}N_4O_5$ 528.61	39.60 163	II B/E	Found	67.98	6.10	10.57	2940, 2800, 1740, 1450,
					Cal.	68.17	6.31	10.60	1270, 700

the corresponding primary amine were added. The solution was refluxed and monitored by TLC. In case the reaction was not completed after one hour, half of the quantities of formaldehyde and amine (given above) were additionally added. The solution was refluxed for 30 minutes and allowed to stand at room temperature for 7 days. The work up procedure was either: The crystals were filtered (A). If after one week no precipitate appeared, the solvent was evaporated in vacuo and the obtained oil dissolved in methanol (M) or ethanol (E). The diazabicyclononanones precipitated within 7 days (B). The raw products were recrystallized from ethanol. Analytical data of the compounds **1-16** and details of either synthesis are given in Table 4, ^{1}H-NMR-and ^{13}C-NMR data in Tables 5 and 6.

NMR Measurements.

All ^{1}H and ^{13}C NMR were performed on a Bruker Avance 400 FT NMR spectrometer operating at 400.132 MHz and equipped with a Kayak XA_b computer using the XWIN-NMR program package Version 3.0. The spectrometer operating at 400.132 MHz (^{1}H) and 100.621 MHz (^{13}C) with a sample temperature of 300 K. 32 Scans with a frequency range of 4006.4 Hz were collected into 32,050 data points, giving a digital resolution of 0.13 Hz/point. An appropriate Gaussian function was applied before Fourier transformation in order to enhance the spectral resolution. Abbreviations for data quoted are: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. ^{1}H and ^{13}C-NMR assignments given for each compound were confirmed by randomly running HETCOR and H,H-COSY experiments using the corresponding Bruker software. NOESY experiments were performed by means of the Bruker software.

Hyperchem Calculations.

In order to calculated the distances of a rotamer of compound **4**, the coordinates of the X-ray structure were taken to build up the molecule and the pyridine rings were rotated by 180°. The geometry was optimized by using the MM2 program implemented in Hyperchem [13] and the distances of interest measured.

Crystal Structure Determination of **4**.

X-ray crystallographic data were collected on a Bruker Smart Apex CCD-diffractometer with D8-goniometer (Mo-Kα radiation, λ=71.073 pm) equipped with a low temperature device at 193 K [14]. Absorption correction was employed (SADABS)

Table 5

^{1}H-NMR Data of Compounds **1-16** [$CDCl_3$; δ (ppm); J (Hz)]

No.	H 2/4	H 6/8	OCH_3	N3-CH_3	N7-Substituent	Aromatic hydrogens
1	4.77 (s)	2.99 (d, 12.4) 2.46 (d, 12.4)	3.84 (s)	2.05 (s)	N-CH_2: 2.38 (d, 2H, 7.3) CH_3: 1.08 (t, 7.2)	7.22 (dq, 2H, 1.2/4.9) 7.79 (dt, 2H 1.8/7.6) 8.14 (d, 2H, 7.6) 8.51 (dd, 2H, 0.9/4.8)
2	4.76 (s)	2.50 (d 12.4) 3.02 (d 12.4)	3.84 (s)	2.05 (s)	N-CH_2: 2.22-2.29 (m, 2H) CH_2: 1.50 (sext, 2H, 7.6) CH_3: 0.95 (t, 7.3)	7.20-7.29 (m, 2H) 7.79(dt, 2H, 1.4/7.9) 8.13 (d, 2H, 7.9) 8.51 (d, 2H, 4.2)
3	4.72 (s)	2.78 (dd, 2H, 5.2, 12.2) 3.0 (d, 2H, 12.2)	3.83 (s)	2.05 (s)	1.06-1.22 (m, 1H); 1.57-1.70 (m, 1H); 2.31-2.53 (m, 1H) 0.83-0.99 (m, 6H)	7.19-7.30 (m, 2H) 7.73-7.81 (m, 2H) 8.6 (t, 2H, 6.9) 8.51 (d, 2H, 4.6)
4	4.65 (s)	2.72 (d, 2H, 12.2) 3.11 (d, 2H, 12.2)	3.77 (s)	2.00 (s)	CH_3: 0.94 (s, 9H)	7.12-7.20 (m, 2H) 7.69 (dt, 2H, 1.7/7.6) 8.09 (d, 2H, 7.8) 8.54 (d, 2H, 0.8/4.8)
5	4.74 (s)	2.49 (d, 2H, 12.1) 3.01-2.9* (m, 4H, +N7-CH_2)	3.81 (s)	2.03 (s)	N-CH_2: 3.01-2.9* (m,4H) CH: 5.86 (m, 1H) CH_2: 5.11 (m, 2H)	7.01 (dq, 2H, 1.1/4.9) 7.76 (dt, 2H, 1.7/7.6) 8.09 (d, 2H, 7.8) 8.48-8.50 (m, 2H)
6	4.77 (s)	2.74 (d, 2H, 12.3) 2.99 (d, 2H, 12.3)	3.88 (s)	2.05 (s)	N-CH_2: 3.30 (d, 2H, 2.4) 2.22-2.25 (m, 1H)	7.17-7.24 (m , 2H) 7.77 (dt, 2H, 1.7/7.7) 8.10 (d, 2H, 7.9) 8.51 (d, 2H, 3.9)
7	4.67 (s)	2.68 (d, 2H, 12.5) 2.99 (d, 2H, 12.5)	3.39 (s)	1.96 (s)	N-CH: 1.31-1.39 (m, 1H) CH_2: 0.32-0.39 (m, 2H) 0.42-0.49 (m, 2H)	7.13-7.18 (m, 2H) 7.72 (dd, 2H, 1.8/7.6) 7.86 (d, 2H, 7.9) 8.42-8.44 (m, 2H)
8	4.77 (s)	2.48 (d, 2H, 12.3) 3.08 (d, 2H, 12.3)	3.83 (s)	2.04 (s)	N-CH_2: 2.15 (d. 2H, 6.7) CH: 0.84-0.92 (m, 1H) CH_2: 0.46-0.53 (m, 4H)	7.20 (dq, 2H, 1.2/4.9) 7.21 (dt, 2H, 1.6/7.7) 8.20 (d, 2H, 7.9) 8.49 (dd, 2H, 0.9/2.4)
9	4.77 (s)	2.30 (d, 2H, 12.6) 2.92 (d, 2H, 12.6)	3.84 (s)	2.07 (s)	N-CH: 2.56-2.71 (m, 1H) 1.54-1.91 (m, 6H)	7.21-7.30 (m , 2H) 7.79-7.87 (m, 2H) 8.22 (d, 2H, 7.8) 8.51 (d, 2H, 4.1)
10	4.76 (s)	2.17-2.51* (m, 3H, +N7-CH) 3.06 (d, 2H, 12.8)	3.85 (s)	2.05 (s)	N-CH_2.17-2.51* (m, 3H) 1.53 (br, 8H)	7.17-7.23(m , 2H) 7.77(dt, 2H, 1.7/7.7) 8.15 (d, 2H, 7.8) 8.47-8.49 (m, 2H)
11	4.72 (s)	2.45 (d, 2H, 12.5) 3.08 (d, 2H, 12.5)	3.84 (s)	2.03* (br, 7H)	2.03* (br, 3H) 1.79 (br, 3H) 1.30 (br, 4H) 0.96 (br, 2H)	7.20 (dq, 2H, 0.9/4.9) 7.76 (dt, 2H, 1.7/7.7) 8.11 (d, 2H, 7.9) 8.50-8.52 (m, 2H)
12	4.65 (s)	2.51-2.54 (m, 2H) 3.1 (br, 2H)	3.76 (s)	1.96 (s)	2.38-2.44 (m, 6H) 1.75 (br, 4H) 1.41 (br, 2H)	7.13-7.20 (m, 2H) 7.70 (dt, 2H, 1.7/7.6) 8.06-8.07 (m, 2H) 8.44 (d, 2H, 4.3
13	4.73 (s)	2.63-2.65 (m, 2H) 3.07-3.10 (m, 2H)	3.84 (s)	2.05 (s)	N-CH_2: 2.47-2.51 (m, 6H) 1.75 (br, 2H) O-CH_2: 3.75 (br, 4H)	7.23 (dq, 2H, 1.1/5.0) 7.24 (dt, 2H, 1.7/7.7) 8.10 (d, 2H, 5.8) 8.25 (d, 2H, 4.0)
14	4.70 (s)	2.55 (d, 2H, 12.4) 3.06 (d, 2H, 12.4)	3.82 (s)	1.98 (s)	N-CH_2: 3.36 (s, 2H)	7.12 (dq, 2H, 1.1/4.8) 7.39-7.48 (m, 7H) 7.88 (d, 2H, 7.8) 8.44 (dd, 2H, 0.9/5.5)
15 DMSO-d_6	4.56 (s)	2.52-2.56# (m, 2H) 3.01 (d, 2H, 12.2)	3.69 (s)	1.89 (s)	N-CH_2: 3.47 (s, 2H)	7.27 (dd, 2H, 3.3/5.2) 7.53 (d, 2H, 8.2) 7.64 (dt, 2H, 1.6/7.7) 7.85(d, 2H, 7.6) 8.03 (d, 2H, 7.9) 8.43 (d, 2H, 3.6)

Table 5 (continued)

No.	H 2/4	H 6/8	OCH_3	N3-CH_3	N7-Substituent	Aromatic hydrogens
16	4.78 (s)	2.59-2.68 (m, 4H + CH_2) 3.11 (d, 2H, 12.5)	3.85 (s)	2.01 (s)	N-CH_2: 2.77-2.82 (m, 2H)	7.18-7.26 (m, 4H) 7.27-7.35 (m, 2H) 7.77 (dt, 2H, 1.7/7.7 8.07 (d, 2H, 7.9) 8.52-8.53 (m, 2H)

*Signal could not be assigned; # Signal hidden by solvent (d_6-DMSO).

Table 6

^{13}C-NMR data of Compounds **1-16** [$CDCl_3$; δ (ppm)]

No.	C1/5	C2/4	C6/8	C9	C=O	OCH_3	N3-CH_3	N7-Substituent	Aromatic carbons
1	62.09	73.65	58.62	203.92	168.79	52.47	43.32	N-CH_2: 51.20 CH_3: 12.15	123.00, 123.61, 149.18, 136.30, 158.88^q
2	62.08	73.61	59.70*	203.77	16.51	52.37	42.20	N-CH_2: 59.05* 19.81 CH_3: 12.91	122.90, 123.52, 149.10, 136.16, 158.71^q
3	63.01	74.05	54.97	204.20	169.43	52.83	43.76	N-CH:61.14 CH_2 :25.37 CH_3: 14.37/12.32	123.32, 124.11, 149.61, 136.56, 159.12^q
4	62.86	73.98	58.40	204.70	169.63	52.88	43.73	N-C: 52.88 -CH_3: 26.27	123.30, 124.49, 149.66, 136.47, 158.96^q
5	62.16	73.59	60.28*	203.51	168.52	52.36	43.20	N-CH_2: 58.49* -CH=: 136.14 =CH_2: 118.89	122.89, 123.53, 149.09, 133.65, 158.73^q
6	62.03	73.59	57.52	202.97	168.25	52.45	43.20	N-CH_2: 45.48 -C: 77.56 CH: 74.01	122.92, 123.10, 149.12, 136.35, 158.67^q
7	59.22	73.41	61.86	204.04	168.65	52.46	43.43	N-CH: 37.78 -CH_2: 6.25	123.02, 123.47, 149.28, 136.15, 158.62^q
8	62.30	73.51	58.94*	203.76	168.52	52.32	43.21	N-CH_2: 62.03* -CH: 8.50 CH_2: 4.04	122.84, 123.50, 148.77, 136.12, 158.77^q
9	61.67	73.40	55.79	203.97	168.61	52.36	43.23	N-CH: 60.10 CH_2: 14.07, 26.74	122.92, 123.74, 149.09, 136.11, 158.58^q
10	58.75	74.28	62.82	204.72	169.46	53.01	43.96	N-CH: 67.85 CH_2: 24.52, 30.25	123.61, 124.55, 149.84, 136.80, 159.42^q
11	62.20	73.84	60.05*	203.91	168.71	52.47	43.38	N-CH_2: 65.89* -CH: 35.05 -CH_2: 26.21, 26.52, 32.62	122.98, 123.67, 149.25, 136.20, 158.55^q
12	62.69	74.16	59.49*	203.96	169.05	52.90	43.63	-CH_2: 24.62 26.20 54.50 55.26 56.46*	123.42, 124.19, 149.62, 136.75, 159.15^q
13	62.14	73.54	58.96*	203.38	168.55	52.46	43.18	53.79 53.86 55.82* O-CH_2: 66.80	122.96, 123.50, 149.20, 136.21, 158.64^q
14	62.14	73.67	58.90*	203.57	168.43	52.33	43.14	N-CH_2: 62.02*	122.79, 130.31, 123.30, 136.04, 127.52, 136.80^q, 128.31, 148.96, 158.33^q
15 DMSO-d_6	62.71	73.87	59.30*	204.48	168.88	53.07	43.50	N-CH_2: 61.67* COOH: 168.18	123.99, 137.31, 124.22, 142.70^q, 130.23, 149.77, 131.04, 158.49^q
16	62.23	73.64	59.01*	203.62	168.67	52.52	43.32	N-CH_2: 58.71* CH_2: 32.87	123.03, 128.60, 123.54, 136.34, 126.27, 139.69^q, 128.49, 149.28, 168.67^q

*Signal assignment is exchangable; "^{q}" indicates quart. carbon.

[15]. The structure was solved by direct methods (SHELXS-97) [16] and refined by full-matrix least squares methods against F^2 (SHELXL-97) [17]. R Values are defined as $R1 = \Sigma||F_o|-|F_c|| / \Sigma|F_o|$, $wR2 = [\Sigma w(F_o^2 - F_c^2)^2/[\Sigma wF_o^2)^2]^{0.5}$, $w = [\sigma^2(F_o^2) + (g_1P)^2 + g_2P]^{-1}$, $P = 1/3[\max(F_o^2,0) + 2F_c^2]$. All non-hydrogen atoms were refined with anisotropic displacement parameters. All hydrogen atoms were refined isotropically using a riding model.

Additional crystallographic data of the refinement, data collection and atomic coordinates with displacement parameters for non-hydrogen atoms are given in Tables 1-2.

Pharmacology.

The human μ, κ, or δ_2 opioid subtype receptor binding assays were performed in microtiter plate assays essentially as described for the human κ opioid subtype [6]. In more detail, cell membrane preparations of CHO-K1 or HEK293 CHO-K1 cells transfected with human recombinant μ or κ opioid receptor (NEN/Receptor Biology Zaventem, Belgium) were used for the opioid subtype specific binding, respecticely. The μ and κ opioid subtype binding was performed as an homogeneous scintillation proximity assay (SPA) in the presence of either 1 or 2 mg of wheatgerm agglutinin coated SPA-beads (Amersham Pharmacia, Freiburg, Germany) per microtiter well. As radioactive ligand 1 nM of [^{3}H]-naloxone (NEN, Zaventem, Belgium) or [^{3}H]-CI977 (Amersham Pharmacia, Freiburg, Germany) were used for the μ or κ opioid subtype binding, whereas 25 μM or 100 μM of naloxone (Sigma, Deisingen, Germany) served as control for unspecific binding, respectively. Incubation of both assays took place at room temperature in 96 well flat bottom microtiterplates (#3632, Corning Inc., Corning, NY, USA) with an incubation time of 90 minutes. Incubation buffer was 50 mM TRIS-HCl , 0.05 % sodium azide, pH 7.4 which was further supplemented for κ opioid binding with 0.02 % bovine serum albumin.

Human δ_2 opioid subtype receptor binding was run as a filter harvest assay with cell membrane preparations of CHO-K1 cells transfected with human recombinant δ_2 opioid receptor (NEN/Receptor Biology Zaventem, Belgium). 1 nM of [^{3}H]-D-Ala-deltorphine II (NEN, Zaventem, Belgium) served as ligand and 10 μM naloxone as control for unspecific binding. 50 mM TRIS-HCl , 5mM $MgCl_2$, pH 7.4 served as incubation buffer for a total incubation time of 120 minutes at room temperature in a total volume of 250 μl per well of microtiter plates. Afterwards the incubates were filtered by means of a 96-well Brandel cell harvester (Hassel, Munich, Germany) onto Unifilter-96 GF/B glass fibre plates (Packard, Dreieich, Germany) presoaked with 50 mM TRIS-HCl , 5mM $MgCl_2$, 0.5 % polyethylene imine, pH 7.4. The filter plates were dried for 1 hour at 55 °C, supplied with 35 μl Ultima Gold MV scintillant (Packard, Dreieich, Germany) per well and sealed. All assays were counted after a time delay of at least 90 minutes in a 1450 MicroBeta^{TM} scintillation counter (Wallac, Freiburg, Germany) . All incubates run as duplicates and all assays were repeated twice. IC_{50} values were calculated according to the law of mass by means of standard computer software (Fig. P, Biosoft, Cambridge, UK) and K_i values were obtained using the Cheng-Prusoff equation.

Acknowledgement.

Thanks are due to the DFG (HO 1368/3-3) and the Fonds der Chemischen Industrie for financial support. We thank Prof. Dr. D. Stalke for a possibility of using a Bruker-CCD-diffractometer and D. Leusser and B. Walfort for the help with the crystal structure determination.

REFERENCES AND NOTES

[1] A. Samhammer and U. Holzgrabe, *Arch. Pharm.* (Weinheim), **322**, 551 (1989).

[2] A. Borsodi, S. Benyhe, U. Holzgrabe, A. Marki and C. Nachtsheim, *Reg. Peptides* **54**, 27 (1994).

[3] W. Brandt, S. Drosihn, M. Haurand, U. Holzgrabe and C. Nachtsheim, *Arch. Pharm. Pharm. Med. Chem.* **329**, 311 (1996).

[4] B. Kögel, T. Christoph, E. Friderichs, H.-H. Hennies, T. Matthiesen, J. Schneider and U. Holzgrabe, *CNS Drug Reviews* **4**, 54 (1998).

[5] T. Siener, A. Cambareri, U. Kuhl, W. Englberger, M. Haurand, B. Kögel and U. Holzgrabe, *J. Med. Chem.* **43**, 3746 (2000).

[6] U. Kuhl, W. Englberger, M. Haurand and U. Holzgrabe, *Arch. Pharm. Pharm. Med. Chem.* **333**, 226 (2000).

[7] R. Caujolle, P. Castera and A. Lattes, *Bull. Soc. Chim.* Fr. 413 (1984).

[8] T. Siener, U. Holzgrabe, S. Drosihn and W. Brandt, *J. Chem. Soc. Perkin Trans.* 2, 1827 (1999).

[9] U. Holzgrabe and E. Erciyas, Arch. Pharm. (Weinheim), **325**, 657 (1992).

[10] U. Kuhl, M. Graf v. Korff, K. Baumann, C. Burschka and U. Holzgrabe, *J. Chem. Soc. Perkin Trans. 2*, 2037 (2001).

[11] U. Ashauer-Holzgrabe and T. Busch, Z. Naturforsch **43b**, 873 (1988).

[12] E. Friderichs, T. Christoph and H. Buschmann, Analgesics and Antipyretics in Ullmann's Encyclopedia of Industrial Chemistry, 6th Edition, Wiley-VCH, Weinheim 2000

[13] Hyperchem release 5.1; Hypercube Inc, Waterloo, Ontario, Canada.

[14] D. Stalke, *Chem. Soc. Rev.*, **27**, 171-178 (1998).

[15] M. Sheldrick SADABS 2.0: program for Bruker/Siemens area detector absorption and other corrections, University of Göttingen 2000.

[16] G. M. Sheldrick, *Acta Crystallogr. Sect.* A **46**,467 (1990).

[17] G. M. Sheldrick, SHELXL-97, Program for Crystal Structure Refinement, University of Göttingen, 1997.

[18] Bruker-AXS Inc., SMART NT and SAINT NT: data collection and processing software for the SMART system X-ray instruments, Madison WI 2000.

[19] G. M. Sheldrick SHELXTL-NT V5.1: program package for solution and refinement of crystal structures, University of Göttingen 2000.