Lipophilicity of Some Substituted Morpholine Derivatives Synthesized as Potential Antinociceptive Agents

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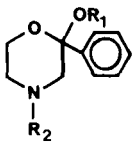
Some substituted 2-alkoxy-morpholines have been synthesized as potential antinociceptive agents. These compounds share some structural characteristics of the piperidine analgesics. Their lipophilicity, expressed as log P (octanol-water) and as R_M values (from reversed phase thin layer chromatography) was determined. Correlation of these two lipophilicity parameters indicated the classification of the tested compounds into two subgroups. Acute toxicity and, for some selected structures, analgesic activity are reported.

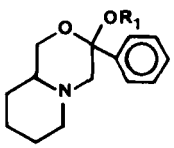
Lipophilie einiger substituierter Morpholinderivate, synthetisiert als mögliche analgetische Wirkstoffe

Einige substituierte 2-Alkoxy-morpholine wurden synthetisiert als mögliche analgetische Wirkstoffe. Diese Verbindungen haben einige Strukturcharakteristika der Piperidin-Analgetika. Ihre Lipophilie, angegeben als log P (Oktanol-Wasser) und als R_M (aus reversed phase DC-Chromatographie) wird bestimmt. Korrelation dieser zwei Lipophilie-Parameter kann die untersuchten Verbindungen in zwei Gruppen einteilen. Akute Toxizität und, für einige ausgewählte Strukturen, analgetische Aktivität sind angegeben.

The synthesized substituted 2-alkoxy-2-phenyl-morpholines **I, II, III** present structural similarities with some centrally acting antinociceptive agents[1,2], for example pethidine, prodines. However, the morpholine oxygen causes some differences, compared to the piperidine analgesics, concerning both chemical and physicochemical properties. We tried to outweigh these effects by proper substitution, principally in positions 2 and 4 of the morpholine ring.



I



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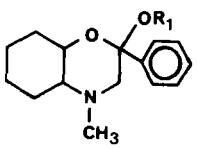

III

In this paper, we studied the synthesis of some morpholine derivatives, we checked their toxicity and, for some representative structures, their analgesic activity. We mainly investigated, however, their lipohilicity, which we expressed as log P from the octanol-water system and as R_M values, derived from reversed phase thin layer chromatography (tlc). We tried to correlate these two expressions of lipophilicity quantitatively. Since morpholine derivatives show a special behaviour concerning their partitioning characteristics[3], such a correlation could more generally contribute to a further elucidation of their lipophilic properties.

Results and Discussion

Synthesis

The formation of the morpholine ring to 2-hydroxy-morpholine-derivatives was accomplished spontaneously by intramolecular nucleophilic attack (Fig. 1). The ring closure depends on a favourable conformation of the ketoaminoalcohol, which seems to be influenced by the N-substituent. The N-methyl derivative was formed readily (72% yield), the N-n-butyl analogue had a yield of 68%, but the piperidine analogue (**7**, Table 1), though having the same number of carbon atoms, was less easily formed. When the piperidine was replaced by a pyrrolidine residue, the morpholine ring closure did not occur; this could be attributed to the prevention of the hydroxy-side chain to take a favourable conformation for the morpholine ring formation.

The preparation of the 2-alkoxy-derivatives, which was considered to occur via an intermediate carbenium-oxonium ion, stabilized by the 2-phenyl group, was influenced by the bulk of the entering alkoxy group. Thus, *t*-butyl- or *iso*-propyl-alcohols did not react. Interestingly, the 2-*iso*-propoxy-analogue **15** was formed. An explanation to this could be that the 5,6-tetramethylene substituent of the morpholine ring may contribute to a conformation of the intermediate ion favourable to the attack even by the *iso*-propyl alcohol.

Physicochemical studies

The pKa values of the tested compounds were between 6.98 and 7.50. These are lower than the ionization constants of most of the (piperidine) analgesics (7.8-8.9)[4,5]. There was a slight increase of pKa with the increase of the size of the N-alkyl chain, evidently due to the inductive effect of these substituents.

Lipophilicity of the tested compounds has been expressed as log P between n-octanol and aqueous buffer (pH 7.4) and as R_M values. log P (octanol-water) determination is the most reliable method for estimating lipophilic characteristics of biologically active compounds[6]. However, R_M determination with reversed phase tlc is considered to be a faster

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and more convenient way for measuring lipophilicity[7,8]. Furthermore, a linear correlation can exist, which may relate R_M to log P values[9]. This correlation depends on many parameters, such as the composition of the eluent, the nature of the stationary phase and their analogy to the octanol and aqueous phases[6,9], as well as the congenerity of the compounds tested.

In this series of morpholine derivatives, the compounds have the same main skeleton with various substituents at different positions of the morpholine ring. It was interesting to investigate the effect of these substituents on lipophilicity, and the possibility of deriving a correlation between log P and R_M values. Both methods were in agreement concerning some general aspects, i.e. the 2-hydroxy compounds were the most hydrophilic, and lipophilicity increased with increasing size of the nonpolar substituents.

Interestingly, monocyclic morpholine derivatives had higher log P than expected, compared to those which have a second ring fused to the morpholine moiety. It has been suggested that the higher than expected lipophilicity of some substituted morpholines could be attributed to an interconversion of the chair conformers via the intermediate boat conformers, which are more lipophilic[3,10]. This may happen to a much lower degree when there is a second fused ring, which results in a less flexible structure.

Attempts to correlate linearly the log P and R_M values of all compounds tested gave a poor regression. The distribution of the points indicated clustering into two almost parallel lines. Thus, the compounds were divided into two subgroups, one containing the monocylic (equation 1) and the other the bicyclic (equation 2) morpholine derivatives, and the corresponding equations were satisfactory (numbers in parentheses are 95% confidence limits):

$$\log P = 0.251\ (\pm 0.076)\ R_M + 2.193\ (\pm 0.023)\quad n=5\ s=0.043\ r=0.885 \qquad (1)$$

$$\log P = 0.274\ (\pm 0.031)\ R_M + 1.847\ (\pm 0.010)\quad n=10\ s=0.025\ r=0.952 \qquad (2)$$

Within each subgroup the number of C-atoms (C) correlated well with lipophilicity (equations 3 and 4):

$$R_M = 0.174\ (\pm 0.020)\ C - 2.615\ (\pm 0.321)\quad n=5\ s=0.063\ r=0.981 \qquad (3)$$

$$R_M = 0.117\ (\pm 0.018)\ C - 1.836\ (\pm 0.324)\quad n=10\ s=0.117\ r=0.914 \qquad (4)$$

This may indicate that, given the basic skeleton, carbon containing substituents have the same effect on lipophilicity, regardless of the specific position of each of them.

Biological studies

LD_{50} values of the synthesized 2-alkoxy derivatives varied from 350 to 995 μmoles per kg. Although toxicity generally was increased with increasing lipophilicity, a significant correlation of toxicity and lipophilicity was not obtained. This is not unexpected, since it is known that toxicity can be considered as a set of complex and often unspecific interactions between xenobiotics and biological systems[11].

The three compounds tested demonstrated a statistically significant antinociceptive activity compared to controls ($P<0.001$), as determined by the Hot Plate method[12]. Approximately the same response was obtained after pethidine injection at a dose about 30% of the doses administered.

Our results indicate that the synthesis of some properly substituted morpholine derivatives could lead to compounds with significant antinociceptive activity. These compounds could be classified into two subgroups, according to their lipophilicity. Within these subgroups, R_M values from reversed phase tlc represent a reliable index of their lipophilic characteristics.

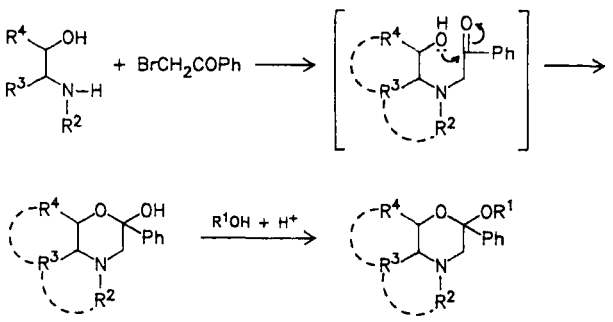


Figure 1: Formula scheme showing the synthetic route followed for the morpholine derivatives (see also Table 1).

R^1: alkyls
R^2: CH_3, $CH_2(CH_2)_2CH_3$
R^2 R^3: $-CH_2CH_2CH_2CH_2-$
R^2: CH_3
R^3, R^4: H
R^4: H
R^3 R^4: $-CH_2CH_2CH_2CH_2-$

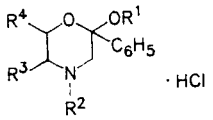
Experimental Part

Melting points (uncorrected): Carl Zeiss melting point microscope. - IR, UV, ^{1}H-NMR (in DMSO-d_6 or $CDCl_3$, TMS as an internal standard) spectra: Perkin Elmer 597 and 554 double beam spectrophotometer, Brucker AW80 spectrometer at 80 MHz, respectively. - Elemental analyses: Perkin Elmer 240 analyzer. - Determination of ionization constants: digital Radiometer PHM63 pH meter, combined Radiometer GK24010 glass electrode. - Tlc: silica gel ($60F_{254}$, Merck) glass plates. - Starting materials and Solvents (Aldrich Chemical Co., USA): analytical grade. - Male Wistar rats (160-220 g). Hot Plate, type Ridi 85 (Greece).

Synthesis

The 2-hydroxy-substituted morpholines, as hydrohalides, were prepared from the corresponding aminoethanols (0.22 mol) and phenacyl bromide (0.10 mol) in dry ether and neutralization of the obtained morpholine bases[13,14]. 2-Hydroxy-morpholines were heated in acidic medium with excess of the appropriate alcohol to give the 2-alkoxy derivatives[14-16]. Starting 2-hydroxymethyl-piperidine, 2-hydroxymethyl-pyrrolidine and 2-methylamino-cyclohexanol were

Table 1: Synthesized morpholine derivatives, yields, melting points and elemental analyses.



Compound	R^1	R^2	R^3	R^4	Yield (%)	m.p. (°C)	Elemental Analyses (%) Calculated C	H	N	Found C	H	N
1	$CH_2CH_2CH_3$	CH_3	H	H	71	168-9	61.9	8.10	5.2	61.5	7.96	5.1
2	$CH_2(CH_2)_2CH_3$	CH_3	H	H	61	156-7	63.1	8.41	4.9	63.0	8.27	4.9
3	H	$CH_2(CH_2)_2CH_3$	H	H	68	136-7	61.9	8.10	5.2	61.8	7.98	5.1
4	CH_2CH_3	$CH_2(CH_2)_2CH_3$	H	H	73	152-3	64.1	8.68	4.7	64.0	8.59	4.6
5	$CH_2CH_2CH_3$	$CH_2(CH_2)_2CH_3$	H	H	77	144-5	65.1	8.93	4.5	65.2	8.85	4.5
6	$CH_2(CH_2)_2CH_3$	$CH_2(CH_2)_2CH_3$	H	H	42	141-2	65.9	9.16	4.27	65.7	9.22	4.1
7	H	$CH_2CH_2CH_2CH_2$		H	33	179-80	62.3	7.43	5.2	62.2	7.5	5.2
8	CH_2CH_3	$CH_2CH_2CH_2CH_2$		H	73	170-1	64.5	8.07	4.7	64.6	7.9	4.6
9*	$CH_2CH_2CH_3$	$CH_2CH_2CH_2CH_2$		H	57	163-4	57.3	6.74	3.9	57.6	7.09	3.9
10	$CH_2(CH_2)_2CH_3$	$CH_2CH_2CH_2CH_2$		H	54	160-1	66.4	6.74	4.3	66.4	8.49	4.4
11	$CH_2CH(CH_3)_2$	$CH_2CH_2CH_2CH_2$		H	56	159-60	66.4	8.60	4.3	66.4	8.50	4.4
12	$CH_2C_6H_5$	$CH_2CH_2CH_2CH_2$		H	50	143-4	70.1	7.23	3.9	69.7	7.11	3.8
13	H	CH_3	$CH_2CH_2CH_2CH_2$		34	148-50	63.5	7.76	4.9	63.2	7.87	4.9
14*	$CH_2CH_2CH_3$	CH_3	$CH_2CH_2CH_2CH_2$		54	133-5	58.4	7.57	3.8	58.6	7.30	3.7
15*	$CH(CH_3)_2$	CH_3	$CH_2CH_2CH_2CH_2$		75	202-4	58.4	7.57	3.8	58.0	7.60	3.8
16	$CH_2(CH_2)_3CH_3$	CH_3	$CH_2CH_2CH_2CH_2$		42	139-40	67.9	9.05	4.0	68.0	8.93	3.8

* As hydrobromides.

prepared from pipecolinic acid[17], proline[17] and cyclohexanol[18], respectively.

The 2-hydroxy-derivatives show a strong band at 3400 cm^{-1} (IR, nujol mull), λmax 210-220 nm, ε = 6200 or 12300 (compounds with one or two phenyls respectively), ε_{240} = 740-1100 (UV, 0.15-0.70 mM in 2-propanol). The 2-alkoxy-derivatives show no band at 3400 cm^{-1}, λmax 210-220 nm, ε = 7500 or 13000, ε_{240} = 170 (0.15-0.70 mM in 2-propanol). - NMR spectra show a triplet at δ = 0.8-0.9 (aliphatic methyl H), multiplets at δ = 2.8-3.8 (methylenic H) and multiplets at δ 7.5 - 7.7 (aromatic H). Furthermore, configuration of compounds **13-16** (Table 1) is postulated to be *trans*, according to the employed route, similar to those reported to have led to *trans* morpholine derivatives[19,20], and to the ^{1}H-NMR spectrum of **16** in $CDCl_3$: C-6-H appeared as a part of a AB system at δ = 3.35 (J = 13 Hz).

Reaction of 2-hydroxymethyl-pyrrolidine with phenacyl bromide gave N-phenacyl-prolinol (26%), m.p. (hydrochloride) 144-145°C, $C_{13}H_{17}NO_2$ calcd. C 71.2 H 7.76 N 6.9 found C 70.9 H 7.93 N 6.5. It gave a broad band at 3350 cm^{-1} and a strong peak at 1680 cm^{-1} (IR, nujol mull).

The synthesized compounds, yields, melting points and elemental analyses are shown in Table 1.

Physicochemical experiments

Ionization constanst were determined by potentiometric titration[21] at 37°C.

Apparent Partition Coefficients were determined between n-octanol and buffer solution (pH 7.4), mutually saturated, after vigorous agitation (10 min), centrifugation and determination of the concentration spectroscopically (220 nm, at least four individual measurements, standard deviation of P less than 10%).

True Partition Coefficients were calculated using the equation $P = P_{app} / (1-\alpha)$, where α is % ionization[22].

Reversed phase tlc was performed on silica gel plates impregnated with 5% (v/v) liquid paraffin in light petroleum, as described[7]. Mobile phase: methanol/water mixture (67/33 v/v) containing 1% aqueous NH_4OH (27%). Plates were developed in closed chromatography tanks, saturated with the polar phase, at 20°C, spots were detected under UV light. R_F values: average of at least ten measurements. R_M values were determined from the corresponding R_F values using the equation $R_M = \log (1/R_F-1)$.

pKa, log P, R_F and R_M values are shown in Table 2.

Biological experiments

Acute toxicity of the 2-alkoxy compounds, administered intraperitoneally, was expressed as LD_{50} values and determined by probit analysis[23]. Confidence limits were within 3 mg. LD_{50} values are given in Table 2.

Antinociceptive activity for compounds **2** and **9** (Table 2) was determined by the Hot Plate test at 60°C[12,24], for 50 min after intraperitoneal administration. Antinociception was evaluated using an arbitrary scale of 1-10[25]. The sum of degrees for 50 min obtained from each group of animals was averaged. The administered doses per kg body weight and the degrees of analgesia were: Compound **1**, 276 μmol,

Table 2: Physicochemical parameters and acute toxicity of the synthesized morpholine derivatives.

Compound*	pKa	log P	R_F (± SD)	R_M (± SD)	LD_{50} (μmol/kg b.w.)
1	7.12	2.13	0.567 (± 0.034)	-0.120 (± 0.062)	670
2	7.09	2.23	0.526 (± 0.030)	-0.063 (± 0.048)	665
4	7.30	2.00	0.442 (± 0.031)	0.100 (± 0.054)	831
5	7.24	2.03	0.306 (± 0.025)	0.355 (± 0.049)	768
6	7.22	2.13	0.224 (± 0.014)	0.539 (± 0.035)	672
7	7.40	1.77	0.652 (± 0.036)	-0.266 (± 0.080)	n.d.
8	7.27	1.87	0.506 (± 0.028)	-0.018 (± 0.033)	995
9	7.21	1.92	0.386 (± 0.024)	0.216 (± 0.034)	915
10	7.19	1.94	0.275 (± 0.018)	0.420 (± 0.040)	676
11	7.22	1.95	0.285 (± 0.027)	0.400 (± 0.060)	680
12	6.98	1.95	0.283 (± 0.016)	0.402 (± 0.035)	723
13	7.50	1.81	0.575 (± 0.040)	-0.134 (± 0.071)	n.d.
14	7.13	1.91	0.367 (± 0.038)	0.235 (± 0.073)	350
15	7.14	1.89	0.327 (± 0.030)	0.312 (± 0.046)	365
16	7.31	2.05	0.205 (± 0.014)	0.589 (± 0.035)	410

* As in Table 1.
n.d.: Not determined.

38.7 degrees; Compound **2**, 210 μmol, 36.4 degrees; Compound **9**, 273 μmol, 36.4 degrees. Controls received water, 7.5 degrees. Pethidine was used as a reference compound at a dose of 90 μmol, which gave about the same response, 39.0 degrees.

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