Synthesis and Quantitative Structure-Activity Relationships of Analeptic Agents Related to Dimefline

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Some new dimefline-type derivatives have been synthesized and their pharmacological activity, as well as their distribution coefficients have been determined. The distribution coefficients of a number of previously published analogue compounds have also been measured and the QSAR analysis of the whole set has been carried out. The results of such analysis allow to point out which factors are influencing the biological activity of this group of compounds.

Synthese und quantitative Struktur-Wirkungs-Beziehungen analeptischer mit Dimeflin verwandter Verbindungen

Es wurden einige neue Derivate nach dem Modell des Dimeflins synthetisiert und die pharmakologischen Wirkungen, zusammen mit den Verteilungskoeffizienten, bestimmt. Weiter wurden die Verteilungskoeffizienten von ähnlichen, vorher veröffentlichten Verbindungen gemessen. Die QSAR-Analyse der ganzen Gruppe wurde erstellt. Solche Analysen erlauben es festzustellen, welche Faktoren auf die biologische Wirkung dieser Gruppe von Verbindungen einwirken.

Following our line of research on the SAR of analeptic drugs, we have recently reported the results of the introduction of halogen substituents (F and Cl) in the 4' position of dimefline[1]. The activity of the new compounds was decreased by the introduction of F or Cl and a change in the pharmacological action pattern was detected; both effects have to be ascribed to the presence of the new substituents and it was suggested that the electronic properties of halogens might have a role in determining them.

The influence of lipophilicity could not be excluded, but could not be assessed either, because of the lacking of measured data.

In a related work on the QSAR (Quantitative Structure-Activity Relationships) of analeptic xanthone derivatives[2], the R_m (thin-layer chromatography retention index) of the compounds was found to be an important parameter in correlating the activity with the lipophilic character, and a parabolic equation was formulated for the dependence of the activity on R_m.

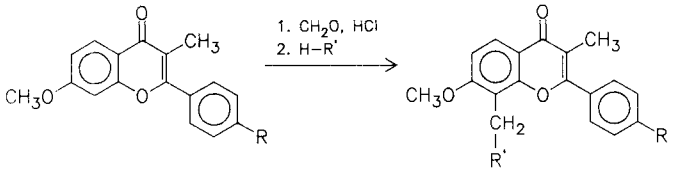
With the aim of estimating quantitatively the effects of the 4' substituents and of the lipophilicity in this class of compounds, we have synthesized a number of new derivatives (Tab. 1), which bear different substituents in the 4' position; these substituents have been chosen in a way to increase the range of the electronic property ($\sigma(CH_3) = -0.17$, $\sigma(NO_2) = 0.78$).

Moreover the distribution coefficient at pH = 7.4 (log D) has been measured for all the compounds of the series; this parameter instead of the partition coefficient of the neutral molecules has been considered, in order to take into account the ionization of the compounds in the pharmacological medium. The partition between n-octanol and phosphate buffer at pH = 7.4 is thought to represent more closely the partition between lipophilic and hydrophilic biological phases.

Results and Discussion

a) Pharmacology

The LD_{50} of each new compound is reported in Table 1. The range of values is from 10 (**2a**) to 330 (**2e**) mg/kg, but it



	R
1a - e	CH_3
2a - e	NO_2
3a - e	H
4a - e	F
5a - e	Cl

	R'
a	NMe_2
b	NEt_2
c	N
d	N
e	N O

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Table 1. Acute toxicity of the analeptic agents related to dimefline. LD_{50} s were estimated following *Weil's* method[17].

	Tested doses (mg/kg, i.p.)	No. dead mice for each group	LD_{50}^{a} (mg/kg)
1a	10	2	22 (14-34)
	20	6	
	40	6	
	80	10	
1b	30	0	75 (58-97)
	60	3	
	120	9	
	240	10	
1c	30	0	69 (44-107)
	60	5	
	120	8	
	240	9	
1d	40	1	112 (79-159)
	80	2	
	160	8	
	320	9	
1e	100	2	242 (156-376)
	200	5	
	400	6	
	800	10	
2a	3	1	10 (7-15)
	6	4	
	12	4	
	24	9	
2b	20	3	45 (24-83)
	40	5	
	80	6	
	160	9	
2c	33	2	95 (63-143)
	66	4	
	132	5	
	264	10	
2d	50	1	140 (99-198)
	100	2	
	200	8	
	400	9	
2e	90	1	330 (229-475)
	180	1	
	360	6	
	720	9	

a) In parenthesis the confidence interval at probability level p = 0.05 is reported.

is evident that the main influence is given by the R' substitution in comparison to R substitution. In fact the decreasing order of toxicity runs parallel from dimethylaminomethyl- to morpholinomethyl-derivatives in both the **1a-e** and **2a-e** subsets.

Following the classification of the patterns reported in "Experimental part", the tonic manifestations observed with all the tested compounds fall within the type 2, thus showing the same symptomatology of the 4'-halogen derivatives previously described[1]. All the **1a-e** and **2a-e** derivatives give rise to paroxysmal seizures, which arise within 3 min after i.p. administration. Their activity appears to be lower than that of the reference compounds **3a-e**[3], with a decreased tonic component in the neuroexcitatory picture (type 2), so that the terminal convulsive attacks are not followed by post-mortem sudden rigidity with "tin soldier" posture. The CNS stimulating potency follows the order of the reference series **3a-e**[3], as well as of the xanthone analogues[4].

b) QSAR

The SAR of the whole series of analeptics (Tab. 2) have been quantitatively studied by means of the *Hansch* method. The parameters screened for the lipophilic, electronic and steric effects were: log D, accounting for the lipophilicity of the whole molecule, σ_p and MR[5], for the electronic and steric effects of the 4' substituents. After preliminary analysis it was realized that the steric effects have no influence on the variation of the activity and that the parameter F[5] works better than σ in accounting for the electronic effects. It was also noted that the dimethylaminomethyl-derivatives have all a characteristic higher activity, so that an indicator variable seems to be needed to quantitate this important effect.

The observed and calculated activities (expressed as log $1/LD_{50}$ Mol/kg) and the parameters used in the correlation equations 1-3 are shown in Tab. 2. The stepwise development of equation 3, that embodies the structure-activity relationships of the series of compounds studied, is the following:

$$\log 1/LD_{50} = 0.88\ (\pm 0.36)\ I + 3.50\ (\pm 0.17) \qquad 1)$$
$$n = 22 \quad r = 0.751 \quad s = 0.341 \quad F_{(1,20)} = 25.93$$

$$\log 1/LD_{50} = 0.72\ (\pm 0.33)\ I - 0.35\ (\pm 0.25) \log D + 4.41\ (\pm 0.67) \qquad 2)$$
$$n = 22 \quad r = 0.835 \quad s = 0.292 \quad F_{(1,19)} = 8.31$$

$$\log 1/LD_{50} = 0.66\ (\pm 0.28)\ I - 0.47\ (\pm 0.22) \log D - 0.62\ (\pm 0.41) F + 4.91\ (\pm 0.64) \qquad 3)$$
$$n = 22 \quad r = 0.897 \quad s = 0.241 \quad F_{(1,18)} = 9.88$$

In the above equations n is the number of compounds on which the equation is based, r is the correlation coefficient, s is the standard deviation and F is the F-test for the significance of the variable added last to the model; I is an indicator variable that takes the value of 1 if the dimethylaminomethyl-moiety is present on the molecule, and 0 if not.

From these equations it comes out that the most important feature of this series of analeptics is the dimethylaminomethyl-group, that contributes substantially to the activity. This can also be verified considering the difference in activity between the dimethylaminomethyl- and the diethylaminomethyl-derivatives of each subset: they can be considered bioisosteric in terms of lipophilicity and electronic properties (see the log D and F values), but their activities are quite different, favouring the dimethylaminomethyl-substituted compound in all the cases.

As far as the electronic properties are concerned, we found that F gives far better correlations than σ. This means that only inductive effects are operating, with no influence of resonance effects. The F term comes last into the equation, and it is actually based on only five values; nevertheless its introduction into the equation is significant ($F_{(1,18;0.01)} = 8.28$). The negative coefficient with F clearly indicates that electron releasing substituents are favoured, thus implying that an increased electron density on the phenyl ring is required for a higher activity.

A striking feature of equation 3 is the negative coefficient with the log D term. We actually expected a parabolic or bilinear function relating the activity to the lipophilic parameter, while what we found might be interpreted at best as a "right side" of such models. In other words, the optimum of lipophilicity (in log D terms) should be at even lower values than the lowest values we reached with our compounds. For drugs acting on the CNS, an optimum log P (or log D) of ca. 2 is often found[6], but the analeptics investigated in this work seem to behave differently. It is interesting, though, to consider the convulsant activities[7] and the log P values[8] of some other well known CNS stimulants, some of which have a mechanism of action similar to that of dimefline:

	CD_{50} (mg/kg)	log P
pentylenetetrazole	40.50	0.14
bemegride	14.10	0.23
strychnine	0.55	1.93
		0.68 (pH = 7.3)
nikethamide	145	0.33
ethamivan	25.50	1.42 (calculated[9])

All the log P values are rather low, suggesting that the lipophilicity could be not so important as in other classes of CNS drugs. Slightly positive log P values still seem to ensure the crossing of the blood-brain barrier at such an extent to allow the penetration into the CNS. More data on the CNS stimulating action of drugs would be needed in order to determine a lower limit of log P.

Experimental Part

Chemistry

3-methyl-7-methoxy-4'-nitroflavone

A mixture of 40 g (0.22 moles) of 2-hydroxy-4-methoxypropiophenone, 80 g of p-nitrobenzoylchloride and 120 g of sodium p-nitrobenzoate was

Table 2. Pharmacological activity and parameters used in the correlation equations.

	log 1/C[a]		Δ	log D	F	I
	Obsd.	Calcd.[b]				
1a	4.19	4.46	0.27	2.41	-0.04	1
1b	3.69	3.78	0.09	2.46	-0.04	0
1c	3.72	3.76	0.04	2.50	-0.04	0
1d	3.53	3.46	0.07	3.13	-0.04	0
1e	3.19	3.19	0.00	3.71	-0.04	0
2a	4.57	4.34	0.23	1.73	0.67	1
2b	3.94	3.70	0.24	1.70	0.67	0
2c	3.62	3.63	0.01	1.83	0.67	0
2d	3.46	3.31	0.15	2.53	0.67	0
2e	3.09	3.08	0.01	3.02	0.67	0
3a	4.83	4.66	0.17	1.93	0.00	1
3b	4.10	3.97	0.13	1.99	0.00	0
3c	-	-	-	-	-	-
3d	3.82	3.67	0.15	2.63	0.00	0
3e	3.63	3.38	0.25	3.25	0.00	0
4a	4.03	4.37	0.34	1.97	0.43	1
4b	3.50	3.71	0.21	1.99	0.43	0
4c	3.18	3.64	0.46	2.13	0.43	0
4d	3.06	3.37	0.31	2.71	0.43	0
4e	-[c]	-	-	3.26	0.43	0
5a	4.30	4.08	0.22	2.62	0.41	1
5b	3.71	3.41	0.30	2.66	0.41	0
5c	3.43	3.36	0.07	2.75	0.41	0
5d	2.76	3.03	0.27	3.46	0.41	0
5e	-[c]	-	-	3.94	0.41	0

a) C = LD_{50} Mol/kg

b) calculated from equation 3

c) activity value not quantitatively determined[1]

heated in an oil bath at 180-190°C for 7-8 h. The reaction mixture was poured into water, washed (NaOH and H_2O) and extracted with $CHCl_3$. Removal of the solvent left a residue which was crystallized from ethanol (24 g, 36%), m.p. 217-220°C. $C_{17}H_{13}NO_5$ (311.3) Calcd. C 65.6 H 4.18 N 4.5 Found C 65.7 H 4.21 N 4.6.

3-methyl-7-methoxy-4'-methylflavone

Following the same procedure as for the previous compound, from 27 g (0.15 moles) of 2-hydroxy-4-methoxypropiophenone, 51 g of p-methylbenzoylchloride and 75 g of sodium p-methylbenzoate, 16.6 g (40%) of crystallized product were obtained (ethanol), m.p. 100-103°C. $C_{18}H_{16}O_3$ (280.3) Calcd. C 77.1 H 5.71 Found C 77.2 H 5.8.

3-methyl-7-methoxy-8-chloromethyl-4'-nitroflavone

To a solution of 24 g (0.08 moles) of 3-methyl-7-methoxy-4'-nitroflavone in 240 ml of acetic acid and 120 ml of conc. HCl, 5.6 g of paraformaldehyde were added and the mixture was stirred at 70-80°C for 5 h, while a stream of HCl was introduced. The reaction mixture was then poured into water and the separated solid was collected, washed with water, dried and crystallized from toluene (80%), m.p. 223-225°C. $C_{18}H_{14}ClNO_5$ (359.8) Calcd. C 60.1 H 3.89 N 3.9 Found C 60.2 H 3.90 N 3.9.

3-methyl-7-methoxy-8-chloromethyl-4'-methylflavone

Following the same procedure as for the previous compound, from 16 g (0.06 moles) of 3-methyl-7-methoxy-4'-methylflavone, the 8-chloromethyl-derivative was obtained (80%) ethanol, m.p. 183-185°C. $C_{19}H_{17}ClO_3$ (328.8) Calcd. C 69.4 H 5.17 Found C 69.5 H 5.20.

General procedure for the 8-aminomethyl-derivatives

A solution of 0.01 moles of the 8-chloromethyl-derivative and an excess of the appropriate amine in 300 ml of benzene was stirred at room temp. for 5-6 h. The reaction mixture was washed with water and the benzene layer was dried over $MgSO_4$. The solvent was then evaporated *in vacuo* and the residue crystallized from the appropriate solvent (Tab. 3).

Table 3. Analytical data of the newly synthesized compounds.

	R	R'		Analysis			m.p. (solvent)	
				%C	%H	%N	°C	
1a	CH_3	NMe_2	Calcd.	74.8	6.82	4.1	100-3	(ligroin)
			Found	74.8	6.94	4.1		
1b	CH_3	NEt_2	Calcd.	74.8	7.65	4.0	103-10	(ligroin)
			Found	74.8	7.69	3.9		
1c	CH_3		Calcd.	76.0	6.89	3.9	135-38	(ligroin)
			Found	76.2	7.07	3.7		
1d	CH_3		Calcd.	76.4	7.16	3.7	159-62	(ligroin)
			Found	76.5	7.41	3.5		
1e	CH_3		Calcd.	72.8	6.60	3.7	181-84	(ligroin)
			Found	72.9	6.57	3.4		
2a	NO_2	NMe_2	Calcd.	65.2	5.43	7.6	191-3	(ethanol)
			Found	65.2	5.66	7.2		
2b	NO_2	NEt_2	Calcd.	66.7	6.06	7.1	168-70	(ethanol)
			Found	66.8	6.12	6.9		
2c	NO_2		Calcd.	67.0	5.58	7.1	180-2	(ethanol)
			Found	67.0	5.61	7.0		
2d	NO_2		Calcd.	67.6	5.88	6.9	182-5	(ethanol)
			Found	67.4	5.97	6.8		
2e	NO_2		Calcd.	64.4	5.37	6.8	224-6	(ethanol)
			Found	64.6	5.41	6.4		

Pharmacology

It is well known that the main acute toxic effects of analeptics may be considered as result of general hyperexcitation of CNS, since these drugs cause both an increase in ventilation and in motor activity; the animals show hyperpnea, hyperexcitability and tonic and clonic convulsions. While clonic convulsions seem mainly an expression of cortical stimulation[10-12], the tonic component usually prevails in true analeptic brain-stem stimulants[13-15].

Therefore in screening tests two general considerations are valuable: 1) drugs which cause clonic convulsions without tonic extension generally exert little or no analeptic activity; 2) the centrally acting drugs produce a particular type of convulsive patterns which are fairly characteristic of their analeptic properties[7,16].

Three different patterns can be outlined after overdosage of this kind of compounds:

1. The animals almost suddenly loose the control of the body posture and struggle vigorously. After one or two generalized clonic tonic attacks, the tonic component prevails and highly characteristic rigidity occurs; the head is flexed on the chest, the forelimbs are extended and rigid, adhering to the torax and the hindlimbs, too, are extended and rigid. Death usually occurs after paroxysmal convulsive excitation or, occasionally, after a deep prostration. This pattern is typical of brain-stem stimulants such as pentylenetetrazole, bemegride and dimefline[16]. In those cases, however, in which the symptomatology is delayed with maximal tonic extension occurring little before dying followed by sudden relaxation, pattern 1 will be indicated as pattern 1a.

2. The animals mantain their posture during the first muscular twitches, but the whole body assumes exaggerated muscular tone: they sit down on their hindlimbs which are rigid, extended and outstretched, whereas forelimbs are lifted up (kangaroo posture). Then the animals struggle with poor coordination and become unable to maintain their posture, a natatory convulsive pattern is observed associated with some gasps and a state of deep depression with respiratory failure, which leads to death, occurs. This picture resembles that described for nikethamide and prethcamide[16].

3. The animals do not maintain their posture and tend to lie at one side or to assume a cathatonic behaviour with a staggered gait. No clonic convulsive attacks are observed and the animals gradually pass from tonic rigidity into a state of deep depression followed by a delayed death.

Since the paroxysmal excitation of CNS is usually the main cause of death and the toxic syndrome is very acute, the reciprocal of LD_{50} may be considered as fairly good index of stimulating activity on CNS and is used to measure the potency ratio with respect to typical brain-stem stimulants such as pentylenetetrazole[7].

Experimental Part

Pharmacology

The acute LD_{50}s, referred to the bases, were estimated following *Weil's* method[17], and the behaviour pattern of animals was observed during one day in groups of mice after i.p. administration of four doses for each compound dissolved in saline as HCl salts.

Groups (n = 10) of female mice (Swiss strain, 20-25g), supplied by Nossan, Italia, were housed, at least for ten days, in groups of ten (Macrolon^R cage) on a 12 h light-dark cycle (8a.m. - 8p.m. light) in a temp. controlled environment (21±1°C) and allowed free access to food (Nossan GP-M pellets) and water until one h before the start of the experiment.

The experiments were performed blind in that during the test the tester was unaware of the compound or dose each individual animal had received. All tests were performed at room temp. and they took place between 10.00 and 15.00 h. Each animal was only used once.

c) **Partition coefficients**

The n-octanol/buffer partition coefficients (distribution coefficients) have been measured by means of the "shake-flask" method[18], using 0.05 molar pH = 7.4 phosphate buffer as the polar phase and n-octanol as the lipid phase; each phase was previously saturated with the other and centrifuged if not clear. A weighted amount of compound was dissolved in the octanol phase, and an appropriate amount of buffer was added; the bottles were then gently shaken for ca. 5 min, and centrifuged for 0.5 h at 2000 r.p.m. A ratio of the octanol and buffer volumes was chosen to give a reliable UV absorbance at the wavelength of maximum absorption. The concentration in the aqueous phase was determined spectrophotometrically by means of a Varian DMS-90 UV-visible spectrophotometer; each reported log D is the average of at least four determinations with $s \leq 0.03$. No dependence of log D on the solute concentration has been detected.

d) **Calculations**

The equations have been developed by means of regression analysis, using the GLM and the STEPWISE procedures of the SAS statistical package[19] running on an IBM 3090 computer of the Centro di Calcolo Interuniversitario C.I.N.E.C.A., Casalecchio di Reno, Bologna.

References

1 P. Valenti, M. Recanatini, P. Da Re, L. Cima, and P. Giusti, Arch. Pharm. *316*, 421 (1983).
2 A.M. Barbaro, M.C. Guerra, G. Cantelli Forti, G. Aicardi, G.L. Biagi, P. Da Re, P. Valenti, and P. Borea, J. Chrom. *242*, 1 (1982).
3 P. Da Re, L. Verlicchi, I. Setnikar, W. Murmann, and M.J. Magistretti, Nature (London) *184*, 362 (1959).
4 P. Da Re, L. Sagramora, V. Mancini, P. Valenti, and L. Cima, J. Med. Chem. *13*, 527 (1970).
5 C. Hansch and A. Leo, Substituent Constants for Correlation Analysis in Chemistry and Biology, Wiley-Interscience, New York 1979.
6 C. Hansch, J.P. Biorkroth, and A. Leo, J. Pharm. Sci. *76*, 663 (1987).
7 I. Setnikar, W. Murmann, M.J. Magistretti, P. Da Re, and L. Verlicchi, J. Med. Pharm. Chem. *3*, 471 (1961).
8 Pomona College Medicinal Chemistry Project Database;
9 Pomona College Medicinal Chemistry Project CLOGP Program Ver.3.42;
10 J. Cheymol, Actual. Pharmacol. *2*, 1 (1950).
11 L.S. Goodman, M.S. Grewal, W.C. Brown, and E.A. Swinyard, J. Pharmacol. *108*, 168 (1953).
12 A. Kreindler, E. Zuckermann, M. Steriade, and D. Chimoion, J. Neurophysiol. *21*, 430 (1958).
13 I. Setnikar, W. Murmann, and M.J. Magistretti, Arzneim. Forsch. *11*, 1109 (1961).
14 T.E. Starzl, W.T. Niemer, and M. Dell, J. Neuropathol. Exp. Neurol. *12*, 262 (1953).
15 V.G. Longo, Electroencephalographic Atlas for Pharmacological Research, Elsevier Publishing Co., Amsterdam 1962.
16 I. Setnikar, Res. Progr. Org. Biol. Med. Chem. *1964*, 423; C.A. *63*, 18506e (1965).
17 C.S. Weil, Biometrics *8*, 249 (1952).
18 T. Fujita, J. Iwasa and C. Hansch, J. Am. Chem. Soc. *86*, 5175 (1964).
19 SAS Institute Inc., SAS User's Guide: Statistics. Version 5 Edition, SAS Institute, Cary, 1985.

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