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Substituted pyrido[3,2-*b*]oxazin-3(4*H*)-ones: synthesis and evaluation of antinociceptive activity

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Abstract

A new series of *N*-substituted pyrido[3,2-*b*]oxazinones has been synthesized, pharmacologically evaluated, and compared with acetyl salicylic acid. The compound with the maximal combination of safety and analgesic efficacy was 4-{3-[4-(4-fluorophenyl-1-piperazinyl)propyl]}-2*H*-pyrido[3,2-*b*]-1,4-oxazin-3(4*H*)-one (**6c**) with ED_{50} values of 12.5 mg/kg po (mouse: phenylquinone writhing test) and 27.8 mg/kg po (rat: acetic acid writhing test), respectively. Compound **6c** proved to be more active than aspirin with a safety index of 5.1.

1. Introduction

The search for a new analgesic agents that are devoid of side effects (such as tolerance, respiratory depression, constipation, physical dependency, and fear of addiction) typical of morphine-like opioid agonists, as well as of the gastro-intestinal problems associated with NSAIDs, has attracted significant attention in recent years. In this regard, a considerable number of benzoxazolinone, benzoxazinone, oxazolopyridine, pyridooxazinone, and oxazolopyridinone derivatives, with analgesic properties, has been reported [1–9]. Among these types of compounds, benzoxazolinones **A** and **B** [1,2], oxazolo[4,5-*b*]pyridin-2(3*H*)-ones **C** [8], and oxazolo [5,4-*b*]pyridin-2(1*H*)-ones **D** [9] have emerged as being of particular interest (Chart 1).

In our continuing study of heteropolycyclic compounds with potential biological activity [8–15], we report here the synthesis and the biological evaluation of a new series of substituted pyrido[3,2-*b*]oxazin-3(4*H*)-ones having the general formula **E** (Chart 2). These compounds possess a 4-arylpiperazinylalkyl chain on the nitrogen atom at the 4-position or an acetyl moiety at the 7-position.

2. Chemistry

Starting materials **2** and **3**, not commercially available, may be prepared by a method reported by Rüfenacht et al. [16] involving the condensation of 2-amino-3-hydroxypyridine **1** and the appropriate chloroacetyl chloride in the presence of sodium hydrogencarbonate (Scheme 1).

Two different general synthetic methods were employed for the preparation of the desired derivatives **4**, **5**, **6**, **10**, **11**, and **12** from the pyrido[3,2-*b*]oxazin-3(4*H*)-ones **2** and **3**.

The first synthetic procedure (method A, Scheme 2) was used to prepare products **4**, **5**, and **6** where *n* stands for two or three carbons. The free nitrogen of the pyrido[3,2-*b*]oxazin-3(4*H*)-ones was alkylated in anhydrous *N*,*N*-dimethylformamide in the presence of sodium ethoxide with (2-chloroethyl)- or (3-chloropropyl)amines, which were prepared according to the procedure described in the literature [17]. When the substituent at the 4-position had more than three carbons, this method

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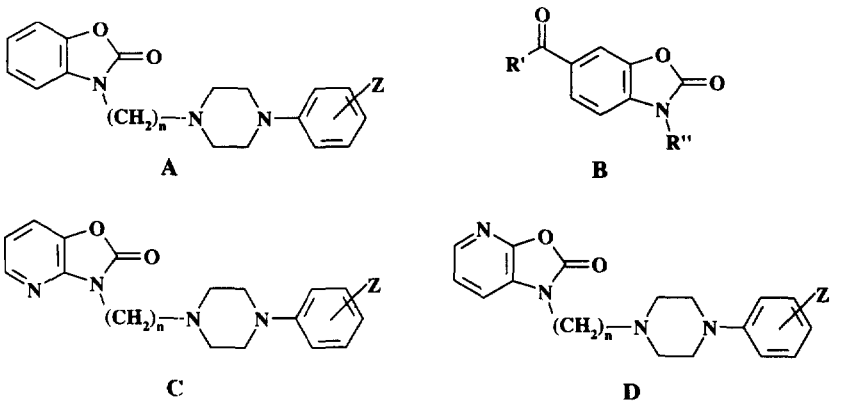
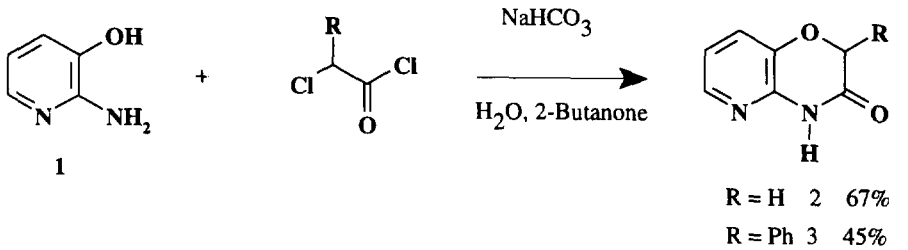
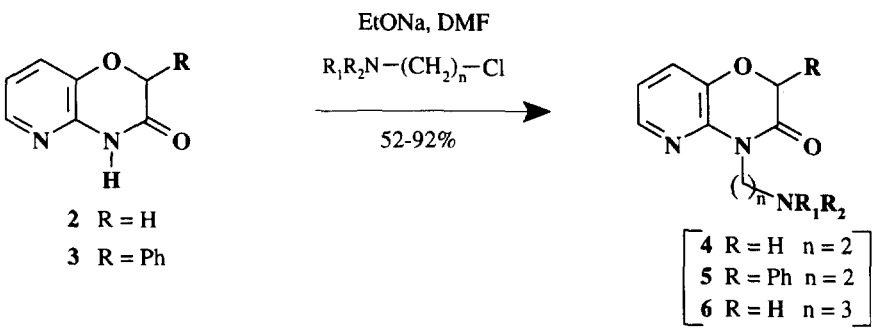


Chart 1.



Scheme 1.



Scheme 2. Method A.

was not satisfactory owing to the intramolecular cyclisation of the 4-(4-chlorobutyl)-1-arylpiperazines into their spiro-fused quaternary chloride salt [18].

The second protocol (method B, Scheme 3) conveniently circumvented this obstacle. The anions of compounds **2** and **3** reacted with dibromoalkanes in DMF to provide **7**, **8**, and **9** in satisfactory yields. Compounds **10**, **11**, and **12** were prepared in excellent yields by alkylation of amine intermediates with the appropriate bromo compounds **7**, **8**, and **9** in an aprotic solvent such as acetonitrile in the presence of diisopropylethylamine.

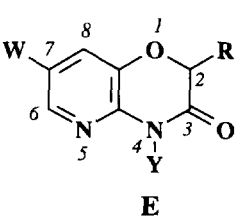
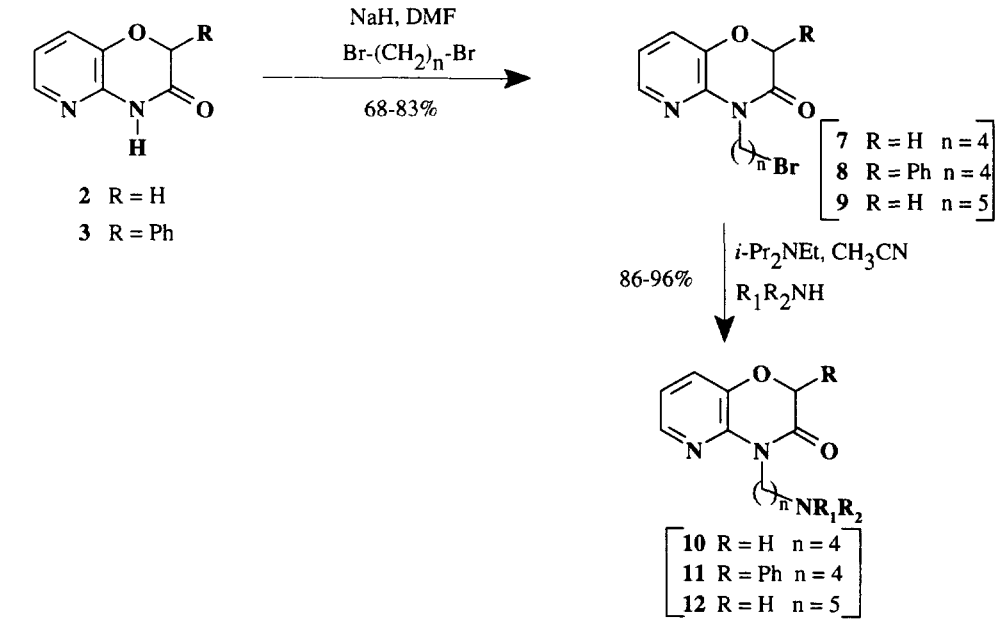


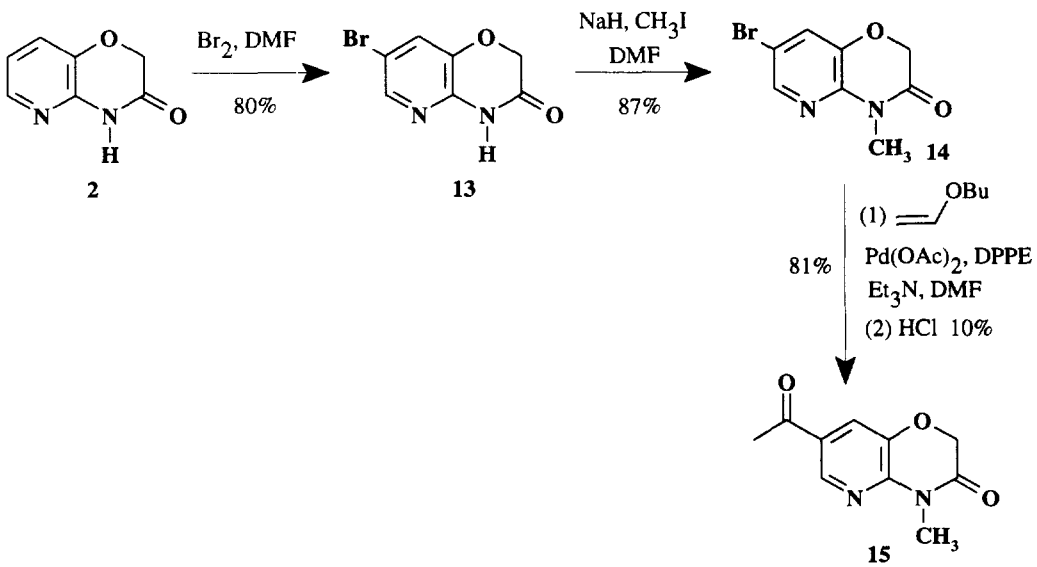
Chart 2.

Preparation of acetyl derivative **15** is reported in Scheme 4 (method C). Treatment of **2** with bromine in DMF, as solvent, according to a procedure described in oxazolopyridine series [19] provides with a 80% yield compound **13**. This compound was converted by reaction with sodium hydride in dry *N,N*-dimethylformamide at room temperature into its anion, which reacted with iodomethane to provide **14** in good yield. Compound **15** was obtained satisfactorily from **14** under a variety of normal Heck conditions [20]. Reaction of **14** with butylvinyl ether in DMF and triethylamine as base, in the presence of 1,2-bis(diphenyl phosphino)ethane as ligand, gave, after acid hydrolysis [21], the acetyl derivative **15** in 81% yield.

Table 1 summarizes the experimental and physical data for the desired compounds. General procedures detailing the synthesis of target stuctures are reported in the Experimental section.



Scheme 3. Method B.

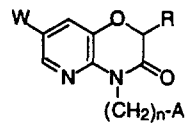


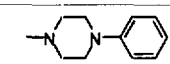
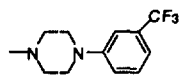
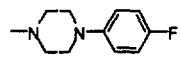
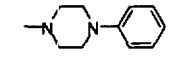
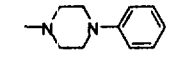
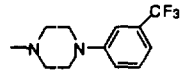
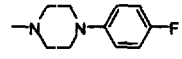
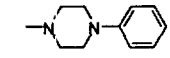
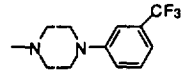
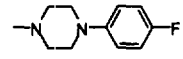
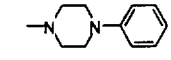
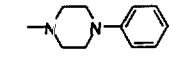
Scheme 4. Method C.

3. Biological results and discussion

According to the structure–activity relationships we previously established within the oxazolo[4,5-*b*]pyridin-(3*H*)-ones [8] and oxazolo[5,4-*b*]pyridin-2(1*H*)-ones [9] series, only substituted or unsubstituted 4-(4-phenylpiperazinylalkyl)pyrido[3,2-*b*]oxazin-3(4*H*)-ones (12 compounds) were synthesized and evaluated. All were first studied for their analgesic activity 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 ratios of activity versus aspirin to correct for slight variations in response between the same evaluation procedures not performed simultaneously (Table 2).

Table 1
1-[4-Arylpiperazinyl)alkyl]pyrido[3,2-*b*]oxazin-3(4*H*)-ones



Compound	n	W	R	A	Method[a]	Yield (%)[b]	mp (°C)	Solvent[c]	Formula	Anal.[d]
4a	2	H	H		A	91	oil		$C_{19}H_{22}N_4O_2$	C,H,N
4b	2	H	H		A	92	oil		$C_{20}H_{21}F_3N_4O_2$	C,H,N
4c	2	H	H		A	92	109–110	*i*-PrOH	$C_{19}H_{21}FN_4O_2$	C,H,N
5	2	H	Ph		A	89	oil		$C_{25}H_{26}N_4O_2$	C,H,N
6a	3	H	H		A	94	oil		$C_{20}H_{24}N_4O_2$	C,H,N
6b	3	H	H		A	70	93–94	*i*-PrOH	$C_{21}H_{23}F_3N_4O_2$	C,H,N
6c	3	H	H		A	89	103–104	*i*-PrOH	$C_{20}H_{23}FN_4O_2$	C,H,N
10a	4	H	H		B	96	86–87	*i*-PrOH	$C_{21}H_{26}N_4O_2$	C,H,N
10b	4	H	H		B	95	85–86	*i*-PrOH	$C_{22}H_{25}F_3N_4O_2$	C,H,N
10c	4	H	H		B	91	69–70	*i*-PrOH	$C_{21}H_{25}FN_4O_2$	C,H,N
11	4	H	Ph		B	90	94–95	*i*-PrOH	$C_{27}H_{30}N_4O_2$	C,H,N
12	5	H	H		B	95	71–72	*i*-PrOH	$C_{22}H_{28}N_4O_2$	C,H,N
15	0	CH_3CO	H	CH_3	C	81	183–184	*i*-PrOH	$C_{10}H_{10}N_2O_3$	C,H,N

[a]Method A is shown in Scheme 2; method B is shown in Scheme 3; method C is shown in Scheme 4; see the Experimental section.
[b]Isolated yields of pure products; no efforts were made to optimize yields. For compound **15**, the yield is calculated from **14**.
[c]Solvent of recrystallization.
[d]Analytical results were within ±0.4% of the theoretical value.

This preliminary evaluation shows that among the unsubstituted 4-(4-phenylpiperazinylalkyl)pyrido[3,2-*b*]oxazin-3(4*H*)-ones (**4a**, **6a**, **10a**, and **12**), the maximal activity is obtained when the alkyl spacer is a butyl function (**10a**).

Substitution of the phenyl ring of the phenyl piperazine moiety by a *m*-trifluoromethyl group (**4b**, **6b**, **10b**) or a *p*-fluorine (**4c**, **6c**, **10c**) leads to activity levels that are surprisingly abnormally low for compounds **4c** and **10b**. As the results for this two compounds appear illogical, and also in discord with those previously obtained [8,9], it is reasonable not to take them into account for the present discussion.

Once that is done, we can conclude that substitution with a *m*-trifluoromethyl group (**4b**, **6b**) appeared to have only little effect on analgesic activity with a slight improvement for **4b** compared to the unsubstituted **4a** (n = 2) and a slight decrease for **6b** compared to **6a** (n = 3). Substitution with a *p*-fluorine (**6c**, **10c**) results in a slight improvement for **6c** (n = 3) and a clear decrease for **10c** (n = 4).

Direct substitution of the pyrido[3,2-*b*]oxazine ring with a 2-phenyl group has almost no effect on analgesic activity when n = 2 (**5**). When n = 4, compound **11** retains a good activity in the acetic acid-induced writhing test (82% inhibition, activity ratio of 1.45) but appears clearly less active in the PBQ test (22% inhibition, activity ratio of 0.35). This discrepancy between the results of the two writhing tests could be explained by differences in the metabolism of compound **11** in rat and mouse.

Only one pyrido[3,2-*b*]oxazin-3(4*H*)-one acylated at 7-position was synthesized and evaluated. This compound (**15**), which was devoid any basic amino moiety, was found half as potent as aspirin.

Seven compounds (**4a**, **4b**, **5**, **6b**, **6c**, and **10a**) were found to be more potent than aspirin itself on the basis of this preliminary evaluation one of them, compound **6c**, which is protected of metabolic hydroxylation by the mean of the fluor group in para position, was selected for further evaluation.

Its ED_{50} were 12.5 (8.7–18.2) mg/kg po and 27.8 (18.1–42.7) mg/kg po in the PBQ induced and the acetic acid induced writhing tests, respectively, compared to 63 (56–75) mg/kg po and 32 (28–46) mg/kg po for acetyl salicylic acid.

Compound **6c** proved also to be significantly active in the hot-plate test performed in mice where it increases the foot licking latency of 5.7 ± 0.3 s ($p < 0.01$) at 32 mg/kg po compared to 9.3 ± 1.2 s ($p < 0.001$) at 16 mg/kg ip for morphine.

Its oral general acute toxicity as well as its 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).

The first behavioural changes are sedation and hypothermia that appear at 64 mg/kg po while the mortality threshold dose is higher than 1024 mg/kg po.

An oral safety index was defined as the ratio of the ED_{50} po in mice with the dose of appearance of the first

Table 2
Analgesic activity screening of 1-[(4-arylpiperazinyl)alkyl]pyrido[3,2-*b*]oxazin-3(4*H*)-ones

Compound	Phenylquinone (PBQ)-induced writhing test (mice)		Acetic acid-induced writhing test (rat)	
	% inhibition at 50 mg/kg po[a]	Activity ratio versus aspirin[b]	% inhibition at 50 mg/kg po[a]	Activity ratio versus aspirin[b]
4a[c]	60%**[e]	1.03	64%**	1.03
4b[c]	74%**	1.28	68%**	1.09
4c	39%***	0.76	12%	0.18
5[c]	54%**	1	48%**	1.22
6a[c]	71%**	1.22	75%**	1.21
6b	86%***	1.25	94%***	1.05
6c	75%***	1.47	91%***	1.40
10a	97%***	1.67	100%***	> 1.6
10b	18%	0.35	ND[d]	—
10c	69%***	1	71%**	0.79
11	22%	0.35	82%**	1.45
12	53%*	0.85	97%**	1.73
15	29%	0.57	28%	0.43

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

[b]Activity ratio: $\frac{\%\text{ of inhibition with compound at 50 mg/kp po}}{\%\text{ of inhibition with aspirin at 50 mg/kg po}}$

[c]These compounds were tested as oxalate.

[d]ND : Not determined.

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

behavioural changes in the IRWIN test. This safety index is relatively good for **6c** with a value of 5.1 if PBQ induced writhing assay in taken into account.

In order to investigate possible mechanism of its action, binding studies were performed and they showed absence of significant affinity for the μ, δ and K opioid receptors. A small affinity was observed for the histamine H_1 and H_2 receptors with inhibition of respectively 99% at 10^{-5} M and 29% at 10^{-7} M for H_1 and 36% at 10^{-5} M and 19% at 10^{-7} M for H_2 receptor. This could explain the sedative effects observed in the IRWIN test.

However a moderate affinity was observed for the serotoninergic 5-HT_{1A} and 5-HT_2 receptors with inhibition of respectively 97% at 10^{-5} M and 32% at 10^{-7} M for 5-HT_{1A} and 92% at 10^{-5} M and 67% at 10^{-7} M for 5-HT_2. Similarly, a mild affinity was obtained for the adrenergic α_1 and α_2 receptors with inhibition of respectively 99% at 10^{-5} M and 56% at 10^{-7} M for α_1 and 100% at 10^{-5} M and 31% at 10^{-7} M for α_2. According to these values, the antinociceptive activity could perhaps result from serotoninergic [22] and/or adrenergic [23] effects.

4. Conclusion

In conclusion, within this class of pyrido[3,2-*b*]oxazin-3(4*H*)-ones, we have shown that some compounds possess potent nonopioid antinociceptive activity. One of the most active compound of this family, 4-{3-[4-(4-fluorophenyl-1-piperazinyl)propyl]}-2*H*-pyrido[3,2-*b*]-1,4-oxazin-3(4*H*)-one **6c** proves to be more active than aspirin with a safety index of 5.1.

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

5. Experimental

5.1 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 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.

5.1.1 2H-Pyrido[3,2-b]-1,4-oxazin-3(4H)-one (2)

2-Amino-3-hydroxypyridine **1** (5.00 g, 45 mmol) was added to a suspension of sodium hydrogenocarbonate (9.10 g, 108 mmol) in a mixture of water (30 ml) and 2-butanone (30 ml). After cooling the solution to 5°C, chloroacetyl chloride (4.2 ml, 51 mmol) diluted with methylethylketone (10 ml) was added dropwise and slowly (for 1 h) so that temperature did not exceed 10°C. The mixture was stirred at room temperature for 30 min and then at 75°C for 30 min. The mixture was cooled and the solvent removed. The resulting precipitate was collected by filtration, washed with water, and dried over P_2O_5 under vacuum. The product **2** was recristallized from CH_3OH/H_2O (1/1:v/v) and obtained in a yield of 67%; mp 204–205°C (CH_3OH/H_2O:1/1) [lit [24].: 205–206°C]; IR (KBr) ν 3200–2400, 1700 cm^{-1}; ^1H NMR ($CDCl_3 + D_2O$) δ 4.68 (s, 2H, OCH_2), 6.97 (dd, 1H, H_7, J=8.1, 5.2), 7.27 (dd, 1H, H_8, J=8.1, 1.5), 8.05 (dd, 1H, H_6, J=5.2, 1.5).

5.1.2 2-Phenyl-2H-pyrido[3,2-b]-1,4-oxazin-3(4H)-one (3)

Compound **3** was prepared by the same procedure as was used for **2**, using 2-chloro-2-phenylacetyl chloride (9.64 g, 51 mmol) as the starting reagent, and obtained in a yield of 45%; mp 196–198°C (CH_3OH/H_2O:1/1); IR (KBr) ν 3200–2400, 1690 cm^{-1}; ^1H NMR ($CDCl_3 + D_2O$) δ 5.75 (s, 1H, OCH), 6.97 (dd, 1H, H_7, J=7.9, 5.4), 7.30–7.47 (m, 6H, H_8 + Harom), 8.02 (dd, 1H, H_6, J=5.4, 1.3). Anal. $C_{13}H_{10}N_2O_2$ (C,H,N).

5.2 4-[n-(4-Aryl-1-piperazinyl)alkyl]-2H-pyrido[3,2-b]-1,4-oxazin-3(4H)-ones and 4-(n-amino-alkyl)-2H-pyrido[3,2-b]-1,4-oxazin-3(4H)-ones. General procedure

5.2.1 Method A (Scheme 1)

Compound **2** (150 mg, 1.0 mmol) was added to a solution of sodium ethylate in ethanol (prepared with

sodium, 28 mg, 1.2 mmol, in anhydrous ethanol, 6 ml). The mixture was stirred at room temperature for 1 h and then ethanol was removed under reduced pressure. The anion was dissolved in *N,N*-dimethylformamide (10 ml) and the solution treated with (2-chloroethyl)- or (3-chloropropyl)amine (1.1 mmol) added dropwise and then refluxed 2 h. After cooling, the solvent was removed and the crude mixture was treated with water and extracted with methylene chloride. The organic layers were dried over magnesium sulfate, evaporated, and chromatographied on silica gel (eluent: CH_2Cl_2/CH_3OH: 8/2).

*5.2.1.1 4-[2-(4-Phenyl-1-piperazinyl)ethyl]-2H-pyrido[3,2-b]-1,4-oxazin-3(4H)-one (**4a**).* IR (neat) ν 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.68–2.75 (m, 6H, $CH_2 + 2\times CH_{2piperaz}$), 3.15 (t, 4H, $2\times CH_{2piperaz}$, $J = 5.1$), 4,34 (t, 2H, $CONCH_2$, $J = 6,2$), 4,66 (s, 2H, OCH_2), 6.83 (t, 1H, H_{arom}, $J = 7.1$), 6.88–6.94 (m. 3H, H_7, H_{arom}), 7.19–7.28 (m, 3H, $H_8 + H_{arom}$), 8.01 (dd, 1H, H_6, $J = 5.0$, 1.2); MS (IE) m/z 338 (M^+).

*5.2.1.2 4-{2-[4-(3-Trifluoromethyl)phenyl-1-piperazinyl]ethyl}-2H-pyrido[3,2-b]-1,4-oxazin-3(4H)-one (**4b**).* IR (neat) ν 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.66–2.76 (m, 6H, $CH_2 + 2\times CH_{2piperaz}$), 3.19 (t, 4H, $2\times CH_{2piperaz}$, $J = 5.4$), 4.33 (t, 2H, $CONCH_2$, $J = 7.0$), 4.65 (s, 2H, OCH_2), 6.92 (dd, 1H, H_7, $J = 7.7$, 4.9), 7.00–7.07 (m, 2H, H_{arom}), 7.08 (s, 1H, H_{arom}), 7.22 (d, 1H, H_8, $J = 7.7$), 7.32 (t, 1H, H_{arom}, $J = 7.7$), 8.01 (dd, 1H, H_6, $J = 4.9$, 0.7); MS (IE) m/z 406 (M^+).

*5.2.1.3 4-{2-[4-(4-Fluorophenyl-1-piperazinyl)ethy]}-2H-pyrido[3,2-b]-1,4-oxazin-3(4H)-one (**4c**).* IR (KBr) ν 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.70–2.75 (m, 6H, $CH_2 + 2\times CH_{2piperaz}$), 3.07 (t, 4H, $2\times CH_{2piperaz}$, $J = 5.2$), 4.34 (t, 2H, $CONCH_2$, $J = 7.1$), 4.67 (s, 2H, OCH_2), 6.82–6.99 (m, 5H, $H_7 + H_{arom}$), 7.22 (dd, 1H, H_8, $J = 7.4$, 1.5), 8.01 (dd, 1H, H_6, $J = 4.4$, 1.5); MS (IC/NH_3) m/z 357 ($M + 1$).

*5.2.1.4 2-Phenyl-4-[2-(4-phenyl-1-piperazinyl)ethyl]-2H-pyrido[3,2-b]-1,4-oxazin-3(4H)-one (**5**).* IR (neat) ν 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.64–2.88 (m, 6H, $CH_2 + 2\times CH_{2piperaz}$), 3.13 (t, 4H, $2\times CH_{2piperaz}$, $J = 4.7$), 4.25–4.36 (m, 1H, CONCH), 4.42–4.53 (m, 1H, CONCH), 5.77 (s, 1H, OCH), 6.82 (t, 1H, H_{arom}, $J = 7.4$), 6.87–6.95 (m, 5H, $H_7 + H_{arom}$), 7.21–7.46 (m, 6H, $H_8 + H_{arom}$), 8.00 (dd, 1H, H_6, $J = 4.4$, 1.3); MS (IE) m/z 414 (M^+).

*5.2.1.5 4-[3-(4-Phenyl-1-piperazinyl)propyl]-2H-pyrido[3,2-b]-1,4-oxazin-3(4H)-one (**6a**).* IR (neat) ν 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.94 (q, 2H, CH_2, $J = 7.4$), 2.51 (t, 2H, CH_2, $J = 7.4$), 2.57 (t, 4H, $CH_{2piperaz}$, $J = 5.3$), 3.19 (t, 4H, $2\times CH_{2piperaz}$, $J = 5.3$), 4.23 (t, 2H, $CONCH_2$, $J = 7.4$), 4.66 (s, 2H, OCH_2), 6.84 (t, 1H, H_{arom}, $J = 6.6$), 6.90–6.95 (m, 3H, $H_7 + H_{arom}$), 7.19–7.32 (m, 3H, $H_8 + H_{arom}$), 8.00 (dd, 1H, H_6, $J = 5.3$, 1.3); MS (IE) m/z 352 (M^+).

*5.2.1.6 4-{3-[4-(3-Trifluoromethyl)phenyl-1-piperazinyl]propyl}-2H-pyrido[3,2-b]-1,4 -oxazin-3(4H)-one (**6b**).* IR (KBr) ν 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.94 (q, 2H, CH_2, $J = 7.7$), 2.52 (t, 2H, CH_2, $J = 7.7$), 2.62 (t, 4H, $2\times CH_{2piperaz}$, $J = 5.5$), 3.21 (t, 4H, $2\times CH_{2piperaz}$, $J = 5.5$), 4.23 (t, 2H, $CONCH_2$, $J = 7.7$), 4.65 (s, 2H, OCH_2), 6.92 (dd, 1H, H_7, $J = 8.2$, 4.6), 7.01–7.11 (m, 3H, H_{arom}), 7.21 (dd, 1H, H_8, $J = 8.2$, 1.5), 7.32 (t, 1H, H_{arom}, $J = 8.2$), 8.01 (dd, 1H, H_6, $J = 4.6$, 1.5); MS (IC/NH_3) m/z 421 ($M + 1$).

*5.2.1.7 4-{3-[4-(4-Fluorophenyl-1-piperazinyl)propyl]}-2H-pyrido[3,2-b]-1,4-oxazin-3 (4H)-one (**6c**).* IR (KBr) ν 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.94 (q, 2H, CH_2, $J = 7.3$), 2.51 (t, 2H, CH_2, $J = 7.3$), 2.60 (t, 4H, $2\times CH_{2piperaz}$, $J = 5.1$), 3.10 (t, 4H, $2\times CH_{2piperaz}$, $J = 5.1$), 4.22 (t, 2H, $CONCH_2$, $J = 7.3$), 4.65 (s, 2H, OCH_2), 6.83–6.98 (m, 5H, $H_7 + H_{arom}$), 7.21 (dd, 1H, H_8, $J = 7.9$, 1.4), 8.00 (dd, 1H, H_6, $J = 4.7$, 1.4); MS (IC/NH_3) m/z 371 ($M + 1$).

5.2.2 Method B (Scheme 3) 4-(n-Bromoalkyl)-2H-pyrido[3,2-b]-1,4-oxazin-3(4H)-ones. General procedure

To a stirred solution of pyrido[3,2-*b*]oxazin-3(4*H*)-one **2** or **3** (1 mmol) in DMF (10 ml) was added NaH (26 mg, 1.1 mmol, 60% in dispersion in oil) at room temperature. The mixture was stirred for 1 h at the same temperature. Then the 1,4-dibromobutane or 1,5-dibromopentane (1.1 mmol) diluted with DMF was added dropwise. The reaction mixture was heated at 110°C for 2 h, cooled to room temperature, and concentrated in vacuo. After addition of water (100 ml), the mixture was extracted with CH_2Cl_2. Organic layers were dried with anhydrous magnesium sulfate and the solvent was removed under vacuum. Purification by column chromatography (eluent:CH_2Cl_2) gave the pure product.

5.2.2.1 4-(4-Bromobutyl)-2H-pyrido[3,2-b]-1,4-oxazin-3(4H)-one (7). Yield 83%; Oil; IR (neat) ν 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.78–2.02 (m, 4H, $2\times CH_2$), 3.45 (t, 2H, CH_2Br, $J = 6.6$), 4.18 (t, 2H, NCH_2, $J = 6.6$), 4.66 (s, 2H, OCH_2), 6.93 (dd, 1H, H_7, $J = 7.9$, 4.8), 7.22 (dd, 1H, H_8, $J = 7.9$, 1.5), 8.01 (dd, 1H, H_6, $J = 4.8$, 1.5). Anal. $C_{11}H_{13}BrN_2O_2$ (C,H,N).

*5.2.2.2 4-(4-Bromobutyl)-2-phenyl-2H-pyrido[3,2-b]-1,4-oxazin-3(4H)-one (**8**).* Yield 68%; Oil; IR (neat) ν 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.72–2.00 (m, 4H, $2\times CH_2$), 3.34–3.50 (m, 2H, CH_2Br), 4.05–4.30 (m, 2H, NCH_2), 5.75 (s, 1H, OCH), 6.87 (dd, 1H, H_7, $J = 8.1$,

4.4), 7.17–7.35 (m, 6H, H_8, + H_{arom}), 7.93 (dd, 1H, H_6, J = 4.4, 1.5). Anal. $C_{17}H_{17}BrN_2O_2$ (C,H,N).

5.2.2.3 4-(5-Bromopentyl)-2H-pyrido[3,2-b]-1,4-oxazin-3(4H)-one (9). Yield 80%; mp 76–78°C; IR (KBr) ν 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.47–1.58 (m, 2H, CH_2), 1.63–1.78 (m, 2H, CH_2), 1.87–1.98 (m, 2H, CH_2), 3.41 (t, 2H, CH_2Br, J = 6.6), 4.14 (t, 2H, NCH_2, J = 6.6), 4.65 (s, 2H, OCH_2), 6.93 (dd, 1H, H_7, J = 8.1, 4.4), 7.21 (dd, 1H, H_8, J = 8.1, 1.5), 8.01 (dd, 1H, H_6, J = 4.4, 1.5). Anal. $C_{12}H_{15}BrN_2O_2$ (C,H,N).

5.2.3 4-[n-(4-Aryl-1-piperazinyl)alkyl]-2H-pyrido[3,2-b]-1,4-oxazin-3(4H)-ones and 4-(n-amino-alkyl)-2H-pyrido[3,2-b]-1,4-oxazin-3(4H)-ones. General procedure

A mixture containing the bromo compound **7**, **8**, or **9** (1 mmol), the appropriate amine (1.5 mmol), diisopropylethylamine (193 mg, 1.5 mmol), and CH_3CN (10 ml) was heated at 60°C for 6 h. After cooling, the mixture was concentrated in vacuo. The resulting residue was poured into water, extracted with CH_2Cl_2, and dried over anhydrous magnesium sulfate. After evaporation of the solvent under vacuum, the desired product was purified by column chromatography (eluent: CH_2Cl_2/MeOH: 95/5).

5.2.3.1 4-[4-(4-Phenyl-1-piperazinyl)butyl]-2H-pyrido[3,2-b]-1,4-oxazin-3(4H)-one (10a). IR (KBr) ν 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.54–1.68 (m, 2H, CH_2), 1.68–1.82 (m, 2H, CH_2), 2.46 (t, 2H, CH_2, J = 7.6), 2.62 (t, 4H, 2×$CH_{2piperaz}$, J = 5.1), 3.22 (t, 4H, 2×$CH_{2piperaz}$, J = 5.1), 4.20 (t, 2H, $CONCH_2$, J = 7.6), 4.68 (s, 2H, OCH_2), 6.85 (t, 1H, H_{arom}, J = 5.5), 6.90–7.00 (m, 3H, H_7 + H_{arom}), 7.20–7.30 (m, 3H, H_8 + H_{arom}), 8.01 (dd, 1H, H_6, J = 5.3, 1.3); MS (IE) m/z 366 (M^+).

5.2.3.2 4-{4-[4-(3-Trifluoromethyl)phenyl-1-piperazinyl]butyl}-2H-pyrido[3,2-b]-1,4-oxazin-3(4H)-one (10b). IR (KBr) ν 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.53–1.67 (m, 2H, CH_2), 1.67–1.79 (m, 2H, CH_2), 2.43 (t, 2H, CH_2, J = 7.3), 2.58 (t, 4H, 2×$CH_{2piperaz}$, J = 6.5), 3.22 (t, 4H, 2×$CH_{2piperaz}$, J = 6.5), 4.17 (t, 2H, $CONCH_2$, J = 7.3), 4.69 (s, 2H, OCH_2), 6.94 (dd, 1H, H_7, J = 7.7, 4.7), 7.05–7.14 (m, 2H, H_{arom}), 7.16 (s, 1H, H_{arom}), 7.22 (dd, 1H, H_8, J = 7.7, 1.2), 7.38 (t, 1H, H_{arom}, J = 8.3), 8.00 (dd, 1H, H_6, J = 4.7, 1.2); MS (IE) m/z 435 (M^+).

5.2.3.3 4-{4-[4-(4-Fluorophenyl-1-piperazinyl)butyl]}-2H-pyrido[3,2-b]-1,4-oxazin-3(4H)-one (10c). IR (KBr) ν 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.55–1.67 (m, 2H, CH_2), 1.68–1.80 (m, 2H, CH_2), 2.45 (t, 2H, CH_2, J = 7.4), 2.58 (t, 4H, 2×$CH_{2piperaz}$, J = 4.9), 3.12 (t, 4H, 2×$CH_{2piperaz}$, J = 4.9), 4.18 (t, 2H, $CONCH_2$, J = 7.4), 4.67 (s, 2H, OCH_2), 6.83–6.98 (m, 5H, H_{arom}), 7.21 (dd, 1H, H_8, J = 8.1, 1.5), 8.01 (dd, 1H, H_6, J = 5.5, 1.5); MS (IC/NH_3) m/z 385 (M + 1).

5.2.3.4 2-Phenyl-4-[4-(4-phenyl-1-piperazinyl)butyl]-2H-pyrido[3,2-b]-1,4-oxazin-3(4H)-one (11). IR (KBr) ν 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.47–1.61 (m, 2H, CH_2), 1.66–1.78 (m, 2H, CH_2), 2.38 (t, 2H, CH_2, J = 7.4), 2.52 (t, 4H, 2×$CH_{2piperaz}$, J = 4.9), 3.13 (t, 4H, 2×$CH_{2piperaz}$, J = 4.9), 4.09–4.25 (m, 2H, $CONCH_2$), 5.69 (s, 1H, OCH), 6.78 (t, 1H, H_{arom}, J = 7.1), 6.83–6.89 (m, 3H, H_7 + H_{arom}), 7.16–7.32 (m, 8H, H_8 + H_{arom}), 7.94 (d, 1H, H_6, J = 4.4); MS (IC/NH_3) m/z 443 (M + 1).

5.2.3.5 4-[5-(Phenyl-1-piperazinyl)pentyl]-2H-pyrido[3,2-b]-1,4-oxazin-3(4H)-one (12). IR (KBr) ν 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.38–1.46 (m, 2H, CH_2), 1.54–1.64 (m, 2H, CH_2), 1.67–1.77 (m, 2H, CH_2), 2.39 (t, 2H, CH_2, J = 7.7), 2.59 (t, 4H, 2×$CH_{2piperaz}$, J = 5.0), 3.20 (t, 4H, 2$CH_{2piperaz}$, J = 5.0), 4.14 (t, 2H, $CONCH_2$, J = 7.7), 4.65 (s, 2H, OCH_2), 6.84 (t, 1H, H_{arom}, J = 7.4), 6.89–6.94 (m, 3H, H_7 + H_{arom}), 7.19–7.28 (m, 3H, H_8 + H_{arom}), 8.01 (dd, 1H, H_6, J = 5.2, 1.5); MS (IC/NH_3) m/z 381 (M + 1).

5.2.4 Method C (Scheme 4)

5.2.4.1 7-Bromo-2H-pyrido[3,2-b]-1,4-oxazin-3(4H)-one (13). To a stirred solution of 2*H*-pyrido[3,2-*b*]-1,4-oxazin-3(4*H*)-one **2** (5.32 g, 23.25 mmol) in DMF (100 ml) was added bromine (1.28 ml, 25.58 mmol) slowly at room temperature and the reaction was stirred at this temperature over 2 h. Addition of water (50 ml) allowed the precipitation of compound **13**. After filtration, washing with water, and drying, **13** was isolated pure in 80% of yield; mp 224-226°C; IR (KBr) ν 3200–3100, 1700 cm^{-1}; ^1H NMR (DMSO + D_2O) δ 4.63 (s, 2H, OCH_2), 7.60 (d, 1H, H_8, J = 1.6); 7.84 (d, 1H, H_6, J = 1.6); MS (IC/NH_3) m/z 229 (M + 1). Anal $C_7H_5BrN_2O_2$ (C,H,N).

5.2.4.2 7-Bromo-2H-4-methylpyrido[3,2-b]-1,4-oxazin-3(4H)-one (14). To a stirred solution of 7-bromo-2*H*-pyrido[3,2-*b*]-1,4-oxazin-3(4*H*)-one **13** (458 mg, 2 mmol) in DMF (10 ml) was added sodium hydride (53 mg, 2.20 mmol, 80% in dispersion in oil) at room temperature and the reaction was stirred at this temperature for 1 h. A solution of iodomethane (426 mg, 3.0 mmol) in DMF (0.5 ml) was then added slowly to the reaction. Then the mixture was stirred 2 h at 110°C. After cooling and concentration, the resulting residue was poured into water, extracted with CH_2Cl_2, and dried over magnesium sulfate. After evaporation the resulting product was purified by silica gel column chromatography (eluent: CH_2Cl_2) to provide 87% of **14** as a crystalline

product; mp 130–132°C; IR (KBr) ν 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.44 (s, 3H, NCH_3), 4.68 (s, 2H, OCH_2), 7.35 (d, 1H, H_8, J=1.5), 8.04 (d, 1H, H_6, J=1.5). Anal. $C_8H_7BrN_2O_2$ (C,H,N).

5.2.4.3 (2H-4-Methyl-3-oxopyrido[3,2-b]-1,4-oxazin-7-yl)ethanone (15). To a stirred solution of 6-bromo-2*H*-4-methylpyrido[3,2-*b*]-1,4-oxazin-3(4*H*)-one **14** (530 mg, 2.18 mmol) in *N*,*N*-dimethylformamide (5 ml) were added triethylamine (0.44 g, 4.36 mmol), butylvinyl ether (1.2 g, 12 mmol), 1,2-bis(diphenylphosphino) ethane (24 mg, 0.06 mmol), and palladium (II) acetate (12 mg, 0.054 mmol). The mixture was heated at reflux, under argon, for 8 h. After cooling to room temperature, HCl (10%) was added and after 1 h of stirring, the mixture was concentrated under reduced pressure. The residue was partitioned between CH_2Cl_2 and water. The organic layer was dried over $MgSO_4$ and concentrated. Purification by flash chromatography (eluent: CH_2Cl_2/AcOEt: 8/2) furnished 81% of **15** as a crystalline product; IR (KBr) ν 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.59 (s, 3H, CH_3), 3.53 (s, 3H, NCH_3), 4.72 (s, 2H, OCH_2), 7.74 (d, H_8, J=1.4), 8.61 (d, H_6, J=1.4); MS (IC/NH_3) m/z 207 (M + 1).

5.3 Pharmacological methods

5.3.1 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 and 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 [25,26].

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 [27].

5.3.2 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 [28]. 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 [27].

5.3.3 Hot-plate test in mice

According to the method described by Eddy [29], 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 was administered 1 h (po) or 30 min (ip) before the test.

5.3.4 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 [30,31].

5.3.5 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, respectively, for receptors μ and σ [32,33]. For receptors H_1 and H_2, membranes were prepared from guinea pig cerebral cortex and incubated with [^3H]pyrilamine and [^3H]tiotidine, respectively [34]. For receptor K, membrane was prepared from guinea pig cerebellum incubated with [^3H]U 69593 [35]. 5-HT_{1A} assays used rat hippocampus membranes, [^3H]-8-OH-DPAT, and buspirone for non specific binding (NSB) [36], 5-HT_2 assays used calf frontal cortex, [^3H]ketanserin, and spiperone for NSB [37]. For α_1 and α_2 receptors, membranes were prepared from rat brain incubated respectively with [^3H]prazosin [38] and [^3H]rauwolscine [39].

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 coun-

ter. Triplicates of each compound were determined at two concentrations (10^{-7} and 10^{-5} M).

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