Department of Pharmaceutical Chemistry[1], Department of Pharmacology and Toxicology[2], and Faculty of Pharmacy, Sofia, Bulgaria

Synthesis, toxicological and pharmacological assessment of morpholinooximes

P. D. Avramova[1], N. D. Danchev[2] and R. T. Buyukliev[3]

The synthesis, pharmacology and toxicology of four morpholine derivatives from 1-(2-arylmorpholino)-3-phenyl-3-propanonoxime and the synthesis of two anilides are described. The structures of the synthesized derivatives were proved by IR, ^{1}H NMR and occasionally with ^{13}C NMR. The acute toxicity of the compounds in mice was determined. A comparative pharmacological study of the *in vivo* effect on the central nervous system was realised by the following screening tests: pentobarbital induced sleeping time, locomotor activity and behaviour despair test for antidepressive activity. The most active compound was 1-(2-phenylmorpholino)-3-phenyl-3-propanonoxime (**2b**) which showed low toxicity and antidepressive activity at a dose of 1/10 LD_{50}.

1. Introduction

Moclobemide and fluvoxamine belong to the class of atypical antidepressants, which are widely used in the therapy of depression [1, 2]. Moclobemide is a reversible MAO-A inhibitor [3], while fluvoxamine is an inhibitor of the 5-HT uptake [4]. The present paper deals with the synthesis and pharmacological activity of four new compounds, chemically related to moclobemide (morpholine ring) and fluvoxamine (oxime group).

2. Investigations, results and discussion

The 2-aryl-4-(2-benzoylethyl)morpholines **1a–d** were obtained through Mannich condensation of morpholine or 2-arylmorpholines with paraformaldehyde and acetophenones. Further the 2-aryl-4-(2-benzoylethyl)morpholines reacted with hydroxylamine to yield the 1-(2-aryl morpholino)-3-phenyl-3-propanone oximes **2a–d** in 31–75% yield.

By a Beckmann rearrangement of ketoximes with phosphorus(V) chloride the aminoanilides **3a, c** were obtained (oil liquids) [5]. They were isolated with methyl iodide as their methoiodides **4a, c** in 70–85% yield (Scheme).

The structure of compounds **2a–d** and **4a, c** was confirmed by IR and ^{1}H NMR spectroscopy. IR spectra of the compounds were recorded on solid state in nujol. The C=N groups absorb in the 1603–1630 cm^{-1} region. All aminooximes have absorption in the 3159–3180 cm^{-1} region, due to the hydroxyl groups. In the proton NMR spectra of **2a–d**, there is a signal in weak field at approx. 11.2 ppm due to the oxime group. The signal is sharp except for compound **2d**. The chemical shift of this signal cannot give a definite answer whether the hydroxyl group is in syn- or anti-configuration to the benzene ring. After addition of the shift reagent ($Eu(FOD)_3$) to the compound **2d** the chemical shifts of the aromatic protons do not change significantly, which is an evidence that this group is in anti-position to the benzene fragment. With the aminoamides **3a, c** a signal between 10.0 and 10.15 ppm is observed, which is the signal of the amide proton. Theoretically, it is possible to obtain two isomers because of the Beckmann's rearrangement (A and B).

The isomers can be distinguished by means of selective decoupling of the amide proton and with model compounds [6]. After selective decoupling of the amide proton in **4a** · CH_3I only a very small increase in the signal intensity of the methylene group at 2.90 ppm was observed and the kind of the signal was not influenced. These circum-

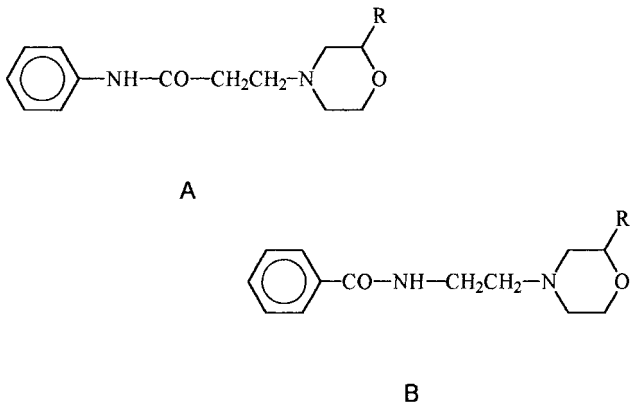
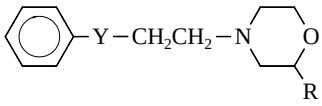
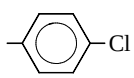
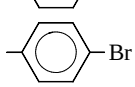
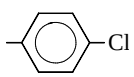


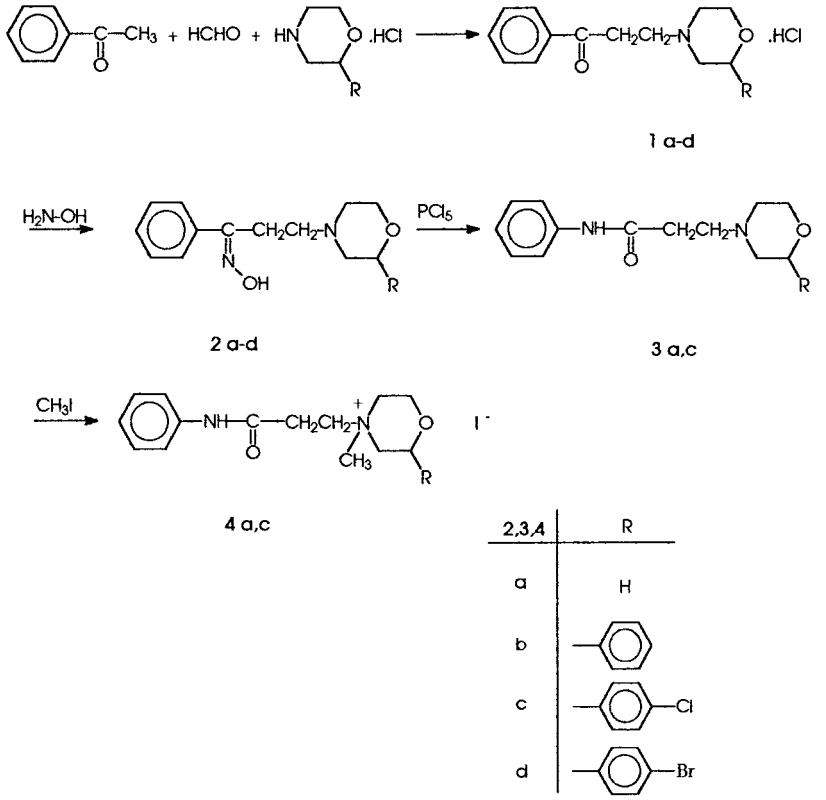
Table 1: Physical and analytical data of compounds 2a–d and 4a–c



Compd.	Y	R	M.p. (°C)	Yield (%)	Formula (molecular mass)
2a	C(=NOH)	H	150–152	40	$C_{13}H_{18}O_2$ (234.3)
2b	C(=NOH)		151–153	82	$C_{19}H_{22}N_2O_2$ (310.4)
2c	C(=NOH)		129–131	46	$C_{19}H_{21}ClN_2O_2$ (344.8)
2d	C(=NOH)		156–158	35	$C_{19}H_{21}BrN_2O_2$ (389.3)
4a*	NH–CO	H	146–148	86	$C_{14}H_{21}IN_2O_2$ (376.2)
4c*	NH–CO		160–161	84	$C_{19}H_{21}ICIN_2O_2$ (486.8)

* as methoiodide salts

Scheme



stances confirm a presence of a Ar–NH–CO–CH_2-, instead of a Ar–CO–NH–CH_2-group, because in the second case the multiplicity and the dimensions of the CH_2 signal should be changed. The weak increase of the signal, due to the Overhauser effect, shows that the methylene group is not located in direct neighbourhood to the –NH-group; otherwise, a greater increase of the signal would be expected.

In the ^{13}C NMR spectra of **2a**–**d**, a good correlation between the positions of the carbon atoms and their chemical shifts is observed. The region of the oxime carbon atom is 153–158 ppm; the aliphatic and aromatic carbon atoms give signals in their usual regions.

The newly synthesized compounds were investigated in mice for acute toxicity and their influence on pentobarbital sleeping time. The compounds were tested for *in vivo* antidepressive activity using the "behaviour despair" test. The compounds were also studied for the influence on spontaneous locomotor activity of mice. Moclobemide was used as a reference compound in all the pharmacological tests.

Determination of the acute toxicity (LD_{50}) showed that compounds **2b, 2c** and **2d** were less toxic than the standard moclobemide. Compound **2a** had a significantly higher acute toxicity than moclobemide (Table 2). The effect of the compounds on pentobarbital induced sleeping time at doses 1/10 and 1/20 of LD_{50} are shown in Table 3. All tested compounds and moclobemide statistically sinnificant caused a prolongation of the pentobarbital sleeping time.

As shown by the influence of the compounds on locomotor activity of mice all tested compounds and moclobemide decreased the locomotor activity (Table 4) except compound **2b**.

The antidepressive activity was determined by the behaviour despair test; the results are shown in Table 5. Compound **2b** showed antidepressive activity at the dose which was 1/10 of LD_{50} (0.32 mmol/kg i.p.). All other compounds did not show any antidepressive activity at doses 1/10 and 1/20 of LD_{50}. The reference compound

Table 2: Acute toxicity (LD_{50}) of the compounds

Compd.	LD_{50} (mmol/kg i.p.) and 95% confidence interval
2a	0.94 (0.90 ÷ 0.99)*
2b	3.18 (2.46 ÷ 3.11)*
2c	>11.61*
2d	>10.27*
Moclobemide	2.22 (1.89 ÷ 2.52)

* $p \leq 0.05$, statistically significant difference in comparison with moclobemide

Table 3: Effect of the compounds on the pentobarbital sleeping time

Compd.	Doses (part of LD_{50})	Sleeping time (min)
Control	–	34 ± 7.63
2a	1/10	59 ± 3.16*
2a	1/20	56.4 ± 3.13*
Control	–	34 ± 7.63
2b	1/10	71.3 ± 4.89*
2b	1/20	> 80*
Control	–	35 ± 5.3
2c	1/10	> 90*
2c	1/20	> 90*
Control	–	34 ± 7.63
2d	–	–
2d	1/20	105.4 ± 17.8*
Control	–	37.6 ± 7.7
Moclobemide	1/10	61.6 ± 11.5*
Moclobemide	1/20	–

* statistically significant difference in comparison with the control group

Table 4: Effect of the compounds on the locomotor activity of mice

Compd.	Doses (mmol/kg i.p.)	Total locomotor activity (arbitrary units ± SD)
Control	–	2650 ± 264
2a	0.094	168 ± 21.4*
2a	0.047	796 ± 84.5*
Control	–	2320 ± 301
2b	0.32	1796 ± 352
2b	0.16	2540 ± 196
Control	–	1948 ± 211
2c	1.16	511 ± 62*
Control	–	2075 ± 241
2d	1.02	711 ± 96*
Control	–	2028 ± 229
Moclobemide	0.22	796 ± 98*

* $p \leq 0.05$ compared with control group

moclobemide exerted antidepressive activity at doses 0.11 and 0.22 mmol/kg b.w.i.p. (Table 5).

The results of the *in vivo* pharmacological screening show that compound **2b** has an antidepressive effect and a significantly lower acute toxicity than the standard drug moclobemide. On the other hand this compound significantly increased the pentobarbital sleeping time and decreased locomotor activity, similar to the effect of Moclobemide. This observation could be explained by interference of the sedative component of **2b** and moclobemide activity. In this regard more compounds should be synthesized in order to find out some structure-activity relationship.

Table 5: Effect of the compounds on the time of immobilization in the behaviour despair test

Compd.	Doses (part of LD_{50})	Time of immobilization (s ± SD)
Control	–	210 ± 18.7
2a	1/10	147.5 ± 95.4
2a	1/20	185 ± 72.3
Control	–	143.0 ± 11.4
2b	1/10	50.0 ± 33.0*
2b	1/20	115.0 ± 72.0
Control	–	251.3 ± 40.1
2c	1/10	188.8 ± 40.1
2c	1/20	219.8 ± 18.4
Control	–	143 ± 11.4
2d	1/10	121.2 ± 31.8
2d	1/20	107.5 ± 51.2
Control	–	242 ± 26
Moclobemide	1/10	127.0 ± 27*
Moclobemide	1/20	163.0 ± 39*

* $p \leq 0.05$ compared with control group

3. Experimental

3.1. Chemistry

Melting points were measured on a Boetius hot plate microscope (Germany) and were corrected. IR spectra (Nujol) were recorded in a UR 20 (Carl Zeiss, Jena, Germany) apparatus. ^{1}H NMR spectra were recorded at room temperature on a Brucker WP 100 (100 MHz) spectrometer in $CDCl_3$ or DMSO-D_6.

Chemical shifts are given in ppm. TMS was used as internal standard. ^{13}C NMR spectra were recorded at room temperature on a Brucker WP 100 (25.18 MHz). TLC was performed on 0.25 mm precoated plates Kieselgel 60 Merck (Germany) with chloroform petroleum ether/acetone/methanol (4 : 4 : 1.5 : 0.5) and detected with Dragendorff reagent. The novel structures were supported by characteristic IR and NMR data and by microanalyses (Microanalytical Unit. Faculty of Pharmacy, Sofia).

3.1.1. General method for 2-aryl-4-(2-benzoylethyl)morpholines **1a–d**

To 30 mmol of the 2-arylmorpholine hydrochloride, solved in 20 ml ethanol, 90 mmol paraformaldehyde, 30 mmol of the respective substituted acetophenone and 1 ml conc. HCl were added. The reaction mixture was refluxed 8 h (water bath) with a reflux condenser. After that the solvent was removed on a rotary vacuum evaporator. The residue was recrystallised from ethanol [7].

3.1.2. General method for 1-(2-arylmorpholino)-3-phenyl-3-propanone oximes **2a–d**

To 20 mmol **1a–d** in 10 ml ethanol 40 mmol hydroxylamine hydrochloride and 80 mmol KOH in 20 ml water were added. The solution was refluxed for 2 h (water bath) and left at room temperature for 12 h. The precipitated 3-(2-arylmorphino-1-phenyl-1-propanone oximes **2a–d** were filtered and recrystallized from ethanol/water 2 : 1 (Table 1).

3.1.3. General method for 3-(2-arylmorpholino)-N-phenylpropionamides **3a, c**

To an anh. benzene solution of 10 mmol **2a, c** in 20 ml benzene, 11 mmol (2.29 g) PCl_5 were added. The mixture was mixed with cooling and allowed to stand at room temperature for 1 h. Ice was then added, the water layer was separated and 4N NaOH was added. The crude product was extracted with ether and the ethereal solution was evaporated. The residue was gently heated in dry ether with CH_3I (10 mmol) for 30 min.

The 3-(2-arylmorpholino)-*N*-phenylpropionamides were isolated as their methoiodides and recrystallised from ethanol (Table 1).

3.2. Pharmacology

The experiments were conducted on 300 male H-albino mice (body weight 18–20 g). The acute toxicity (LD_{50}) of the studied compounds was assessed after solubilisation in saline with 1–2 drops Tween 80 and i.p. administration in mice. LD_{50} was calculated according the lethality of mice at for 4 or 5 different doses (Litchfield-Wilcoxon). At least 6 animals per experimental group were used [8].

3.2.1. Influence on pentobarbital sleeping time

The investigated compounds were administered to male mice i.p. at doses 1/10 of LD_{50} (the same volume – 0.1 g · b.w. of the solvent 0.9% NaCl – was administered to the animals in control group). The solution of pentobarbital sodium at a dose of 50 mg/kg b.w. was administered i.p. to the animals 30 min after administration of the solutions of the investigated compounds. The sleeping time was measured in min by observing the righting reflex recovery.

3.2.2. Influence on locomotor activity

A group of 6 animals was put into an actometer (Activity Gage, Ugo Basile, Italy) and the locomotor activity in arbitrary units was measured at 10 min intervals during 90 min. The tested compounds were administered at a dose 1/10 of LD_{50} to the animals and their effect on the locomotor activity was measured during 120 min under analogical conditions. The total locomotor activity was compared to that of the control (vehicle-treated) group.

The antidepressive activity was examined using the "behaviour despair" screening test [9], calculating the time of immobilisation of the mice in seconds over a 5 min observation.

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

References

1 Burkard, W. P.; Bonetti, E. P.; Da Prada, M.; Martin, G. R.; Polc, P.; Shaffner, R.; Sherschlict, R.; Hefti, F.; Muller, R. K. M.; Wyss, P. C.; Haefely, W.: J. Pharm. Exp. Ther. **2448**, 391 (1989)
2 De Wilde, J. E.; Doogan, D. P.: J. Affective Disord. **4**, 249 (1982)
3 Burrows, C. D.; Da Prada, M.: J. Neural. Trans. **28** (suppl.), 1 (1989)
4 Claasen, V.; Davies, J. E.; Herting, G.; Placheta, P.: J. Pharmacol. **60**, 505 (1977)
5 Scott, F. L.; McConaill, R. G.; Riodam, J. C.: J. Chem. Soc. (C), 44 (1967)
6 Aldrich Library of NMR-spectra, ed. 1984
7 Avramova, P. D.; Buyukliev, R. T.; Dantchev, N. D.: unpublished results.
8 Litchfield, Y.; Wilcoxon, F.: J. Pharmacol. Exp. Ther. **96**, 99 (1949)
9 Porsolt, R.; Bertin, A.; Golfer, M.: Arch. Int. Pharmacodyn. **229**, 2, 1112 (1977)

Received October 6, 1998
Accepted December 3, 1998

Prof. Dr. Petya Avramova
Dept. of Pharmaceutical Chemistry
2 Dunav St.
1000 Sofia
Bulgaria