Synthesis, Toxicological, and Pharmacological Assessment of Derivatives of 2-Aryl-4-(3-arylpropyl)morpholines

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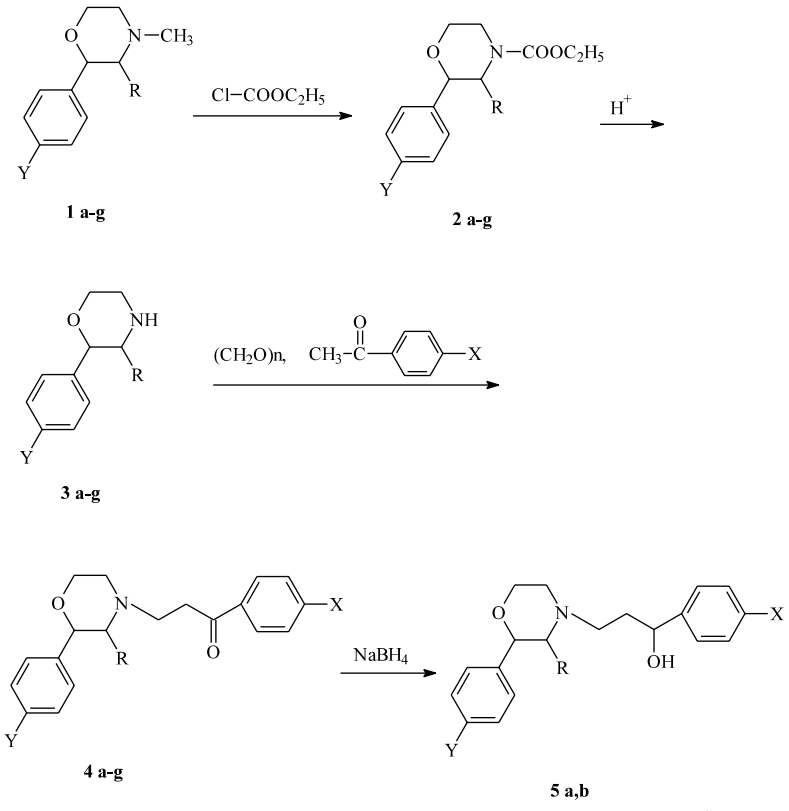
***Key Words**: 2-Aryl-4-(3-arylpropyl) morpholine derivatives; acute toxicity; antidepressive activity; MAO inhibition*

Summary

The synthesis of nine original morpholine derivatives, i.e.2-aryl-4-(3-arylpropyl)morpholines, is described. The structure of all synthesised derivatives was proved by IR and ^{1}H-NMR, and some of them by ^{13}C-NMR. Acute toxicity studies of the compounds were performed on mice. A comparative pharmacological study of the in vivo effects on the central nervous system was undertaken using the screening tests: hexobarbital induced sleeping time; locomotor activity; and behaviour despair (for antidepressive activity). The most active compound 4-(2-benzoylethyl)-2-phenyl-3-methyl) morpholine **4e** was studied for MAO-A and MAO-B inhibition in vitro in rat brain mitochondria preparations.

Introduction

Recent years have seen a revival of interest in antidepressants with MAO inhibitory activity. This was made possible after introduction into clinical practice of new selective reversible MAO-A inhibitors such as moclobemide, brofaromine, and others [1,2]. Some drugs of this new group of compounds contain a substituted morpholine ring in their structure [3,4]. We describe here the synthesis, pharmacological, toxicological, and biochemical investigations of nine new 2-aryl-4-(3-arylpropyl) morpholine derivatives.



Scheme 1

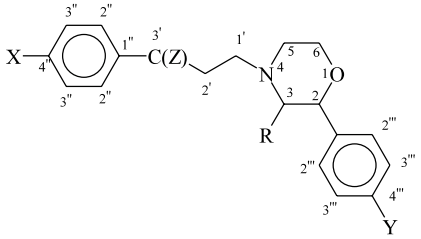
  0365-6233/98/1111/0342 $17.50 +.50/0

Results and Discussion

Synthesis

The starting 2-arylmorpholines **3a–g** were obtained from phenacyl chloride or from its halogenated derivatives, 2-methylaminoethanol and formic acid, cyclized to *N*-methylmorpholines [5]. *N*-Demethylation through interaction with ethyl chloroformate and subsequent hydrolysis led to 2-arylmorpholines [6]. The 4-(2-aroylethyl)-2-arylmorpholines **4a–g** were obtained by Mannich condensation of 2- or 2,3-disubstituted morpholines **3** with paraform and substi-

Table 1. ^{1}H chemical shifts (δ values) of the compounds.



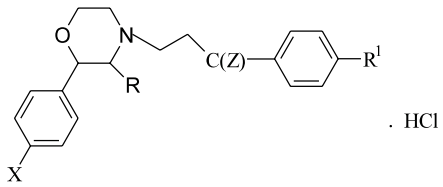
4: Z= C=O

5: Z= CH–OH

Compound	H-2	4H(3H)-3,5	2H-6	2H-1′	2H-2′	1H-3′	H-R
4a	4.20d	2.19–2.60m	3.88–4.04m	3.22t	2.84t	–	
4b	4.16d	2.18–2.60m	3.85–4.03m	3.22t	2.85t	–	
4d	4.14d	2.20–2.60m	3.84–4.02m	3.20t	2.85t	–	
4e	4.12d	2.90(H-3)	3.83–4.02m	3.21t	2.88t	–	
		2.54–2.78(2H-5)					
4g	4.12d	2.88d(H-3)	3.82–4.04m	3.22t	2.89t	–	0.90dd A_2B_2
		2.54–2.81(2H-5)					
5a	4.68dd	2.20–2.65m	3.83–4.04m	2.81	2.33t	4.87t	
5b	4.84d	2.53–2.77m(2H-5)	3.84–3.99m	2.84t	2.10t	4.90t	0.90dd A_2B_2

*Examples of coupling *J* (Hz): 4a: H-2, dd, J_1 = 10.5, J_2 = 2.0; 2H-1′, t, *J* = 7.0; 2H-2′, q, *J* = 7.0; H-2″,2‴, d, *J* = 6.9; H-3″, 3‴, d, *J* = 6.9.

Table 2. Amino ketones and amino alcohols.



. HCl

Compound	Z	X	R	R^1	Mp (°C)	Yield (%)
4a	C=O	Br	H	Br	182–186	41
4b	C=O	Cl	H	H	235–237	43
4c	C=O	Cl	H	Cl	175–178	78
4d	C=O	Cl	H	Br	189–192	40
4e	C=O	H	CH_3	H	148–151	63
4f	C=O	H	CH_3	Cl	187–190	51
4g	C=O	H	CH_3	Br	169–171	21
5a	CHOH	Br	H	Br	141–145	27
5b	CHOH	H	CH_3	H	80–82	61

Table 3. Acute toxicity (LD_{50}) of the compounds and moclobemide.

Compound	LD_{50} (mg/kg ip.) and 95% confidence interval
4a	>20.04
4b	1.11 (1.06–1.17)*
4c	3.74 (2.23–4.98)*
4d	6.23 (4.43–8.74)*
4e	10.42 (8.55–11.45)*
4f	6.45 (5.42–7.58)*
4g	21.27 (19.24–23.17)
5a	9.23 (8.46–10.16)*
Moclobemide	20.31 (17.31–23.09)

* $p \leq 0.05$, statistically significant different compared to moclobemide.

Table 4. Effect of the compounds on the hexobarbital sleeping time.

Compound	Doses 1/10 of LD_{50} (mmol/kg ip.)	Sleeping time (min) ($x \pm SD$)
Control group	--	37.6–7.7
4a	2.0	38.0–7.3
4b	0.11	47.5–12.3
4d	0.62	49.0–12.4
4e	0.10	73.4–6.2*
4g	2.13	23.8–8.5 *
5a	0.92	54.8–16.4
Moclobemide	2.03	61.6–11.5*

* $p \leq 0.05$, statistically significant compared with the control group.

tuted acetophenones in ethanol and heating for 5–6 h. Further, the 4-(2-aroylethyl)-2-arylmorpholines reacted with sodium borohydride in methanol-water solution to give the 4-(3-aryl-3-hydroxy)-2-arylmorpholines **5a–b** in 27–96% yield.

The structure of the compounds **4a–g** and **5a,b** was confirmed by IR. and ^{1}H-NMR spectroscopy. The data of the ^{1}H-NMR spectra of the compounds are shown in Table 1. The hydroxyl protons were as broad singlets in the region of 5.70–6.30 ppm. They were not well observed.

That is why we decided to look for a method to make these protons more sharp and observable. We recorded a number of spectra of the compounds with graduated addition of shift reagent – $Eu(FOD)_3$. The main result of the application of the shift reagent was that the hydroxyl groups were clearly observed as a singlet in the region of 12–14 ppm. These spectra showed that the europium complex was formed with the oxygen from the hydroxyl group and it was not attached to O- or N-atom from the morpholine ring (the chemical shifts of the morpholine protons were not strongly influenced). This phenomenon could be explained by the relatively low basicity of the nitrogen in the morpholine ring and by steric factors [7](Table 1).

The structures of some of the compounds were also confirmed by ^{13}C-NMR spectra. They were in accordance with the data of the other spectral methods and confirmed the structures. Table 2 lists data pertaining to chemical structure, mp, and yield of the synthesised compounds.

Pharmacology

The newly synthesised compounds were assayed for acute toxicity, influence on locomotor activity. and hexobarbital sleeping time. Compounds were tested also for in vivo antidepressive activity using the "behaviour despair" test. The most active compound **4e** was studied to ascertain its possible influence on MAO-A and MAO-B activity of rat brain mitochondria. Moclobemide was used as a reference compound.

Analysis of the experimental data on the acute toxicity (LD_{50}) of the compounds as compared to moclobemide showed that the acute toxicity of some of the compounds was statistically significantly higher than that of moclobemide (Table 3). The effects of the compounds in doses 1/10 of LD_{50} on hexobarbital induced sleeping time are shown in Table 4. Compounds **4e** and moclobemide statistically significantly increased the hexobarbital sleeping time.

As shown by the experiments on the influence of the compounds on locomotor activity in mice, all the tested compounds, except compound **4e**, decreased the locomotor activity (Table 5).

Compounds **4e** and **5a** exerted antidepressive activity in vivo according to the behaviour despair test. The compound **5a** showed antidepressive activity only in a dose which was 1/10 of LD_{50}. Compound **4e** dose-dependently decreased the time of immobilisation in doses 1/20 and 1/10 of LD_{50}. All other compounds did not show antidepressive activity at doses 1/10 of LD_{50}. The reference compound moclobemide expressed antidepressive activity in doses 1.01 (1/20 of LD_{50}) and 2.03 mmol/kg (1/10 of LD_{50}) body weight ip (Table 6).

Compound **4e** showed a low degree of MAO-A and MAO-B inhibitory activity. The IC_{50} was 221 and 238 mmol/l respectively (Table 7). These values were less than those of moclobemide – 54 mmol/l and 464 mmol/l.

Conclusions

The results of the in vivo pharmacological screening experiments showed that compound **4e** had an appreciable antidepressive activity comparable to that of moclobemide in equitoxic doses. The acute toxicity of compound **4e** was statistically significantly higher then that of the standard drug moclobemide. On the other hand this compound failed to decrease significantly locomotion (sedative effects), which could be an advantage. Compound **4e** is a poor MAO-A and MAO-B inhibitor in vitro. Bearing in mind similar data for moclobemide [8], we suggest, that this good antidepressive activity in vivo may be due to an active product of liver metabolism with potent MAO inhibition. On the other hand, it is possible that the mechanism of action of these compounds is related with receptor antagonism, for example 5HT and adrenergic receptors [1]. More compounds have to be synthesised in order to evaluate structure-activity relationships.

Table 5. Effects of the compounds of the total locomotor activity on mice over 90 min observation.

Compound	Doses 1/10 of LD_{50} (mmol/kg ip.)	Total locomotor activity in arbitrary units ± SD
Control group	–	1951 ± 216
4a	2.0	533 ± 45*
4b	0.11	1051 ± 61*
4d	0.62	1604 ± 101*
4e	0.10	1766 ± 197
4g	2.13	834 ± 52*
5a	0.92	1236 ± 156*
Moclobemide	2.03	562 ± 68.1*

* $p \leq 0.05$, statistically significant difference compared with the control group.

Table 6. Effect of the compounds on the time of immobilization by behaviour despair test.

Compound	Doses (mmol/kg ip.)	Time of immobilization (s) ± SD
Control group	–	167.2 ± 39.4
4a	1.02	242.1 ± 44.4
4b	0.055	180.0 ± 38.1
4d	0.31	236.0 ± 34.2
4e	0.10	22.5 ± 6.4[ab]
4e	0.05	59.0 ± 18.1[a]
4g	1.07	227.5 ± 9.6
5a	0.46	68.3 ± 21.4[a]
Moclobemide	2.03	76.1 ± 14.0[ab]
Moclobemide	1.01	106.2 ± 15.3[a]

[a] $p \leq 0.05$, statistically significant compared with the control group.
[b] $p \leq 0.05$, statistically significant compared with the control group treated with dose 1/20 LD_{50}.

Experimental

Chemistry

Melting points were measured on a Boetius hot plate microscope (Germany) and were corrected. IR spectra (Nujol) were recorded on a UR 20 (Karl Zeiss, Jena) apparatus. ^{1}H-NMR spectra were recorded at room temperature on a Bruker WP 100 (100 MHz) spectrometer in $CDCl_3$. Chemical shifts are given in ppm; TMS was used as internal standard. ^{13}C-NMR spectra were recorded at room temperature on a Bruker WP 100 (25.18 MHz). TLC was performed on 0.25 mm precoated plates Kiselgel 60 Merck (Germany) with chloroform/ light petroleum/acetone/methanol (4:4:1.5:0.5) and detected by Dragendorf reagent. The novel structures were supported by microanalyses (Micro analytical Unit, Faculty of Pharmacy, Sofia) and the characteristic IR and NMR data quoted. All the elemental analyses (C,H,N) were within ±0.4% of the calculated values.

General Method for 2-Arylmorpholines **3a–g**

The respective 4-methyl-2-arylmorpholine (6 mmol) was dissolved in 10 ml of anhydrous benzene and ethyl chloroformate (12 mmol) dissolved in 5 ml anhydrous benzene was added dropwise. The reaction mixture was stirred for 2 h. After that, the mixture was treated with 50–60 ml water and conc. hydrochloric acid was added to pH 3–4. The benzene solution was dried over Na_2SO_4, filtered and evaporated on a rotary evaporator. The crude product was added to 30 ml 60% sulphuric acid. The reaction mixture was heated for 3 h, 50 ml water was added, and the water solution was treated with charcoal and filtered. The filtrate was treated with 50% NaOH to alkaline (pH 8–9), extracted with ether and the ethereal solution dried over Na_2SO_4. After filtration and evaporation to the crude product was added saturated HCl/ether (pH 5). The separated precipitate was filtered and recrystallized from ethanol.

General Method for 2-Aryl-4-(3-aroylpropyl)morpholines **4 a–g**

The respective 2- or 2,3-disubstituted morpholine hydrochloride (14 mmol) was dissolved in 15 ml ethanol, paraform (28 mmol), accordingly substituted acetophenone(14 mmol) and 1 ml conc. HCl was added. The mixture was heated under reflux for 5–6 h. Ethanol was distilled under vacuum and the crude product was recrystallized from ethanol (Table 2).

Preparation of 4-(2-Benzoylethyl)-2-(4-chlorophenyl)morpholine Hydrochloride **4b**

To 1.00 g (4.3 mmol) 2-(4-chlorophenyl) morpholine hydrochloride, dissolved in 10 ml ethanol, was added 0.38 g (12.9 mmol) paraform, 0.52 g (4.3 mmol) acetophenone and 1 ml conc. HCl. The reaction mixture was refluxed for 7 h. The solvent was removed on a rotary evaporator. The crude 4-(2-benzoylethyl)-2-(4-chlorophenyl) morpholine hydrochloride was recrystallized from ethanol to give 0.68 g (43%) **4b**, mp 235–237 °C.

Preparation of 4-[3-(4-Bromophenyl)-3-hydroxypropyl]-2-(4-bromophenyl)morpholine **5a**

4-{2-(4-Bromobenzoyl)ethyl}-2-(4-bromophenyl) morpholine hydrochloride (1.7 g, 3.3 mmol) was dissolved in 35 ml methanol and a solution of 0.18 g (3.3 mmol) KOH in 7 ml methanol was added. To the mixture was added dropwise a solution of 0.27 g (7 mmol) $NaBH_4$ dissolved in 5 ml of

Table 7. IC50 values (μM) for MAO inhibition (rat brain mitochondria) by derivate **4b**, moclobemide, and deprenil.

Substrate	**4e**	Moclobemide	Deprenil
5-Hydroxytryptamine	221 (148–297)	54 (37–72)	4.1 (3.2–4.9)
Thyramine	238 (167–306)	464 (396–527)	39 (18–51)
Betaphenylethylamine	127 (84–171)	270 (221–319)	0.92 (0.71–1.10)

Data represent the mean values for 3–5 independent experiments.

water. The mixture was heated under reflux for 10 h. The solvents were evaporated on a rotary evaporator. To the crude product was added dilute HCl (1:1) to pH 3–4. The non-basic products were extracted with ether. The ethereal solution was dried and the ether removed. The crude product was recrystallized from acetone. Yield of **5a** – 0.41 g (27%); mp 142–145 °C (Table 2).

Preparation of 4-[3-Phenyl)-3-hydroxypropyl]-2-(4-phenyl)-3-methylmorpholine **5b**

From 3.45 g (10 mmol) **4e**, 0.56 g (10 mmol) KOH, and 0.74 g (20 mmol) $NaBH_4$ in 20 ml methanol, after refluxing for 10 h was obtained 1.89 g (61%) of the product **5b**, recrystallized from acetone. Mp 80–82 °C (Table 2).

Pharmacology

Materials and Methods

The experiments were performed on 368 male white mice with body weight 18–22 g and 5 male Wistar rats (160–200 g body weight). Acute toxicity (LD_{50}) of the studied compounds was assessed by dissolving them in saline (0.9% NaCl), and administering to mice by the intraperitoneal (ip) route in 4 or 5 different doses (6 animals in group for each dose) and calculated by the method of Litchfield-Wilcoxon [9].

Influence on Hexobarbital Sleeping Time (HbST)

The studied compounds were administrated to male mice ip at doses 1/10 of LD_{50} (the same volume – 0.1 ml/10 g b/w of the solvent – 0.9% NaCl, was administered to the controls). The solution of hexobarbital sodium (dose 80 mg/kg body weight) was administered ip to the animals 30 min after the administration of the compounds. Sleeping time was measured in minutes by observing the righting reflex recovery.

Influence on Locomotor Activity

A control group of 6 animals was put in an actometer (Activity Cage, Ugo Basille, Italy) and the locomotor activity was determined in arbitrary units at 10 min intervals for 90 min. The tested compounds in dose 1/10 of LD_{50} were administered to the animals and they were tested under analogous conditions. The total locomotor activity was compared to that of the control (vehicle-treated) group.

The antidepressive activity of the compounds was examined using the screening test "behaviour despair" [10], calculating the time of immobilisation of the mice in seconds over a 5 min observation.

Biochemical Evaluations

Evaluation of the Degree of MAO Inhibition

Rat brain mitochondria preparations and MAO assay procedure were performed according to Meyerson et al. [11]. The ammonia formed was measured according to fluorimetric assay, proposed by Taylor et al. [12].

Substrate concentrations in the MAO inhibition assay were 1 mmol/l serotonine (type A), 0.1 mmol/l betaphenylethylamine (type B), 1 mmol/l tyrosine (type A+B). Enzyme preparation were preincubated with the inhibitors for 15 min at 37 °C. IC_{50} values represent mean values of determination in 3 to 5 rat brain preparations. Inhibitor concentrations over the range 0.01 –100 mmol/l were used .

The MAO inhibition activity in the absence of the tested compounds amounted to 6.12 ± 0.55 (MAO-A) 2.62 ± 0.25 (MAO–B), 6.98 ± 0.30 (MAO-A+B) mmol/l NH_4^+/min/mg of protein. Protein concentrations were determined by the method of Lowry et al. [13].

The results of pharmacological and biochemical experiments underwent statistical processing by the Student-Fisher t-test at level of significance $p \leq 0.05$.

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