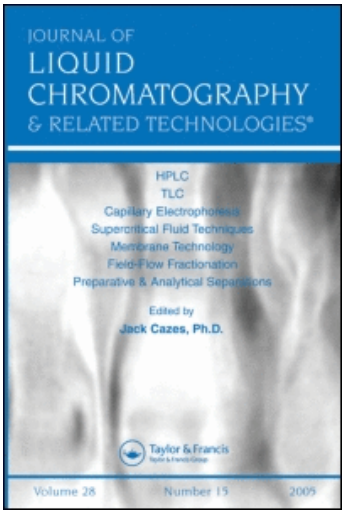

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RP-HPLC OF NEW ANTIDEPRESSANT 2-AMINO-2-OXAZOLINES: A COMPARATIVE STUDY OF THEIR LIPOPHILICITY

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ABSTRACT

A comparative study of lipophilicity in a series of 5-(1-aryl-4-piperazino)methyl-2-amino-2-oxazolines with antidepressant activity has been carried out using a RP-HPLC technique. This chromatographic method allowed the determination of log k'_w values through extrapolation to 100% water from capacity factors data. The partition coefficients (log $P_{o/w}$) and ionization constants (pK_a) were measured by classical methods. A good correlation between log $P_{o/w}$ and log k'_w was found, confirming the feasibility of using the latter as a lipophilicity descriptor. In this homogeneous chemical series the nature and the position of the substituents on the aromatic ring did not induce important variations on the pK_a values, whereas they accounted for a great part in lipophilicity data.

INTRODUCTION

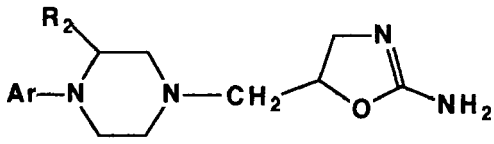
In order to extend our research on bioactive compounds we reported recently the synthesis and the antidepressant activity of a series of 5-(1-aryl-4-piperazino)methyl-2-amino-2-oxazolines (1, 2). One compound (COR 3224) was selected for further clinical studies and is presently under phase II clinical trials (3). One of the most important influence on *in vivo* pharmacological activities of drugs seems to be related to their lipophilicity. Thus the need for lipophilic descriptors in the search for quantitative structure-activity relationships (QSAR) led to the development of the partition coefficient P between n-octanol and water (4). For a number of years, chromatographic methods especially RP-HPLC have been proposed to determine the lipophilicity of drugs (5). An interrelationship between the partition coefficient P and the chromatographic column capacity factor k' in RP-HPLC has been established in a Collander-type equation (6,7). We developed recently such a chromatographic approach of the lipophilicity of new diuretic agents (8).

The purpose of this paper is to study the lipophilicity of the 5-(1-aryl-4-piperazino)methyl-2-amino-2-oxazolines by means of a RP-HPLC technique. The reliability of this methodology is checked by correlation of the k' data with the parameters P measured by the classical "shake-flask" method and calculated using fragmental constants

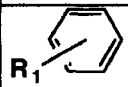
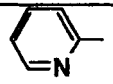
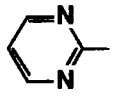
MATERIALS AND METHODS

Apparatus and chromatographic conditions.

Chromatography was performed with a Waters Assoc. apparatus equipped with a Model M45 pump, a Lambda-Max Model ultraviolet detector operating at 235 nm and an U_6K manuel injector. The compounds were chromatographied on a μBondapak C_{18} stainless-steel column (300mm x 3.9 mm, 10μm particle size)(Waters Assoc.). The mobile phase composition ranged from 20 to 80 % (v/v) methanol with 0.06 M phosphate buffer at pH 7.4. The flow rate was 1.5 ml/min. The detector output was recorded on a Model 730 data Module integrator



5-(1-aryl-4-piperazino)methyl-2-amino-2-oxazolines

Comp.	Ar	R_2	Comp.	Ar	R_2
1-17 21-25		H	18		H
20		CH_3	19		H

Standards and reagents

The synthesis of 5-(1-aryl-4-piperazino)methyl-2-amino-2-oxazolines is described elsewhere (1,2). All compounds were characterized from spectral data (IR, ^1H and ^{13}C NMR). An EI mass spectra study of some structurally related 2-amino-2-oxazolines shows that the main fragments are derived from the arylpiperazine moiety (9).

Stock solutions containing 1mg/ml of each drug were prepared in methanol and stored at -20°C. All other chemicals and solvents were of analytical reagent or HPLC grade.

Methanol (Prolabo) was used without further purification to prepare the mobile phase and water was glass-distilled deionized. The phosphate buffer solution at pH 7.4 was prepared with potassium dihydrogen phosphate and dipotassium hydrogen phosphate trihydrate (Merck). Before use for HPLC, the mobile phase was filtered through a 0.45 μm membrane filter.

Measurement of log k'

The column dead-time of the system (t_0) was measured as the time from injection to the first distortion of the baseline after injection of pure water.

Consequently, the stock solutions of tested compounds were diluted with water to the final injected concentrations of 50 µg/ml. According to their chromatographic behaviour, the retention time (t_r) of each compound was determined at six different methanol-phosphate buffer mixtures ranged from 20 to 80%. The compounds were injected separate from each other three times and the mean value of the retention time was retained. The log k'_w value for each compound was obtained by regression analysis of log k' data, expressed from the retention times t_r, through the formula : $k' = (t_r - t_0) / t_0$, and extrapolation to 0% methanol content.

The correlation/regression analysis were carried out with a statistical program on a Vectra computer (Hewlett Packard).

Measurement of pK_a

The 5-(1-aryl-4-piperazino)methyl-2-amino-2-oxazolines present two basicity centers. The pK_a's determinations were performed using a classical potentiometric method described elsewhere (8,10). A X-ray crystallographic study for a related 2-amino-2-oxazoline as dihydrochloride showed that the first protonation (pK_{a1}) occured on the oxazoline endo nitrogen atom of the amidine moiety (11). The second basicity center was found to be the N(4) piperazine nitrogen atom.

Determination of log P

The octanol-water partition coefficients ($P_{o/w}$) were determined by the classical "shake-flask" technique using a conventional methodology. Samples in a weight range of 5-10 mg were partitioned between 5 ml of n-octanol satured with water and 50 ml of water satured with n-octanol. The pH of the water phase was adjusted at 11, ensuring that all compounds were more than 99% unionized.

Calculated octanol/water partition coefficients (P_{calc}) were determined according to Broto's fragmental method (12). As 2-amino-2-oxazolines are tautomeric with 2-iminooxazolidines, two related values can be calculated for each compound. In Table 1 are reported P_{calc} for the amino tautomeric form.

TABLE 1. Analytical data of 5-(1-aryl-4-piperazino)methyl-2-amino-2-oxazolines

N°	SUBSTITUENT R_1	log K'W	Slope S	pKa_1	pKa_2	log $P_{o/w}$	log D	log P_{cal}
1	H	1.61	-0.023	8.88	4.92	0.85	-0.64	0.50
2	4-Cl	2.41	-0.03	8.47	4.8	1.71	0.60	1.12
3	3,4-diCl	2.63	-0.031	8.07	5.03	2.40	1.64	1.75
4	4-CH_3	2.47	-0.033	8.17	5	1.23	0.40	0.92
5	4-OCH_3	1.86	-0.028	8.05	5.22	0.68	-0.06	0.63
6	3-Cl	2.52	-0.032	8.81	4.85	1.83	0.40	1.12
7	4-$N(CH_3)_2$	1.52	-0.022					1.33
8	3-CH_3	2.3	-0.03					0.91
9	4-OH	1.47	-0.031					0.11
10	4-$OCOCH_3$	1.51	-0.024					0.23
11	4-$OCH_2CH_2CH_3$	2.56	-0.034					1.43
12	3-OCH_3	1.81	-0.026	8.38	5.04	0.87	-0.15	0.63
13	2-Cl	2.53	-0.032	8.10	5.41	1.87	1.09	1.12
14	4-CF_3	2.53	-0.033					1.47
15	4-$OCH(CH_3)_2$	2.28	-0.031					1.26
16	4-$OCH_2C_6H_5$	2.46	-0.028					1.99
17	3,4-diOCH_3	1.24	-0.02					0.77
18	2-Pyridyl	1.95	-0.032	8.51	5.42	0.48	-0.54	-0.38
19	2-Pyrimidyl	1.33	-0.023	8.76	4.51	0.21	-1.17	-1.27
20	4-OCH_3 CH_3 piper	1.68	-0.024					0.83
21	2-OCH_3	1.72	-0.027	8.32	5.35	0.76	-0.21	0.63
22	4-F	1.54	-0.02	8.82	5.1	1	-0.44	0.63
23	2-OCH_2CH_3	2.08	-0.026	8.8	5.15	1.21	-0.23	0.97
24	3-CF_3	2.89	-0.034	8.76	5.03	2.21	0.83	1.47
25	2,3-diCH_3	2.9	-0.032	8.38	5.55	2.19	1.16	1.33

RESULTS AND DISCUSSION

The chemical formulae of the tested 5-(1-aryl-4-piperazino)methyl-2-amino-2-oxazolines are given in Table 1. All compounds contain a benzenic ring bearing at least one substituent, except 18 and 19 with an isosteric pyridyl or pyrimidyl moiety. The synthesis strategy was partially based on the TOPLISS approach (13), the choice of the nature and the position of the substituents on the phenyl ring derived from a structure activity study (2).

Determination of log k'_w and S (slope of the regression analysis)

In this study we have chosen to measure the log k' value extrapolated to 0% of the organic modifier in the mobile phase (log k'_w). For many authors (7,14), this technique (polycratic method) allows more adequate evaluation of the hydrophobic nature of the solute and provides a scale of lipophilic parameters normalized to one set of conditions more closely related to log $P_{o/w}$. In these conditions, the test compounds were chromatographied under a variety of conditions in which the percentage of methanol varied from 20 to 80%. For all compounds, linear relationships ($r > 0,99$) were proved to exist between the log k' values and methanol concentrations, allowing the calculation of log k'_w and S through extrapolation (Table 1). A statistically significant correlation was noted between these two parameters ($r = 0.901$, $p < 0.001$). This result may be related to the structural similarity of all tested compounds in regard to their partition behaviour in this RP-HPLC system.

pK_a's determination

For all tested compounds it appears a better homogeneity for the pK_{a1} values (8.49±0.30, variation coefficient 3%) than for the pk_{a2} ones (5.08±0.07, variation coefficient 5%). Nevertheless no particular influence of the nature and the position of the substituent on the phenyl ring was found on the pK_{a2} values. The distance between the aromatic ring and the N-4 nitrogen atom is too large (5.7Å) to introduce electronic effects.

Correlation between lipophilic indexes

The log k'_w values were correlated with log $P_{o/w}$, according equation I for the experimental data listed in Table 1.

$$\log k'_w = 0.666\ (\pm 0.085)\ \log P + 1.285\ (\pm 0.124)$$
$$(n = 15,\ r = 0.909,\ s = 0.218,\ F = 61.70,\ p \leq 0.0001)$$

2-amino-2-oxazolines are basic molecules. In order to take into account the effects of ionization we calculated the distribution coefficient D :

$$D = \frac{P}{1 + [(H^+)/Ka_1] + [(H^+)^2/Ka_1.Ka_2]}$$

The correlation between log k'_w and log D was established (equation II)

$$\log k'_w = 0.585\ (\pm 0.077)\ \log D + 2.045\ (\pm 0.056)$$
$$(n = 15,\ r = 0.904,\ s = 0.223,\ F = 58.47,\ p \leq 0.0001)$$

The substitution of log D instead of log P does not improve the correlation with log k'_w. Therefore, it seems that the ionization effects observed at the experimental pH value (7.4) are quite similar for all the compounds.

A correlation can be calculated between log k'_w and the calculated log P_{Broto} (equation III) :

$$\log k'_w = 0.505\ (\pm 0.114)\ \log P_{calc} + 1.643\ (\pm 0.124)$$
$$(n = 25,\ r = 0.680,\ s = 0.68,\ F = 19.66,\ p \leq 0.005)$$

The sp^2 nitrogen atoms in the pyridyl and pyrimidyl rings (compounds 18 and 19) diminishes the precision for the corresponding calculated log P_{Broto} values. Removal of these compounds from the regression leads to a better correlation (equation IV) :

$$\log k'_w = 0.805\ (\pm 0.16)\ \log P_{calc} + 1.303\ (\pm 0.177)$$
$$(n = 23,\ r = 0.741,\ s = 0.349,\ F = 25.44,\ p \leq 0.0001)$$

CONCLUSION

The present work shows that a good correlation exists between the HPLC data and the log P values measured by the classical shake flask method. Equations I and II account for 83 % of the total variance in the data (r^2). The correlation established with log P calculated from fragmental constants is worse (55% of the total variance in the data). This result could be explained by the inaccuracy of the calculated P values. The amidine group of 2-amino-2-oxazolines induces a tautomeric phenomenon which occurs especially in solution (15). Thus P_{calc} should take into account the respective percentages of the two tautomeric forms. By comparing the capacity factor of substituted compounds versus the non-substituted one, one notices the great influence of this substitution on lipophilicity. The lipophilic behaviour from the log k'_w values can be explained through the probable retention mechanism of 2-amino-2-oxazolines. Like the nature and position of the substituents present a poor influence on the respective basicity strength, one can deduce that the chromatographic behaviour is not greatly related to ionization.

As usual, the substituent position dependance in lipophilicity has smaller effect than the nature of substituent because the field effect of substituents is more important than the resonance effects (16). This result may be observed for the three methoxy compounds (4, 12 and 21) and for the three chloro substituted ones (2, 6 and 13) the log k'_w of which are similar. This influence of the nature of substituent accounts for a great part in lipophilicity. For example one can consider the 4-alkyloxy substituted 2-amino-2-oxazolines 5, 11, 15, 16 and 23. The increase of the carbon chain length leads to a concomitant variation of log k'_w.

In conclusion the results noticed for log k'_w justify the use of this parameter in order to describe the lipophilicity of these antidepressant 5-(1-aryl-4-piperazino)methyl-2-amino-2-oxazolines in relation to their passage through membranes hydrophobic barriers.

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