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6-Aminoalkyloxazolo[4,5-*b*]pyridin-2(3*H*)-ones: Synthesis and Evaluation of Antinoceptive Activity

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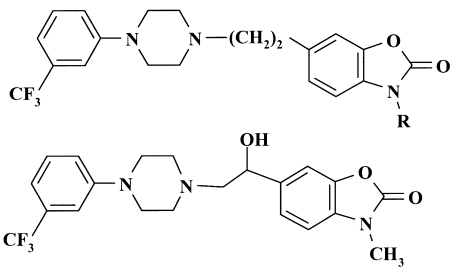
Abstract—Series of 6-aminoalkyloxazolo[4,5-*b*]pyridin-2(3*H*)-ones incorporating structural modifications both in the alkyl chain and basic amino moiety were tested for their analgesic efficacy and safety in mice and rats. Two of the synthesised compounds, **4a** (3-methyl-6-[(4-phenyl-1-piperazinyl)methyl]oxazolo[4,5-*b*]pyridin-2(3*H*)-one) and **12a** (3-methyl-6{1-[2-(4-phenyl-1-piperazinyl)ethan-1-ol]}oxazolo[4,5-*b*]pyridin-2(3*H*)-one) were found to be more potent than aspirin with ED_{50} values of 26 (16.1–42.4) and 15.5 (11.4–21.2) mg/kg po (mouse, phenylquinone writhing test) respectively and 6 (3.1–9.8) and 5.5 (3.5–8.8) mg/kg po (rat, acetic acid writhing test). Compounds **4a** and **12a** proved to be potent nonopioid nonantiinflammatory analgesics but unfortunately have sedative properties at relatively low doses (respectively 64 and 16 mg/kg po, mice)

Introduction

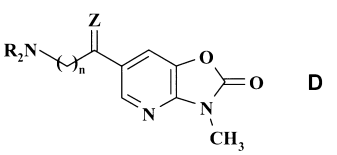
Two main groups of analgesics on the market are the opioids such as morphine and codeine and the non steroidal antiinflammatory agents including aspirin, paracetamol and ibuprofen.[1]

The opioids 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 and physical dependence.[2] The non steroidal antiinflammatory drugs act mainly by inhibiting the prostaglandin synthesis peripherally, but unfortunately they induce gastrointestinal lesions.[3]

Previous work have shown that benzoxazolinones substituted in position 6 by an (arylpiperazinyl)alkyl[4a] (compounds A and B) or an (arylpiperazinyl)hydroxy alkyl[4b] (compound C) are good analgesics with ED_{50} respectively of 2.8, 3.9 and 9 mg/kg po in the phenylquinone induced writhing test performed with mice.



With regard to the bio-isostere relationships, replacing the carbon by a heteroatom in a drug template is a common strategy in medicinal chemistry. Thereby, in connection with our previous studies on heterocyclic compounds with analgesic activity,[5–10] we report here the synthesis and the biological evaluation of oxazolo[4,5-*b*]pyridin-2(3*H*)-ones substituted at the 6-position by an aminoalkyl unit functionalised or not (compounds D).



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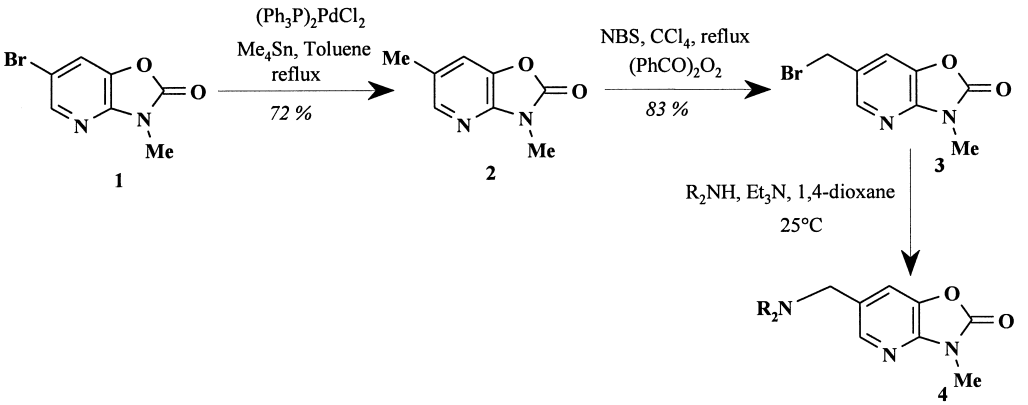
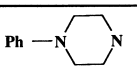
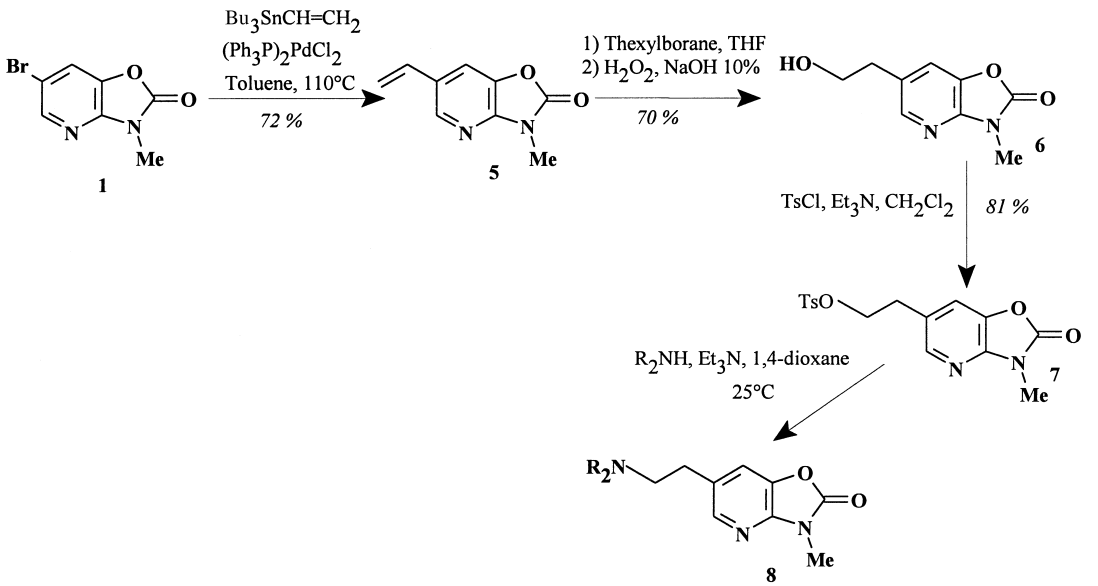
Chemistry

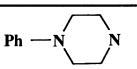
The synthesis of aminooxazolopyridinones **4** is illustrated in Scheme 1. Compound **2** was obtained with a satisfactory yield starting from the brominated derivative **1**[11] using Stille's reaction[12] with tetramethyltin in toluene. This compound, after introduction of the methyl group, was treated with *N*-bromosuccinimide in carbon tetrachloride with a catalytic amount of radical initiator. The brominated derivative **3**, obtained



Compounds	R_2N	Yield %
4a		96%
4b		90%

Scheme 1.



Compound	R_2N	Yield %
8a		91%

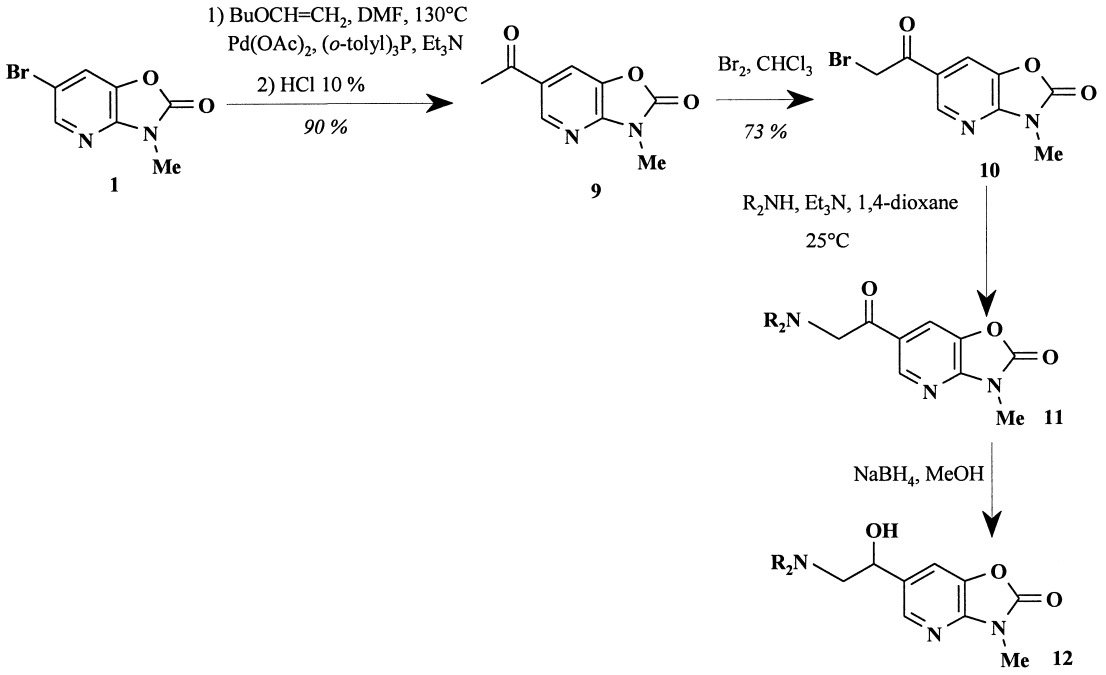
Scheme 2.

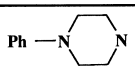
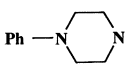
in 83% yield, was submitted to reaction with 1-phenylpiperazine or morpholine in 1,4-dioxane in the presence of triethylamine to provide products **4** in excellent yields.

In Scheme 2 is reported the synthesis of compound **8**. Vinylation of bromo derivative **1** with tributyl(vinyl)tin under palladium catalysis[12] gave a reproducible isolated yield of 72% of the expected product **3**. The treatment of this product with thexylborane in THF followed by addition of hydrogen peroxide and aqueous sodium hydroxide solution afforded the alcohol **6** in 70% yield. Tosylation of **6** with *p*-toluenesulfonyl chloride in the presence of triethylamine in dichloromethane led to the corresponding *p*-toluenesulfonate **7** in 81% yield. The conversion of **7** into the final compound **8a** was accomplished in 91% yield without any difficulty by reaction of **7** with 1-phenylpiperazine in 1,4-dioxane.

Preparation of compounds **11** and **12** is reported in Scheme 3. Compound **9** was easily obtained from bromo derivative **1** under Heck's conditions.[13] Reaction of **1** with butyl vinyl ether in *N,N*-dimethylformamide and triethylamine as base, in the presence of tri-*o*-tolylphosphine as ligand, gave, after acid hydrolysis,[14] the ketone **9** in 90% yield. This compound was converted into bromo acetyl derivative **10** by treatment with bromine in chloroform (73%). The amino compounds **11** were prepared in 81–84% yields by alkylation of 1-phenylpiperazine or morpholine with the bromo ketone **10** in an aprotic solvent such as 1,4-dioxane in the presence of triethylamine. Finally, the reduction of **11** with sodium borohydride in methanol furnished the amino alcohols **12** in excellent yields.

The synthesis of the target oxazolopyridinones **16** is shown in Scheme 4. The coupling reaction[12] of **1** with



Compounds	R_2N	Yield %
11a		84%
11b		86%
12a		95%
12b		98%

Scheme 3.

1-tributylstannyl-3,3-diethoxyprop-1-ene[15] in the presence of tetrakis(triphenylphosphine) palladium(0) and lithium chloride in tetrahydrofuran gave, after acid hydrolysis, the unsaturated aldehyde **13** in 72% yield. Catalytic hydrogenation of this compound over 10% Pd–C afforded the saturated alcohol **14** in 92% yield and its treatment with *p*-toluenesulfonyl chloride in dichloromethane furnished the corresponding tosylate **15** in 78% yield. Finally, the *N*-alkylation of 1-phenylpiperazine or morpholine with **15** afforded the desired amino derivatives **16** in satisfactory yields.

Biological Results and Discussion

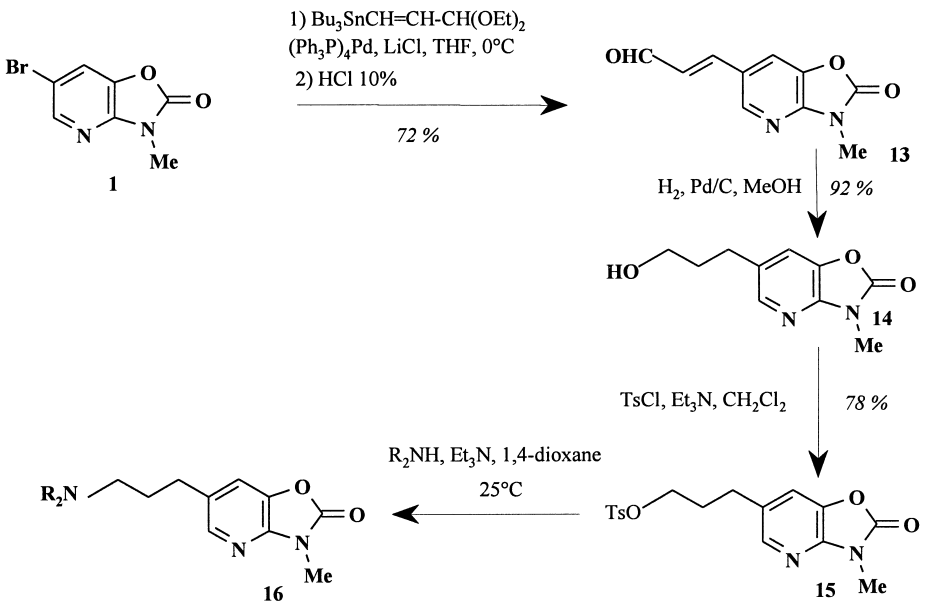
Nine 6-aminoalkyloxazolo[4,5-*b*]pyridin-2(3*H*)ones were evaluated for their analgesic activity. All were first prescreened at a standard dose of 50 mg/kg po using the phenylquinone (PBQ) and acetic acid writhing tests performed respectively in mice and rats. Results were expressed as percentages of inhibition of the writhing and also as an activity ratio versus aspirin to correct for slight variations in response between the same evaluation procedures not performed simultaneously (Table 1).

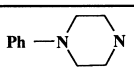
This preliminary evaluation shows that, whatever the length (1–3 carbon atoms) and the nature (alkyl, hydroxyalkyl, alkylcarbonyl) of the spacer between the oxazolo[4,5-*b*]pyridin-2(1*H*)one and the basic amino moiety, phenylpiperazino derivatives (**4a, 11a, 12a**, and **16a**) are always clearly more active than their morpholino counterpart (**4b, 11b, 12b**, and **16b**).

Surprisingly, the length of the alkyl spacer (from 1 to 3 carbon atoms) has not clear influence on the analgesic properties in the PBQ writhing test. The best results are however obtained with a 3 carbon atoms spacer (compound **16a**) with an activity ratio higher than 1.18 versus aspirin, compared to 1.02 for **4a** (methylene spacer) and 1.04 for **8a** (ethyl spacer).

Replacement of the ethyl spacer (compound **8a**) by a hydroxyethyl spacer (compound **12a**) results in a clear increase of the analgesic activity in the PBQ writhing test (activity ratio of 1.04 compared to 1.56), whilst its replacement by an acetyl linker (compound **11a**) leads to a clear decrease in potency with an activity ratio of 0.79.

On the basis of the prescreening results, compounds **4a** and **12a**, which were found more potent than aspirin in



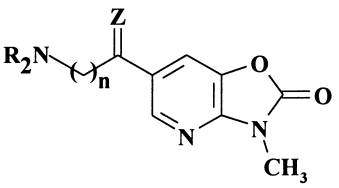
Compounds	R_2N	Yield %
16a		81%
16b		83%

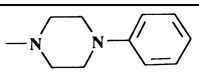
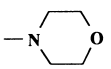
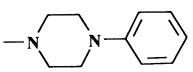
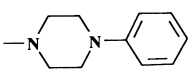
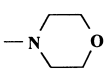
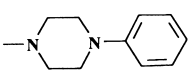
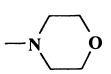
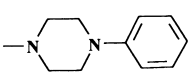
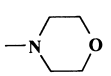
Scheme 4.

both the PBQ and acetid acid-included writhing test, were selected for further investigations. Their ED_{50} were of 26 (16.1–42.4) mg/kg po for **4a** and 15.5 (11.4–21.2) mg/kg po for **12a** in the PBQ writhing test and of 6 (3.7–9.8) mg/kg po **4a** and 5.5 (3.5–8.8) mg/kg po for **12a** in the acetic acid writhing test compared to, respectively, 63 (56–73) and 32 (28–46) mg/kg po for aspirin. Compound **12a** proved to be slightly less active than its benzo analogue **C** which have a ED_{50} of 9 mg/kg po in the PBQ writhing test.[4b]

The oral general acute toxicity of **4a** and **12a** as well as their behavioural effects were investigated via an Irwin test performed in mice (a careful observation of the general behaviour is also useful to avoid possible false positives in the writhing tests). For both compounds, the first behavioural changes are sedation and hypothermia that appear at 16 mg/kg po for **12a** and 64 mg/kg po for **4a**. The mortality threshold dose is higher than 512 mg/kg po for the two compounds. An oral safety index was defined as the ratio of the ED_{50} po in

Table 1. Analgesic activity screening of 6-aminoalkyloxazola[4,5-*b*]pyridin-2(3*H*)ones



Compd[a]	n	Z	R_2N	Phenylquinone(PBQ)-induced writhing test (mice)		Acetic acid-induced writhing test (rat)	
				% inhibition at 50 mg/kg po[b]	activity ratio versus aspirin[c]	% inhibition at 50 mg/kg po[b]	activity ratio versus aspirin
4a	0	H,H		57%**[d]	1.02	98%***	1.38
4b	0	H,H		32%	0.57	33%*	0.47
8a	1	H,H		86.3%**	1.04	ND[e]	
11a	1	O		53%**	0.79	96.8%***	1.01
11b	1	O		18.8%	0.28	26%	0.28
12a	1	H,OH		97%***	1.56	86%**	1.52
12b	1	H,OH		20%	0.33	38%	0.68
16a	2	H,H		100%***	1.18	ND	
16b	2	H,H		32%**	0.38	23%	0.37

[a]These compounds were tested as oxalate.

[b]Five animals were used for each compound, seven animals for the control group.

[c]Activity ratio $= \frac{\%\text{ of inhibition with the compound at 50mg/kg p.o.}}{\%\text{ of inhibition with aspirin at 50mg/kg p.o.}}$

[d]*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

[e]ND: not determined.

mice with the dose of appearance of the first behavioural changes in the Irwin test. This safety index is clearly insufficient with values of 2.5 for **4a** and 1 for **12a**.

In order to investigate a possible morphinic mechanism of action, studies of binding were investigated and showed, for both compounds, an absence of affinity ($K_i < 10^{-5}$ M) for the opioid μ, *K*, and δ, receptors. Affinities for the α_1, α_2, H_1, H_2, D_1, D_2, $5HT_{1A}$, $5HT_{1B}$, $5HT_{1D}$, $5HT_{2A}$, $5HT_{2C}$, and $5HT_3$ receptors were also investigated. Compounds **12b** proved to have moderate affinity for H_1 ($K_i = 1.4\times10^{-7}$ M), $5HT_{1A}$ ($K_i = 9.7\times10^{-8}$ M), $5HT_{1B}$ ($K_i = 7.7\times10^{-6}$ M), $5HT_{2A}$ ($K_i = 3.3\times10^{-6}$ M) receptors whilst **4a** showed same affinity for α_1 ($K_i = 6\times10^{-6}$ M), α_2 ($K_i = 7\times10^{-6}$ M) and $5HT_{1A}$ ($K_i = 8\times10^{-6}$ M). These affinities could explain some of the side effects observed in the Irwin test.

The inhibitory properties of compounds **4a** and **12a** on cyclooxygenase and 5-lipoxygenase were evaluated in vitro by measuring the inhibition of the production of PGE_2 and LTB_4 from rabbit granulocytes cells stimulated by calcium ionophore A 23187.[16] Both compounds appeared to be devoid of any inhibitory activity at a concentration of 10^{-5} M.

Conclusion

In conclusion within this class of 6-aminoalkyloxazolo[4,5-*b*]pyridin-2(3*H*)-ones, we have shown that some compounds possess potent nonopioid antinociceptive activity without antiinflammatory properties. Two of them, **4a** and **12a** proved to be more active than aspirin but unfortunately with a clearly insufficient safety index.

Experimental

Chemistry

Melting points were determined on a Köfler hot-stage apparatus and are uncorrected. Proton NMR were recorded on a Bruker 300 spectrometer. The coupling constants are recorded 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 R 10-10 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 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 visualised with UV light or an alcohol solution of ammonium cerium(IV) nitrate. Column chromatography was performed with Kieselgel 60 (70–230 mesh) silica gel (Merck) for gravity columns and Kieselgel 60 (230–400 mesh) silica gel (Merck) for flash columns. Where analyses are indicated by symbols of the elements, 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. Tetrakis (triphenylphosphine) palladium(0) was prepared by using the literature procedure.[17]

3,6-Dimethyloxazolo[4,5-*b*]pyridin-2(3*H*)-one (2). To a stirred solution of 6-bromo-3-methyloxazolo[4,5-*b*]pyridin-2(3*H*)-one **1** (414 mg, 1.8 mmol) in toluene (10 mL) was added successively tetramethyltin (0.3 mL, 2.2 mmol) and bis(triphenylphosphine)palladium(II) chloride (70 mg, 0.1 mmol). Then, the mixture was stirred for 8 h at reflux. After cooling, the solvent was evaporated and the residue was quenched with water. After extraction with dichloromethane, organic layers were washed, dried over magnesium sulfate and evaporated. The crude product was purified by silica gel column chromatography (eluent:dichloromethane) to provide **2** (416 mg) in 72% yield.; mp 115–116 °C; ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 3H, CH_3), 3.46 (s, 3H, NCH_3), 7.23 (d, 1H, $J = 1.5$ Hz, H_7), 7.93 (d, 1H, $J = 1.5$ Hz, H_5); IR (KBr) ν 1780 cm^{-1}. Anal. $C_8H_8N_2O_2$ (C,H,N).

6-Bromomethyl-3-methyloxazolo[4,5-*b*]pyridin-2(3*H*)-one (3). Under argon atmosphere, compound **2** (0.5 g, 3.05 mmol) was dissolved in carbon tetrachloride (50 mL). To this solution was added *N*-bromosuccinimide (597 mg, 3.35 mmol) and a trace of benzoyl peroxide, then the mixture was stirred at reflux for 3 h. After cooling and filtration, the solvent was removed under reduce pressure and the residue was purified by flash chromatography (eluent:dichloromethane) to give compound **3** (617 mg) in 83% yield; mp 105–106 °C; ^{1}H NMR ($CDCl_3$) δ 3.49 (s, 3H, NCH_3), 4.53 (s, 2H, CH_2), 7.46 (d, 1H, $J = 1.5$ Hz, H_7), 8.13 (d, 1H, $J = 1.5$ Hz, H_5); IR (KBr) ν 1785 cm^{-1}. Anal. $C_8H_7BrN_2O_3$ (C,H,N).

3-Methyl-6-[(4-phenyl-1-piperazinyl)methyl]oxazolo[4,5-*b*]-pyridin-2(3*H*)-one (4a). Compound **3** (1.0 g, 4.1 mmol) was dissolved, under argon atmosphere, in 1,4-dioxane (25 mL) at room temperature, then was added successively at this same temperature 1-phenyl-piperazine (0.70 g, 4.3 mmol) and triethylamine (0.62 g, 6.2 mmol). After 5 h of stirring the crude mixture was concentrated in vacuo and water was added to the residue.

The mixture was extracted with dichloromethane and the extracts were washed with water and dried over magnesium sulfate. After evaporation, the crude product was purified by silica gel column chromatography (eluent: dichloromethane/methanol, 98/2) to provide **4a** (1.28 g) in 96% yield; mp 131–132 °C (*i*-PrOH); ^{1}H NMR ($CDCl_3$) δ 2.61 (dd, 4H, J = 5.2, 4.4 Hz, $CH_{2piperaz}$), 3.19 (dd, 4H, J = 5.2, 4.4 Hz, $CH_{2piperaz}$), 3.48 (s, 3H, NCH_3), 3.58 (s, 2H, CH_2), 6.86 (t, 1H, J = 7.3 Hz, H_{arom}), 6.92 (d, 2H, J = 8.8 Hz, H_{arom}), 7.20–7.28 (m, 2H, H_{arom}), 7.51 (d, 1H, J = 1.8 Hz, H_7), 8.05 (d, 1H, J = 1.8 Hz, H_5); IR (KBr) ν 1775 cm^{-1}; MS (IC/NH_3) *m/z* 325 (M + 1). Anal. $C_{18}H_{20}N_4O_2$ (C,H,N).

3-Methyl-6-[(1-morpholino)methyl]oxazolo[4,5-*b*]pyridin-2(3*H*)-one (4b). This compound was prepared according to the procedure above from product **3** and morpholine. After column chromatography (eluent: dichloromethane/methanol, 98/2) the expected product **4b** was obtained in 90% yield; mp 144–145 °C (*i*-PrOH); ^{1}H NMR ($CDCl_3$) δ 2.46 (dd, 4H, J = 5.2, 4.3 Hz, CH_{2morph}), 3.49 (s, 3H, NCH_3), 3.52 (s, 2H, CH_2), 3.71 (dd, 4H, J = 5.2, 4.3 Hz, CH_{2morph}), 7.50 (d, 1H, J = 1.8 Hz, H_7), 8.03 (d, 1H, J = 1.8 Hz, H_5); IR (KBr) ν 1775 cm^{-1} MS (IC/NH_3) *mz* 250 (M + 1). Anal. $C_{12}H_{15}N_3O_3$ (C,H,N).

3-Methyl-6-vinyloxazolo[4,5-*b*]pyridin-2(3*H*)-one (5). To a stirred solution of 6-bromo-3-methyloxazolo[4,5-*b*]pyridin-2(3*H*)-one **1** (414 mg, 1.8 mmol) in toluene (10 mL) was added successively tributyl(vinyl)tin (697 mg, 2.2 mmol), and bis(triphenylphosphine)palladium (II) chloride (70 mg, 0.1 mmol). The mixture was stirred at reflux for 8 h. After cooling, evaporation of solvent and hydrolysis with water, the product was extracted with dichloromethane. The organic layer was dried over magnesium sulfate and the solvent was removed under reduce pressure. The crude product was purified by silica gel column chromatography (eluent: dichloromethane) to afford **5** (274 mg) in 86% yield; mp 124–125 °C; ^{1}H NMR ($CDCl_3$) δ 3.48 (s, 3H, NCH_3), 5.34 (d, 1H, J = 11.1 Hz, CH_2), 5.72 (d, 1H, J = 17.4 Hz, = CH_2), 6.71 (dd, 1H, J = 11.1, 17.4 Hz, = CH), 7.51 (d, 1H, J = 1.3 Hz, H_7), 8.08 (d, 1H, J = 1.3 Hz, H_5); IR (KBr) ν 1790 cm^{-1}. Anal. $C_9H_8N_2O_2$ (C,H,N).

6-[2-(Hydroxyethyl)]-3-methyloxazolo[4,5-*b*]pyridin-2(3*H*)-one (6). To a solution of thexylborane, previously prepared according to the literature procedure,[18] was added at 0 °C the vinyl compound **5** (880 mg, 5.0 mmol) dissolved in tetrahydrofuran (20 mL). After stirring for 2 h at 0 °C, 10% aqueous NaOH (2.40 mL) then hydrogen peroxide (2.00 mL) were successively added and reaction mixture stirred for an additional 1 h at room temperature. The solvent was removed under pressure and the product was extracted from the resulting crude reaction mixture, after aqueous hydrolysis, with dichloromethane. The organic layer was dried ($MgSO_4$) and evaporated to dryness under reduced pressure. The residual oil was chromatographed on a silica gel column (eluent: dichloromethane/methanol, 95/5) to give **6** (679 mg) in 70% yield as a white solid; mp 143–144 °C; ^{1}H NMR ($CDCl_3 + D_2O$) δ 2.89 (t, 2H, J = 6.6 Hz, CH_2), 3.47 (s, 3H, NCH_3), 3.88 (t, 2H, J = 6.6 Hz, CH_2O), 7.35 (s, 1H, H7), 8.00 (s, 1H, H_5); IR (KBr) ν 3400–3100, 1775 cm^{-1}. Anal. $C_9H_{10}N_2O_3$ (C,H,N).

3-Methyl-6-[2-(*p*-toluenesulfonyloxy)ethyl]oxazolo[4,5-*b*]-pyridin-2(3*H*)-one (7). To a stirred solution of alcohol **6** (194 mg, 1.0 mmol) in dichloromethane (5 mL) were added successively *p*-toluenesulfonyl chloride (286 mg, 1.5 mmol) and triethylamine (0.4 mL, 3 mmol) at 0 °C under argon atmosphere. The reaction mixture was allowed to reach room temperature. After hydrolysis with water, the crude was extracted with dichloromethane. The organic layers were washed, dried over magnesium sulfate and concentrated in vacuo. The residue was purified by column chromatography (eluent: dichloromethane) to provide **7** (282 mg) in 81% yield; mp 141–142 °C; ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 3H, CH_3), 2.97 (t, 2H, J = 6.6 Hz, CH_2), 3.46 (s, 3H, NCH_3), 4.23 (t, 2H, J = 6.6 Hz, OCH_2), 7.13 (d, 1H, J = 1.5 Hz, H_7), 7.27 (d, 2H, J = 8.1 Hz, H_{arom}), 7.68 (d, 2H, J = 8.1 Hz, H_{arom}), 7.89 (d, 1H, J = 1.5 Hz, H_5); IR (KBr) ν 1790 cm^{-1}. Anal. $C_{16}H_{16}N_2O_5S$ (C,H,N).

3-Methyl-6-[2-(4-phenyl-1-piperazinyl)ethyl]oxazolo[4,5-*b*]-pyridin-2(3*H*)-one (8a). To a solution of **7** (210 mg, 0.6 mmol) in 1,4-dioxane (8 mL) were added successively 1-phenylpiperazine (0.10 mL, 1.38 mmol) and triethylamine (0.18 mL, 1.3 mmol) at room temperature. Then, the mixture was stirred at the same temperature for 24 h. After evaporation of solvent under reduced pressure, the residue was quenched with water. The aqueous mixture was extracted with dichloromethane and was dried over magnesium sulfate. The organic solution was concentrated and the residue was purified by column chromatography (eluent: dichloromethane/methanol, 95/5) to give **8a** (185 mg) in 91% yield; mp 81–82 °C (Et_2O); ^{1}H NMR ($CDCl_3$) δ 2.64–2.72 (m, 6H, CH_2 and $CH_{2piperaz}$), 2.88 (dd, 2H, J = 8.0, 7.1 Hz, CH_2), 3.24 (dd, 4H, J = 5.6, 4.8 Hz, $CH_{2piperaz}$), 3.49 (s, 3H, NCH_3), 6.88 (t, 1H, J = 7.1 Hz, H_{arom}), 6.95 (d, 2H, J = 7.9 Hz, H_{arom}), 7.24–7.32 (m, 2H, H_{arom}), 7.35 (d, 1H, J = 1.6 Hz, H_7), 8.01 (d, 1H, J = 1.6 Hz, H_{arom}); IR (KBr) ν 1790 cm^{-1}; MS (IC/NH_3) *m/z* 339 (M + 1). Anal. $C_{19}H_{22}N_4O_2$ (C, H, N).

(3-Methyl-2-oxooxazolo[4,5-*b*]pyridin-6-yl)ethanone (9). To a stirred solution of brominated compound **1** (0.50 g, 2.18 mmol) in *N,N*-dimethylformamide (5 mL) were added successively triethylamine (0.44 g, 4.36 mmol), butyl vinyl ether (1.2 g, 12 mmol), 1,2-bis(diphenyl-

phosphino)ethane (24 mg, 0.06 mmol) and palladium(II) acetate (12 mg, 0.054 mmol). The mixture was stirred at reflux for 8 h under inert atmosphere. After cooling, the solution was hydrolysed with a solution of 10% HCl and stirring was maintained for 1 h. The solvent was evaporated and the residue treated with water and extracted with CH_2Cl_2. The combined organic extracts were washed with water, dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by flash chromatography (eluent: dichloromethane/ethyl acetate, 8/2) to afford **9** (377 mg) in 90% yield; mp 163–164 °C; ^{1}H NMR ($CDCl_3$) δ 2.53 (s, 3H, CH_3), 3.63 (s, 3H, NCH_3), 7.91 (d, 1H, J = 1.5 Hz, H_7), 8.72 (d, 1H, J = 1.5 Hz, H_5); IR (KBr) ν 1790, 1670 cm^{-1}. Anal. $C_9H_8N_2O_3$ (C,H,N).

2-Bromo-1-(3-methyl-2-oxooxazolo[4,5-*b*]pyridin-6-yl)-ethanone (10). To a stirred solution of **9** (500 mg, 2.6 mmol) in chloroform (15 mL) was added dropwise bromine (0.2 mL, 2.6 mmol). After stirring at room temperature for 5 h, the solution was evaporated under reduced pressure. The residue was chromatographed on silica gel column (eluent: dichloromethane) to provide **10** (515 mg) in 73% yield; mp 133–134 °C; ^{1}H NMR ($CDCl_3$) δ 3.54 (s, 3H, NCH_3), 4.41 (s, 2H, CH_2), 7.97 (d, 1H, J = 1.5 Hz, H_7), 8.81 (d, 1H, J = 1.5 Hz, H_5); IR (KBr) ν 1770, 1670 cm^{-1}. Anal. $C_9H_7BrN_2O_3$ (C,H,N).

1-(3-Methyl-2-oxooxazolo[4,5-*b*]pyridin-2-yl)-2-(4-phenyl-1-piperazinyl)ethanone (11a). This compound was prepared according to the same experimental procedure described for compounds **4** using **10** and 1-phenylpiperazine as starting materials. Compound **11a** was obtained in 84% yield; mp 202–203 °C (Et_2O); ^{1}H NMR ($CDCl_3$) δ 2.75 (dd, 4H, J = 5.1, 4.4 Hz, $CH_{2piperaz}$), 3.24 (dd, 4H, J = 5.1, 4.4 Hz, $CH_{2piperaz}$), 3.52 (s, 3H, NCH3), 3.78 (s, 2H, CH_2), 6.85 (t, 1H, J = 7.4 Hz, H_{arom}), 6.92 (d, 2H, J = 8.1, H_{arom}), 7.21–7.30 (m, 2H, H_{arom}), 8.05 (d, 1H, J = 1.5, H_5), 8.99 (d, 1H, J = 1.5, H_5); IR (KBr) ν 1775 cm^{-1}; MS (IC/NH_3) m/z 353 (M + 1). Anal. $C_{19}H_{20}N_4O_3$ (C,H,N).

1-(3-Methyl-2-oxooxazolo[4,5-*b*]pyridin-6-yl)-2-morpholinylethanone (11b). This compound was prepared according to the same experimental procedure described for compounds **4** using **10** and morpholine as starting materials. Compound **11b** was obtained in 86% yield; mp 186–187 °C (Et2O); ^{1}H NMR ($CDCl_3$) δ 2.57 (dd, 4H, J = 5.2, 4.4 Hz, CH_{2morph}), 3.51 (s, 3H, NCH_3), 3.71 (s, 2H, CH_2), 3.73 (dd, 4H, J = 5.2, 4.4 Hz, CH_{2morph}), 8.01 (d, 1H, J = 1.5, H_7), 8.93 (d, 1H, J = 1.5, H_5); IR (KBr) ν 1775 cm^{-1}; MS (IC/NH_3) m/z 280 (M + 1). Anal. $C_{13}H_{15}N_3O_4$ (C,H,N).

3-Methyl-6-{1-[2-(4-phenyl-1-piperazinyl)ethan-1-ol]}-oxazolo[4,5-*b*]pyridin-2(3*H*)-one (12a). To a solution of ketone **11a** (352 mg, 1.0 mmol) in methanol (15 mL) was added sodium borohydride (42 mg, 1.1 mmol) and the mixture was stirred for 5 h at room temperature. After hydrolysis, the resulting precipitate was collected, dried and crystallised from diethyl ether to give the product **12a** (336 mg) in 95% yield; mp 183–184 °C (Et_2O); ^{1}H NMR ($CDCl_3$ + D_2O) δ 2.47–2.60 (m, 2H, CH_2), 2.61–2.70 (m, 2H, $CH_{2piperaz}$), 2.88–2.98 (m, 2H, $CH_{2piperaz}$), 3.18–3.33 (m, 4H, $CH_{2piperaz}$), 3.48 (s, 3 H, NCH_3), 4.83 (dd, 1H, J = 10.3, 4.4 Hz, CH), 6.88 (t, 1 H, J = 7.4, H_{arom}), 6.94 (d, 2H, J = 8.1 H_{arom}), 7.25–7.33 (m, 2H, H_{arom}), 7.53 (d, 1H, J = 1.5, H_7), 8.10 (d, 1H, J = 1.5, H_5); IR (KBr) ν 3400−3100, 1775 cm^{-1}; MS (IC/NH_3) m/z 355 (M + 1). Anal. $C_{19}H_{22}N_4O_3$ (C,H,N).

3-Methyl-6-[1-(2-morpholinyl)ethan-1-ol]oxazolo[4,5-*b*]-pyridin-2(3*H*)-one (12b). The compound **12b** was prepared as above from the ketone **11b** in 98% yield; mp 146–147 °C (Et_2O); ^{1}H NMR ($CDCl_3$ + D_2O) δ 2.36–2.46 (m, 4H, CH_2 and CH_{2morph}), 2.68–2.78 (m, 2H, CH_{2morph}), 3.48 (s, 3H, NCH_3), 3.66–3.80 (m, 4H, CH_{2morph}), 4.76 (dd, 1H, J = 10.4, 3.9, CH), 7.47 (d, 1H, J = 1.5, H_7), 8.04 (d, 1H, J = 1.5, H_5); IR (KBr) ν 3400–3100, 1775 cm^{-1}; MS (IC/NH_3) m/z 280 (M + 1). Anal. $C_{13}H_{17}N_3O_4$ (C,H,N).

(*E*)-3-(3-Methyl-2-oxooxazolo[4,5-*b*]pyridin-6-yl)propenal (13). The corresponding diethylacetal of the compound **13** was prepared according to the same experimental procedure described for product **2** using 1-tributylstannyl-3,3-diethoxyprop-1-ene[15] as the reagent and tetrahydrofuran as the solvent. The crude diethylacetal obtained was dissolved in water and treated with few drops of 10% HCl. The mixture was stirred for 1 h at room temperature, then extracted several times with dichloromethane. After drying over $MgSO_4$ followed by evaporation of the solvent, the residue was purified by silica gel column chromatography (eluent: dichloromethane/methanol, 98/2) to provide the aldehyde **13** in 72% yield; mp 211–212 °C; ^{1}H NMR ($CDCl_3$) δ 3.51 (s, 3H, NCH_3), 6.68 (dd, 1H, J = 15.4, 7.4 Hz, =CH), 7.49 (d, 1H, J = 15.4 Hz, =CH), 7.60 (d, 1H, J = 2.2 Hz, H_7), 8.29 (d, 1H, J = 2.2 Hz, H_5), 9.72 (d, 1H, J = 7.4 Hz, CHO); IR (KBr) ν 1790, 1680 cm^{-1}. Anal. $C_{10}H_8N_2O_3$ (C,H,N).

3-Methyl-6-[2-(propan-1-ol)]oxazolo[4,5-*b*]pyridin-2(3*H*)-one (14). Compound **13** (500 mg, 2.45 mmol) was dissolved in methanol (35 mL). Then palladium on charcoal (10%) (50 mg) was added. The solution was kept under hydrogen pressure in a Parr shaker at 40 psi for 6 h. The catalyst was removed by filtration, then the solvent was evaporated under reduce pressure. The crude product was purified by silica gel column chromatography (eluent: dichloromethane/methanol, 95/5) to give the alcohol **14** (470 mg) in 92% yield; mp 218–219 °C;

^{1}H NMR ($CDCl_3 + D_2O$) δ 1.84–1.94 (m, 2H, CH_2), 2.77 (dd, 2H, $J = 8.1$, 7.0 Hz, CH_2), 3.47 (s, 3H, NCH_3), 3.70 (dd, 2H, $J = 7.0$, 5.9 Hz, CH_2), 7.29 (d, 1H, $J = 1.5$ Hz, H_7), 7.98 (d, 1H, $J = 1.5$ Hz, H_5); IR (KBr) ν 3400–3100, 1770 cm^{-1}. Anal. $C_{10}H_{12}N_2O_3$ (C,H,N).

3-Methyl-6-[3-(*p*-toluenesulfonyloxy)propyl]oxazolo[4,5-*b*]-pyridin-2(3*H*)-one (15). This compound was prepared according to the same experimental procedure described for compound **7** using **14** as the starting material. Compound **15** was obtained in 78% yield; mp 151–152 °C; ^{1}H NMR ($CDCl_3$) 1.91–2.03 (m, 2H, CH_2), 2.47 (s, 3H, CH_3), 2.77 (t, 2H, $J = 7.4$ Hz, CH_2), 2.47 (s, 3H, CH_3), 2.77 (t, 2H, $J = 7.4$ Hz, CH_2), 3.46 (s, 3H, NCH_3), 4.05 (t, 2H, $J = 7.4$ Hz, CH_2), 7.11 (d, 1H, $J = 1.5$ Hz, H_7), 7.36 (d, 2H, $J = 8.1$ Hz, H_{arom}), 7.79 (d, 2H, $J = 8.1$ Hz, H_{arom}), 7.88 (d, 1H, $J = 1.5$ Hz, H_5); IR (KBr) ν 1790 cm^{-1}. Anal. $C_{17}H_{18}N_2O_5S$ (C,H,N).

3-Methyl-6-[3-(4-phenyl-1-piperazinyl)propyl]oxazolo[4,5-*b*]-pyridin-2(3*H*)-one (16a). This compound was prepared according to the same procedure described for product **8a** using **15** as the starting material. Compound **16a** was obtained in 81% yield; amorphous solid; ^{1}H NMR ($CDCl_3$) δ 1.79–1.90 (m, 2H, CH_2), 2.41 (t, 2H, $J = 7.4$ Hz, CH_2), 2.59 (dd, 4H, $J = 5.1$, 4.4 Hz, $CH_{2piperaz}$), 2.71 (t, 2H, $J = 7.4$ Hz, CH_2), 3.21 (dd, 4H, $J = 5.1$, 4.4 Hz, $CH_{2piperaz}$), 3.49 (s, 3H, NCH_3), 6.85 (t, 1H, $J = 7.4$ Hz, H_{arom}), 6.92 (d, 2H, $J = 8.1$ Hz, H_{arom}), 7.22–7.29 m (m, 3H, $H_7 + H_{arom}$), 7.96 (d, 1H, $J = 15$ Hz, H_5); IR (KBr) ν 1785 cm^{-1}; MS (IC/NH_3) m/z 353 (M + 1). Anal. $C_{20}H_{24}N_4O_2$ (C,H,N).

3-Methyl-6-[3-(1-morpholino)propyl]oxazolo[4,5-*b*]pyridin-2(3*H*)-one (16b). The compound **16b** was prepared as above from the tosylate **15** in 83% yield; amorphous solid; ^{1}H NMR ($CDCl_3$) δ 1.75–1.90 (q, 2H, $J = 7.4$ Hz, CH_2), 2.37 (t, 2H, $J = 7.4$ Hz, CH_2), 2.44 (t, 4H, $J = 4.4$ Hz, $CH_{2piperaz}$), 2.71 (t, 2H, $J = 7.4$ Hz, CH_2), 3.49 (s, 3H, NCH_3), 3.74 (t, 4H, $J = 4.4$ Hz, CH_{morph}), 7.28 (d, 1H, $J = 1.5$ Hz, H_7), 7.97 (d, 1H, $J = 1.5$ Hz, H_7); IR (KBr) ν 1790 cm^{-1}; MS (IC/NH_3) m/z 278 (M + 1). Anal. $C_{14}H_{19}N_3O_3$ (C,H,N).

Pharmacological methods

Phenylquinone (PBQ)-induced writhing in mice. Male CD1 mice in the weight range of 20–35 g were used for the study after an overnight fast. The test compounds were suspended in 0.5% carboxymethyl cellulose 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. For the screening evaluation at 50 mg/kg, 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 of 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 minutes after phenylquinone administration was recorded.[19,20]

The ratio of the mean writhes of the control animals versus the mean writhes of the treated animals was calculated, and the results are 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.[21]

Acetic acid-induced writhing in rats

Male Wistar rats in the weight range of 140–160 g were used for the study after an overnight fast. The test compounds were suspended in 0.5% carbomethyl cellulose 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. For the screening evaluation at 50 mg/kg, 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 and 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 was recorded.[22] The ratio of the mean writhes of the control animals versus the mean writhes of the treated animals was calculated, and the results are 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.[21]

Evaluation of toxic, physiological, and behavioural effects in mice (Irwin)

Three animals per dose were treated po with the test compound (dispersed in a 5% acacia gum suspension at a volume of 0.25 mg/20 g) and observed through a standardised observation grid at regular intervals for up to 24 h. The presence or absence and the intensity of various symptoms were noted.[23,24]

Receptor binding assays

Receptor binding assays were performed by incubating membranes prepared from the rat central nervous system with [^{3}H]DAMGO, [^{3}H]-*p*-Cl-Phe-DPDPE, respectively, for receptors μ and δ.[25,26] For receptors H_1 and H_2, membranes were prepared from guinea pig cerebral cortex and incubated with [^{3}H]pyrilamine and [^{3}H]tiotidine, respectively.[27] For receptor K, membrane was prepared from guinea pig cerebellum incubated with [^{3}H]U 69593.[28] $5HT_{1A}$ assays used rat hippocampus

membranes, [^{3}H]-8-OH-DPAT, and buspirone for non-specific binding (NSB),[29] $5HT_{1B}$ assays used rat cortex, [^{125}I]CYP, and serotonin for NSB. $5HT_2$ assays used calf frontal cortex, [^{3}H]ketanserin, and spiperone for NSB.[30] $5HT_3$ assays used NLE-115 cells, [^{3}H]BRL 43694 for NSB.[31] D_1 assays used rat striatum, [^{3}H]SCH 23390 for NSB.[32] D_2 assays used rat striatum, [^{3}H]YM 09151-2 for NSB.[33] For α_1 and α_2 receptors, membranes were prepared from rat brain incubated respectively with [^{3}H]prazosin[34] and [^{3}H]rauwolscine.[35]

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

Cyclooxygenase and 5-lipoxygenase activities

Isolated rabbit granulocytes were precubated during 15 min at 37 °C with tested compound at a concentration of 10^{-5} M in DMSO. Calcic ionophore A 32187 (5×10^{-6} M in DMSO) was added during 15 min. For each compound, cycloxygenase and 5-lipoxygenase inhibition were evaluated by dosing respectively PGE_2 and LTB_4 formation using the enzymoimmunoassay (EIA) method.[16] The reference compounds used were indomethacine ($IC_{50}=2.7\times10^{-9}$ M) and NDGA ($IC_{50}=4\times10^{-7}$ M), respectively, for inhibition of PGE_2 and LTB_4 formation.

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