Pharmacological Studies and Synthesis of Morpholino Alkyl Derivatives

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Seven morpholin derivatives were synthesized (compounds 1—7) and their behavioural effects were evaluated; the elements considered were locomotor activity, motor coordination, catalepsy, stereotyped behaviour and antinociception. All the compounds, at doses of 10—20—40 mg/kg/i.p., induced a reduction of all behavioural elements without a significant antinociceptive effect. These results indicate that these morpholin derivatives affect the central nervous system.

Key words morpholine; behavioural effect; central nervous system

It is well known that 2-(4-morpholino)ethyl-1-phenylcyclohexane-1-carboxylate is a σ receptors ligand.[1)] Although the physiological roles of σ receptors are not well understood at present, several potential "atypical" anti-psychotic drugs have been found to have high affinity for σ receptors.[2)] One of those atypical anti-psychotics, remoxipride, has been found in double-blind studies in acute schizophrenic patients to be superior than haloperidol.[2)] The above evidences given, a set of morpholin derivatives, **1**—**7**, represented in Fig. 1, has been synthesized and structurally characterized,[3)] bringing in chemical modifications to some compounds previously prepared.[4)]

The seven compounds were tested and found active on some behavioural elements related to the central nervous system. Although the ligand–receptor interaction is not clearly understood, here we interpreted the pharmacological results in terms of molecular level interaction.

We examined the neuropharmacological effects induced by the morpholin derivatives (**1**—**7**); the results of this study indicate that compounds **1**—**7** induce a significative reduction of the mice behaviour such as locomotor activity, motor coordination, catalepsy and stereotyped behaviour (see Figs. 2—4 and Table 1). An increase of pentobarbital-induced sleep was also observed, thus indicating that compounds **1**—**7** exert important depressant effects on the central nervous system. Furthermore compounds **1**—**7** did not induce analgesia (data not reported).

The comparative study performed with haloperidol indicates that although neuropharmacological profile is different, compounds **1**—**7** are endowed with pharmacological properties very close to the antipsycothic agent effects.[5)]

In fact, haloperidol induced both neurosedative and antinociceptive activity whereas compounds **1**—**7** did not induce antinociception.

If the reduction of motor coordination and stereotyped behaviour registered are considered together with those induced in locomotor activity and on pentobarbital-induced sleep, the hypothesis that compounds **1**—**7** might act as a mild neurosedative drug is supported.

In conclusion, the results demonstrate that these morpholine derivatives induce significant effects on the central nervous system. Further studies are in progress to understand the molecular level mechanisms underlying these effects, to explain the observed correlation between structure and activity. These include a structural and conformational analysis of the morpholin derivatives at higher levels of calculations and a simulation of their interaction with a model of the σ receptor by computational methods.

Experimental

Analytical thin-layer chromatography (TLC), was used to monitor reactions. Plates 5×10 cm K6F silica gel 60A (Whatman) were used. The compounds were detected by UV light and exposure to iodine vapors. Melting points were determined by a Gallenkamp apparatus and are uncorrected. The elementary analyses were in accord with the theoretical values within the range±0.4%. All IR spectra (KBr pellets) were recorded on FT-IR Matthson 1000 Spectrophotometer.

All ^{1}H-NMR was recorded on Bruker WM 600 and chemical shifts (δ) were reported in parts per million (ppm).

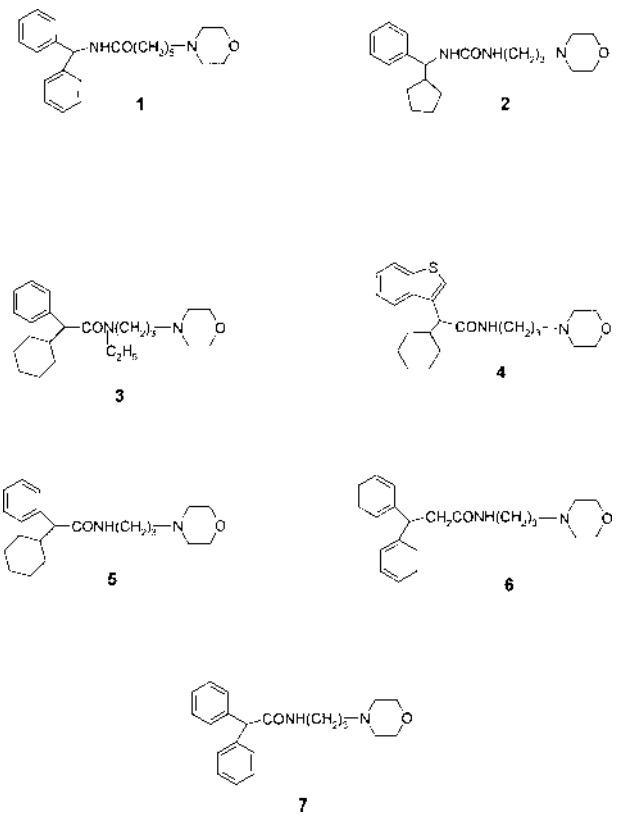

Fig. 1. Structures of Compounds **1**—**7**

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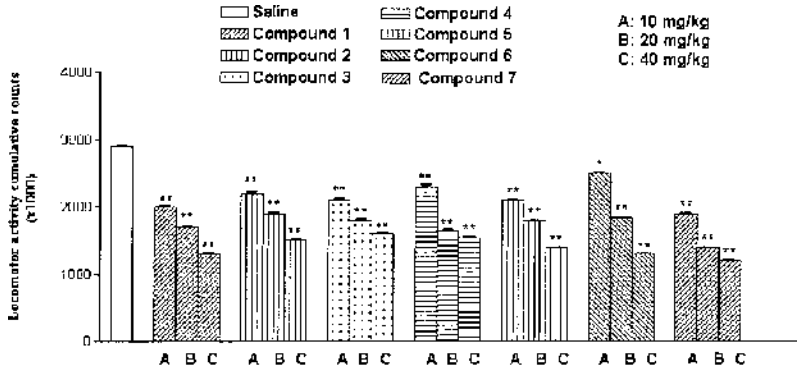

Fig. 2. Time and Dose–Effect Curves of Compounds **1**—**7** on Locomotor Activity in Mice

Results are mean±S.E.M. (n=6).

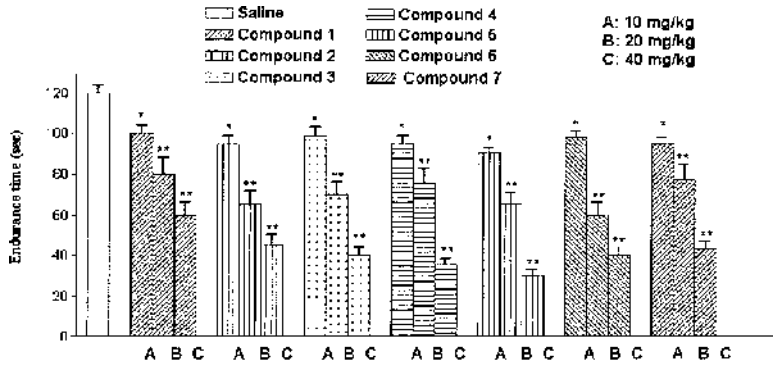

Fig. 3. Time and Dose–Effect Curves of Compounds **1**—**7** on Motor Coordination in Mice

Abscissa: time in minute; ordinate: endurance time in second(s). Results are mean±S.E.M. (n=6); $*p<0.05$, $**p<0.01$.

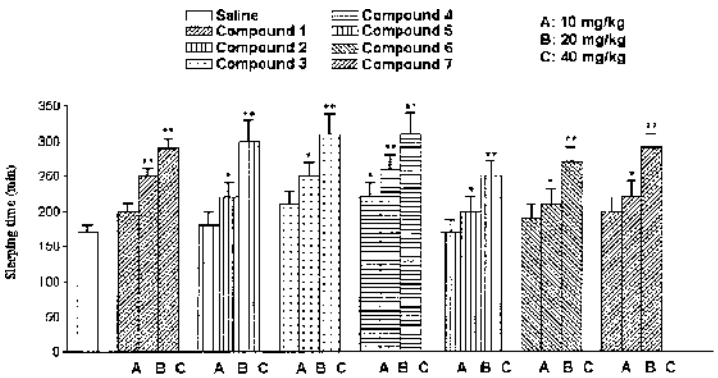

Fig. 4. Effect of Compounds **1**—**7** on Pentobarbital-Induced Sleep in Mice after Intraperitoneal Injection

Each bar indicates minutes elapsed between loss and recovery of righting reflex.

Synthesis scheme of the compounds **1**—**7** is reported in Chart 1. Chemical-physical data are reported in Table 2. The IR and ^{1}H-NMR data are reported respectively in Tables 3 and 4.

Synthetic Procedures for Compounds 1—7 a) To a stirred solution of aminodiphenylmethane (2 g, 0.0179 mol) and 6-chlorohexanoylchloride (3 g, 0.0179 mol) in benzene Na_2CO_3 was added (2.8 g, 0.0264 mol). The mixture was refluxed for 4 h and concentrated at reduced pressure. The residue was suspended in water and extracted with diethyl ether. The combined organic layers were dried over $MgSO_4$, filtered and evaporated *in vacuo* to yield the corresponding chlorohexylamide, which then reacted with morpholine in *N*,*N*-dimethylformamide (DMF) solution in the presence of Na_2CO_3 by refluxing for 2 h. After concentrating under reduced pressure the residue was suspended in water and extracted with diethyl ether. The combined organic layers were dried over $MgSO_4$. Removal of solvent at reduced pressure yielded the base (**1**) as an oil which was then converted in to the crystalline oxalate salt (see Chart 1).

b) To a stirred solution of 1-phenyl-1-cyclopentylisocyanate (2 g, 0.0099 mol) and 3-(4-morpholine)-1-propylamine (1.43 g, 0.0099 mol) in toluene triethylamine (TEA) (1 g, 0.0099 mol) was added. The mixture was refluxed for 3 h. Removal of solvent at reduced pressure and crystallization from petroleum ether/diethyl ether (4 : 1) provided the desired compound **2** (see Chart 1).

c) The suitable carboxylic acid was refluxed with $SOCl_2$ in benzene for 4 h and then evaporated *in vacuo* to yield the crude acyl chloride which then reacted with *N*-(3-aminopropyl)morpholine in benzene in presence of Na_2CO_3 for 4—5 h to give derivatives (**3**—**7**). The corresponding bases were isolated as oxalate (**6**) or chloridrate (**3**—**5**, **7**) salt (see Chart 1).

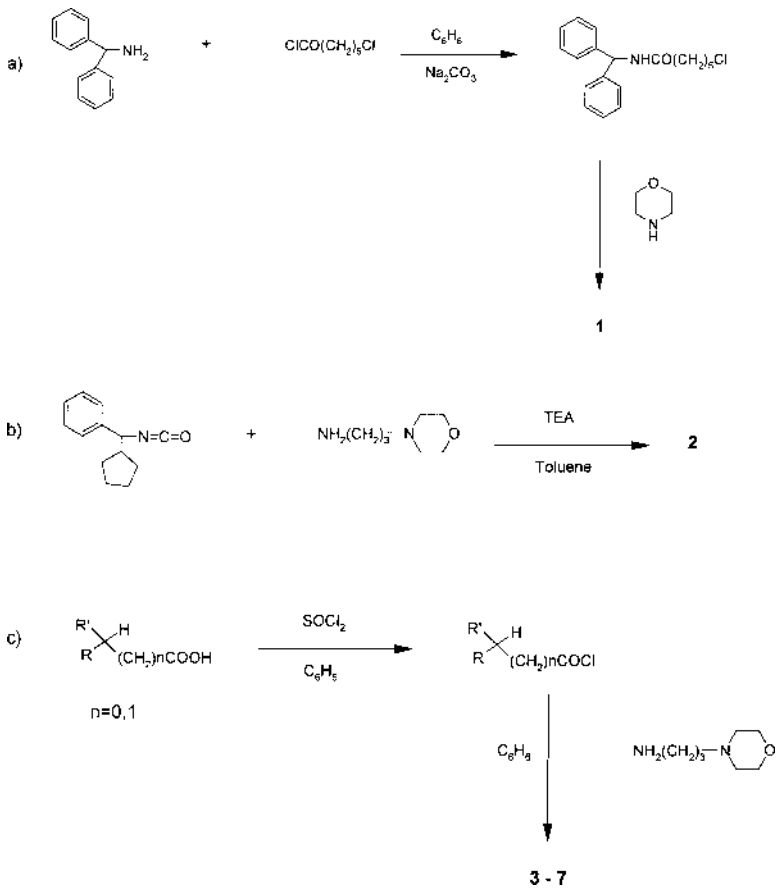

Chart 1. Synthesis of the Compounds **1**—**7**

Table 1. Effect of Saline and Compounds **1**—**7** (10—20—40 mg/kg/i.p.) on the Stereotyped Behaviour of Mice

	RE	GR	SRT	CR	SM	WF	SC	BH
Saline	79±9.0	69±7.0	61±6.0	57±8.0	75±9.0	56±7.0	51±6.0	54±8.0
1 10 mg	47±8.0	42±7.0	49±9.0	50±9.0	65±4.0	40±8.0	44±9.0	45±7.0
1 20 mg	35±5.0	30±6.0	30±5.0	45±4.0	43±6.0	30±5.0	30±3.0	33±4.0
1 40 mg	27±4.0	23±5.0	20±3.0	26±5.0	32±7.0	16±3.0	11±6.0	17±3.0
Saline	75±8.0	58±9.0	67±6.0	59±5.0	66±9.0	75±6.0	53±5.0	48±5.0
2 10 mg	47±4.0	41±9.0	35±9.0	41±7.0	57±5.0	69±4.0	47±9.0	36±8.0
2 20 mg	39±6.0	33±8.0	23±9.0	37±6.0	32±7.0	41±5.0	33±3.0	28±2.0
2 40 mg	22±4.0	12±2.0	13±4.0	25±6.0	22±3.0	25±3.0	24±2.0	15±3.0
Saline	77±9.0	60±7.0	59±6.0	52±7.0	57±8.0	69±9.0	55±8.0	53±7.0
3 10 mg	59±3.0	45±9.0	37±9.0	41±8.0	46±9.0	44±6.0	41±5.0	40±7.0
3 20 mg	35±3.0	33±5.0	30±4.0	26±3.0	30±4.0	37±3.0	32±4.0	34±4.0
3 40 mg	22±5.0	11±4.0	10±7.0	15±5.0	22±4.0	28±2.0	27±3.0	29±5.0
Saline	58±9.0	60±7.0	50±8.0	45±6.0	65±7.0	60±8.0	47±7.0	45±6.0
4 10 mg	40±3.0	47±9.0	45±9.0	46±8.0	56±9.0	47±8.0	52±9.0	47±5.0
4 20 mg	35±5.0	34±5.0	29±5.0	30±9.0	45±5.0	30±9.0	24±6.0	26±9.0
4 40 mg	21±3.0	14±4.0	21±3.0	26±5.0	24±4.0	27±6.0	21±5.0	24±4.0
Saline	89±7.3	79±9.3	61±6.4	67±7.7	80±8.8	66±6.7	63±6.9	74±6.9
5 10 mg	50±4.7	41±5.5	42±4.8	35±2.7	33±3.6	36±2.9	38±3.1	41±3.6
5 20 mg	44±3.5	36±2.5	31±2.8	29±2.2	46±3.3	39±3.8	27±2.1	36±3.1
5 40 mg	25±1.6	15±1.2	18±1.6	15±1.7	12±1.0	16±1.2	12±1.0	11±1.4
Saline	85±7.8	78±7.4	77±6.8	79±6.4	86±7.2	65±6.8	63±5.7	57±6.1
6 10 mg	46±4.9	42±4.5	35±3.1	40±4.6	37±3.9	31±3.9	30±2.7	24±1.9
6 20 mg	23±2.6	27±3.1	25±3.7	21±1.9	31±3.7	20±3.2	17±1.9	19±2.4
6 40 mg	10±1.2	15±1.7	11±1.6	10±1.8	11±1.5	15±1.7	13±1.4	16±1.9
Saline	80±9.4	78±8.4	79±8.2	62±7.3	77±8.2	69±7.3	75±7.7	73±8.1
7 10 mg	49±5.3	43±4.9	37±4.1	41±5.2	33±3.7	37±3.5	33±4.3	20±2.6
7 20 mg	25±2.7	26±3.5	22±3.6	20±2.5	32±3.3	21±2.6	12±1.7	14±1.8
7 40 mg	12±1.9	11±2.4	10±1.6	15±1.8	10±1.7	10±2.3	11±1.7	12±1.8

Stereotyped behavior are dose-dependently and significantly reduced by compounds **1**—**7**. Results are mean±S.E.M. (n=6) of the number frequency of the stereotyped behaviour during all the recording period (60 min). The abbreviations are as follows: RE=rearing, CR=crossing, GR=grooming, SM=smelling, SC=scratching, SRT=social response test, WF=washing face, BH=bar holding.

Table 2. Physical and Analytical Data of Compounds **1**—**7**

Compound	mp (°C)	Yield (%)	Formula
1	146	48	$C_{25}H_{32}N_2O_6$
2	93	73	$C_{20}H_{31}N_3O_2$
3	138	85	$C_{23}H_{37}ClN_2O_2$
4	210	45	$C_{23}H_{33}SClN_2O_2$
5	120	80	$C_{21}H_{33}ClN_2O_2$
6	142	62	$C_{24}H_{30}N_2O_6$
7	170	74	$C_{21}H_{27}ClN_2O_2$

Table 3. IR Data of Compounds **1**—**7**

Compound	Ir (KBr) ν cm^{-1}
1	3282 (NH); 2330, 2450, 1730 $(COOH)_2$; 1636 (CO); 1540, 1200, 1110, 709 lead band
2	3360, 3300 (NH); 1530 (CO); 1305, 1125, 924, 709 lead band
3	1634 (CO); 1457, 1269, 1132, 704, 708 lead band
4	3280 (NH); 1635 (CO), 1430, 1250, 710 lead band
5	3280 (NH); 1635 (CO), 1433, 1240, 709 lead band
6	3280 (NH); 2300, 2400, 1730 $(COOH)_2$, 1640 (CO), 1540, 1400, 1200, 1100, 710 lead band
7	3240 (NH), 1635 (CO), 1530, 1115, 710, 700 lead band

Table 4. ^{1}H-NMR Data (δ ppm) of Compounds **1**—**7** (DMSO-d_6)

Compound	
1	8.11 (1H, NH), 7.38—7.00 (m, 10H, HAr), 3.00 (1H, CH), 2.98, 2.87, 2.71, 1.64, 1.42 (m, $(CH_2)_5$)
2	7.09—6.98 (m, 5H, HAr), 6.22, 6.21 (d, 1H, NH), 5.62—5.60 (t, 1H, NH), 4.28 (d, 1H, CH), 3.36 (m, 2H, NH–$\underline{CH_2}$–), 3.34—2.79 (m, 2H, $\underline{CH_2}$–N), 2.76—2.08 (m, 2H, CH_2–$\underline{CH_2}$–CH_2), 2.00—1.23 (m, 8H, CH_2), 1.13—1.11 (m, 5H, CH_2 cyclopenthyl)
3	7.21—6.99 (m, 5H, HAr), 3.12 (1H, CH), 3.30, 3.16, 2.66, 2.10 (m, $(CH_2)_3$), 1.70 (q, 2H, CH_2), 0.82 (t, 3H, CH_3, J=7 Hz)
4	8.32 (1H, NH), 7.67, 7.65 (d, 2H, HAr), 7.66, 7.56 (d, 2H, HAr), 7.12, 7.06 (m, 2H, HAr), 7.03 (s, H, thienyl), 3.40, 3.32, 2.78, 2.66, 2.10 (m, 18H (CH_2))
5	8.23 (1H, NH), 7.24—7.16 (m, 5H, HAr), 4.12 (1H, CH), 3.80., 3.70, 2.66, 1.06 (m, 18H$(CH_2)_n$)
6	8.12 (1H, NH), 7.40—7.02 (m, 10H, HAr), 4.47 (d, 2H, CH), 2.98—2.50 (m, 6H, CH_2), 2.71, 2.68, 1.64, 1.59 (m, 8H, $(CH_2)_4$)
7	8.00 (1H, NH), 7.56, 7.50, 7.40, 7.38 (m, 10H, HAr), 4.00 (1H, CH), 3.12, 2.07 (m, $(CH_2)_3$), 3.13, 2.99 (m, 2H, morpholine), 1.55, 1.23 (m, 2H, morpholine)

Pharmacology

Male Swiss mice weighing 20 to 25 g, supplied by Charles River (Italy), were housed in colony cages (10 mice each) under standard light (light on from 7.00 a.m. to 7.00 p.m.), temperature (22±1 °C) and room humidity (60±10%) conditions for at least 1 h before experimentation. Food and water were available *ad libitum*.

On the testing day the compounds (**1**—**7**) used in the experimental sessions were dissolved in saline for administration. Drugs were injected in a volume of 10 ml/kg, i.p. Compounds were administered at doses of 10—20—40 mg/kg/i.p. 1 h before the beginning of the tests.

Locomotor Activity, Motor Coordination, Pentobarbital Sleeping time, Stereotyped Behavior, Catalepsy, Nociceptive Assays were performed as previously reported.[6—14)]

All data (expressed as mean±S.E.M.) were analysed by the analysis of variance (ANOVA) and Dunnett's procedure for multiple comparisons with a single control group. When the analysis was restricted to two means, Student's *t*-test (Two-tailed) was used. The Fisher exact test was used to analyse the rotarod data. Significance was assumed at a 5% level.

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