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N-Substituted Aminohydroxypyridines as Potential Non-opioid Analgesic Agents

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Abstract—A series of new N-substituted aminohydroxypyridines have been synthesized, pharmacologically evaluated and compared with their N-substituted oxazolopyridone analogs. The compound with the maximal combination of safety and analgesic efficacy was 3-{2-[4-(4-fluorophenyl)-1-piperazinyl]ethyl}amino-2-hydroxypyridine (compound **10a**), with ED_{50} values 0.4 mg kg^{-1} po (mouse: phenylquinone writhing test) and 0.5 mg kg^{-1} po (rat: acetic acid writhing test). Compound **10a** possesses a potent non-opioid antinociceptive activity with moderate anti-inflammatory properties.

Introduction

The three main groups of analgesic on the market are the opioids such as morphine, codeine and dextropropoxyphen, the nonsteroidal anti-inflammatory agents including aspirin, ibuprofen, indomethacin and paracetamol.

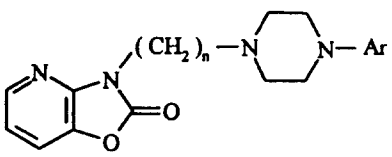
The non-steroidal anti-inflammatory drugs act mainly by inhibiting prostaglandin synthesis peripherally, but unfortunately they induce gastrointestinal lesions.[1] The opioid analgesics with clinical indications for moderate to severe pain produce their action by interacting with specific receptors in the central nervous system but their therapeutic use is limited by adverse side effects including tolerance, constipation, respiratory depression, physical dependence and fear of addiction.[2] The main objective for current research in pain therapy is to develop new, improved non-opioid analgesics having the efficacy of opioid analgesics but without their side effects.

In connection with our studies on heteropolycyclic compounds with potential biological activity, we had synthesized 3-[(4-aryl-1-piperazinyl)alkyl]oxazolo[4,5-*b*]pyridin-2-(3*H*)-ones **A** which have been shown to possess potent analgesic activity[3] but low bioavailability due to an intense and rapid metabolism.[4] This metabolism results in the opening of the oxazolopyridinone moiety in 3-hydroxy-2-aminopyridine derivatives and a cleavage of the aminoalkyl side chain in carboxyalkyl derivatives. Compounds of the type **B**, 1-[(4-aryl-1-piperazinyl)alkyl]oxazolo[5,4-*b*]pyridin-2-(1*H*)-ones, were also synthesized and proved to be more potent as analgesics than their oxazolo[4,5-*b*]-pyridin-2-(3*H*)-one analogues.[5]

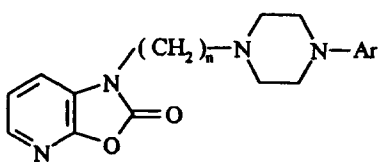
In this paper, we report the synthesis and the biological evaluation of a new series of aminohydroxypyridines having the general formula **C**, which are one of the metabolites of the above-mentioned oxazolopyridinone analgesics **A** and **B**.[4] According to the structure–activity relationships previously established in the oxazolopyridinone series,[3,5] only aminohydroxypyridines with phenylpiperazine substituents were synthesized and their analgesic properties compared to those of their oxazolopyridinone analogues.

Chemistry

The desired compounds were prepared according to Schemes 1–3. One general procedure was used in preparing the desired N-substituted pyridines **8**, **9**, **10a–c**

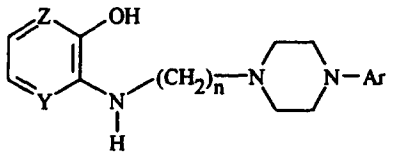


A

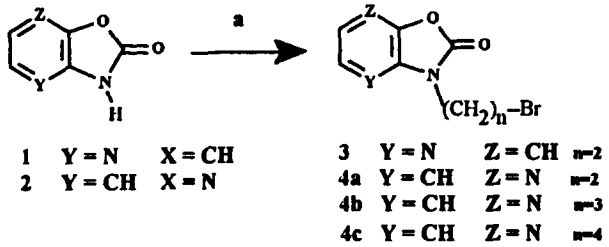


B

and **12** (Schemes 1 and 2). Oxazolo[4,5-*b*]pyridine-2(3*H*)-one (**1**)[3] and oxazolo[5,4-*b*]pyridine-2(1*H*)-one (**2**)[5] were converted into their anions by reaction with sodium hydride in dry DMF at room temperature which reacted with dihalogenoalkanes to provide **3** and **4a–c** in good yields. Alkylation of **3** and **4a–c** with piperazine intermediates, in acetonitrile as solvent, gave compounds **5**, **6** and **7a–c** in good yields which were treated under basic conditions to give the final compounds **8**, **9** and **10a–c** (Scheme 1). The reaction of compound **4a** with potassium cyanide gave the nitrile **11**, which was treated with 10% sodium hydroxide to provide **12** in good yield (Scheme 2). The reactions in Scheme 3 were used to synthesize compound **15**. The anion of compound **2** was obtained by reaction with sodium ethoxide in dry ethanol. After evaporation of the solvent, it was alkylated in DMF with 4-bromo-1-butene, giving **13** in good yield. Ozonolysis of **13**, using the standard procedure, gave the aldehyde **14** which was oxidized to **15** with potassium permanganate in *t*-butanol.

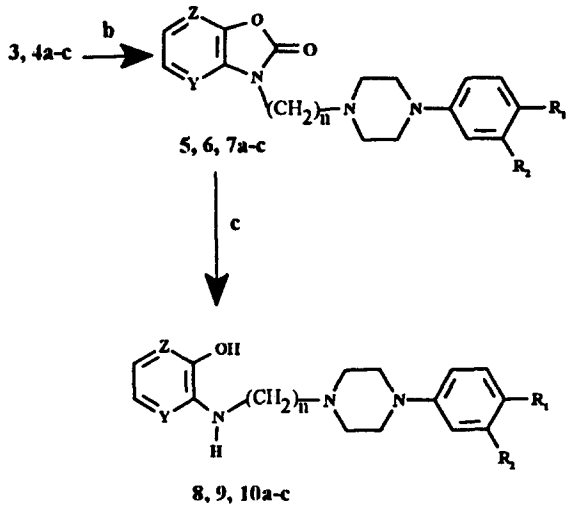


C



1 Y = N X = CH
2 Y = CH X = N

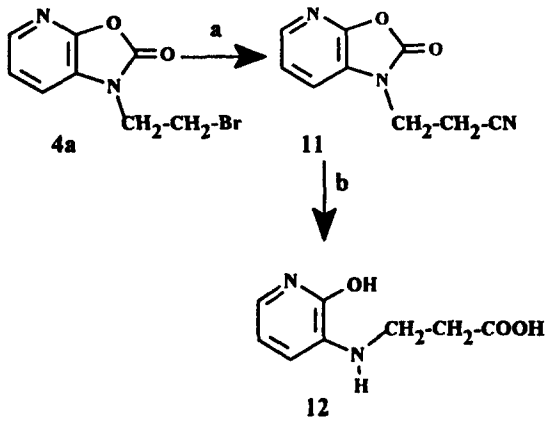
3 Y = N Z = CH n=2
4a Y = CH Z = N n=2
4b Y = CH Z = N n=3
4c Y = CH Z = N n=4



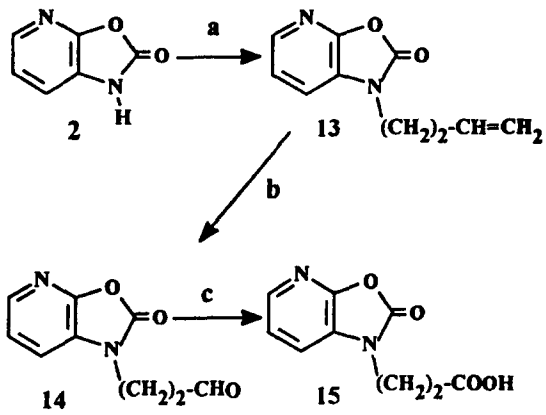
5, 6, 7a-c

8, 9, 10a-c

Scheme 1.
Reagents (a) NaH, DMF, Br–$(CH_2)_n$–Br, 110 °C, (64–67%);
(b) arylpiperazines, $(iPr)_2NEt$, CH_3CN, 85 °C, (79%);
(c) NaOH 10%, reflux then HCl, (95%).



Scheme 2.
Reagents (a) KCN, KI, DMSO, rt, (87%);
(b) NaOH 10%, reflux then HCl, (88%).



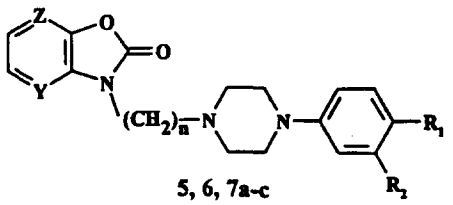
Scheme 3.
Reagents (a) EtONa, DMF, Br–$(CH_2)_2$–CH=CH_2, (79%);
(b) O_3, CH_2Cl_2, then Me_2S, (75%);
(c) $KMnO_4$, *t*-BuOH, (67%).

Biological Results and Discussion

Five N-substituted aminohydroxypyridine derivatives (**8**, **9** and **10a–c**, Table 1) have been prepared, evaluated and compared with their N-substituted oxazolopyridinone analogs (**5**, **6** and **7a–c**, Table 1). All these compounds (except for compound **6**) were first evaluated in mice at 50 mg kg^{-1} po for their analgesic activity via a screening procedure with the phenylquinone (PBQ) induced writhing test and then, for the most active of them, also at the same dose in rat with the acetic acid induced writhing test. The results shown in Table 2, include, for each compound and test (except for compound **6**), an activity ratio versus aspirin, in order to take into account the slight response variations observed between same evaluation procedures not carried out simultaneously.

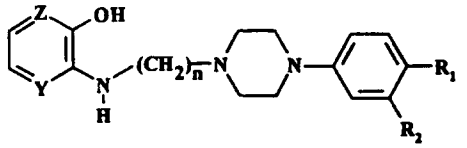
This preliminary screening shows that: (a) 3-hydroxy-2-aminopyridine derivatives (compounds **8** and **9**) having a two carbon long alkyl side chain (n = 2) are less active than their oxazolo[4,5-*b*]pyridin-2(3*H*)-one analogs (compounds **5** and **6**); (b) compound **9** substituted by a *m*-trifluoromethyl is less active than its

Table 1.



5, 6, 7a–c

Compd	Y	Z	n	R_1	R_2
5	N	CH	2	H	H
6	N	CH	2	H	CF_3
7a	CH	N	2	F	H
7b	CH	N	3	F	H
7c	CH	N	4	F	H



Compd	Y	Z	n	R_1	R_2
8	N	CH	2	H	H
9	N	CH	2	H	CF_3
10a	CH	N	2	F	H
10b	CH	N	3	F	H
10c	CH	N	4	F	H

unsubstituted analogue **8**; (c) 3-amino-2-hydroxypyridine derivatives **10a–c** proved to be very active and whatever the length of the alkyl side chain (n = 2, 3, 4) gave a 100% inhibition in both writhing tests, seeming in this screening evaluation procedure to be similarly or more active as their oxazolo[5,4-*b*]pyridin-2(1*H*)-one analogs **7a–c**; (d) compounds **12** and **15**, which are carboxy derivatives and thus possibly metabolites[4] of **7b** and **10b** by cleavage of the aminoalkyl side chain, both proved to be almost inactive in the phenylquinone induced writhing test. The compounds which were more potent than aspirin in both screening tests (Table 2) were selected for additional investigations in order to choose compounds with the highest analgesic activity and safety index.

The following parameters have been determined and are shown in Table 3: (a) determination of the ED_{50} po in both phenylquinone and acetic acid induced writhing tests; (b) evaluation of the general acute toxicity and of the behavioural modifications via an Irwin test (a careful observation of the general behaviour is useful to avoid possible false positives in the writhing tests); (c) determination of a safety index defined as the ratio of the ED_{50} po in the mice with the dose of appearance of the first behavioural change in the Irwin test. The data obtained for the ED_{50} confirm the good analgesic properties of 3-amino-2-hydroxy pyridine derivatives **10a–c** and show some slight differences in the structure–activity relationships between 3-amino-2-

Table 2. Screening of analgesic activity

	Phenylquinone (PBQ) induced writhing test (mice)		Acetic acid induced writhing test (rat)	
Compd	% of inhibition versus 50 mg/kg po (a)	Activity ratio versus aspirin (b)	% of inhibition at 50 mg/kg po (a)	Activity ratio versus aspirin (b)
5	73 % ** (d)	1.05	64 % **	0.93
6	50 % at 12 mg/kg po		ND (c)	
7a	83 % ***	1.19	85 % **	1.23
7b	85 % ***	1.59	79 %	2.02 **
7c	100 % ***	> 1.87	87 % ***	2.21
8	43 % ***	0.50	ND	
9	16 %	0.19	ND	
10a	100 % ***	> 1.87	100 % ***	> 2.54
10b	100 % ***	> 1.83	100 % ***	>1.98
10c	100 % ***	> 1.83	100 % ***	>1.98
12	13 %	0.19	ND	
15	20 %	0.30	ND	

(a) Five animals were used for each compound, seven animals for the control group.

(b) Activity ratio = $\frac{\%\text{ of inhibition with the compound at 50 mg kg}^{-1}\text{ po}}{\%\text{ of inhibition with aspirin at 50 mg kg}^{-1}\text{ po}}$

(c) ND: not determined.

(d) $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

hydroxypyridine and oxazolo[5,4-*b*]pyridin-2(1*H*)-one series. For all the tested compounds, sedation and hypothermia were the first behavioural change observed in the Irwin test and appeared between 4 and 8 mg kg^{-1} po.

In the 3-amino-2-hydroxy pyridine series, the best analgesic properties and highest safety index were obtained for compound **10a** (n = 2) while in the oxazolo[5,4-*b*]pyridin-2(1*H*)-one series[5] the best analgesic properties were obtained for **7b** (n = 3) or **7c** (n = 4) and the highest safety index for **7b** (n = 3).

3-Amino-2-hydroxypyridine compounds were more active than their oxazolopyridinone analogues when n = 2 (**10a** > **7a**) while they were less active when n = 3 or 4 (**7b** > **10b**, **7c** > **10c**).

The length of the alkyl side chain proved to be an essential parameter, a dramatic decrease of both analgesic activity and safety index being observed between compound **10a** (n = 2) and **10b** (n = 3). The mortality threshold po in mice of **10a** proved to be around 1024 mg kg^{-1} (highest value of the series) and equivalent to that of its oxazolo[5,4-*b*]pyridin-2(1*H*)-one analogs **7a**.

Table 3. Antinociceptive potency, orientative acute toxicity and safety index of preselected compounds

Compd	Phenylquinone (PBQ) induced writhing test (8 mice per dose) ED_{50} (mg/kg po) (a)	Acetic acid induced writhing test (8 rats per dose) ED_{50} (mg/kg po) (a)	Orientative acute toxicity IRWIN test (3 mice per dose) First adverse effect (b)	Mortality threshold (c)	Safety Index (d)
7a	6.7 (3.0 - 14.3)	3.6 (1.6 - 6.4)	sedation (8 mg/kg ip)	1024 mg/kg po (33%)	≥ 19 (e)
7b	5.6 (4.4 - 7.2)	0.5 (0.2 - 0.8)	sedation (64 mg/kg po)	1024 mg/kg po (33%)	11.43
7c	2.9 (2.2 - 4.0)	0.5 (0.3 - 0.8)	sedation (8 mg/kg po)	512 mg/kg po (100%)	2.76
10a	0.40 (0.30 - 0.53)	0.65 (0.31 - 1.2)	sedation (4 mg/kg po) hypothermia	1024 mg/kg po (33%)	10
10b	7.9 (6.4 - 9.9)	5.3 (4.2 - 6.6)	sedation (8 mg/kg po) hypothermia	> 256 mg/kg po	1.01
10c	3.5 (2.5 - 4.9)	10.2 (6.8 - 15.5)	sedation (4 mg/kg po) hypothermia	256 mg/kg po (66%)	1.14
Acetyl salicylic acid	63 (56 - 75)	32 (28 - 46)			

(a) Values in parenthesis are confidence intervals determined at 95% (p = 0.005).
(b) Values in parenthesis represent the dose of appearance of the first adverse effect.
(c) Values in parenthesis represent the % of mortality.
(d) Safety Index = $\frac{\text{Dose of appearance of the first effect in the Irwin test (mice po)}}{ED_{50}\text{ PBQ writhing test in the mice (po)}}$
(e) Safety Index calculated with the ip dose of appearance of sedation and therefore possibly underestimated.

Table 4. Complementary analgesic studies, anti-inflammatory and effect on motor-coordination evaluation

Compd	Hot Plate variation of the foot licking latency (a)	Rota Rod Test Variation of the performance time of the treated group compared to the time of the control group (10 mice per dose) (a)	Carrageenan-induced rat paw edema (6 rats per dose) Dose	Variation of edema volume	Inhibition of PGE_2 synhesis	Inhibition LTB_4 synthesis
10a	+ 38 % (1 mg/kg ip)*(b)	- 54 % (10 mg/kg po)***	50 mg/kg po	- 73 %	54 %	77 %
Morphine sulphate	+ 289 % (16 mg/kg ip)***					
Mephenesin		- 94 % (100 mg/kg po)***				
Phenidone			200 mg/kg po	- 68 %	100 %	100 %
Indomethacin			10 mg/kg po	- 57 %	100 %	18 %

(a) Values in parenthesis are the dose used for the test.
(b) *p < 0.05; **p < 0.01; ***p < 0.001.

Due to its potent analgesic activity, high mortality threshold and good safety index, compound **10a** was selected for further pharmacological investigation, the results of which are reported in Table 4.

Compound **10a** was evaluated in the hot plate test in mice and showed a significant activity evaluated as an increase of the foot licking latency (+38% at 1 mg kg^{-1} ip). Unfortunately, **10a** was also found to have important deleterious effects on motor-coordination as shown in the rotarod test where it very significantly decreases the performances of the mice (–54% at 10 mg kg^{-1} po). At 50 mg kg^{-1} po (a relatively high dose compared to its ED_{50} in the writhing tests), **10a** was found to have anti-inflammatory properties in a carrageenan rat paw edema test giving a 73% decrease in edema volume and 54 and 77% inhibitions, respectively, of PGE_2 and LTB_4 productions.

In order to investigate possible mechanisms of action, binding studies were performed and showed an absence of affinity for the opioid and neurokinin receptors (μ, δ, κ, NK_1, NK_2 and NK_3). On the other hand **10a** proved to have moderate affinity for H_1 ($IC_{50} = 10^{-7}$ M), α_1 ($IC_{50} = 3 \times 10^{-7}$ M), α_2 ($IC_{50} = 4 \times 10^{-7}$ M), σ ($IC_{50} = 5 \times 10^{-6}$ M), and D_2 ($IC_{50} = 9 \times 10^{-6}$ M) receptors. Affinities for these receptors could explain some of the side effects observed in the Irwin test and possibly the activity in the rotarod test.

Conclusion

In conclusion, within the family of *N*-[(4-phenyl-1-piperazinyl)alkyl]amino hydroxy pyridines and more particularly among the 3-amino-2-hydroxypyridine derivatives, we have shown some compounds possess potent non-opioid antinociceptive activity with moderate anti-inflammatory properties.

Our most active compound, 3-{2-[4-(4-fluorophenyl)-1-piperazinyl]ethyl}amino-2-hydroxy pyridine **10a** proved to be more active than its oxazolo[5,4-*b*]pyridin-2(1*H*)-one analogue **7a** also with a better safety index.

Complementary safety pharmaceutical evaluation is currently under investigation to determine if this compound could be a good candidate for clinical development.

Experimental

Chemistry

Melting points were determined on a Köfler hot-stage apparatus and were uncorrected. Proton NMR were recorded on a Bruker 300 spectrometer. The coupling constants are in Hertz (Hz) and the chemical shifts are reported in parts per million (δ, ppm) downfield from tetramethylsilane (TMS), which was used as an internal standard. Infrared spectra were obtained with a Perkin–Elmer spectrophotometer 297. Mass spectra were recorded on a R10-10 C Nermag (70 eV) apparatus. Organic solvents were purified when necessary by the methods described by D. D. Perrin, W. L. F. Armarego and D. R. Perrin (*Purification of Laboratory Chemicals*, Pergamon; Oxford, 1986) or were purchased from Aldrich Chimie. All solutions were dried over anhydrous magnesium sulfate and evaporated on a Büchi rotatory evaporator. Analytical thin-layer chromatography (TLC) was carried out on precoated plates (silica gel, 60 F_{254}), and spots were visualized with UV light or alcohol solution of ammonium cerium(IV) nitrate. Column chromatography was performed with Kieselgel 60 (70–230 mesh) silica gel for gravity columns and Kieselgel 60 (230–400 mesh) silica gel (Merck) for flash columns. Analytical results obtained for those elements were ±0.4% of the theoretical values. All anhydrous reactions were performed in oven-dried glassware under an atmosphere of argon. The column chromatography solvents employed were distilled and solvent mixtures were reported as volume to volume ratios.

3-(2-Bromoethyl)oxazolo[4,5-b]pyridine-2(3H)-one (3). To a stirred solution of oxazolo[4,5-*b*]pyridin-2(3*H*)-one (**1**)[3] (1.00 g, 7.35 mmol) in DMF (30 mL) was added sodium hydride (0.26 g, 11.02 mmol, 60% in dispersion in oil) at room temperature and the reaction was stirred at 60 °C over 40 min. After cooling, a solution of 1,2-dibromoethane (3.8 mL, 44.10 mmol) in DMF (5 mL) was added slowly to the reaction. Then the mixture was stirred during 1.5 h at 110 °C. After cooling, the resulting residue was poured into water (50 mL), extracted with CH_2Cl_2 (2 × 50 mL) and dried over magnesium sulfate, and after evaporation the resulting product was purified by flash chromatography (eluent: MeCN:CH_2Cl_2 5:95) to provide 1.36 g (77%) of **3**; mp 84 °C. ^{1}H NMR ($CDCl_3$) δ: 3.78 (2H, *t*, *J* = 6.3 Hz, CH_2), 4.36 (2H, *t*, *J* = 6.3 Hz, CH_2), 7.10 (1H, *dd*, *J* = 8.2, 5.6 Hz, C_6-H), 7.43 (1H, *dd*, *J* = 8.2, 0.5 Hz, C_7-H), 8.13 (1H, *dd*, *J* = 5.6, 0.5 Hz, C_5-H); IR 2900–3100, 1760, 1600 cm^{-1}. Anal. $C_8H_7BrN_2O_2$ (C, H, N).

1-(2-Bromoethyl)oxazolo[5,4-b]pyridin-2(1H)-one (4a). Compound **4a** was prepared similarly to **3** using oxazolo[5,4-*b*]pyridin-2(1*H*)-one (**2**)[5] and obtained with a yield of 75%; mp 111 °C. ^{1}H NMR ($CDCl_3$) δ: 3.48 (2H, *t*, *J* = 6.6 Hz, CH_2), 4.26 (2H, *t*, *J* = 6.6 Hz, CH_2), 7.15 (1H, *dd*, *J* = 8.2, 5.6 Hz, C_6-H), 7.35 (1H, *d*, *J* = 8.2 Hz, C_7-H), 8.08 (1H, *d*, *J* = 5.6 Hz, C_5-H); IR 3000–2900, 1760, 1620 cm^{-1}. Anal. $C_8H_7BrN_2O_2$ (C, H, N).

1-(3-Bromopropyl)oxazolo[5,4-b]pyridin-2(1H)-one (4b). Compound **4b** was prepared similarly to **4a**, using 1,3-dibromopropane as starting reagent, and obtained with a yield of 55%; mp 64 °C. ^{1}H NMR ($CDCl_3$) δ: 2.45 (2H, *m*, CH_2), 3.48 (2H, *t*, *J* = 6.6 Hz, CH_2), 4.10 (2H, *t*, *J* = 6.6 Hz, CH_2), 7.18 (1H, *dd*, *J* = 8.1, 5.2 Hz, C_6-H), 7.40 (1H, *dd*, *J* = 8.1, 2.2 Hz, C_7-H), 8.15 (1H, *dd*, *J* = 5.2, 2.2 Hz, C_5-H); IR 3000, 2900, 1760, 1600 cm^{-1}. Anal. $C_9H_9BrN_2O_2$ (C, H, N).

1-(4-Bromobutyl)oxazolo[5,4-b]pyridin-2(1H)-one (4c). Compound **4c** was prepared similarly to **4a**, using 1,4-dibromobutane as starting reagent, and obtained with a yield of 64%; mp 50 °C. ^{1}H NMR ($CDCl_3$) δ: 1.92–2.03 (4H, *m*, 2 × CH_2), 3.46 (2H, *m*, CH_2), 3.91 (2H, *m*, CH_2), 7.16 (1H, *dd*, *J* = 8.2, 5.2 Hz, C_6-H), 7.26 (1H, *dd*, *J* = 8.1, 2.2 Hz, C_7-H), 8.05 (1H, *dd*, *J* = 5.2, 2.2 Hz, C_5-H); IR 3100–2900, 1760, 1620 cm^{-1}. Anal. $C_{10}H_{11}BrN_2O_2$ (C, H, N).

1-[(4-Aryl-1-piperazinyl)alkyl]oxazolo[5,4-b]pyridin-2(1H)-ones and 3-[(4-aryl-1-piperazinyl)alkyl]oxazolo-[4,5-b]pyridin-2(3H)-ones. General procedure (Scheme 1). A mixture containing **3** and **4a–c**, (4.12 mmol), appropriate amine (6.17 mmol), diisopropylethylamine (0.80 g, 6.17 mmol), and MeCN (30 mL) was heated at 85 °C for 12 h. After cooling, the mixture was poured into water (100 mL), extracted with CH_2Cl_2 (2 × 100 mL), and dried over anhydrous magnesium sulfate. After evaporation of the solvent, the desired product was purified by flash chromatography (eluent: MeOH:CH_2Cl_2 5:95) and recrystallized from a suitable solvent.

3-[2-(4-Phenyl-1-piperazinyl)ethyl]oxazolo[4,5-b]-pyridin-2(3H)-one (5). Yield 97%; mp 110–112 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ: 2.66–2.72 (4H, *m*, $CH_{2\ piperaz}$), 2.85 (2H, *t*, *J* = 6.6 Hz, CH_2), 3.07–3.13 (4H, *m*, $CH_{2\ piperaz}$), 4.10 (2H, *t*, *J* = 6.6 Hz, CH_2), 6.79–6.91 (3H, *m*, H_{arom}), 7.03 (1H, *dd*, *J* = 7.8, 5.4 Hz, C_6-H), 7.19–7.27 (2H, *m*, H_{arom}), 7.34 (1H, *dd*, *J* = 7.8, 1.1 Hz, C_7-H), 8.1 (1H, *dd*, *J* = 5.4, 1.1 Hz, C_5-H); IR 3100–2700, 1700, 1590 cm^{-1}. MS m/z 325 (M + 1). Anal. $C_{18}H_{20}N_4O_2$ (C, H, N).

3-[2-[4-(3-Trifluoromethylphenyl)-1-piperazinyl]ethyl]-oxazolo[4,5-b]pyridin-2(3H)-one (6). Yield 86%; mp 95 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ: 2.65–2.75 (4H, *m*, $CH_{2\ piperaz}$), 2.85 (2H, *t*, *J* = 6.6 Hz, CH_2), 3.00–3.2 (4H, *m*, $CH_{2\ piperaz}$), 4.10 (2H, *t*, *J* = 6.6 Hz, CH_2), 6.90–7.15 (4H, *m*, C_6-H + 3H_{arom}), 7.25–7.35 (2H, *m*, H_{arom}), 7.45 (1H, *dd*, *J* = 7.8, 1.1 Hz, C_7-H), 8.10 (1H, *dd*, *J* = 5.4, 1.1 Hz, C_5-H); IR 3100–2700, 1700, 1590 cm^{-1}. MS m/z 393 (M + 1). Anal. $C_{19}H_{19}F_3N_4O_2$ (C, H, N).

1-[2-[4-(4-Fluorophenyl)-1-piperazinyl]ethyl]oxazolo-[5,4-b]pyridin-2(1H)-one (7a). Yield 89%; mp 136 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ: 2.65–2.70 (4H, *m*, $CH_{2\ piperaz}$), 2.77 (2H, *t*, *J* = 6.2 Hz, CH_2), 3.03–3.087 (4H, m, $CH_{2\ piperaz}$), 4.00 (2H, *t*, *J* = 6.2 Hz, CH_2), 6.81–6.87 (2H, *m*, H_{arom}), 6.92–6.98 (2H, *m*, *J* = 1.4 Hz, H_{arom}), 7.13 (1H, *dd*, *J* = 7.7, 5.3 Hz, C_6-H), 7.30 (1H, *dd*, *J* = 7.7, 5.3 Hz, C_6-H), 7.30 (1H, *dd*, *J* = C_7-H), 8.04 (1H, *dd*, *J* = 5.3, 1.4 Hz, C_5-H); IR 3100–2800, 1760, 1600 cm^{-1}. MS m/z 343 (M + 1). Anal. $C_{18}H_{19}FN_4O_2$ (C, H, N).

1-[3-[4-(4-Fluorophenyl)-1-piperazinyl]propyl]oxazolo-[5,4-b]pyridin-2(1H)-one (7b). Yield 97%; mp 129 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ: 1.95–2.05 (2H, *m*, $CH_{2\ piperaz}$), 2.46 (2H, *t*, *J* = 6.1 Hz, CH_2), 2.50–2.55 (2H, *m*, $CH_{2\ piperaz}$), 3.01–3.06 (2H, *m*, $CH_{2\ piperaz}$), 3.96 (2H, *t*, *J* = 6.1 Hz, CH_2), 6.82–6.88 (2H, *m*, H_{arom}), 6.91–6.99 (2H, *m*, H_{arom}), 7.13 (1H, *dd*, *J* = 7.7, 5.1 Hz, C_6-H), 7.32 (1H, *dd*, *J* = 7.7, 1.3 Hz, C_7-H), 8.03 (1H, *dd*, *J* = 5.1, 1.3 Hz, C_5-H); IR 3100–2700, 1760, 1600 cm^{-1}. MS m/z 356 (M + 1). Anal. $C_{19}H_{21}FN_4O_2$ (C, H, N).

1-[4-[4-(4-Fluorophenyl)-1-piperazinyl]butyl]oxazolo-[5,4-b]pyridin-2(1H)-one (7c). Yield 81%; mp 130 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ: 1.55–1.70 (2H, *m*, CH_2), 1.80–1.91 (2H, *m*, CH_2), 2.44 (*t*, *J* = 6.6 Hz, CH_2), 2.54–2.59 (4H, *m*, $CH_{2\ piperaz}$), 3.07–3.12 (4H, *m*, $CH_{2\ piperaz}$), 3.89 (2H, *t*, *J* = 6.6 Hz, CH_2), 6.82–6.98 (4H, *m*, H_{arom}), 7.14 (1H, *dd*, *J* = 6.4, 5.5 Hz, C_6-H), 7.25 (1H, *dd*, *J* = 6.4, 1.3 Hz, C_7-H), 8.04 (1H, *dd*, *J* = 5.5, 1.3 Hz, C_5-H); IR 3100–2700, 1750, 1600 cm^{-1}. MS m/z 370 (M + 1). Anal. $C_{20}H_{23}FN_4O_2$ (C, H, N).

2-[4-(Aryl-1-piperazinyl)alkylamino]pyridin-3-ols and 3-[4-(aryl-1-piperazinyl)alkylamino]pyridin-2-ols. General procedure (Scheme 2). Each of the compounds of **5**, **6** and **7a–c** (6 mM) was dissolved in 24 mL of NaOH (10%) and refluxed during 3 h. After cooling, HCl (37%) was added until pH = 1. The precipitate was washed with water three times then with methylene chloride two times. Compounds **8**, **9** and **10a–c** were obtained in excellent yields (95%).

2-[2-(4-Phenyl-1-piperazinyl)ethylamino]pyridin-3-ol (8). mp 191 °C. ^{1}H NMR (D_2O) δ: 3.40–3.60 (8H, *m*, CH_2 + $CH_{2\ piperaz}$), 3.80–3.92 (4H, *m*, $CH_{2\ piperaz}$), 6.72 (1H, *dd*, *J* = 7.5, 5.3 Hz, C_5-H), 7.00–7.20 (4H, *m*, C_4-H + H_{arom}), 7.38–7.55 (3H, *m*, H_{arom} + C_6-H); IR 3400, 1600 cm^{-1}. MS m/z 299 (M + 1). Anal. $C_{17}H_{22}N_4O$ (C, H, N).

2-[2-[4-(3-Trifluoromethylphenyl)-1-piperazinyl]-ethyl-amino]pyridin-3-ol (9). mp 155 °C. ^{1}H NMR (DMSO-d_6) δ: 2.59–2.63 (8H, *m*, CH_2 + $CH_{2\ piperaz}$), 3.21–3.30 (4H, *m*, $CH_{2\ piperaz}$), 3.45–3.52 (2H, *m*, CH_2), 5.80 (1H, *bs*, NH), 6.35 (1H, *dd*, *J* = 7.5, 5.3 Hz, C_5-H), 6.79 (1H, *dd*, *J* = 7.5, 1.5 Hz, C_4-H), 7.01–7.24 (3H, *m*, H_{arom}), 7.40 (1H, *m*, H_{arom}), 7.48 (1H, *dd*, *J* = 5.3, 1.5 Hz, C_6-H), 9.74 (1H, *m*, OH); IR 3400, 1600 cm^{-1}. MS m/z 367 (M + 1). Anal. $C_{18}H_{21}F_3N_4O$ (C, H, N).

3-[2-[4-(4-Fluorophenyl)-1-piperazinyl]ethylamino]-pyridin-2-ol (10a). mp 178 °C. ^{1}H NMR ($CDCl_3$) δ: 2.62–2.71 (4H, *m*, CH_2), 2.74 (2H, *t*, *J* = 6.0 Hz, CH_2), 3.10–3.17 (4H, *m*, CH_2), 3.22 (2H, *q*, *J* = 6.0 Hz, CH_2), 5.26 (1H, *bs*, NH), 6.20 (1H, *t*, *J* = 7.4, 5.3 Hz, C_5-H), 6.28 (1H, *t*, *J* = 7.4, 2.2 Hz, C_4-H), 6.69 (1H, *dd*, *J* = 5.3, 2.2 Hz, C_6-H), 6.83–7.00 (4H, *m*, H_{arom}), 11.20 (1H, *bs*, OH); IR 3400, 3300–2600, 1635, 1565 cm^{-1}. MS m/z 317 (M + 1). Anal. $C_{17}H_{21}FN_4O$ (C, H, N)

3-[3-[4-(4-Fluorophenyl)-1-piperazinyl]propylamino]-pyridin-2-ol (10b). mp 236 °C. ^{1}H NMR ($CDCl_3$) δ: 1.90–2.00 (2H, *m*, CH_2), 2.48–2.50 (2H, *m*, CH_2), 2.52–2.54 (4H, *m*, CH_2), 3.23–3.30 (6H, *m*, CH_2), 5.50 (1H, *bs*, NH), 6.25 (1H, *dd*, *J* = 7.4, 5.2 Hz, C_5-H), 6.33 (1H, *d*, *J* = 7.4 Hz, C_4-H), 6.50 (1H, *d*, *J* = 5.2 Hz, C_6-H), 6.80–7.00 (4H, *m*, H_{arom}), 10.70 (1H, *bs*, OH); IR 3400, 3300–2600, 1635, 1535 cm^{-1}. MS m/z 331 (M + 1). Anal. $C_{18}H_{23}FN_4O$ (C, H, N).

3-[4-[4-(4-Fluorophenyl)-1-piperazinyl]butylamino]-pyridin-2-ol (10c). mp 144 °C. ^{1}H NMR ($CDCl_3$) δ: 1.60–1.70 (4H, *m*, CH_2), 2.48–2.50 (2H, *m*, CH_2), 2.55–2.60 (2H, *m*, CH_2), 3.20–3.30 (4H, *m*, CH_2), 4.98 (1H, *bs*, NH), 6.25 (1H, *dd*, *J* = 7.5, 5.3 Hz, C_5-H), 6.33 (1H, *d*, *J* = 7.5 Hz, C_4-H), 6.65 (1H, *d*, *J* = 2.2 Hz, C_6-H), 6.80–7.00 (4H, *m*, H_{arom}), 11.50 (1H, *bs*, OH); IR 3400, 3300–2600, 1635, 1535 cm^{-1}. MS m/z 345 (M + 1). Anal. $C_{19}H_{25}FN_4O$ (C, H, N).

1-(2-Cyanoethyl)oxazolo[5,4-b]pyridin-2(1H)-one (11). To a stirred solution of compound **4a** (200 mg, 0.82 mmol) in DMSO (5 mL) was added potassium cyanide (161 mg, 2.4 mmol) and potassium iodide (68 mg, 0.41 mmol) at room temperature during 4 h. After evaporation on the Kügelrohr apparatus, the crude product was poured into water, extracted with methylene chloride and dried over magnesium sulfate. After evaporation the resulting product was purified by flash chromatography (eluent: AcOEt) to provide 130 mg (84%) of **11**; mp 150 °C. ^{1}H NMR ($CDCl_3$) δ: 2.88 (2H, *t*, *J* = 6.6 Hz, CH_2), 4.12 (2H, *t*, *J* = 6.6 Hz, CH_2), 7.17 (1H, *dd*, *J* = 7.4, 5.2 Hz, C_6-H), 7.38 (1H, *d*, *J* = 7.4 Hz, C_7-H), 8.07 (1H, *d*, *J* = 5.2 Hz, C_5-H); IR 1750, 1600 cm^{-1}. Anal. $C_9H_7N_3O_2$ (C, H, N).

3-(2-Hydroxypyridin-3-ylamino)propionic acid (12). This compound was obtained from **11** according to the general procedure described for the preparation of **8**, **9** and **10a–c**; mp 217 °C. ^{1}H NMR (DMSO-d_6) δ: 2.51–2.53 (2H, *m*, CH_2), 3.12–3.26 (2H, *m*, CH_2), 5.25 (1H, *bs*, NH), 6.01 (1H, *dd*, *J* = 7.4, 5.9 Hz, C_5-H), 6.17 (1H, *d*, *J* = 7.4 Hz, C_4-H), 6.53 (1H, *d*, *J* = 5.9 Hz, C_6-H), 11.20 (1H, *bs*, OH), 12.10 (1H, *bs*, OH); IR 3400, 3300–2800, 1700, 1635 cm^{-1}. MS m/z 183 (M + 1). Anal. $C_8H_{10}N_2O_3$ (C, H, N).

1-(3-Butenyl)oxazolo[5,4-b]pyridin-2(1H)-one (13). To a solution of sodium ethoxide, prepared by addition of sodium (0.46 g, 20 mmol) to 200 mL of anhydrous EtOH, was added oxazolo[5,4-*b*)]pyridin-2(1*H*)-one (**2**) (2.72 g, 20 mmol), and the resulting solution was stirred at room temperature for 1 h. The solvent was removed by rotatory evaporation yielding the sodium salt. The resulting solid was dissolved in 20 mL of DMF, and this solution was added dropwise to a solution of 4-bromo-1-butene (2.03 mL, 20 mmol) in 8 mL of DMF. The reaction mixture was heated at 110 °C for 1 h, at which time the reaction was cooled to room temperature, and concentrated. After addition of water (2 × 100 mL) the mixture was extracted with CH_2Cl_2 (2 × 100 mL). The extracts were dried with $MgSO_4$, and the solvent was removed *in vacuo*. Flash chromatography (eluent: CH_2Cl_2) of the residue gave 2.71 g of pure **13** (70%). ^{1}H NMR ($CDCl_3$) δ: 2.48 (2H, *q*, *J* = 6.6 Hz, CH_2), 3.85 (2H, *t*, *J* = 6.6 Hz, CH_2), 5.02 (1H, *d*, *J* = 11.8 Hz, CH), 5.03 (1H, *d*, *J* = 15.5 Hz, CH), 5.65–5.80 (1H, *m*, CH), 7.07 (1H, *dd*, *J* = 5.2, 8.1 Hz, C_6-H), 7.17 (1H, *dd*, *J* = 1.5, 8.1 Hz, C_7-H), 7.97 (1H, *dd*, *J* = 5.2, 1.5 Hz, C_5-H); IR 3100–2900, 1760 cm^{-1}. Anal. $C_{10}H_{10}N_2O_2$ (C, H, N).

3-[Oxazolo[5,4-b]-2(1H)-one-pyridin-1-yl]propanal (14). Compound **13** (0.9 g, 4.71 mmol) was dissolved in a solution of (CH_2Cl_2:MeOH 4:1) at –78 °C and placed in an ozonolysis apparatus. After 10 min of reaction, the excess of ozone was removed by nitrogen stream and ten equivalents of methyl sulfite were added to the solution, which was then allowed to warm up to room temperature. The solvents were evaporated. Flash chromatography (eluent: MeOH:CH_2Cl_2 5:95) of the residue gave 0.6 g of pure **14** (75%). ^{1}H NMR ($CDCl_3$) δ: 3.07 (2H, *t*, *J* = 6.1 Hz, CH_2), 4.10 (2H, *t*, *J* = 6.1 Hz, CH_2), 7.16 (1H, *dd*, *J* = 5.2, 7.7 Hz, C_6-H), 7.47 (1H, *dd*, *J* = 7.7, 1.1 Hz, C_7-H), 8.01 (1H, *dd*, *J* = 5.2, 1.1 Hz, C_5-H), 9.80 (1H, *s*, CHO); IR 1760, 1720, 1600, 1420, 1350 cm^{-1}. MS m/z 193 (M + 1). Anal. $C_9H_8N_2O_3$ (C, H, N).

3-[Oxazolo[5,4-b]-2(1H)-one-pyridin-1-yl]propanoic acid (15). Compound **14** (1.0 g, 5.2 mmol) was dissolved in *t*-BuOH (25 mL). To this solution, a 1 M solution of $KMnO_4$ (25 mL) was added at room temperature. The mixture was stirred for 3 h at the same temperature. Addition of a solution of Na_2SO_3, followed by filtration and evaporation, gave a residue which was poured into water (50 mL) and extracted with methylene chloride (2 × 100 mL). The aqueous layer was adjusted to pH 4. Filtration gave 0.725 g of pure **15** (67%). ^{1}H NMR (DMSO-d_6) δ: 2.68 (2H, *t*, *J* = 6.6 Hz, CH_2), 3.97 (2H, *t*, *J* = 6.6 Hz, CH_2), 7.22 (1H, *dd*, *J* = 5.9, 8.1 Hz, C_6-H), 7.67 (1H, *dd*, *J* = 8.1, 1.3 Hz, C_7-H), 7.91 (1H, *dd*, *J* = 5.9, 1.3 Hz, C_5-H), 12.4 (1H, *s*, COOH); IR 3500–2500, 1760, 1700 cm^{-1}. MS m/z 209 (M + 1). Anal. $C_9H_8N_2O_4$ (C, H, N).

Pharmacological methods.

Phenylquinone (PBQ) induced writhing in mice. Male CD1 mice in the weight range of 20 to 25 g were used for the study after an overnight fast. The test compounds were suspended in 0.5% carboxymethylcellulose at each of the required doses immediately prior to dosing. The mice were dosed orally with either test compound or vehicle using a constant dose volume of 10 mL kg^{-1}. For the screening evaluation at 50 mg kg^{-1}, there were seven animals in the control group and five animals in each of the treated groups. Thirty minutes after oral treatment, each mouse received an intraperitoneal injection of 0.25 mL or a solution containing 0.01% phenylquinone in 5% ethanol. The number of writhes elicited in each mouse during the period between the 5th and 25th minute after phenylquinone administration was recorded.[6,7]

The ratio of the mean writhes of the control animals versus the mean writhes of the treated animals was calculated and the results expressed as a percentage of inhibition (Table 2). For the ED_{50} determination, eight animals were used per dose and the 95% confidence limits were estimated using the probit method of Finney.[8]

Acetic acid-induced writhing in rats. Male Wistar rats in the weight range of 140 to 160 g were used for the study after an overnight fast. The test compounds were

suspended in 0.5% carboxymethylcellulose at each of the required doses immediately prior to dosing. The rats were dosed orally with either the test compound or the vehicle using a constant dose volume of 10 mL kg^{-1}. For the screening evaluation at 50 mg kg^{-1}, there were seven animals in the control group and five animals in each of the treated groups. Thirty minutes after oral treatment, each rat received an intraperitoneal injection of 1.0 mL of a solution containing 1% acetic acid in distilled water. The number of writhes elicited in the following 25-min period were recorded.[9] The ratio of the mean writhes of the control animals versus the mean writhes of the treated animals was calculated and the result expressed as a percentage of inhibition (Table 2). For the ED_{50} determination, eight animals were used per dose and the 95% confidence limits were estimated using the probit method of Finney.[8]

Examination of the time course of analgesic effects in the acetic acid writhing test in rats. Using the same methodology, a group of sixty rats (male Wistar) were used. Subgroups of ten rats were examined 15, 30, 60 min, 2, 3 and 6 h after oral administration of the compound or vehicle. At the post-dose times indicated, each rat received an intraperitoneal injection of 1.0 mL of a 1.0% solution of acetic acid. The number of writhes elicited in each rat in the following 25-min period was recorded.[9]

Hot-plate test in mice. According to the method described by Eddy,[10] mice were placed on a heated plate (55 °C) inside a plexiglass cylinder. The latency before the animals started to lick their feet was measured. If no reaction was noted the test was terminated after 120 s.

Ten animals were studied per dose (dispersed in a 5% acacia gum suspension at a volume of 0.25 mg/20 g). The compound administration was performed 1 h (po) or 30 min (ip) before the test.

Evaluation of toxic, physiological and behavioural effects in the mice (Irwin). Three animals per dose were administered po with the test compound (dispersed in a 5% acacia gum suspension at a volume of 0.25 mg/20 g) and were observed according to a standardized observation grid at regular intervals for up to 24 h. The presence or absence and the intensity of various symptoms were noted.[11,12]

Carrageenin paw edema in rats. Wistar male rats (200 ± 20 g) were used for the study (six animals per group). One hour after oral administration of the compound (suspended in 2% gelatine), 0.1 mL of 2% solution of carrageenan in saline was injected into the plantar area of the hind paw. Three hours later, the inflammation was characterized by the increased volume of the hind paw (measure with a plethysmometer UGO BASILE) compared to the value obtained before carrageenan injection.[13] The potential anti-inflammatory activity of the compounds was evaluated by the reduction of the increased volume compared to the control animals (results are expressed as the percentage of the increased volume of the control animals). Afterwards, animals were sacrificed and anti-inflammatory mediators were isolated. After prostaglandin and leukotriene extraction, the cyclooxygenase and 5-lipoxygenase activities were determined by a radio-immunoassay measuring the level of PGE_2 and LTB_4 respectively (results are expressed as the percentage of control animal activity).[14,15,16]

Receptor binding assay. Receptor binding assays were performed by incubating membranes prepared from the rat central nervous system with ^3H DAMGO, ^3H *p*Cl-Phe-DPDPE, ^3H [Sar^9,$Met(O_2)^{11}$]-SP and ^3H Senktide respectively for μ, δ, NK_1 and NK_3 receptors.[17,18,19,20] For the κ receptor, membrane was prepared from guinea-pig cerebellum incubated with ^3H U-69593.[21] For the NK_2 receptor, membrane was prepared from rat duodenum incubated with ^{125}I-His-NKA.[22] For the H_1 and σ receptors, membranes were prepared from guinea-pig cerebellum incubated respectively with ^3H pyralamine[23] and ^3H (+)-PPP.[24] For α_1 and α_2 receptors, membranes were prepared from rat brain incubated respectively with ^3H prazosin[25] and ^3H rauwolscine.[26] For the D_2 receptor, membrane was prepared from rat striatum incubated with ^3H YM-09151-2.[27]

After the incubation period, bound and unbound radioligand were separated by filtration. Radioactivity bound to membranes in the absence and presence of compounds was counted in a liquid-scintillation counter. Each compound was studied in triplicate at two concentrations (10^{-7} and 10^{-5} M).

Rotarod test in mice. Female ICR (CD1) mice in the weight range of 18 to 24 g were made to walk in an accelerating rotarod consisting of a rotating rod which is accelerated uniformly during a 5-min period from 4 revolutions min^{-1} to a maximum of 40 revolutions min^{-1} during the 2 days before dosing. Mice were placed on the rotarod whilst it was turning at the lowest speed and the 'perfomance' time of an animal was taken as the time during which it was able to remain on the accelerating rotarod.[28] On the morning of the day of the test, the pre-dose perfomance time was measured for each mouse and mice were randomized into a study-group of 10 animals using a random numbers table so that the mean pre-dose performance times were approximately equal. Each mouse received either test compound, reference standard or vehicle (0.5% w/v CMC) orally using a constant dose volume of 10 mL kg^{-1}. One hour after oral administration, each mouse was given three successive trials in the accelerating rotarod and the maximum performance time was used for calculation of the mean results. Final results are expressed as the percentage of variation in the 'performance time' of the treated animals.

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