Synthesis and Opioid Receptor Affinity of a Series of 2,4-Diaryl-Substituted 3,7-Diazabicylononanones

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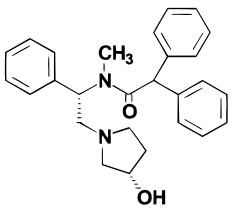
3,7-Diazabicyclo[3.3.1]nonan-9-ones having aryl rings in positions 2 and 4 with systematically varied substituents were synthesized using a double Mannich procedure. Radioligand binding assays were performed to measure the affinity of the compounds to the μ-, δ-, and κ-opioid receptors. The affinity of all 2,4-diphenyl-substituted 3,7-diazabicyclo[3.3.1]nonan-9-ones to the μ- and δ-receptors was found to be low. In contrast, with exception of the nitro- and cyanophenyl-substituted compounds, most of the diazabicycles showed considerable affinity for the κ-receptor. In particular, the *m*-fluoro-, *p*-methoxy-, and *m*-hydroxy-substituted compounds have an affinity in the submicromolar range. Due to solubility problems in aqueous media, salts of **HZ2** were synthesized. The methiodide shows high κ-affinity and may, thus, be a promising candidate for development of a peripheral κ-agonist, e.g. for use in the case of rheumatoid arthritis.

Introduction

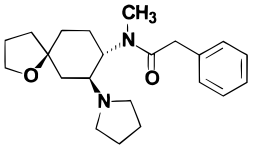
The search for compounds which can effectively treat strong pain, and not show side effects associated with the use of morphine, remains a challenge in drug development. Besides a wide variety of emerging targets connected with analgesia,[1−3] the three major subtypes of the opioid receptors, μ, κ, and δ, are still an important focus of efforts identifying subtype-selective ligands.[4] κ-Agonists were originally believed to be free of dependence, tolerance, and respiratory depression.[5] However, the first compounds in clinical trials for postsurgical pain, spiradoline and enadoline, have been abandoned due to dose-limiting dysphoria.[6] To avoid the side effects associated with the CNS, peripherically acting κ-agonists recently were targeted for use in inflammatory hyperalgesia, such as asimadoline (Chart 1) which has potential utility in treating rheumatoid arthritis.[1] In the case of the arylacetamide derivative U-69,593 (Chart 1), a peripheral component could be also detected in a rat model of unilateral neuropathy.[7] In addition, it was reported that κ-agonists can downregulate the expression of the human immunodeficiency virus (HIV-1) in human microglial cells.[8] With respect to δ-agonists, preclinical results of the highly active TAN-67 (Chart 1) showed that ligands of the δ-receptor may be effective and safe analgesics.[9]

Recently, the 2,4-di-2-pyridine-substituted 3,7-dimethyl-3,7-diaza-9-oxobicyclo[3.3.1]nonane-1,5-dicarboxylate, **HZ2**, was reported to have selective κ-affinity[10,11] combined with strong antinociceptive activity comparable to that of morphine and a potent action in inflammatory and persistent pain.[12] As a part of our efforts in opioid ligand design, the purpose of the present study was to assess the influence of substitution on the 2,4-aryl rings on the affinity for the three opioid receptor subtypes. To enhance water solubility and to direct the compounds to the peripheral receptors, **HZ2** was methylated at the nitrogen in position 7 and the product was used to form various salts (Table 1).

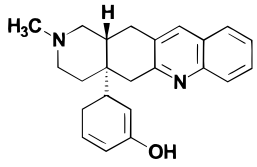
Chart 1. Structures of Asimadoline, U-69,593, and TAN-67



Asimadoline



U-69,593



TAN-67

Results and Discussion

Chemistry. To study structure−activity relationships (SAR) in the series of 3,7-diazabicyclo[3.3.1]nonan-9-ones, it was decided to systematically vary the substitution on the aryl rings in the 2- and 4-positions with regard to lipophilicity and electronegativity. Using the Craig plot,[13] the following substitution pattern was chosen: fluorine, chlorine, hydroxy, methoxy, nitro, cyano, and methyl substituents on either position of the phenyl ring (Table 1). In addition, the phenyl ring was replaced with various pyridine, quinoline, and naphthalene rings.

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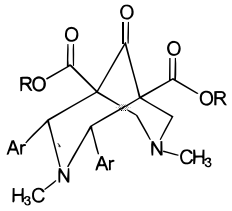
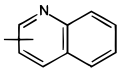
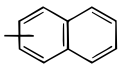
Table 1. Structural Variations of the Diazabicyclo[3.3.1]nonan-9-ones



Compound	Ar	R
11	 **a:** X = p-F **b:** X = m-F **c:** X = o-F	$R = CH_3$
12	 **a:** X = p-OH **b:** X = m-OH **e:** X = m-OH	**a,b:** $R = CH_3$ **e:** $R = C_2H_5$
13	 **a:** X = p-OCH_3 **b:** X = m-OCH_3	$R = CH_3$
14	 **a:** X = p-Cl **b:** X = m-Cl **c:** X = o-Cl **d:** X = 3,4-diCl **e:** X = p-Cl	**a-d:** $R = CH_3$ **e:** $R = C_2H_5$
15	 **a:** X = p-NO_2 **b:** X = m-NO_2 **c:** X = o-NO_2 **e:** X = m-NO_2	**a-c:** $R = CH_3$ **e:** $R = C_2H_5$
16	 **a:** X = p-CN **b:** X = m-CN	$R = CH_3$
17	 **a:** 2-quinolyl **b:** 4-quinolyl	$R = CH_3$
18	 **a:** 1-naphthyl **b:** 2-naphthyl	$R = CH_3$
19	 **a:** 3-pyridyl **b:** 4-pyridyl	$R = CH_3$
HZ2		$R = CH_3$

The 3,7-diazabicyclo[3.3.1]nonan-9-one skeleton could be obtained by a double-step Mannich reaction. In analogy to Petrenko-Kritschenko and Zoneff,[14] the condensation of 1 mol of dialkyl oxoglutarate, 1 mol of methylamine, and 2 mol of the corresponding arylaldehyde led to the 2,6-diaryl-substituted γ-oxopiperidine-3,5-dicarboxylates **1**−**9**. With exception of the *o*- and *p*-tolyl-, *o*-hydroxy-, and *o*-cyanophenyl-substituted piperidinones, all other compounds could be obtained in mostly good yields. Subsequently, the piperidin-4-ones were converted with methylamine and formaldehyde in methanol to give the 3,7-diaza-3-methylbicyclo[3.3.1]-nonan-9-ones **11**−**19** (compare with ref 15). With the exception of the *o*-methoxyphenyl-substituted compound **13c**, which was not possible to synthesize, all bicyclics were obtained in acceptable yields.

NMR spectroscopic measurements of the first fraction of crystals isolated from the reaction mixture often showed cis/trans isomerism with regard to the aryl rings. After recrystallization from hot ethanol, all diazabicycles were converted to the cis configuration (for details, see ref 16). Only in the case of the 2-quinolyl-substituted compound **17a** were the aryl substituents found to have a trans arrangement to each other. In the case of the *p*-nitrophenyl-substituted diazabicycles **5a**,**e**, both the cis and trans isomers could be isolated. Previous studies of representative diazabicyclic compounds (**15a,b**, **17a,b**, and **18a,b**) showed[16] that the compounds are configurationally stable under the course of radioligand binding studies. In addition, the formation of rotational isomers was observed.[16]

To determine whether the diazabicycles were protonated under physiological conditions, the pK_a values were representatively measured for **HZ2**, **11b**, **12b**, **13a**, and **14b** using a Sirius PCA 101 apparatus and a Yasuda−Shedlovsky plot[17] (Table 3). Due to solubility

Table 2. Opioid Receptor Binding Data of the Diazabicyclononanones **11**−**20**: Inhibition Constants (K_i) or Percent Inhibition of the Compounds at ^{3}H-Radiolabeled Opioid Receptors in Rat Brain Membranes[a]

compd	Ar	μ	δ_1	δ_2	κ
HZ3	-phenyl	14.5% (1)[a]	−0.3% (10)	−1.6% (1)	$K_i = 0.21\ \mu$M
11a	*p*-F-phenyl	10.1% (1)		0.4% (1)	$K_i = 0.14\ \mu$M[b]
11b	*m*-F-phenyl	5% (1)		3.2% (1)	$K_i = 0.026\ \mu$M[b]
11c	*o*-F-phenyl	−9.2% (1)		1.5% (1)	47.7% (10)[b]
12a	*p*-OH-phenyl	14.2% (1)		7.3% (1)	8.4% (10)[b]
12b[c]	*m*-OH-phenyl	35.6% (1)	27% (1)		$K_i = 0.02\ \mu$M[d]
12e	*m*-OH, HCl	10.5% (1)		6.9% (1)	92.7% (10)[b]
13a	*p*-OCH_3-phenyl	26.5% (1)		3% (1)	$K_i = 0.018\ \mu$M[b]
13b[c]	*m*-OCH_3-phenyl	−16.2% (1)		−1.6% (1)	52.2% (10)[b,e]
14a	*p*-Cl-phenyl	2.2% (1)		−2.1% (1)	63% (10)[b]
14b[c]	*m*-Cl-phenyl	8.8% (1)	−0.8% (10)	−11.8% (1)	42.3% (10)[b]
14c	*o*-Cl-phenyl	2.7% (1)		1.9% (1)	65.4% (10)[b]
14d	3,4-diCl-phenyl	6.1% (1)		−5.3% (1)	38.9% (10)[b]
14e	*p*-Cl-phenyl	−5% (1)		1.8% (1)	$K_i = 1.98\ \mu$M[b]
15a cis	*p*-NO_2-phenyl	4.6% (1)		−3.1% (1)	22.6% (10)[d]
15a trans	*p*-NO_2-phenyl	4.8% (1)		−4% (1)	27.8% (10)[d]
15b[c]	*m*-NO_2-phenyl	−23.5% (1)	14% (1)	−7.9% (1)	10% (10)[b]
15c	*o*-NO_2-phenyl	2.9% (1)		−1.1% (1)	3.4% (10)[b]
15e	*p*-NO_2-phenyl	2.3% (1)		6.1% (1)	5.9% (10)[d]
16a	*p*-CN-phenyl	5.6% (1)		3.2% (1)	17.2% (10)[b]
16b	*m*-CN-phenyl	9.3% (1)		5% (1)	35.5% (10)[b]
17a	2-quinolyl	36.6% (1)		11.8% (1)	$K_i = 0.19\ \mu$M[b]
17b	4-quinolyl	−7.1% (1)		1.2% (1)	25.8% (10)[b]
18a	1-naphthyl	−14.4% (1)		1.8% (1)	7.8% (10)[b]
18b	2-naphthyl	−23.1% (1)		−2.1% (1)	18.6% (10)[b,e]
19a	3-pyridyl	30.7% (1)		1.1% (1)	54.8% (10)[b]
19b	4-pyridyl	3.1% (1)		−9% (1)	12.5% (10)[b]
HZ2	2-pyridyl	20% (1)	5% (10)	−6.6% (1)	$K_i = 0.015\ \mu$M[d] 61.7% (10)[b]
HZ2 oxalate	2-pyridyl	37.6% (1)		−16.1% (1)	62.6% (10)[b]
HZ2 perchlorate	2-pyridyl	32.4% (1)		−5.9% (1)	65.0% (10)[b]
HZ2 methoiodide	2-pyridyl	29.2% (1)		3.3% (1)	96.4% (10)[d]
20[c]	*m*-Me-phenyl	−5.9% (1)	3.4% (1)		$K_i = 0.82\ \mu$M[d]

[a] The respective test concentration is indicated in parentheses as μmol/L. [b] Human κ-opioid site ([^{3}H]diprenorphine). [c] Ref 18. [d] Rat κ-opioid site ([^{3}H]CI 977). [e] Solubility problems.

Table 3. pK_a Values

compd	pK_{a1}	pK_{a2}	pK_{a3}	pK_{a4}
HZ2	10.99 ± 0.10	8.05 ± 0.06	3.47 ± 0.07	2.03 ± 0.01
11b	13.08 ± 0.09	10.72 ± 0.15		
12b	10.29 ± 0.24	7.97 ± 0.01	9.12 ± 0.10	9.21 ± 0.26
13a	9.05 ± 0.23	5.51 ± 0.58		
14b	10.63 ± 1.16	9.51 ± 0.58		

problems in water, the determination was performed in a mixture of water/dioxane and an extrapolation to 0% dioxane had to be applied (compare with ref 18). Two pK_a values were found: the first one in a range of 10−12 and the second between 8 and 10. Thus, at the pH occurring under physiological conditions (7.4), a notable amount of the diazabicycles will be once and mostly twice positively charged. In case of a double protonation the two additional hydrogens are in direct proximity in a chair/chair conformation and, therefore, the diazabicycle may adopt a chair/boat conformation with a boat in the less substituted piperidin-4-one ring.[11]

Pharmacology and SARs. To evaluate the affinity to either opioid receptor subtype, all compounds were subjected to radioligand binding assays using [^{3}H]-naloxone, [^{3}H]Cl-DPDPE, [^{3}H]-D-Ala-deltorphine, and [^{3}H]CI 977 or [^{3}H]diprenorphine for μ, δ_1, δ_2, and κ (rat or human κ-opioid site) receptor subtypes, respectively. The K_i values and percent inhibition of the radioligand binding are displayed in Table 2. None of the diazabicyclic compounds showed an affinity for the δ_1- or δ_2-receptors. The ability to displace the μ-radioligand naloxone was also limited and was always surpassed by the affinity to the κ-receptor. The affinity for the κ-receptor subtype ranged from nanomolar to micromolar concentrations. However, the affinity of some compounds was found to be too low to perform a full dose−effect curve. In these cases, the percentage of inhibition of the radioligand binding was given. Thus, qualitative SAR will be discussed in the following section.

Comparing the different types of substituents on the aryl ring in the 2- and 4-positions, it can be stated that nitro and cyano groups on the phenyl rings, as well as naphthyl rings, are disadvantageous to the affinity for the κ-receptor. Moreover, the cis and trans configurations of **15a** do not influence the affinity for the κ-receptor. With respect to the halogenated compounds, a fluorine atom creates a much higher affinity to the κ-receptor than a chlorine atom. Even the 3,4-dichloro substitution, which can be often found in potent arylacetamide derivatives, such as U-50488, DuP 747, and BRL 52580,[5] does not enhance the affinity for the κ-receptor. However, even though slightly different test systems were used, it can be stated that the *m*-fluorophenyl compound **11b** has as high an affinity for the κ-receptor as does the parent compound **HZ2**. Unfortunately, the poor water solubility of this compound prevented the intravenous application route in the in vivo experiments.

Among the hydroxy- and methoxyphenyl-substituted series **12** and **13**, compounds of high affinity were found. It is worth pointing out that the OH group in the *meta* position (**12b**) and the OCH_3 group (**13a**) in the *para* position create the highest affinities. Taking additionally the *m*-fluorophenyl compound into account, no rule

Table 4. Binding Methods

	[^{3}H]naloxone (1 nM)	[^{3}H]Cl-DPDPE (0.25 nM)	[^{3}H]-D-Ala-deltorphine (1 nM)	[^{3}H]CI 977 (1 nM)
rat brain area	brain without cerebellum	brain without cerebellum, pons, and medulla oblongata	brain without cerebellum, pons, and medulla oblongata	brain without cerebellum, pons, medulla oblongata, and cortex
assay volume	2 mL	2 mL	1 mL	1 mL
nonspecific binding	naloxone, 10^{-5} M	naloxone, 10^{-3} M	naloxone, 10^{-5} M	naloxone, 10^{-4} M
preincubation	37 °C, 40 min	25 °C, 30 min	none	37 °C, 45 min
incubation buffer	50 mM Tris-HCl, pH 7.4	50 mM Tris-HCl, pH 7.4, containing 900 μg BSA,[a] 45 μM PMSF,[b] and 4.5 mM $MgCl_2$ per assay	50 mM Tris-HCl, pH 7.4, containing 900 μg BSA[a] and 4.5 mM $MgCl_2$ per assay	50 mM Tris-HCl, pH 7.4
incubation	25 °C, 30 min	25 °C, 240 min	37 °C, 90 min	25 °C, 70 min
filter presoak	incubation buffer	incubation buffer	incubation buffer	incubation buffer

[a] BSA = bovine serum albumin. [b] PMSF = phenylmethanesulfonyl fluoride.

concerning the locus of substitution can be given from the data set. However, for each type of substitution, the affinity is sensitive to the position of the substituent. This is also true for the pyridine-substituted compounds **19** and **HZ2**. **HZ2** shows the highest affinity. The shift of the nitrogen in the aryl ring to the 3-position results in a slight loss, and the shift to the 4-position provides an almost complete loss of affinity.

The 2-quinolyl compound **17a** corresponding to **HZ2** shows a reduced but considerable affinity for the κ-receptor, whereas the 4-quinolyl-substituted diazabicycle **17b** corresponding to **19b** is almost inactive. However, a writhing test of compounds **17** and **19** resulted in the exhibition of some analgesic activity.

Taken together, within this series of compounds, three diazabicycles were found to have a considerable affinity for the κ-receptor which is comparable to that of **HZ2**. Despite their nanomolar affinity for the κ-opioid receptor, none of the compounds tested, however, exhibits as high an antinociceptive potency as **HZ2**.[12] In addition, due to relatively poor solubility, not all compounds could be tested by the intravenous route in the tail-flick or writhing test in vivo. To improve the solubility of the compounds, oxalates and perchlorates of **HZ2** were synthesized. These salts showed almost the same affinity for the κ-receptor and exerted a strong analgesic activity as did **HZ2** (i.e. 100% writhing inhibition at a dose of 10 mg/kg iv).

To direct **HZ2** to the peripheral κ-opioid receptors only, **HZ2** was permanently charged by formation of a methiodide which carries the additional methyl group on the nitrogen in position 7. This methiodide analogue retains complete inhibition in the radioligand binding, indicating high affinity and selectivity toward the κ-opioid site. In addition, it exerts a strong antinociceptive activity in the tail-flick assay (ED_{50} 6.7 mg/kg iv) and writhing test (ED_{50} 1.05 mg/kg iv) performed in mice. Furthermore pharmacological investigations are in progress in order to prove whether the site of action for the methiodide compound is predominantly peripheral.

Taken together, the results of the SAR study revealed some interesting compounds. The quaternization of the nitrogen in position 7 offers the perspective to develop peripheral κ-agonists which might be used against pain of rheumatic and osteoarthritic origin (compare with ref 5). Due to the high stability, the fluorine-substituted compound **11b** seems to be a promising candidate for the development of a peripheral κ-agonist.[20]

Experimental Section

Synthesis. Melting points were determined with a Dr. Tottoli melting point apparatus (Büchi) and were not corrected. ^{1}H spectra were recorded on a Varian XL 300 (^{1}H NMR: 299.956, 75 MHz) spectrometer. The centers of the peaks of $CDCl_3$ and DMSO-d_6 were used as internal references. Abbreviations for data quoted are d, doublet; t, triplet; and m, multiplet. Coupling constants are given in hertz (Hz). TLC was performed using Merck silica gel F_{254} plates, 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. The analytical results of almost all compounds are within ±0.4% of the theoretical values. The deviation within the theoretical and calculated values for some compounds such as **12e**, **16a**,**b**, and **17a,b** are caused by the presence of different solvents (molar ratios of DMSO, ethanol, or methanol in the crystals).

General Procedure for Synthesis of 2,6-Diaryl-Substituted 3,5-Dialkyl-*N*-methyl-4-piperidone-3,5-dicarboxylates 1−9 (modified after procedures in ref 19). Exactly 0.04 mol of the corresponding arylaldehyde and 1.5 mL of a 40% aqueous solution of methylamine were dissolved in 20 mL of methanol and cooled to 0 °C. Over a course of about 1 h, 0.02 mol of a dialkyl oxoglutarate was added dropwise to the mixture at 0 °C. The solution was allowed to stand overnight at 5 °C. The product was obtained by filtration of the precipitate formed. When no precipitate appeared, the solvent was removed in vacuo at 40−50 °C, and the remaining oil was dissolved in ethanol or treated with diethyl ether. The crystals obtained could be washed with a mixture of ethanol/diethyl ether and recrystallized from ethanol.

General Procedure for Synthesis of 2,4-Diaryl-Substituted 1,5-Dialkyl-3,7-dimethyl-3,7-diaza-9-oxobicyclo-[3.3.1]nonane-1,5-dicarboxylates 11−19 (modified after procedures in ref 19). Exactly 0.0025 mol of the piperidin-4-one was dissolved in 20 mL of methanol by refluxing. To this solution, was added 0.6 mL of a 35% aqueous solution of HCHO, followed by 0.4 mL of a 40% aqueous solution of methylamine. The mixture was refluxed for 7−8 min, and afterward it was allowed to stand for 3 h at ambient temperature. Three different workup procedures were possible. First, the obtained precipitate was filtered and washed with a mixture of ethanol/diethyl ether. When no precipitate appeared within 24 h, the solvent was removed in vacuo at 40−50 °C, and the remaining oil was dissolved in a small amount of ethanol and diethyl ether. Crystals usually appeared within 2 days. When no crystals were obtained during this time, the solvent was removed again, and the remaining oil was treated with diethyl ether. The raw product can be recrystallized from ethanol. The perchlorates and oxalates of **HZ2** were formed according to known procedures.[20]

Synthesis of the Methiodide of HZ2. Exactly 1 mmol of **HZ2** was dissolved in 10 mL of tetrahydrofuran, and 16 mmol methyliodide was added. The solution was allowed to stand for 24 h at ambient temperature and was protected from light. The so obtained crystals were separated, washed with tet-

rahydrofuran, and recrystallized from a mixture of methanol/diethyl ether: yield 79%, mp 115 °C dec.

pK_a Values. The pK_a values were measured potentiometrically in water using a Sirius PCA101 apparatus. Exactly 0.002−0.005 g of the substances was dissolved in 8−12 mL of aqueous dioxane (50%) and diluted to 20.0 mL with a 0.15 M KCl solution. The titration was performed starting from pH 11. Using the Yasuda−Shedlovsky plot,[17] the pK_a values could be calculated and extrapolated to 0% dioxane.

Pharmacology. All animal testing was performed in accordance with the recommendations and policies of the International Association for the Study of Pain and the national law on the care of animals in experiments (German Animal Welfare Law). All study protocols were approved by the local government committee for animal research, which is also an ethics committee.

Receptor Binding Studies. The binding studies with membranes from rat brains for the μ-, δ_1/δ_2-, and κ-opioid receptor sites were done as essentially described by Brandt et al.[11] In brief, Wistar SPF rats, weighing about 200 g, were killed by decapitation. Membrane suspensions were prepared essentially as described by Wood et al.[21] Protein concentration was determined by the method of Lowry et al.,[22] using bovine serum albumin as the standard. Incubation was initiated by addition of the membrane suspension. Details of the binding studies for the rat membrane can be found in Table 3 (compare with ref 12). All incubations were run in triplicate and terminated by rapid filtration under mild vacuum (Brandel cell harvester type M-24R) and three washes with 5 mL of ice-cold buffer using FP-100 Whatman GF/B filter mats. The radioactivity of the samples was counted, after a stabilization and extraction period of at least 15 h, by use of Ready Protein scintillation fluid (Beckman). The radioligands for μ-, δ_1/δ_2-, and κ-opioid receptor sites (rat brain membranes) sites were [^{3}H]naloxone, [^{3}H]Cl-DPDPE/[^{3}H]-D-Ala-deltorphine, and [^{3}H]-CI 977, respectively. The receptor binding toward the human κ-opioid receptor was done with membranes from CHO-K1 cells transfected with the human κ-opioid receptor (Receptor Biology, Inc., Beltsville, MD). The membranes were thawed rapidly, diluted 90-fold with 50 mM Tris-HCl pH 7.4 and resuspended by homogenization. Threefold incubations were carried out in 50 mM Tris-HCl pH 7.4 at 20 °C for 1.5 h in a total volume of 250 μL, containing 200 μL of a membrane suspension with 45 μg of protein and 2 nM [^{3}H]diprenorphine as the radioactive ligand. Nonspecific binding was defined in the presence of 100 μM naloxone. Incubations were started by the addition of the membrane suspension and terminated by rapid filtration with a Brandel 96 sample harvester using GF/B-UniFilterplates (Packard) presoaked with 50 μL of 50 mM Tris-HCl (pH 7.4/well), followed by washing three times with 250 μL of 50 mM Tris-HCl (pH 7.4). After the filter plates had dried at 50 °C for 1 h, 35 μL of Ultima Gold MV scintillant (Packard) was added per well, and the radioactivity was determined using a Wallac 1450 MicroBeta scintillation counter.

IC_{50} values were calculated using the computer software Figure P (version 6.0c; Biosoft, Cambridge, U.K.), and K_i values were obtained using the Cheng−Prusoff equation.[23]

Nociceptive Testing Methods. The experiments were carried out in male NMRI mice, weighing 23−26 g (tail-flick) and 31−37 g (writhing test). The animals were kept under standard laboratory conditions (group housing, approximately 22 °C, 12-h light−dark cycle), with free access to standard laboratory food and tap water. Both were withdrawn during the tests. Writhing was induced by ip injection of 0.35 mL of a 0.02% solution of phenylquinone (PQ) according to the method described by Hendershot and Forsaith.[24] The characteristic writhing response was observed and counted from 5 to 20 min after PQ administration. The tail-flick test (TF) was carried out by a modification of the method described by D'Amour and Smith.[25] A TF analgesy meter (tail-flick type 55/12/10.fl; Labtec, Dr. Hess, Germany) was used to measure TF latency. The time it took a mouse to withdraw its tail from a radiant heat source (bulb, 12 V/55 W) focused on the dorsal surface of the tail was measured. The heat source was adjusted to produce a baseline tail-flick latency of 3−5 s prior to any of the experiments and was left at a constant setting thereafter.

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Supporting Information Available: Analytical data (molecular formula, yield, and melting point), elemental analyses, and spectroscopic data (IR, ^{1}H) of the intermediates **1**−**9** and final products **11**−**19** are available free of charge via the Internet at http://pubs.acs.org.

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