Eur J Med Chem (1994) 29, 431–439

Synthesis and analgesic activities of urea derivatives of α-amino-*N*-pyridyl benzene propanamide

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(Received 18 October 1993; accepted 14 March 1994)

Summary — New urea L-phenyl alanine derivatives of 4-aminopyridine have been synthesized and evaluated for analgesic activity with the PBQ writhing test in mice and the Randall–Selitto test in rats. Potent oral activity (ID_{50} < 10 mg/kg) and good tolerance were found in alkyl, arylalkyl and carboxyalkyl urea derivatives. The analgesic activity was found to be totally dependent on the pyridine moiety and was at least partly inhibited by pretreament with (α) methyltyrosine, as was the case for 4-aminopyridine. These compounds are therefore pharmacologically interesting as new analgesic derivatives of 4-aminopyridine. They have a higher oral activity and a better activity/tolerance profile.

analgesic activity / 4-aminopyridine / urea derivative

Introduction

The pharmacological activity of 4-aminopyridine (4AP) has been known for many years (for a review, see references [1] and [2]). For example, birds ingesting 4AP become disoriented and emit distress calls that cause other, nonintoxicated birds to leave the area. This 'frightening effect' has been used to reduce the damage caused by urban or rural population of many species of birds since 1965 [3, 4]. For this reason, the toxicity of 4AP has been widely investigated in birds, mammals and even man [5, 6]. In animals the po LD_{50} was found to be generally less than 10 mg/kg. In man, nausea, ataxia, confusion and even convulsions were observed at doses ranging from 0.9 to 3.5 mg/kg [5]. The most-documented biochemical effects of 4AP are potassium-channel blockage and increase of the release of neurotransmitters, especially acetylcholine, evoked by nerve impulses [2, 7–10]. When 4AP is used alone or in combination with neostigmine or pyridostigmine, it can reverse the neuromuscular blockage induced by tubocurarine or pancuronium [1, 2, 11]. It has been used for more than 10 years in Bulgaria, under the trade name Pymadin, as an anticurare agent in anesthetic practice. Treatment with 4AP has been attempted for various severe clinical conditions in which impaired cholinergic transmission is thought to be involved: flunitrazepam overdose [1]; botulism [1, 12]; Eaton–Lambert syndrome; and myasthena gravis [13], Alzheimer's disease [14]. Results were generally positive but the narrow therapeutic index of 4AP precluded its routine clinical use. 4AP has also been shown to restore conduction in demyelinated nerves [15] and is currently in phase-2 trials in the USA for the relief of symptoms of multiple sclerosis [16]. Rupreht and Dzoljic [17] found that 4AP had a pronounced analgesic effect in rats (3 mg/kg) in the paw pressure test (Randall–Selitto method). As this effect was unaffected by atropine and naloxone but was suppressed by a pretreatment with α-methyltyrosine, they suggested the involvement of a noradrenergic mechanism.

More recently, Roussel Uclaf have patented compounds I and II (fig 1), among others, as enkephalinase inhibitors and orally active analgesics [18, 19]. Compound I was found to be very active orally in the Randall–Selitto test (DA_{50} between 4 and 20 mg/kg) [18], a feature not commonly reported for enkephalinase inhibitors. We suspected that the 4AP moiety could be partly responsible for the activity of compound I and this led us to study L-phenylalanine derivatives of 4AP as part of our programme in the search for new analgesics. We wish to report the results obtained with urea derivatives (compounds III), chosen because of their resemblance to compounds I and II and because of the stability of ureas towards peptidases.

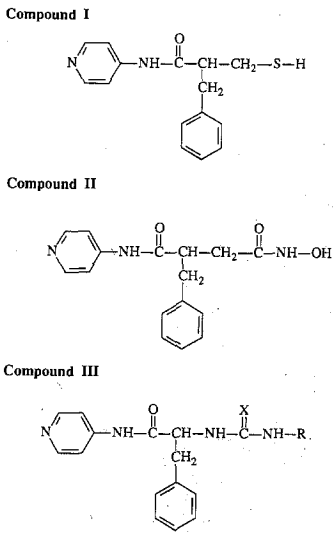
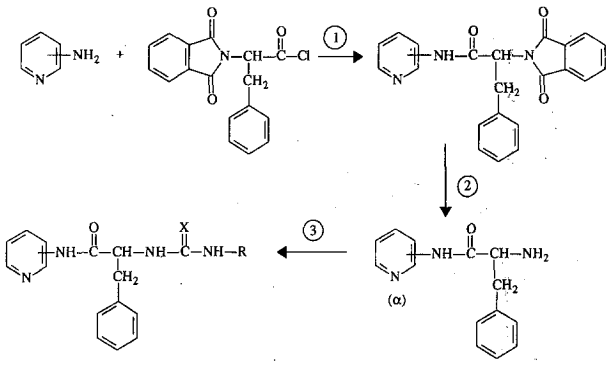
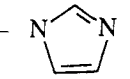


Fig 1. Structures of compounds I, II and III.



Scheme 1. Synthesis of compounds III. 1. $N(Et)_3$, THF (*Method A*); 2. NH_2NH_2EtOH (*Method B*) then L-tartaric acid (*Method C*); 3. R–N = C = X or compound IV



(*Method D*).

Chemistry

The preparation of the 2-, 3- and 4-pyridine derivatives of L-phenylalanine was described by Altman *et al* [20], but for the large quantities that we required, we preferred the method described in scheme 1. Phthaoyl-L-phenylalanine chloride, prepared by condensation of L-phenylalanine with phthalic anhydride and chloration with PCl_5 [21], was condensed with 4AP to obtain α-phthalimido-*N*-(4-pyridyl) benzenepropanamide in high yield (83%, *Method A*). The phthalimido protecting group was then removed by reaction with hydrazine (*Method B*). Some racemization occurred during this synthesis and so the amine obtained was purified by crystallization with L-tartaric acid (*Method C*). A single crystallization and basification gave an amine with $[\alpha]_D = 23.8°$ (EtOH, 1%), which could not be modified by further crystallization with L-tartaric acid. This amine was then condensed with commercial isothiocyanates or isocyanates, or with compounds IV (*N*-(1*H*-imidazol-1-yl carbonyl) alkylamines, aralkylamines or amino-acid esters). The preparation of *N*-(1*H*-imidazol-1-yl carbonyl) alkylamine compounds by condensation of an alkylamine with carbonyldiimidazole and their use as surrogates for isocyanates was described by Staab and Benz in 1961 [22], but does not seem to have been reported since. Compounds IV were synthesized as described by Staab and Benz (*Method E*), except for amino-acid ester derivatives, for which 2 equivalents of carbonyl diimidazole had to be used (*Method F*). Compound III-44 (table I) was obtained by hydrogenolysis of III-43 (*Method G*). The synthesis described in scheme 1 worked equally well starting from amino-2- and amino-3-pyridine, or from methyl-2- and methyl-3-amino-4 pyridine (prepared by nitration of *N*-oxide [23]) to yield compounds III-51–III-54. Compound III-55 was obtained by *N*-oxidation of III-3 with *m*-chloroperbenzoic acid (*Method H*).

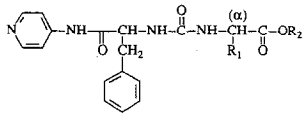
Pharmacological results and discussions

Each compound was tested at 2 doses (generally 10 and 30 mg/kg po) for the inhibition of phenylbenzoquinone (PBQ)-induced writhing in mice ($n = 6$), after the determination of a maximal acceptable dose (the first dose without disturbing effect [24]).

For the most active compounds, an ID_{50} was then determined independently (dose for which the number of nociceptive reaction was decreased by 50%). Some of the compounds III were also tested by the rat paw pressure test (Randall–Selitto). The observed increases in pain threshold are reported in table II.

The inhibition of PBQ-induced writhing by aryl and aralkyl derivatives of compounds III are reported in table III. Thioureas were found to be less active than the corresponding ureas (III-2 *vs* III-1 and III-4 *vs* III-3) and their investigation was abandoned because of their tendency to cyclize into thiohydantoins when heated with loss of 4AP. Comparison between compounds III-1–III-10 showed that the best R was benzyl (III-3), although phenyl, phenyl-2-ethyl and phenyl-1-ethyl derivatives (III-1, 5, 9, 10) were also potent. *Para*-substitution of the benzyl moiety (III-11–III-16) yielded compounds that were less active than the lead compound, as did *meta*-substitution (III-21–III-24). The only substituted benzyl compounds that were nearly as potent as compound III-3 were III-18 (*o,o*-difluoro) and III-20 (*o,o*-

Table I. Inhibition of PBQ-induced writhing in mice by amino-acid derivatives of compounds III.



Compound	R_1	R_2	α (4)	mp°C	Inhibition of PBQ-induced writhing (1) 10 mg/kg	30 mg/kg	ID_{50} (2)	MAD (3)
III-43	H	benzyl	-	142	18	58	7.4 (3.9-14)	30
III-44	H	H	-	197	64	81	5 (2.4-10,8)	nd
III-45	H	ethyl	-	164	nd	43	nd	30
III-46	benzyl	ethyl	(L)	115	65	77	3.4 (1.2-9.1)	100
III-47	phenyl	ethyl	(D)	138	31	72	13.9 (8.1-23.9)	100
III-48	phenyl	ethyl	(L)	145	38	84	ndd	30
III-49	isopropyl	ethyl	(L)	91	0	57	ndd	30
III-50	methyl	ethyl	(L)	124	35	63	4.6 (1.8-11.7)	30

[1]Percentage of inhibition of painful reactions at tested doses (compounds administered po, relative SEM ± 20%); [2]dose for which the number of nociceptive reactions was decreased by 50% and 95% confidence limits; [3]maximum acceptable dose (first dose without disturbing effect); [4]stereochemistry at carbon α; ndd: not dose-dependent; nd: not measured.

dichloro). *Para*-substitution of phenethyl (III-29, 30) or phenyl derivatives (III-31, 32) also yielded compounds that were less active than their unsubstituted counterparts.

Replacement of the benzyl by heteroaromatic methyl groups (III-25–III-28) was not found to be favorable, except for 4-pyridylmethyl (III-25).

A marked analgesic activity was also found in alkyl derivatives (table IV). Butyl (III-3), propyl (III-35) and isopropyl (III-39) were the most potent. Lipophilic compounds with long unbranched side chains (III-37, III-38) were still active but less so.

In order to explore very different structural and physicochemical properties, we also synthesized amino-acid derivatives (table I). Some ester derivatives (III-46,50) and one acidic compound (III-44) were found to be very active. With an ID_{50} of 3.4 mg/kg for the inhibition of PBQ-induced writhing in mice and a maximal acceptable dose of 100 mg/kg, compound III-46 was one of the most interesting in the series.

Analgesic activity was found to be totally dependent on the pyridine moiety (table V). The 2- and 3-aminopyridine analogues of compound III-3 (III-51, III-52) were almost inactive (less than 50% inhibition of PBQ-induced writhing at 100 mg/kg), as were the 4-amino-2-methyl analogue (III-54) and the *N*-oxide derivative of III-3 (III-55). In sharp contrast, III-53 (4-amino-3-methyl) was slightly more active than III-3.

The activities of some of the compounds III in the Randall–Selitto test are reported in table II. All the tested compounds that were active in the PBQ-induced writhing test were also found to be active in the Randall–Selitto test. As no systematic or significant differences were observed between inflamed and noninflamed paws, compounds III seem devoid of antiinflammatory properties.

We checked the ability of compound III-3 to inhibit purified rabbit kidney enkephalinase, using [^{3}H]-D-Ala^2-Leu enkephalin as a substrate [25] and found it to be inactive. Compound III-3 was also found to have

Table II. Activity of selected compounds III in the rat-yeast paw test (Randall–Selitto).

Compound	ID_{50} mg/kg [1] p.o. non-inflamed paw	ID_{50} mg/kg [1] p.o. inflamed paw	Compound	ID_{50} mg/kg p.o. non-inflamed paw	ID_{50} mg/kg p.o. inflamed paw
III-1	5 (3-9)	8 (5-13)	III-25	4.5 (2-10)	2 (1-3)
III-3	6 (4-9)	7 (5-9)	III-33	6 (4-10)	9 (5-14)
III-5	3.1 (2-5)	1.3 (0.5-4)	III-36	4 (3-6)	5 (4-7)
III-9	3 (2-6)	10 (7-15)	III-39	6 (4-9)	3 (2-6)
III-18	6.5 (4-11)	5.5 (4-9)	4AP[2]	0.2 (0.1-0.4)	0.1 (0.08-0.3)

[1]Dose for which the pain threshold was increased by 50% and 95% confidence limits; [2]intraperitoneal administration.

no affinity for opioid receptors (μ, δ and κ) using [^{3}H]-DAGO, [^{3}H]-DPDPE and [^{3}H]-U69593 as radioligands.

The analgesic activities of compounds III were compared with that of 4AP. The appearance of toxic effects at very low doses (≥ 1 mg/kg) precluded the observation of analgesic activity of 4AP in the PBQ-induced writhing test in mice. In a single experiment (10 rats), 4AP was given 1 mg/kg po 1 h before testing. This increased the pain threshold by 56% in a Randall–Selitto type test (without inflammation of the paw). In the same conditions, compounds III-3 (10 mg/kg po) provoked an increase in the pain threshold of 76%. After pretreatment with α-methyltyrosine (300 mg/kg ip, 5 h before testing), the observed increase of pain threshold was only 22% with 4AP and 29% with III-3 (10 rats each). This suggests that the mechanisms by which 4AP and compounds III exert their antinociceptive activities are the same and involve a noradrenergic system (α-methyltyrosine is known to deplete noradrenaline stores [17, 26]. Compounds III are then new pharmacologically interesting analgesic derivatives, which are more active orally and less toxic than 4-aminopyridine, and with an activity/toxicity profile that can be modulated by large structural variations of the urea moiety.

Experimental protocols

Chemistry

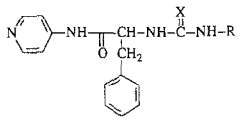
^{1}H-NMR spectra were measured at 200 MHz on a Brucker 200 spectrometer and recorded in $CDCl_3$ or DMSO-d_6. Chemical shifts were reported in δ (ppm) units relative to internal reference Me_4Si (S: singlet, d: doublet, t: triplet, m: multiplet). Melting points were recorded on an electrothermal digital capillary melting point apparatus and were uncorrected. All final compounds were analyzed on Carlo Erba MOD-106 elemental analyser and results obtained were within ± 0.4% of the theoretical values. Starting materials were commercially available and were of synthetic grade, or known and synthesized according to indicated references. α-Methyltyrosine was purchased from Coger, Paris, France.

Method A. Example 1. (S)-α-phthalimido-N-(4-pyridyl)benzene propanamide

4-Aminopyridine (55.4 g, 0.6 mol) was dissolved in 250 ml THF and 85.8 ml triethylamine (0.6 mol). Phthaloyl-L-phenylanine chloride [21] (184 g, 0.6 mol) in 600 ml THF was rapidly added and the reaction mixture was refluxed for 6 h. THF was evaporated and the residue was taken up with water, filtered, washed with ether and dried to yield 180.8 g (80%) of the expected product.

Mp 256°C, ^{1}H-NMR (DMSO-d_6): 10.3 (s, 1H); 8.4 (d, 2H, J = 5 Hz); 7.8 (s, 4H); 7.6 (d, 2H, J = 5 Hz); 7.1 (s, 5H); 5.3 (dd, 1H, J = 3 Hz, 7 Hz); 3.6–3.4 (ABX, 3.60, 1H, J = 3 Hz, 7 Hz; 3.40, 1H, J = 7 Hz, 7 Hz).

Table III. Inhibition of PBQ-induced writhing in mice by aryl and aralkyl derivatives of compounds III (compounds III, R = aryl or aralkyl).



Compound	R	X	mp°C	Inhibition of PBQ-induced writhing (1) 10 mg/kg	30 mg/kg	ID_{50} (2)	MAD (3)
III-1	phenyl	O	163	28	78	9 (7.3-11)	30
III-2	phenyl	S	138	15	46	32 (26-40)	300
III-3	benzyl	O	178	79	83	4 (1.7-13)	30
III-4	benzyl	S	122	17	88	ndd	30
III-5	phenyl 2-ethyl	O	158	64	86	6 (4.1-9.7)	30
III-6	phenyl 3-propyl	O	178	17	53	18 (13-23)	30
III-7	diphenyl 2,2-ethyl	O	193	41	55	13 (6.5-24)	100
III-8	diphenyl methyl	O	206	nd	51	nd	
III-9	phenyl-1 ethyl (R)	O	159	40	87	7.1 (3.2-15.8)	30
III-10	phenyl-1 ethyl (S)	O	162	60	nd	9 (2-42)	10
III-11	p-fluoro benzyl	O	197	20	30	ndd	30
III-12	p-chloro benzyl	O	197	13	65	ndd	30
III-13	p-methoxy benzyl	O	184	44	71	ndd	100
III-14	p-methyl benzyl	O	192	51	63	ndd	30
III-15	p-dimethyl amino benzyl	O	176	48	77	7.5 (4.1-13.8)	10
III-16	p-nitro benzyl	O	207	nd	50	nd	nd
III-17	o-fluoro benzyl	O	199	18	32	nd	nd
III-18	o,o difluoro benzyl	O	184	65	93	6 (4-8.8)	nd
III-19	o-methyl benzyl	O	182	14	48	30 (19-46)	100
III-20	o,o dichloro benzyl	O	204	38	82	7.2 (4.1-12.6)	30
III-21	m-trifluoromethyl benzyl	O	173	45	62	13 (4-46)	300
III-22	m-methyl benzyl	O	168	62	79	7.6 (3.8-15.3)	nd
III-23	m-chloro benzyl	O	172	10	52	ndd	nd

Table III. *(Continued.)*

Compound	R	X	mp°C	Inhibition of PBQ-induced writhing (1) 10 mg/kg	30 mg/kg	ID_{50} (2)	MAD (3)
III-24	m-methoxy benzyl	O	174	49	81	7.4 (3-16)	10
III-25	4-pyridyl methyl	O	191	53	83	4 (2.6-6.2)	30
III-26	3-pyridyl methyl	O	159	4	81	10 (8-13)	30
III-27	2-furanyl methyl	O	216	17	18	ndd	30
III-28	2-thienyl methyl	O	198	35	8	ndd	30
III-29	p-fluoro phenyl-2 ethyl	O	186	46	nd	nd	10
III-30	p-methoxy phenyl-2 ethyl	O	179	57	67	8.1 (5-14)	30
III-31	p-chloro phenyl	O	216	25	9	nd	30
III-32	p-tolyl	O	171	30	76	ndd	30

[1]Percentage of inhibition of painful reactions at tested doses (compound administered po, relative SEM ± 20%); [2]dose for which the number of nociceptive reaction was decreased by 50% and 95% confidence limits; [3]maximum acceptable dose (first dose without disturbing effect); ndd: not dose-dependent; nd: not measured.

Method B. Example 2 (S)-α-amino-N-(4-pyridyl)benzene propanamide

The product of example 1 (297 g, 0.8 mol), and 40 g (0.8 mol) hydrazine hydrate were refluxed in ethanol for 6 h. After return to ambient temperature, the precipitate was filtered and the ethanol evaporated. The residue was taken up in HCl 2 N and the phthalylhydrazide filtered off. The aqueous phase was then basified with sodium hydroxide and extracted with ethyl acetate to yield 180 g (93%) of an oil.

^{1}H-NMR ($CDCl_3$): 9.89 (s, 1H); 8.3 (d, 2H, J = 5 Hz); 7.4 (d, 2H, J = 5 Hz); 7.2 (m, 5H); 3.65 (dd, 1H, J = 4 Hz, 7 Hz); 3.2–2.6 (ABX, 3.2, 1H, J = 3 Hz, 13 Hz; 2.7, 1H, J = 8 Hz, 13 Hz); 2.2 (broad s, 2H).

Method C. Optical purification of example 2

The product of example 2 (180 g, 0.74 mol) was dissolved in 700 ml EtOH, and 112 g (0.74 mol) tartaric acid in 300 ml EtOH were added. After standing for 2 h, the precipitate obtained was filtered and dissolved in water. After basification with NaOH and extraction with ethyl acetate, 139 g of an oil with an NMR spectrum identical to that of the product of example 2 was obtained. $[\alpha]_D = 23.8°$ (EtOH, 1%).

Method D. Example 3 (S)-N'-benzyl, N''-[N-(pyridinyl-4)-benzene propanamidyl-2]urea (compound III-3)

To 12 g (0.05 mol) of the amine of example 2 in 150 ml CH_2Cl_2, was added 6.6 g benzyl isocyanate. After stirring for overnight, the precipitate obtained was filtered, washed with ether and dried. Yield 11.8 g (63%).

Mp 178°C. ^{1}H-NMR (DMSO-d_6): 10.6 (1H, s); 8.5 (2H, d, J = 5 Hz); 7.6 (2H, d, J = 5Hz); 7.3 (10H, m); 6.6 (1H, t, J = 5 Hz); 6.45 (1H, d, J = 7 Hz); 4.7 (1H, q, J = 6 Hz); 4.2 (2H, d, J = 5 Hz); 3.1–2.8 (ABX, 3.1, 1H, J = 5 Hz, 15 Hz; 2.6, 1 H, J = 9 Hz, 15 Hz).

Method E. Example 4 N-(1H-imidazol-1-ylcarbonyl)-4-methoxybenzylamine

4-Methoxybenzylamine (20 g, 0.145 mol) was dissolved in THF and was slowly added to a suspension of carbonyldiimidazole in THF (23.6 g, 0.145 mol); the temperature was maintained below 10°C. After stirring for 3 h, the temperature was allowed to return at ambient levels. THF was then evaporated and the residue was taken up in CH_2Cl_2, washed with water, dried and CH_2Cl_2 was evaporated. The residue was crystallized in ether to yield 22.7 g (65%) of a white product.

Mp 151°C, ^{1}H-NMR ($CDCl_3$): 8.5 (1H, t, J = 5 Hz); 8.1 (1H, s); 7.5 (1H, s); 7.2 (2H, d, J = 8 Hz); 6.9 (1H, s); 6.8 (2H, d, J = 8 Hz); 4.5 (2H, d, J = 5 Hz); 3.75 (3H, s).

Method F. Example 5 L-N-(1H-imidazol-1-ylcarbonyl)phenylalanine, ethyl ester

L-Phenylalanine ethyl ester (25 g, 0.13 mol) in CH_2Cl_2 was slowly added to a suspension of 41.8 g (0.26 mol) carbonydi-

imidazole in CH_2Cl_2, the temperature being maintained below 10°C. After the end of the addition, the temperature was allowed to return to ambient levels. After stirring overnight, the reaction mixture was washed with water, dried and evaporated to yield 36 g (100 %) of an oil which was used further without purification.

^{1}H-NMR ($CDCl_3$): 8.0 (1H, s); 7.3 (5H, m); 7.1 (2H, m); 7.0 (1H, s); 6.75 (1H, d, J = 6 Hz); 4.85 (1H, dd, J = 6 Hz, 7 Hz); 4.2 (2H, q, J = 7 Hz); 3.3–3.1 (ABX, 3.3, 1H, J = 6 Hz, 13 Hz; 3.2, 1 H, J = 7 Hz, 13 Hz); 1.3 (3H, t, J = 7 Hz).

Method G. Example 6 (S)-N-[2-[-[N-(4-pyridyl)benzene propanamide]amino]carbonyl]glycine (compound III-44)

Compound III-43 (3 g, 0.007 mol) (prepared from glycine benzyl ester by *Methods F* and *D*) was dissolved in MeOH and hydrogenated at atmospheric pressure with 10% Pd/C as catalyst. After the theoretical amount of hydrogen had been taken up, the catalyst was filtered off, washed with DMF, and the combined organic phases were evaporated. The residue was crystallized in ether and recrystallized in ethanol (800 mg, 40%).

Mp 197°C, ^{1}H-NMR (DMSO-d_6): 10.2 (s, 1H); 8.3 (d, 2H, J = 5 Hz); 7.5 (d, 2H, J = 5 Hz); 7.2 (m, 5H); 6.6 (d, 1H, J = 8 Hz); 6.3 (t, 1H, J = 2 Hz); 4.6 (dd, 1H, J = 8 Hz, 6 Hz); 3.7 (d, 2H, 2 Hz); 3.0–2.5 (ABX, 3.0, 1H, J = 6 Hz, 14 Hz; 2.9 1H, J = 8 Hz, 14 Hz).

Method H. Example 7 (S)-N'-benzyl, N''-[N-[(N-oxopyridinyl-4)]benzene propanamidyl-2]urea (compound III-55)

m-Chloroperbenzoic acid (3.6 g, 70%, 0.0133 mol) was added in portions to a solution of 5 g of compound III-3 (0.0133 mol) in acetone; the temperature was maintained by external cooling below 10°C. After the addition, the temperature was allowed to return to ambient levels and stirring was continued for 3 h. The acetone was then evaporated and the residue was taken up in ethyl acetate and washed with carbonated water. The white solid that separated was filtered and washed with ethyl acetate and dried (4.6 g, 88%).

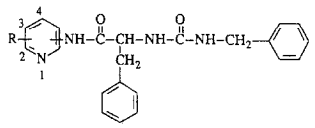
Mp 152°C, ^{1}H-NMR (DMSO-d_6): 10.8 (s, 1H); 8.1 (d, 2H, J = 7 Hz); 7.65 (d, 2H, J = 7 Hz); 7.2 (m, 5H); 6.6 (m, 2H); 4.6 (dd, 1H, J = 5 Hz, 1.5 Hz); 4.2 (d, 2H, J = 6 Hz); 3.0–2.5 (ABX, 3.0, 1H, J = 7 Hz, 1.5 Hz; 2.8, 1H, J = 7 Hz, 5 Hz).

Table IV. Inhibition of PBQ-induced writhing in mice by alkyl derivatives of compounds III (compounds III, R = alkyl).

Compound	R	mp°C	Inhibition of PBQ-induced writhing (1) 10 mg/kg	30 mg/kg	ID_{50} (2)	MAD (3)
III-33	CH_3	171	61	nd	9	10
III-34	CH_3-CH_2	173	32	68	13.5 (8-22.5)	10
III-35	CH_3-$(CH_2)_2$-	172	49	82	6.4 (2.6-15.7)	30
III-36	CH_3-$(CH_2)_3$-	162	79	nd	3.6 (2.6-12.8)	30
III-37	CH_3-$(CH_2)_8$-	147	44	86	29	100
III-38	CH_3-$(CH_2)_{11}$-	124	51	88	27	100
III-39	$(CH_3)_2$-CH-	172	41	79	6.5 (4.3-9.7)	30
III-40	$(CH_3)_3$-C	203	48	67	13.3 (10.6-16.7)	nd
III-41	$(CH_3)_2$-CH-CH_2-	176	77	64	ndd	nd
III-42	$(CH_3)_2$-CH-$(CH_2)_2$-	174	44	64	8	30

[1]Percentage of inhibition of painful reactions at tested doses (compound administered po, relative SEM ± 20%); [2]dose for which the number of nociceptive reactions was decreased by 50% and 95% confidence limits; [3]maximum acceptable dose (first dose without disturbing effect); ndd: not dose-dependent; nd: not measured.

Table V. Inhibition of PBQ-induced writhing in mice by compounds III (pyridine modified derivatives).



Compound	Pyridine	R	mp°C	Inhibition of PBQ-induced writhing (1) p.o. administration				MAD (3)
				100 mg/kg	30 mg/kg	100 mg/kg	ID_{50} (2)	
III-51	2 amino pyridine	H	156	nd	43	45	nd(4)	300
III-52	3 amino pyridine	H	172	nd	40	40	nd	300
III-53	4 amino pyridine	3-CH_3	193	85	98	nd	2.9 (1.2-7)	30
III-54	4 amino pyridine	2-CH_3	215	nd	42	47	nd	300
III-55	4 amino pyridine N-oxyde	H	152	nd	36	43	nd	nd

[1]Percentage of inhibition at tested doses (compound administered po, relative SEM ± 20%); [2]dose for which the number of nociceptive reactions was decreased by 50% and 95% confidence limits; [3]maximum acceptable dose (fiirst dose without disturbing effect); [4]nd: not done.

Pharmacology

Phenylbenzoquinone-induced writhing assay in male mice

The nociceptive reaction was induced following the method of Siegmund *et al* [27]. One hour after oral administration of the test compound, the mice (male mice CD-1 strain, Charles River France) received intraperitoneally 0.2 ml of a hydroalcoholic 0.02% phenylbenzoquinone solution. The number of nociceptive reactions (writhings and stretches) were counted from the 5th to the 10th minute. The percentage of painful reactions was calculated for each dose and for each group (n = 6) from unpaired values in comparison with the mean control value. Each compound was first tested at 2 doses (generally 10 and 30 mg/kg po) and, if it was found to be interesting, the ID_{50} was determined. According to the MAP and the results from the 2 doses, mice were given 5 or 6 doses (generally 0–1–3–10–30–100 mg/kg or 0–0.3–1–3–10–30 mg/kg, 6 animals for each dose) and ID_{50} values (doses for which the number of nociceptive reactions was decreased by 50%) were determined by linear regression on values.

Randall–Selitto paw pressure assay

Paw pressure [28] was assayed on male rats Iffa OFA, Iffa Credo, France. Two hours after intraplantar yeast administration (0.05 ml of 20% brewer's yeast), the pain threshold in the inflamed (injected) and noninflamed paw was determined by applying increasing pressure on the paw with an analgesimeter until withdrawal or struggle or vocalization was obtained (withdrawal was the most frequently observed response). The tested compounds were administered orally 1 h before the test. The percentage increase in the pain threshold was calculated for each dose and each group (n = 10) from unpaired values in comparison with the mean control value. ID_{50} values (doses for which the pain threshold was increased by 50%) were determined by linear regression.

Maximum acceptable dose

A maximum acceptable dose (MAD), which is the maximum dose that does not disturb effect, was evaluated on a group of 5 male CD-1 strain mice (Charles River, France), according to a standardized observation grid similar to that described by Irwin [24].

Animals were given 5 doses (5 animals for each dose) and observed frequently on the day of the test and daily for 7 d. The MAD was determined 24 h after a single oral administration of the test compound.

Acknowledgments

We thank A Gourvil for analytical determinations, and MF De Oliveira and R Dudley for the manuscript preparation.

References

1 Agoston S, Langreh D, Newton DEF (1985) *Seminars in Anesthesia* 6, 1, 81–86
2 Bowman WC (1982) *Trends Pharmacol Sci* 183–185
3 Goodhue LD, Baugartner FM (1965) *J Wildl Manage* 29, 830–837
4 De Grazio JW, Besser JF, De Cino TJ, Guarino JL, Stan RI (1971) *J Wildl Manage* 35, 565–569
5 Uges RA, Buirs B, Sangster B (1984) *Pharm Acta Helv* 59, 5–6, 172–176
6 Schafer WE, Brunton RB, Cummingham DJ (1973) *Toxicol Appl Pharmacol* 26, 532–538
7 Rudy B (1988) *Neuroscience* 25, 3, 729–749
8 Starke K (1987) *Rev Physiol Biochem Pharmacol* 107, 73–146
9 Fredholm BB (1990) *J Neurochem* 54, 4, 1386–1391
10 Damsma G, Biessels PTM, Westerink BHC, De Vries JB, Horn AS (1988) *Eur J Pharmacol* 145, 15–20
11 Miller RP, Booy LHDJ, Agoston S (1979) *Anesthesiology* 50, 416–420
12 Ball AP, Hopkinson RB, Fanel JD (1979) *Q J Med New Series* XLVIII, 191, 473–491
13 Bowman WC, Savage AO (1981) *Rev Pure Appl Pharmacol Sci* 2, 317–371
14 Wesseling H, Agoston S, Van Dam GBP (1984) *N Engl J Med* 310, 988–989
15 Sherrat RM, Bostock H, Sears TA (1980) *Nature (Lond)* 283, 570–572
16 (1992) *Drug Data Report* 14, 6, 489
17 Rupreht J, Dzoljic MR (1983) *Arch Int Pharmacodyn* 265, 203–210
18 Delevallée F, Vevert JP, Gasc JC (1987) *French Patent* 2, 597, 865, *Chem Abstr* 109 110262
19 Vevert JP, Delevallée F, Deraedet RF (1984) *European Patent* 115997, *Chem Abstr* 101 230000
20 Altman J, Gorecki M, Wilchreck M, Votano JR, Rich A (1984) *J Med Chem* 27, 596–600
21 Sheehan JC, Chapman DW, Roth RW (1952) *J Am Chem Soc* 74, 3822–3825
22 Staab HA, Benz W (1961) *Liebigs Ann Chem* 648, 72–82
23 Essery JM, Schofield K (1960) *J Chem Soc* 4953–4959
24 Irwin S (1968) *Psychopharmalogia* 13, 222–257
25 Llorens C, Malfroy B, Schwartz JC, Gacel G, Roques B, Roy J, Morgat JL, Javay Agid F (1982) *J Neurochem* 39, 1081
26 Spector S, Sjoerdsma A, Udenfried S (1965) *J Pharmacol Exp Ther* 147, 86–95
27 Siegmund E, Cadmus R, Lu G (1957) *Proc Soc Exp Biol Med* 95, 729–731
28 Randall LO, Selitto JJ (1957) *Arch Int Pharmacodyn Ther* 111, 409–419