Synthesis, Structural, Conformational and Pharmacological Study of New Fentanyl Derivatives of the Camphidine System

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A series of *N*-phenethyl-8-β-amidocamphidines **4a-f** (3-phenethyl-8-β-(*N*-arylamido)-3-azabicyclo-[3.2.1]octane) has been designed, synthesized and stereochemically characterized as semirigid analogous of the 4-anilidopiperidine analgesics in an attempt to study the influence of certain stereochemical factors on analgesia in this class of compounds. In deuteriochloroform and deuteriobenzene solution, compounds **4a-f** display the same preferred conformation. The cyclopentane and piperidine rings adopt an envelope and distorted chair conformation respectively flattened at N-3, with the N and C-8 substituents in equatorial and axial positions with respect to the piperidine ring.

In vivo pharmacological testing demonstrated that compounds **4a-f** were inactive in the analgesic test, with the exception of compound **4f** which showed an ED_{50} of 250 mg/kg p.o.

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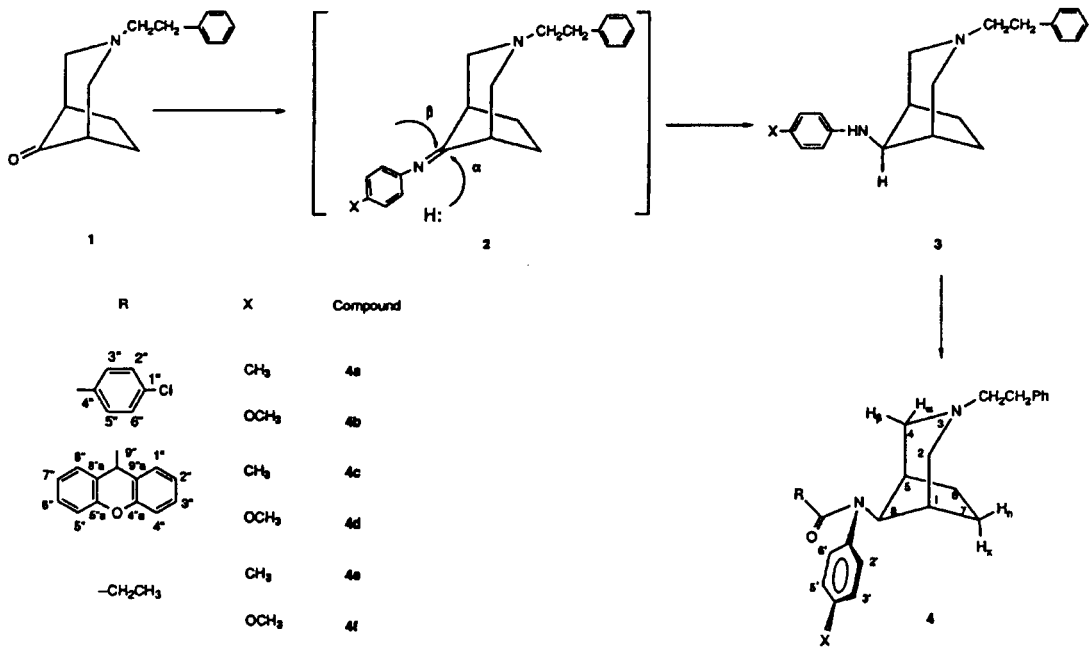
Introduction.

In the search for the ideal analgesic devoid of the typical side effects common to all morphinomimetic compounds, investigations have modified the fentanyl structure [1] in various ways. Fentanyl is a well-known analgesic, 80-100 times more potent than morphine with a fast onset and short duration of action. However, as with earlier opioids, it produces profound respiratory depression, muscle rigidity, postoperative nausea and physical dependence. As a result of the ongoing effort to develop the ideal analgesic, there is today an interesting number of novel compounds of the fentanyl family, all exhibiting an array of analgesic profiles.

In an attempt to design analogues of the 4-anilidopiperidine analgesics that would have measurable analgesic activity and would allow for a determination of conformational influences on this activity, we have undertaken a study of camphidine analogues of this class of basic anilide analgesics. In this line and in connection with our previous studies about camphidine derivatives [1-6] we report in this paper the synthesis and structural analysis carried out with the aid of ^1H and ^{13}C nmr spectroscopy of a series of *N*-phenethyl-8-β-[*N*-arylamido]camphidine derivatives (compounds **4a-f**, Scheme 1).

Scheme I



In order to gain additional information concerning the effects of stereochemical factors on analgesic activity, a pharmacological study of compounds **4a-f** has been carried out.

Synthesis.

The schematic synthesis pathways for **4a-f** are represented in Scheme 1. Reaction of *N*-phenethylcamphidin-8-one (3-phenethyl-3-azabicyclo[3.2.1]octan-8-one (**1**)) [1] with the corresponding aromatic amine at *p*H ≈ 7 in the presence of sodium cyanoborohydride led to the amines **3** *via* reductive amination [7]. Bearing in mind kinetic grounds and assuming that the more stable conformation of the intermediate ketimine **2** is similar to that found for **1** in solution [1] (Scheme 1), the amines **3** arise from the more favorable α-attack by the hydride anion (the β-attack is more hindered by H2(4)β than α-attack by H6(7)x, as can be seen with molecular models). These results are in quite good agreement with those observed for related compounds [2-6].

The amides **4a-f** were prepared by treatment of the corresponding amine with the appropriate acyl chloride.

Results and Discussion.

NMR Spectra.

The ^1H and ^{13}C nmr data of compounds **4a-f** are summarized in Tables 1-3.

The assignment of proton and carbon resonances has been made on the basis of double resonance experiments, and on previous studies of related compounds [2,3].

Conformational Study.

The ^1H and ^{13}C parameters of compounds **4a-f** are in good agreement with previously reported values for related bicyclic systems in which the cyclopentane and piperidine rings have a flattened C8 envelope and a distorted chair conformation puckered at C8 and flattened at N3 respectively. In the ^1H nmr spectra of compounds **4a-f**, $W_{1/2}$ values for the H1(5) signal (12 Hz) correspond to a

Table 1

^1H-NMR Chemical Shifts in C_6D_6 for Compounds **4a-4f**

δ (ppm) [a]	**4a**	**4b**	**4c**	**4d**	**4e**	**4f**
H6 (7) x (m)	1.63	1.64	1.51	1.52	1.62	1.63
H6 (7) n (m)	1.97	1.81	1.72	1.71	1.74	1.77
H1 (5) (brs) [b]	2.74 [c]	2.73	2.51 [c]	2.52 [c]	2.62 [c]	2.60 [c]
H2 (4) α	2.46 (m) [d]	2.38 (m) [d]	2.34 (d)	2.34 (d)	2.26 (m) [d]	2.27 (m) [d]
H2 (4) β (d)	2.46 (m) [d]	2.38 (m) [d]	2.26 (dd)	2.27 (dd)	2.26 (m) [d]	2.27 (m) [d]
H8 (t)	3.92	3.92	3.70	3.72	3.92	3.92
CH_2Ph (m) [d]	2.58	2.49	2.51 [c]	2.52 [c]	2.44	2.45
CH_2N (m) [d]	2.74 [c]	2.62	2.62	2.63	2.57 [c]	2.60 [c]
Ph (m) [d]	7.15	7.09 [c]	7.10 [e]	7.10 [e]	7.08 [e]	7.08 [e]
H2' (6') (m) [d]	6.63	6.69	6.82	6.84 [c]	6.73	6.71
H3' (5') (m) [d]	6.72	6.38	6.94 [c]	6.53	6.79	6.57
CH_3 (s)	1.85		2.04		2.00	
OCH_3 (s)		3.02		3.26		3.21
CH_3 (t)					1.08	1.10
CH_2 (q)					1.93	1.94
H2" (6") (m) [d]	7.11	7.08 [c]				
H3" (5") (m) [d]	6.86	6.83				
H1" (8")			7.18 (dd)	7.17 (dd)		
H2" (7")			6.97 (td)	6.93 (td)		
H3" (6")			6.90 (td) [c]	6.87 (td) [c]		
H4" (5")			7.06 (dd)	7.02 (dd)		
H9" (s)			5.27	5.30		

[a] Abbreviations: br, broad; d, doublet; dd, doublet of doublets; m, multiplet; q, quartet; s, singlet; t, triplet. δ Values were deduced by first order analysis of the spectra; error ± 0.05 ppm. [b] $w_{1/2}$ ≈ 12 Hz. [c] Signals are partially overlapped. [d] Multiplets of low resolution; tabulated chemical shifts correspond to the center of the multiplets [e] This signal is partially concealed by the solvent signal.

Table 2

^1H-NMR Multiplicities in C_6D_6 for Compounds **4a-4f**

J (Hz) [a]	**4a**	**4b**	**4c**	**4d**	**4e**	**4f**
H1 (5) - H8	4.0	4.1	4.1	4.1	4.1	4.0
H2 (4) α - H2 (4) β			-11.0	-10.9		
H2 (4) β - H1 (5)			3.5	3.7		
CH_3-CH_2					7.4	7.4
H2' (6') - H3' (5')	8.2	8.8	7.9	8.4	8.4	8.8
H2" (6") - H3" (5")	8.7	8.7				
H1" (8") - H2" (7")			7.2	7.2		
H1" (8") - H3" (6")			1.7	2.1		
H2" (7") - H3" (6")			7.2	7.8		
H2" (7") - H4" (5")			1.7	2.0		
H3" (6") - H4" (5")			7.3	7.2		

[a] Error ± 0.2Hz.

Table 3

^{13}C-NMR Chemical Shifts in $CDCl_3$ for Compounds **4a-4f**

δ (ppm)	**4a**	**4b**	**4c**	**4d**	**4e**	**4f**
C1 (5)	36.9	36.7	36.7	36.6	36.9	36.8
C2 (4)	53.3	53.2	53.1	53.1	53.1	53.1
C6 (7)	26.2	26.1	26.1	26.0	26.2	26.2
C8	62.5	62.3	62.7	62.7	61.5	61.4
CH_2Ph	33.4	33.2	33.3	33.2	33.4	33.3
CH_2N	59.8	59.9	59.9	59.8	59.8	59.9
C1 (Ph)	140.1	140.7	139.4	140.8	140.3	140.9
C2 (6) (Ph)	128.7	129.0	128.6	128.6	129.6	128.7
C3 (5) (Ph)	128.2	128.6	128.4	128.1	129.5	128.1
C4 (Ph)	125.8	125.8	125.8	125.7	125.7	125.8
C=O	172.4	172.5	175.0	175.0	176.3	176.6
CH_3	20.9		21.1		21.0	
CH_3O		55.2		55.5		55.4
C1'	140.9	135.3	140.9	134.4	141.0	135.6
C2' (6')	129.9	131.0	130.2	131.3	129.4	130.7
C3' (5')	129.1	113.8	129.9	114.3	128.6	114.1
C4'	136.6	158.2	138.1	158.9	137.8	158.6
CH_2 (Et)					28.9	28.9
CH_3 (Et)					9.4	9.4
C1"	134.6	134.5				
C2" (6")	129.4	128.1				
C3" (5")	127.8	127.8				
C4"	137.2	136.5				
C1" (8")			127.7	127.6		
C2" (7")			122.8	122.8		
C3" (6")			128.2	128.4		
C4" (5")			117.1	117.0		
C4"a (5"a)		151.7	151.5			
C8"a (9"a)		120.6	120.5			
C9"		43.9	44.2			

piperidine ring in a flattened chair conformation [8]; ^{3}JH2(4)β-H1(5) is greater than ^{3}JH2(4)α-H1(5), and consequently, the dihedral angle H2(4)β-C-C-H1(5) is smaller than H2(4)α-C-C-H1(5) according to the Karplus relationship [9]. This is also more consistent with a chair flattened conformation than with a boat conformation for the piperidine ring since the latter form should not only give a value of *ca.* 10 Hz for ^{3}JH2(4)β-H1(5) but also the signal corresponding to H1(5) should appear as an apparent doublet, a common feature in previously reported systems that adopt the boat conformation [8].

The relative spatial disposition of the amido group was deduced by comparing the values of δC2(4), δH2(4)α, δH2(4)β and ^{3}JH1(5)-H8 with those found for the α and β-epimers of 3-phenethyl-3-azabicyclo[3.2.1]octan-8-ol [2,3]. These parameters in amides **4a-f** are similar to those observed for the 3-phenethyl-3-azabicyclo[3.2.1]octan-8β-ol [2]: δH2(4)α ≈ δH2(4)β, δC2(4) = 52-53 ppm and ^{3}JH1(5)-H8 ≥ 4Hz, while the α-epimer exhibits the following values: Δδ[H2(4)β-H2(4)α] = 0.6 ppm, δC2(4) = 58.4 ppm and ^{3}JH1(5)-H8 ≤ 2 Hz [3]. In consequence, it can be assigned an axial arrangement with respect to the piperidine ring for the *N*-arylamido group. On this basis, the value of δC2(4) can be attributed to: a) the γ-effect exerted by the axial amido group on H2(4)β and b) the increased eclipsing between H2(4)β and H1(5) in a flattened chair conformation of the piperidine ring.

Keeping in mind the aromatic δ ^{1}H and ^{13}C values it can be deduced that a) the aryl moiety bonded to the carbonyl group occupies a semicoplanar disposition with respect to the amido group in **4a,b** and b) the *N*-aryl group occupies a non-coplanar (near perpendicular) arrangement with respect to the amido group (Scheme 1).

The δC6(7), C1' and C2' values of **4a-f** are in close agreement with an equatorial disposition of the *N*-substituent [2,3,8].

Summarizing, ^{1}H and ^{13}C data of compounds **4a-f** in solution show the same predominant chair-envelope conformation. The cyclopentane ring adopts an envelope conformation and the piperidine ring a distorted chair conformation puckered at C8 and flattened at N3, with the 3-phenethyl group and the 8-*N*-arylamido in equatorial and axial dispositions, respectively, with respect to the piperidine ring.

Pharmacology.

Treatment with compound **4f** prevented acetic-acid induced writhing in the mouse (ED_{50}, 250 mg/Kg p.o., as compared to an ED_{50} of 9 mg/Kg for diclofenac; $p < 0.01$ in both cases) but failed to prolong reaction time in the hot plate assay in the rat. This antinociceptive activity could not be explained by an opioid mechanism since the compound did not inhibit electrically stimulated twitch contraction of the guinea-pig myenteric plexus preparation. All other compounds were inactive in the writhing assay and were not further evaluated.

Discussion.

First order ^1H nmr approximations of a series of *N*-substituted 4-anilidopiperidine derivatives and 3-methyl-4-propanylidopiperidines imply that the preferred conformation is that of a piperidine chair with the 4-propanilido moiety in the equatorial orientation [10]. Analysis of the crystal structure of fentanyl and its structural analogues indicate this is the case [11,12]. In addition, the *N*-phenethyl and propanylido moieties are extended in the crystal structure [12]. The amide function is planar and at 90° angle to the mean plane of the piperidine ring. Also, the amide group is nearly perpendicular to the anilido phenyl group [13]. The preferred conformation can be depicted as in Figure 1, this conformation is also the probable solute-state conformation of fentanyl [14].

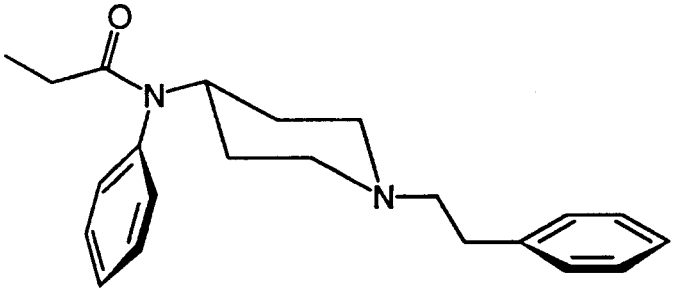


Figure 1

As was deduced from the nmr studies of compounds **4a-f**, the steric requirement presented above are met in these compounds, with the exception that the amido moiety occupies an axial disposition.

In vivo pharmacological testing demonstrated that compounds **4** were inactive in the analgesic test, with exception of compound **4f** which showed an ED_{50} of 250 mg/Kg p.o.

In the case **4a-d** the removal of the propionyl group would explain the lack of activity [15]. In the case of compound **4e** the lack of activity would be due to a): The axial position of the anilido group, b) the C6-7 ethylene unit in this part of the molecule would hinder an adequate binding to the opiate μ receptor.

The above reasonings together with the experimental pharmacological results support the hypothesis that the analgesic activity of **4f** cannot be accounted for by an interaction with the opiate receptor.

On the other hand, the only differences between **4f** and **4e** are the steric and electronic parameters of the arylanilido moiety and this latter compound showed no antinociceptive effect. Consequently, this fact remains unclear and demands further investigations.

EXPERIMENTAL

All melting points were taken in open capillary tubes in Electrothermal IA6304 apparatus, and are uncorrected. The elemental analysis were made in a Perkin-Elmer Elemental Analyzer Model 240E. The ir spectra wre recorded with a Perkin-Elmer 883 spectrophotometer in the solid state (potassium bromide).

The ^1H nmr spectra of 4% (w/v) deuteriochloroform or deuteriobenzene solutions of compounds **4a-f** were recorded at 300 MHz using a Varian UNITY-300 spectrometer. Spectral parameters included sweep widths of 4000 Hz in 24 K memory and acquisition times of 3.0 s over 64 transients. Resolution enhancement using LB = -0.80, GF = 0.50 and GFS = 0.20 was followed by zero filling into 32K memory prior to Fourier transformations. Conventional irradiation was used for the double resonance experiments.

The ^{13}C nmr spectra were obtained at 75.429 MHz on a Varian UNITY-300 spectrometer at a spectral width of 16501 Hz in 64 K memory, acquisition time of 1 s and relaxation delay of 1 s, using *ca.* 20% (w/v) deuteriochloroform solution. Two types of spectra were recorded: proton-noise decoupled spectra (to determine the chemical shifts) and off-resonance decoupled spectra (to help assign the signals).

All measurements were carried out at 298°K using TMS as the internal reference.

Synthesis of Amides **4a-f**.

General Procedure.

To a solution of 3-phenethyl-3-azabicyclo[3.2.1]octan-8-one (1.37 g, 6 mmoles) [1] and the corresponding amine (36 mmoles) in absolute methanol (15 ml) was added methanolic hydrogen chloride (5 *N*, 24 ml) and sodium cyanoborohydride (0.22 g, 36 mmoles). The mixture was stirred at room temperature for 3 days, acidified with concentrated hydrochloride acid (12*N*) at *p*H <2, and concentrateed under reduced pressure. The residue was extracted with water (50 ml) and ether (2 x 25 ml). The aqueous layer was neutralized with potassium hydroxide at *p*H >10, and extracted with methylene chloride (5 x 25 ml), dried (anhydrous magnesium sulfate) and concentrated under reduced pressure to give an oil. The excess primary amine was removed by distillatioin. Then, the residual oil was purified on a silica gel column. Elution with ethyl acetate/hexane (7:3) gave an oil which was further utilized.

To a stirred solution of the corresponding amine (3 mmoles)

and triethylamine (3 mmoles) in anhydrous methylene chloride (15 ml), with external cooling, was added dropwise a solution of the corresponding acyl chloride (4.5 mmoles) in anhydrous methylene chloride (15 ml.). The mixture was refluxed for 4 hours then a solution of potassium carbonate was added, and the resulting mixture was extracted with methylene chloride. The organic layer was dried (anhydrous magnesium sulfate), the solvent removed *in vacuo* and the resulting solid was recrystallized.

3-Phenethyl-8-β[*N*-(*p*-tolyl)-*p*-chlorobenzamido]-3-azabicyclo[3.2.1]octane (**4a**).

This compound was obtained in 62% yield, mp 120-122° (from hexane); ir (potassium bromide): υ CO, 1662 cm^{-1}; ^1H nmr: see Table 1; ^{13}C nmr: see Table 2.

Anal. Calcd. for $C_{29}H_{31}ClN_2O$: C, 75.87; H, 6.81; N, 6.10. Found: C, 76.19; H, 6.61; N, 6.23.

3-Phenethyl-8-β[*N*-(*p*-methoxyphenyl)-*p*-chlorobenzamido]-3-azabicyclo[3.2.1]octane (**4b**).

This compound was obtained in 65% yield, mp 128-129° (from hexane); ir (potassium bromide): υ CO, 1643 cm^{-1}; ^1H nmr: see Table 1; ^{13}C nmr: see Table 2.

Anal. Calcd. for $C_{29}H_{31}ClN_2O_2$: C, 73.32; H, 6.57; N, 5.89. Found: C, 73.14; H, 6.61; N, 6.10.

3-Phenethyl-8-β[*N*-(*p*-tolyl)-xanthen-9-carboxamido]-3-azabicyclo[3.2.1]octane (**4c**).

This compound was obtained in 75% yield, mp 153-155° (from ethanol); ir (potassium bromide): υ CO, 1665 cm^{-1}; ^1H nmr: see Table 1; ^{13}C nmr: see Table 2.

Anal. Calcd. for $C_{36}H_{36}N_2O_2$: C, 81.78; H, 6.86; N, 5.30. Found: C, 81.64; H, 6.63; N, 5.42.

3-Phenethyl-8-β[*N*-(*p*-methoxyphenyl)xanthen-9-carboxamido]-3-azabicyclo[3.2.1]octane (**4d**).

This compound was obtained in 80% yield, mp 155-157° (from hexane); ir (potassium bromide): υ CO, 1659 cm^{-1}; ^1H nmr: see Table 1; ^{13}C nmr: see Table 2.

Anal. Calcd. for $C_{36}H_{36}N_2O_3$: C, 79.38; H, 6.66; N, 51.4. Found: C, 79.67; H, 6.83; N, 4.79.

3-Phenethyl-8-β[*N*-(*p*-tolyl)propanamido]-3-azabicyclo-[3.2.1]octane (**4e**).

This compound was obtained in 61% yield, mp 80-82° (from hexane-acetone); ir (potassium bromide): υ CO, 1664 cm^{-1}; ^1H nmr: see Table 1; ^{13}C nmr: see Table 2.

Anal. Calcd. for $C_{25}H_{32}N_2O$: C, 79.53; H, 8.54; N, 7.42. Found: C, 79.66; H, 8.22; N, 7.57.

3-Phenethyl-8-β[*N*-(*p*-methoxyphenyl)propanamido]-3-azabicyclo[3.2.1]octane (**4f**).

This compound was obtained in 59% yield, mp 78-80° (from hexane); ir (potassium bromide): υ CO, 1662 cm^{-1}; ^1H nmr: see Table 1; ^{13}C nmr: see Table 2.

Anal. Calcd. for $C_{25}H_{32}N_2O_2$: C, 76.49; H, 8.21; N, 7.14. Found: C, 76.19; H, 8.51; N, 6.97.

Pharmacological Methods.

Writhing Assay.

For assessment of antinociceptive activity, the title compounds were suspended in 1% carboxymethylcellulose aqueous solution and administered p.o. to15-hour fasted male Swiss-Webster mice weighing 22-25 g (n = 5 per treatment group) followed 60 minutes later by i.p. administration of 10 ml/Kg of 7.5% acetic acid aqueous solution [15]. Writhing was observed during a subsequent 20-minute interval. A writhe was defined as abdominal stretching, downward arching of the back and full extension of the hind legs. The number of writhing responses exhibited by each animal was determined and the mean ± s.e. for each experimental group was calculated. Data were expressed as percentage of inhibition of the mean number of writhes in the vehicle-treated control group. ED_{50} represents the dose that produces 50% inhibition of response and was calculated in accord with the method of Litchfield and Wilcoxon [16]. Acetyl salicyclic acid and diclofenac were used as reference drugs.

Hot Plate.

Mice were placed on a copper plate kept at 55° [17]. The pain threshold was taken as the time a mouse began to lick its hind forepaw.

Assessment of Potential Opioid Properties.

Female guinea-pig (weighing 300-400 g) were killed by a blow in the head and then exsanguinated by cutting the jugular vein. Segments of distal ileum about 20 cm long were quickly removed (excision was performed about 10 cm above the ileocecal junction) and immediately placed in aerated Ringer solution of the following composition (m*M*): NaCl, 154; KCl, 5.66; $CaCl_2$, 2.54; $NaHCO_3$, 5.95; glucose, 2.77, and chlorine hydrochloride, 0.002. Strips of guinea-pig ileum myenteric plexus were mounted in a organ bath, superfused with Ringer solution, gassed with carbon dioxide and warmed at 37°, following Puig *et al* [18]. The plexus was connected to an isotonic transducer under a resting tension of 0.5 g and electrically stimulated by square wave pulses (5 ms) of 70 V at frequencies of 0.15 Hz. The preparation was allowed to equilibrate for 60 minutes before addition of the drugs. Morphine hydrochloride was used as the reference compound.

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