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Quantitative Structure-activity Relationships in Minor Tranquilizers Benzodiazepinooxazole Derivatives

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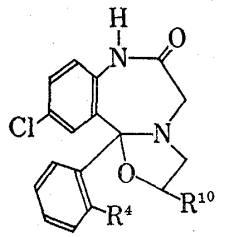
Quantitative structure-activity relationships (QSAR) for benzodiazepinooxazoles were formulated in 9 equations correlating chemical structure with 9 types of biological activities. The equations describing the relationships are log $1/C = a\pi\text{-}7 + bF\text{-}3 + cF\text{-}4 + dI\text{-}1 + eI\text{-}2 + fI\text{-}3 + g$ where C is the molar concentration causing 50% biological activity, π-7 is the hydrophobic constant for substituents on the N^7 position of 1,4-diazepine (II), F-3 and F-4 refer to the electronic effects of R^3 and R^4, respectively, and the indicator variable I-1, I-2 and I-3 refer to the methyl function of the R^8, R^9 and R^{10}, respectively.

Keywords——QSAR (Quantitative Structure-Activity Relationships); minor tranquilizer; benzodiazepinooxazole; benzodiazepine; central nervous system drugs; linear free energy relationships; behavioral pharmacology; anxiolytic sedative activity; anticonvulsant activity; sleep-inducing activity

Since the first paper on the pharmacology of chlordiazepoxide (methaminodiazepoxide), a benzodiazepine, was published by Randall, *et al.* in 1960,[2] extensive studies of 1,4-benzodiazepines have been proceeded. In our laboratories, studies on psychotherapeutic agents have been carried out for many years.[3,4] Finally we found an excellent, anxiolytic sedative activity in 1,4-benzodiazepinooxazole derivatives with a new tricyclic ring system.[5,6] Oxazolam (Serenal®) (Ia) and Cloxazolam (Sepazon®) (Ib) were chosen for the market based on the pharmacological screenings (Fig. 1).



Ia : R^4=H, R^{10}=CH_3
Ib : R^4=Cl, R^{10}=H

Fig. 1

In this paper the formulation of the QSAR for nine kinds of biological assay is shown. The application of QSAR method using linear free energy relationships to central nervous system (CNS) drugs has been challenging for medicinal scientists because the practical examples of the success have been very few[7] and none of them has treated with benzodiazepines. We obtained excellent correlation equations accounting for the behavioral pharmacology.

Method

The substituent-constants used in this work are from the compilation by Pomona College[8] or were

1) Location: *1–2–58, Hiromachi, Shinagawa-ku, Tokyo, 140, Japan.*
2) L.O. Randall, W. Schellek, G.A. Heise, H.F. Keith, and R.E. Bagdon, *J. Pharmacol. Exptl. Therap.*, **129**, 163 (1960).
3) G. Sunagawa and T. Ichii, *Yakugaku Zasshi*, **79**, 1401 (1959).
4) T. Ichii, *Yakugaku Zasshi*, **82**, 992, 999 (1962).
5) T. Miyadera, A. Terada, M. Fukunaga, Y. Kawano, T. Kamioka, C. Tamura, H. Takagi, and R. Tachikawa, *J. Med. Chem.*, **14**, 520 (1971).
6) H. Takagi, S. Kobayashi, and T. Kamioka, *Ann. Sankyo Res. Labo.*, **23**, 1 (1971).
7) C. Hansch, A.R. Steward, S.M. Anderson, and D. Bentley, *J. Med. Chem.*, **11**, 1 (1968); R.I. Mongovius, *Eur. J. Med. Chem.*, **10**, 474 (1975).
8) C. Hansch, A. Leo, S.H. Unger, K.H. Kim, D. Nikaitani, and E.J. Lien, *J. Med. Chem.*, **16**, 1207 (1973).

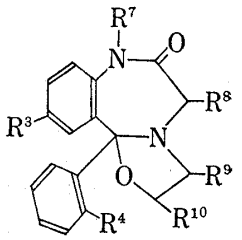
calculated from these values. Many examples of calculations of π values have been reported.[9] The nine test methods were employed to evaluate the pharmacological activities. Animals used were male mice of the ddY-strain, weighing 20—25 g. The compounds were administered orally as a suspension in 0.85% saline containing 0.5% gum tragacanth. Each effect was measured 1 hour later after administration. The ED_{50} was calculated by the method of Litchfield and Wilcoxon.[10] C in the correlation equations is the mole/kg description of ED_{50}. Most of the data come from the published papers[5,6] and a few data from the unpublished.

Results

Anti-bemegride Activity

The studies on an anxiolytic sedative activity were carried out on groups of 10 or 20 male mice at a minimum of 3 dose levels. The test compounds were given orally 1 hour before administration of bemegride (30 mg/kg, *s.c.*) and the animals were observed 30 min after bemegride injection. The ED_{50} was calculated as the dose to prevent 50% of animals from clonic convulsions.

In order to formulate the correlation equation for II (Fig. 2) the examination of the physicochemical constants for substituents on each position was carried out. The comparison of the average deviation of log $1/C$ with the electronic substituent constants for R^3 in Table I suggested the best fit to F values, which are measures of inductive effects.[8] The estimation of the parameter for R^4 was proceeded in the same way as that of R^3 and the good fit of the activities to δ_m or F values was found. We preferred F values to σ_m from a physicochemical point of view : R^4 located on *ortho*-position and not on *meta*-position of the phenyl ring shown in II. The appropriate constants for R^7 were π and MR from a preliminary tabular



II

Fig. 2

Table I. Estimation of the Variables for R^3: the Comparison of log $1/C$ with Substituent Constants

R^4, R^7, R^8, R^9, R^{10}	R^3 H	Cl	Br	NO_2
7-CH_3 10-CH_3		4.80	4.92	5.17
10-CH_3	4.32	5.16	5.34	5.49
H		5.20	5.35	5.62
7-CH_3		4.86	5.09	5.53
8-CH_3		4.71	4.80	
7-C_2H_5		4.96	5.25	5.21
9-CH_3		5.87	5.96	6.23
4-Cl 10-CH_3	3.96	6.00	6.43	
4-Cl		6.18	6.45	
4-F		5.98	6.45	
4-Cl 8-CH_3		5.91	6.01	
4-Cl 9-CH_3		6.52	6.61	
4-Cl 7,10-$(CH_3)_2$		5.11	5.55	
4-Cl 7-C_2H_5		5.84	5.93	
4-F 9-CH_3		6.50	6.73	
4-Cl 7-CH_3		5.84	6.11	
Average deviation	1.44	0.22	0.22	
σ_m	0.0	0.37	0.39	0.71
σ_p	0.0	0.23	0.23	0.78
F	0.0	0.41	0.44	0.67

9) C. Hansch and M. Yoshimoto, *J. Med. Chem.*, **17**, 1160 (1974); M. Yoshimoto, C. Hansch, and P.Y.C. Jow, *Chem. Pharm. Bull.* (Tokyo), **23**, 437 (1975).

10) J.T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exptl. Therap.*, **96**, 99 (1949).

Table II. Estimation of I–1 for R^8: the Comparison of log $1/C$ between the Compounds with $R^8=CH_3$ and Those with $R^8=H$

R^8	Other subst. 3–Cl	3–Br	3,4–Cl_2 10–CH_3	3–Br 4–Cl	3–Cl 4–Cl	Average deviation	I–1
H	5.20	5.35	6.00	6.45	6.18		0.0
CH_3	4.71	4.80	5.13	6.01	5.91		1.0
Deviation	−0.49	−0.55	−0.87	−0.44	−0.27	−0.52	

Table III. Estimation of I–2 for R^9: the Comparison of log $1/C$ between the Compounds with $R^9=CH_3$ and Those with $R^9=H$

Other subst.	R^9 H	CH_3	Deviation
3–Cl	5.20	5.87	0.67
3–Br	5.35	5.96	0.61
3–NO_2	5.62	6.23	0.61
3,4–Cl_2	6.18	6.52	0.34
3–Cl 4–F	5.98	6.50	0.52
3,4–Br_2	6.26	6.48	0.22
3–Br 4–Cl	6.45	6.61	0.16
3–Br 4–F	6.45	6.73	0.28
3–Cl 7–CH_3	4.86	5.60	0.74
3,4–Cl_2 7–CH_3	5.84	6.23	0.39
Average deviation			0.45
I–2	0.0	1.0	

Table IV. Estimation of I–3 for R^{10}: the Comparison of log $1/C$ between the Compounds with $R^{10}=CH_3$ and Those with $R^{10}=H$

Other subst.	R^{10} H	CH_3	Deviation
3–Cl	5.20	5.16	−0.04
3–Br	5.35	5.34	−0.01
3–NO_2	5.62	5.49	−0.13
3,4–Cl_2	6.18	6.00	−0.18
3–Cl 4–F	5.98	5.70	−0.28
3–Br 4–Cl	6.45	6.43	−0.02
3–Cl 7–CH_3	4.86	4.80	−0.06
3–Cl 7–C_2H_5	4.96	4.93	−0.03
3–Br 7–CH_3	5.09	4.92	−0.17
3–NO_2 7–CH_3	5.51	5.17	−0.34
3,3–Cl_2 7–CH_3	5.84	5.11	−0.73
3–Br 4–Cl 7–CH_3	6.11	5.55	−0.56
3,4–Cl_2 8–CH_3	5.91	5.13	−0.78
Average dev.			−0.26
I–3	0.0	1.0	

examination. For R^8, R^9, and R^{10} we had unsubstituted and methyl-substituted compounds to correlate and the employment of indicator variables[11] was assumed to be appropriate: $I-1=1.0$ for $R^8=CH_3$, $I-1=0.0$ for $R^8=H$, $I-2=1.0$ for $R^9=CH_3$, $I-2=0.0$ for $R^9=H$, $I-3=1.0$ for $R^{10}=CH_3$ and $I-3=0.0$ for $R^{10}=H$ were assigned. Table II, III, and IV

11) C. Daniel and F.S. Wood, "Fitting Equations to Data," Wiley-Interscience, New York, N.Y., 1971, p. 55, 169, 203.

Table V. Biological Data Correlated in Eq(1) and Constants for Deriving Eq(1)—(9)

No.	Substituents	log 1/C Obsd	log 1/C Calcd	Δlog 1/C	π-7	I-1	I-2	I-3	F-3	F-4
1	4-Cl 10-CH_3	3.96	4.83	−0.87	0.0	0.0	0.0	1.0	0.0	0.41
2	10-CH_3	4.32	4.02	0.30	0.0	0.0	0.0	1.0	0.0	0.0
3	3-Cl 7-$CH_2C_6H_4$-2′-Cl	4.33	4.47	−0.14	2.72	0.0	0.0	0.0	0.41	0.0
4	3-Cl 7-C_4H_9 10-CH_3	4.35	4.39	−0.04	2.00	0.0	0.0	1.0	0.41	0.0
5	3-Cl 4,10-$(CH_3)_2$	4.63	4.88	−0.25	0.0	0.0	0.0	1.0	0.41	−0.04
6	3-Cl 8-CH_3	4.71	4.86	−0.15	0.0	1.0	0.0	0.0	0.41	0.0
7	3-Cl 7-$CH_2CH=CH_2$ 10-CH_3	4.77	4.64	0.13	1.10	0.0	0.0	1.0	0.41	0.0
8	3-Cl 7-$CH_2C_6H_5$ 10-CH_3	4.77	4.38	0.39	2.01	0.0	0.0	1.0	0.41	0.0
9	3-Cl 7,10-$(CH_3)_2$	4.80	4.82	−0.02	0.50	0.0	0.0	1.0	0.41	0.0
10	3-Br 8-CH_3	4.80	4.93	−0.13	0.0	1.0	0.0	0.0	0.44	0.0
11	3-Cl 7-CH_3	4.86	5.11	−0.25	0.50	0.0	0.0	0.0	0.41	0.0
12	3-Cl 7-CH_2CH_2Cl	4.90	4.85	0.05	1.39	0.0	0.0	0.0	0.41	0.0
13	3-Br 7,10-$(CH_3)_2$	4.92	4.88	0.04	0.50	0.0	0.0	1.0	0.44	0.0
14	3-Cl 7-C_2H_5 10-CH_3	4.93	4.67	0.26	1.00	0.0	0.0	1.0	0.41	0.0
15	3-Cl 7-C_2H_5	4.96	4.97	−0.01	1.00	0.0	0.0	0.0	0.41	0.0
16	3-Br 7-CH_3	5.09	5.18	−0.09	0.50	0.0	0.0	0.0	0.44	0.0
17	3,4-$(Cl)_2$ 7,10-$(CH_3)_2$	5.11	5.62	−0.51	0.50	0.0	0.0	1.0	0.41	0.41
18	3,4-Cl_2 8,10-$(CH_3)_2$	5.13	5.37	−0.24	0.0	1.0	0.0	1.0	0.41	0.41
19	3-Cl 10-CH_3	5.16	4.96	0.20	0.0	0.0	0.0	1.0	0.41	0.0
20	3-NO_2 7,10-$(CH_3)_2$	5.17	5.41	−0.24	0.50	0.0	0.0	1.0	0.67	0.0
21	3-Cl	5.20	5.25	−0.05	0.0	0.0	0.0	0.0	0.41	0.0
22	3-NO_2 7-C_2H_5	5.21	5.56	−0.35	1.00	0.0	0.0	0.0	0.67	0.0
23	3-Br 7-C_2H_5	5.25	5.03	0.22	1.00	0.0	0.0	0.0	0.44	0.0
24	3-Br 10-CH_3	5.34	5.03	0.31	0.0	0.0	0.0	1.0	0.44	0.0
25	3-Br	5.35	5.32	0.03	0.0	0.0	0.0	0.0	0.44	0.0
26	3-NO_2 10-CH_3	5.49	5.55	−0.06	0.0	0.0	0.0	1.0	0.67	0.0
27	3-NO_2 7-CH_3	5.53	5.70	−0.17	0.50	0.0	0.0	0.0	0.67	0.0
28	3-Br 4-Cl 7,10-$(CH_3)_2$	5.55	5.69	−0.14	0.50	0.0	0.0	1.0	0.44	0.41
29	3-Cl 7,9-$(CH_3)_2$	5.60	5.58	0.02	0.50	0.0	1.0	0.0	0.41	0.0
30	3-NO_2	5.62	5.85	−0.23	0.0	0.0	0.0	0.0	0.67	0.0
31	3-Cl 4-F 10-CH_3	5.70	5.80	−0.10	0.0	0.0	0.0	1.0	0.41	0.43
32	3-Cl 4-F 7-CH_3	5.81	5.95	−0.14	0.50	0.0	0.0	0.0	0.41	0.43
33	3,4-Cl_2 7-CH_3	5.84	5.91	−0.07	0.50	0.0	0.0	0.0	0.41	0.41
34	3,4-Cl_2 7-C_2H_5	5.84	5.77	0.07	1.00	0.0	0.0	0.0	0.41	0.41
35	3-Cl 9-CH_3	5.87	5.72	0.15	0.0	0.0	1.0	0.0	0.41	0.0
36	3,4-Cl_2 8-CH_3	5.91	5.67	0.24	0.0	1.0	0.0	0.0	0.41	0.41
37	3-Br 4-Cl 7-C_2H_5	5.93	5.84	0.09	1.00	0.0	0.0	0.0	0.44	0.41
38	3-Br 9-CH_3	5.96	5.79	0.17	0.0	0.0	1.0	0.0	0.44	0.0
39	3-Cl 4-F	5.98	6.10	−0.12	0.0	0.0	0.0	0.0	0.41	0.43
40	3,4-Cl_2 10-CH_3	6.00	5.76	0.24	0.0	0.0	0.0	1.0	0.41	0.41
41	3-Br 4-Cl 8-CH_3	6.01	5.73	0.28	0.0	1.0	0.0	0.0	0.44	0.41
42	3-Br 4-Cl 7-CH_3	6.11	5.98	0.13	0.50	0.0	0.0	0.0	0.44	0.41
43	3,4-Cl_2	6.18	6.06	0.12	0.0	0.0	0.0	0.0	0.41	0.41
44	3-NO_2 9-CH_3	6.23	6.31	−0.08	0.0	0.0	1.0	0.0	0.67	0.0
45	3,4-Cl_2 7,9-$(CH_3)_2$	6.23	6.38	−0.15	0.50	0.0	1.0	0.0	0.41	0.41
46	3,4-Br_2	6.26	6.19	0.07	0.0	0.0	0.0	0.0	0.44	0.44
47	3-Br 4-Cl 10-CH_3	6.43	5.83	0.60	0.0	0.0	0.0	1.0	0.44	0.41
48	3-Br 4-F	6.45	6.17	0.28	0.0	0.0	0.0	0.0	0.44	0.43
49	3-Br 4-Cl	6.45	6.13	0.32	0.0	0.0	0.0	0.0	0.44	0.41
50	3,4-Br_2 9-CH_3	6.48	6.65	−0.17	0.0	0.0	1.0	0.0	0.44	0.44
51	3-Cl 4-F 9-CH_3	6.50	6.56	−0.06	0.0	0.0	1.0	0.0	0.41	0.43
52	3,4-Cl_2 9-CH_3	6.52	6.52	0.00	0.0	0.0	1.0	0.0	0.41	0.41
53	3-Br 4-Cl 9-CH_3	6.61	6.59	0.02	0.0	0.0	1.0	0.0	0.44	0.41
54	3-Br 4-F 9-CH_3	6.73	6.63	0.10	0.0	0.0	1.0	0.0	0.44	0.43

exhibited the excellent correlation of these parameters to the activities. The correlation equation (1) with least standard deviation was formulated from the data in Table V. The figures in parentheses indicate 95% confidence intervals. The number of compounds used in the correlation is n, correlation coefficient r and

$$\log 1/C = -0.286(\pm 0.13)\,\pi\text{-}7 + 2.288(\pm 0.63)F\text{-}3 + 1.965(\pm 0.37)F\text{-}4 - 0.392(\pm 0.26)\,I\text{-}1 + 0.466(\pm 0.20)\,I\text{-}2 - 0.294(\pm 0.16)\,I\text{-}3 + 4.314(\pm 0.35) \quad (1)$$

n	r	s
54	0.938	0.254

standard deviation s. The hydrophobic substituents in R^7 decrease the activities. For R^3 and R^4 the compounds with the stronger electron-attracting groups are the more active. The methyl groups of R^8 and R^{10} decrease the activities, while the methyl of R^9 increases the activities. The relatively large deviation between the observed and the predicted activity of compound (**1**) in Table V might be ascribed to the presence of only one compound with 4-halo-substituent and without 3-substituent, because all of other 4-halo-substituted compounds (No. 17, 18, 28, 31—34, 36, 37, 39—43, 45—54 in Table V) have 3-substituents and are very active. In other words, the contribution of F-4 is enhanced by the effect of coexistent R^3. We need more compounds with 4-halo-substituents and without 3-substituents to know whether the enhancement is due to a true physicochemical perturbation or a mathematical quantitative structure-activity relationships (QSAR) method.

The QSAR exhibited in eq (1) is very good from a statistical point of view. The standard deviation shows that the compounds correlated are predicted in the range of 1.8 times (anti-log of 0.254) in average. According to Takagi, *et al.*[12] this method can serve as an excellent screening test for the anxiolytic minor tranquilizers. We could support their evaluation from the standpoint of QSAR.

Table VI. Squared Correlation Matrix Showing Degree of Collinearity (r^2) between the Important Variables Used in Anti-bemegride Test Correlation Analysis

	π-7	MR-7	F-3	F-4	I-1	I-2	I-3
π-7	1.00	0.95	0.00	0.10	0.05	0.06	0.00
MR-7		1.00	0.00	0.09	0.04	0.05	0.01
F-3			1.00	0.04	0.00	0.00	0.04
F-4				1.00	0.01	0.02	0.02
I-1					1.00	0.02	0.01
I-2						1.00	0.12
I-3							1.00

Table VII. Development of QSAR for Anti-bemegride Test, Eq(1)

Intercept	F-4	F-3	I-2	π-7	I-3	I-1	MR-7	r	s	$F_{1,x}$[a]
5.11	2.13							0.650	0.530	38.1
3.83	2.46	2.80						0.799	0.423	30.5
3.83	2.27	2.58	0.72					0.895	0.318	40.4
3.97	2.06	2.57	0.65	−0.22				0.912	0.295	9.29
4.20	1.97	2.34	0.55	−0.23	−0.25			0.925	0.275	8.14
4.31	1.96	2.29	0.47	−0.29	−0.29	−0.39		0.938	0.254	9.50
4.30	2.00	2.28	0.49		−0.27	−0.36	−0.22	0.934	0.262	

a) $F_{1,40}(\alpha=0.001)=12.6$, $F_{1,40}(\alpha=0.01)=7.31$

12) H. Takagi, T. Kamioka, S. Kobayashi, Y. Suzuki, and R. Tachikawa, *Folia Pharmacol. Jap.*, **66**, 107 (1970).

TABLE VIII. Biological Data Correlated in Eq(2)—(9)

No.	Eq(2) log 1/C		Eq(3) log 1/C		Eq(4) log 1/C		Eq(5) log 1/C		Eq(6) log 1/C		Eq(7) log 1/C		Eq(8) log 1/C		Eq(9) log 1/C	
	Obsd	Calcd	Obsd	Calcd	Obsd	Calcd	Obsd	Calcd	Obsd	Calcd	Obsd	Calcd	Obsd	Calcd	Obsd	Calcd
9	4.66	4.45	4.06	4.01	3.30	3.54	3.60	3.80	3.65	3.83	3.86	3.98	3.84	3.93	4.46	4.40
11	4.72	4.72	4.12	4.24			3.56	3.80	3.79	3.94	4.19	4.30	4.12	4.33	4.07	4.40
13	4.59	4.50	4.11	4.05	3.53	3.54	3.81	3.86	3.91	3.90	4.00	4.03	4.03	3.97	4.38	4.46
16	4.82	4.76	4.17	4.28	3.87	3.81	4.02	3.86	4.14	4.01	4.34	4.35	4.57	4.37	4.57	4.46
17	4.88	5.20	4.35	4.50	3.38	3.71	3.88	4.11	3.90	4.13	4.53	4.41	4.32	4.41	4.79	5.00
19	4.79	4.80	4.17	4.46	3.94	4.02	3.61	3.80	3.66	3.83	4.44	4.27	4.40	4.25	4.44	4.69
20	4.75	4.85	4.21	4.35	3.81	3.54	4.10	4.33	4.34	4.41	4.76	4.40	4.76	4.29	4.99	4.87
21	4.81	5.07	4.50	4.69	4.08	4.29	3.86	3.80	3.91	3.94	4.73	4.59	4.73	4.65	4.90	4.69
24	4.86	4.84	4.74	4.50	4.14	4.02	4.11	3.86	4.25	3.90	4.40	4.32	4.37	4.29	4.75	4.74
25	5.06	5.11	4.99	4.73	4.30	4.29	4.16	3.86	4.19	4.01	4.75	4.64	4.76	4.70	5.06	4.74
26	5.12	5.20	4.88	4.81	4.05	4.02	4.30	4.33	4.38	4.41	4.30	4.69	4.25	4.61	4.93	5.15
27	5.33	5.12	4.53	4.58	4.08	3.81	4.42	4.33	4.53	4.52	4.63	4.73	4.49	4.69	4.89	4.87
28	5.03	5.24	4.55	4.53	3.60	3.71	4.12	4.18	4.26	4.19	4.30	4.46	4.30	4.45	4.88	5.06
30	5.37	5.46	5.01	5.04	4.01	4.29	4.37	4.33	4.43	4.52	5.02	5.01	4.89	5.01	5.10	5.15
31	5.69	5.58	4.75	4.97	4.26	4.19	3.97	4.13	3.98	4.14	4.16	4.72	4.08	4.75	5.20	5.32
33	5.41	5.46	4.78	4.73	3.76	3.98	4.04	4.11	4.20	4.23	4.56	4.73	4.71	4.81	5.22	5.00
35	5.24	5.26	5.01	4.90	4.29	4.29	4.19	4.17	4.24	4.23	4.61	4.59	4.61	4.65	4.92	4.97
36	5.33	5.45	5.04	5.18	4.11	4.06	4.20	4.11	4.16	4.23	4.36	4.50	4.48	4.65	4.85	4.96
38	5.34	5.31	5.04	4.94	4.34	4.29	4.23	4.23	4.29	4.29	4.49	4.64	4.59	4.70	5.12	5.02
39	5.90	5.85	5.08	5.21	4.32	4.46	4.16	4.13	4.20	4.25	4.58	5.04	4.74	5.16	5.24	5.32
40	5.64	5.54	5.14	4.95	4.04	4.19	4.10	4.11	4.20	4.13	4.78	4.70	4.71	4.73	5.41	5.29
41	5.61	5.49	5.13	5.22	4.01	4.06	4.31	4.18	4.30	4.30	4.68	4.54	4.87	4.70	5.13	5.02
42	5.61	5.51	5.13	4.77	4.21	3.98	4.38	4.18	4.43	4.30	4.99	4.78	4.94	4.85	5.31	5.06
43	5.67	5.81	5.14	5.18	4.13	4.46	4.04	4.11	4.24	4.23	4.99	5.02	5.07	5.13	5.08	5.29
47	5.77	5.59	5.15	4.99	4.61	4.19	4.43	4.18	4.53	4.19	5.18	4.74	5.38	4.77	5.36	5.34
49	5.92	5.85	5.25	5.22	4.75	4.46	4.42	4.18	4.52	4.30	5.15	5.07	5.39	5.17	5.60	5.34
48	5.98	5.89	5.42	5.24	4.76	4.46	3.88	4.19	4.34	4.31	5.36	5.09	5.66	5.20	5.34	5.37
51	6.11	6.04	5.29	5.41	4.34	4.46	4.50	4.50	4.54	4.53	5.34	5.04	5.43	5.16	5.67	5.60
52	5.98	6.01	5.30	5.39	4.60	4.46	4.48	4.48	4.52	4.52	4.82	5.02	4.91	5.13	5.33	5.57
53	6.01	6.05	5.43	5.43	4.64	4.46	4.53	4.55	4.57	4.59	5.13	5.07	5.27	5.17	5.74	5.62
32					4.06	3.99			4.94	4.25						
46					4.16	4.47			4.34	4.32						

TABLE IX. Squared Correlation Matrix Showing Degree of Collinearity (r^2) between the Variables Used inEq(2)

	π–7	F–3	F–4	I–1	I–2	I–3
π–7	1.00	0.02	0.03	0.04	0.10	0.04
F–3		1.00	0.18	0.01	0.03	0.01
F–4			1.00	0.06	0.00	0.01
I–1				1.00	0.01	0.04
I–2					1.00	0.12
I–3						1.00

Table VI shows that except for (π, MR) other vectors used in the correlation are quite orthogonal. Substituting MR-7 for π-7 in eq (1) gives a correlation with r=0.926 which is very close to r=0.931 in eq (1) (See Table VII). Considering the high collinearity between π and MR (r^2=0.95 from Table VI) the similarity of the both equations is very reasonable. From the above reason the examination of MR was omitted in eq (2)—(9), but the priority of π or MR is unknown until the polar R^7 compounds are synthesized to break the collinearity

Table X. Develeopment of QSAR for Eq(2)

Intercept	F–4	π–7	I–3	F–3	I–1	I–2	r	s	$F_{1,x}$[a]
4.96	1.70						0.757	0.314	37.6
5.13	1.52	−0.80					0.855	0.253	15.9
5.23	1.46	−0.70	−0.27				0.898	0.219	10.2
4.56	1.69	−0.73	−0.28	1.37			0.926	0.191	9.01
4.57	1.80	−0.78	−0.32	1.40	−0.43		0.952	0.158	12.6
4.44	1.81	−0.69	−0.27	1.53	−0.36	0.20	0.962	0.144	5.98

a) $F_{1,28}$ (α=0.001)=13.5, $F_{1,26}$ (α=0.005)=9.41, $F_{1,25}$ (α=0.01)=7.77 $F_{1,23}$ (α=0.025)=5.75

between π and MR. The development of the QSAR for eq (1) is given in Table VII and each stepwise improvement is statistically very important.

Anti-pentylenetetrazole Test

This test was proceeded as a measure of the central nervous system depressant effect. The ED_{50} was calculated as the dose which prevents 50% of animals from clonic convulsions after the subcutaneous injection of 100 mg/kg of pentylenetetrazole. The correlation equation with least standard deviation was formulated from the data in Table V and VIII. Each term in eq (2) is statistically meaningful and eq (2) shows the excellent correlation of the activities with the chemical structure. The standard deviation shows that the compounds correlated are predicted in the range of 1.4 times (anti-log of 0.144) in average and the correlation coefficient is very high (0.962).

$$\begin{aligned}\log 1/C = &-0.693(\pm0.25)\pi\text{-}7 + 1.528(\pm0.27)F\text{-}3 + 1.814(\pm0.30)F\text{-}4\\ &-0.361(\pm0.24)I\text{-}1 + 0.200(\pm0.17)I\text{-}2 - 0.266(\pm0.12)I\text{-}3\\ &+4.438(\pm0.38) \qquad (2)\end{aligned}$$

n	r	s
30	0.962	0.144

Considering the comparison of eq (2) with eq (1), the intercepts and the coefficients of F–3, F–4, I–1, I–2, and I–3 are identical within 95% confidence intervals, while the coefficients of π–7 are somewhat different. The hydrophobic functions of R^7 decrease the activity in anti-pentylenetetrazole test to a greater extent than in anti-bemegride test, although this is not a solid conclusion because of the poor variation of π–7 in Table VIII. Anti-pentylene-tetrazole test is assumed to be similar to anti-bemegride test from a pharmacological point of view[12,13] and our QSAR studies afforded the good evidence for this assumption.

Table IX shows that the vectors employed in eq (2) are quite orthogonal. Most of the compounds used in deriving eq (3)—eq (9) are common to those in eq (2) and the correlation matrices of the constants of the formers are identical with that in Table IX to be omitted from the tabulation. The development of the QSAR for eq (2) is exhibited in Table X. Each stepwise improvement of the QSAR is very important from a statistical point of view.

Anti-fighting Test

This test was employed as a measure of the taming ability. The animals were stimulated by an electric current (60 Vpp, 1 mA, 3 cps) which was applied through a grid to the feet of the animals. The ED_{50} was determined by the ability to abolish the fighting behavior. The correlation equation was formulated from the data in Table V and VIII. A most interesting

13) L.H. Sternbach, L.O. Randall, R. Banziger, and H. Lehr, "Drugs Affecting the Central Nervous System," Medicinal Research Series 2, Marcell Dekker, Inc., New York, 1968, p. 237.

Table XI. Development of QSAR for Eq(3)

Intercept	π–7	F–4	I–3	F–3	I–2	I–1	r	s	$F_{1,x}$[a]
5.02	−1.24						0.690	0.319	25.4
4.77	−1.07	1.02					0.844	0.241	22.1
4.86	−0.97	0.96	−0.27				0.894	0.205	11.4
4.29	−1.00	1.16	−0.28	1.18			0.919	0.184	7.32
4.15	−0.91	1.19	−0.23	1.19	0.21		0.934	0.170	4.94
4.17	−0.94	1.23	−0.25	1.32	0.18	−0.15	0.937	0.170	1.22

a) $F_{1,26}(\alpha=0.001)=13.5$, $F_{1,26}(\alpha=0.005)=9.41$, $F_{1,25}(\alpha=0.025)=5.69$, $F_{1,24}(\alpha=0.05)=4.26$

aspect of eq (3) is the absence of I–1 term which accounts fro the effect of $R^8=CH_3$. The QSAR shown in eq (3) is statistically very good: the correlation coefficient (0.934) is reasonably high and the standard deviation shows the compounds correlated

$$\log 1/C = -0.911(\pm0.29)\,\pi\text{-}7 + 1.324(\pm0.85)F\text{-}3 + 1.194(\pm0.35)F\text{-}4 + 0.208(\pm0.19)\,I\text{-}2 - 0.230(\pm0.14)\,I\text{-}3 + 4.148(\pm0.44) \quad (3)$$

n	r	s
30	0.934	0.170

to be predicted in the range of 1.5 times (anti-log of 0.170) in average.

The relative importance of the variables in eq (3) can be appreciated by following its stepwise development (Table XI). Adding a term in I–1 to eq (3) improved the correlation coefficient (r=0.937) slightly better, while the variance in log $1/C$ was not reduced at all. Therefore eq (3) was selected as the best correlation formula.

Antimaximal Electroshock Test

This test was employed as a measure of anticonvulsant activity. The electroshock was applied through both corneas with bimolar electrodes with parameters of 12.5 mA, 1000 V for 0.2 sec. Prevention of tonic hind limb extension was assumed as an anti-convulsant effect. The QSAR for benzodiazepinooxazoles causing 50% prevention has been formulated from the data in Table V and VIII.

Table XII. Development of QSAR for Eq(4)

Intercept	π–7	I–3	I–1	F–4	F–3	I–2	r	s	$F_{1,x}$[a]
4.28	−1.04						0.679	0.272	24.8
4.36	−0.93	−0.25					0.764	0.243	8.26
4.40	−0.98	−0.30	−0.34				0.796	0.232	3.71
4.29	−0.96	−0.27	−0.40	0.41			0.827	0.220	4.15
3.96	−0.98	−0.27	−0.40	0.53	0.66		0.838	0.217	1.54
3.88	−0.94	−0.24	−0.37	0.55	0.74	0.11	0.844	0.219	0.76

a) $F_{1,26}(\alpha=0.001)=13.4$. $F_{1,28}(\alpha=0.01)=7.64$, $F_{1,27}(\alpha=0.10=2.90$

$$\log 1/C = -0.957(\pm0.36)\,\pi\text{-}7 + 0.405(\pm0.41)F\text{-}4 - 0.396(\pm0.35)\,I\text{-}1 - 0.271(\pm0.18)\,I\text{-}3 + 4.290(\pm0.16) \quad (4)$$

n	r	s
31	0.827	0.220

The coefficient of F–4 in eq (4) is much smaller than those in eq (1)—(3) and eq (4) lacks a term in F–3. This shows that the effect of the electron-attracting groups of R^3 and R^4 are not so important as those in the preceding three tests. Another characteristic aspect of eq (4) is the lack of I–2 term, which means no role for $R^9=CH_3$ found in the correlation. The cor-

relation coefficient (0.827) is not so good as those in eq (1)—(3), but the correlation is still excellent from the standpoint of standard deviation. The stepwise improvement of QSAR for eq (4) is given in Table XII. The addition of a term in F–3 to eq (4) gave a marginal improvement.

Inclined Plane Test

This test measures the sedative and muscle-relaxant effects. The apparatus consisted of 35° inclined rough plastic plane. The ED_{50} was calculated as the dose which caused half the animals to slide off the plane within 10 sec. The QSAR for II has been formulated from the data in Table V and VIII. The remarkable characteristics of eq (5) are shown in no

$$\log 1/C = 2.039(\pm 0.85)F\text{-}3 + 0.766(\pm 0.34)F\text{-}4 + 0.371(\pm 0.18)\ I\text{-}2 + 2.964(\pm 0.44) \quad (5)$$

n	r	s
30	0.799	0.172

hydrophobic effect of R^7, no effect of I–3, no role in I–1, strong electron-attracting effect of R^3 and weak electron-attracting effect of R^4. The correlation equation is not good in view of r, while the value of s is very small. This means that the observed log $1/C$ from the data in Table VIII is in small variance (standard deviation from the mean value is 0.270). The stepwise improvement of QSAR was afforded in Table XIII. Adding a term in I–3 to eq (5) improved the correlation coefficient and the variance in log $1/C$ slightly better.

Table XIII. Development of QSAR for Eq(5)

Intercept	I–2	F–3	F–4	I–3	π–7	I–1	r	s	$F_{1,x}$[a]
4.07	0.31						0.438	0.247	6.66
3.50	0.23	1.02					0.587	0.226	6.28
2.96	0.37	2.04	0.77				0.799	0.172	21.1
3.02	0.32	2.04	0.74	−0.11			0.819	0.167	2.54
3.06	0.29	2.05	0.70	−0.10	−0.20		0.836	0.163	2.17
3.05	0.30	2.05	0.69	−0.09	−0.19	0.05	0.837	0.166	0.15

a) $F_{1,28}(\alpha=0.025)=5.61$, $F_{1,26}(\alpha=0.001)=13.7$, $F_{1,25}(\alpha=0.25)=1.39$

Rotating Rod Test

This test measures the ataxic and muscle-uncoordinating effects. The rotating rod was made of plastic rod (3 cm in diameter) with a gause-lined surface revolving (10 rev./min). The ED_{50} was calculated as the dose which caused half the animals to fall off the rod within one minute. The QSAR was formulated from the data in Table V and VIII. Eq (6) is identical with eq (5) within 95% confidence interval of each term. From the statistical examination the inclined plane test is assumed to have the same character as the rotating rod test as well as from the pharmacological estimation. The correlation equation is not so good based on r, while it is still excellent in view of standard deviation.

The stepwise improvement of QSAR was given in Table XIV. Adding a term in π–7 to eq (6) improved the correlation coefficient and the variance in log $1/C$ slightly better.

$$\log 1/C = 2.211(\pm 0.76)F\text{-}3 + 0.713(\pm 0.30)F\text{-}4 + 0.286(\pm 0.16)\ I\text{-}2 - 0.108(\pm 0.13)\ I\text{-}3 + 3.035(\pm 0.40) \quad (6)$$

n	r	s
32	0.826	0.155

Traction Test

This test was employed as a severe measure of the muscle-relaxant effect in body and

Table XIV. Development of QSAR for Eq(6)

Intercept	F–3	F–4	I–2	I–3	π–7	I–1	r	s	$F_{1,x}$[a]
3.69	1.15						0.377	0.241	4.98
3.17	1.93	0.72					0.655	0.200	14.5
2.98	2.21	0.75	0.33				0.804	0.160	17.2
3.03	2.21	0.71	0.29	−0.11			0.826	0.155	3.07
3.08	2.21	0.68	0.25	−0.10	−0.19		0.844	0.150	2.65
3.09	2.21	0.70	0.24	−0.11	−0.21	−0.08	0.847	0.152	0.54

a) $F_{1,30}(\alpha=0.05)=4.17$, $F_{1,28}(\alpha=0.001)=13.5$, $F_{1,27}(\alpha=0.10)=2.90$

limbs. The apparatus consisted of a slippery glass rod (1.3 cm in diameter) held horizontally at a height of 30 cm. The animals were suspended by their front paws beneath the rod and forced to pull themselves up on this rod. The ED_{50} was calculated as the dose which caused half the animals to fall within 5 sec. The correlation equation has been formulated from the

$$\log 1/C = -0.576(\pm0.42)\,\pi\text{-}7 + 1.624(\pm1.23)F\text{-}3 + 1.040(\pm0.52)F\text{-}4 - 0.521(\pm0.40)\,I\text{-}1 - 0.321(\pm0.20)\,I\text{-}3 + 3.925(\pm0.62) \quad (7)$$

n = 30, r = 0.807, s = 0.249

data in Table V and VIII. Eq (7) lacks a term in I–2 which accounts for $R^9=CH_3$ and is not similar to any of eq (1)—(6). This QSAR suggests the unique pharmacological aspect of this test different from the preceding six tests.

The correlation coefficient is to some extent poor, while the standard deviation is still good. The stepwise improvement of the QSAR was given in Table XV.

Table XV. Development of QSAR for Eq(7)

Intercept	F–4	I–3	F–3	π–7	I–1	I–2	r	s	$F_{1,x}$[a]
4.47	0.82						0.453	0.348	7.22
4.60	0.74	−0.31					0.598	0.319	6.41
3.86	0.99	−0.32	1.50				0.671	0.301	4.39
3.91	0.91	−0.28	1.59	−0.51			0.738	0.279	5.17
3.92	1.04	−0.32	1.62	−0.58	−0.52		0.807	0.249	7.31
3.92	1.04	−0.32	1.63	−0.57	−0.51	0.01	0.807	0.255	0.009

a) $F_{1,28}(\alpha=0.025)=5.61$, $F_{1,26}(\alpha=0.05)=4.23$

Balance Test

This test was employed as a measure of the effect on the unbalance (a lack of muscular coordination and vestibular function). The apparatus used was the same as that in the traction test. The ED_{50} was calculated as the dose which caused half the animals to fall within one minute when they were placed on the rod. The correlation equation was formulated from the data in Table V and VIII. The quality of eq (8) as well as eq (7) is a little poor not only in view of the correlation coefficient, but also based on the standard deviation. Eq (8)

$$\log 1/C = -0.648(\pm0.49)\,\pi\text{-}7 + 1.384(\pm1.44)F\text{-}3 + 1.171(\pm0.61)F\text{-}4 - 0.479(\pm0.49)\,I\text{-}1 - 0.403(\pm0.24)\,I\text{-}3 + 4.085(\pm0.75) \quad (8)$$

n = 30, r = 0.803, s = 0.293

is completely identical with eq (7) considering 95% confidence interval of each term. The balance test is assumed to have the same characteristics as the traction test from the view-

Table XVI. Development of QSAR for Eq(8)

Intercept	F–4	I–3	π–7	I–1	F–3	I–2	r	s	$F_{1,x}$[a]
4.46	1.05						0.495	0.395	9.10
4.63	0.93	−0.40					0.661	0.347	9.20
4.73	0.82	−0.35	−0.56				0.722	0.326	4.58
4.76	0.94	−0.39	−0.62	−0.47			0.765	0.310	3.84
4.09	1.17	−0.40	−0.65	−0.48	1.38		0.802	0.293	3.93
4.09	1.17	−0.40	−0.65	−0.48	1.38	0.0004	0.802	0.299	0.00

a) $F_{1,28}(\alpha=0.01)=7.64$, $F_{1,26}(\alpha=0.05)=4.23$, $F_{1,25}(\alpha=0.10)=2.92$

point of statistics as well as pharmacology. The stepwise improvement of QSAR was afforded in Table XVI.

Anesthesia-potentiating Test

This was employed as a measure of the sleep-inducing activity in mice. One hour after the dosage, 30 mg/kg of sodium thiopental was injected intravenously. The animals anesthetized longer than twice the control animals were regarded as effectively potentiated. The correlation equation was formulated from the data in Table V and VIII. Eq (9) is of good

$$\log 1/C = -0.570(\pm0.33)\,\pi\text{-}7 + 1.780(\pm0.94)F\text{-}3 + 1.464(\pm0.39)F\text{-}4 - 0.324(\pm0.30)\,I\text{-}1 + 0.282\,(\pm0.21)\,I\text{-}2 + 3.957(\pm0.49) \quad (9)$$

n	r	s
30	0.898	0.189

Table XVII. Development of QSAR for Eq(9)

Intercept	F–4	π–7	F–3	I–2	I–1	I–3	r	s	$F_{1,x}$[a]
4.75	1.22						0.662	0.296	21.8
4.89	1.08	−0.62					0.760	0.261	8.99
4.12	1.34	−0.66	1.56				0.822	0.233	7.85
3.93	1.38	−0.50	1.80	0.33			0.874	0.203	9.41
3.96	1.46	−0.57	1.78	0.28	−0.32		0.898	0.189	4.85
4.02	1.45	−0.56	1.78	0.22	−0.38	−0.12	0.908	0.183	2.64

a) $F_{1,28}(\alpha=0.001)=13.5$, $F_{1,25}(\alpha=0.01)=7.77$, $F_{1,24}(\alpha=0.05)=4.26$

quality based on the correlation coefficient as well as the standard deviation. The compounds correlated are predicted within the range of 1.5 times (anti-log of 0.189) in average. The terms in I–1 and I–3 are not very important in view of 95% confidence intervals as well as F-test (Table XVII). The stepwise improvement of the QSAR was shown in detail in Table XVII.

Discussion

The overview of the pharmacological activities of benzodiazepinooxazoles presented in this paper, when taken with other studies,[7] constitutes convincing evidence that one can expect to be able to formulate the structure-activity relationships of CNS drugs in numerical terms. Although a final decision remains to be made on π-7 or MR-7 on statistical grounds, all other parameters were determined definitely. Eq (1)—(9) were summarized in Table XVIII. The similarities and differences between the equations are clear from the table. Since the sign of the coefficients in each term is identical in all parameters, the optimization of the activities in all equations are discussed in common. The smaller π-7, the larger F-3 and the larger F-4 are desirable for the more active compounds. R^8=H (I–1=0.0), R^9=CH_3

TABLE XVIII. Summary of Eq(1)—(9): Coefficients of Parameters

Eq No.	Intercept	π–7	F–3	F–4	I–1	I–2	I–3	Test No.	Pharmacological test
1	4.31	−0.28	2.29	1.98	−0.40	0.47	−0.30	1	anti-bemegride
2	4.44	−0.69	1.53	1.81	−0.36	0.20	−0.27	2	anti-pentylene tetrazole
3	4.15	−0.91	1.32	1.19		0.21	−0.23	3	anti-fighting
4	4.29	−0.96		0.41	−0.40		−0.27	4	anti-electroshock
5	2.96		2.04	0.77		0.37		5	inclined plane
6	3.04		2.21	0.71		0.29	−0.11	6	rotating rod
7	3.93	−0.58	1.62	1.04	−0.52		−0.32	7	traction
8	4.09	−0.65	1.38	1.17	−0.48		−0.40	8	balance
9	3.96	−0.57	1.78	1.46	−0.32	0.28		9	anesthesia-potentiating

TABLE XIX. Simple Correlation Coefficients Matrix Showing Degree of Collinearity (r) between the Nine Test Data

Test No.	1	2	3	4	5	6	7	8	9
1	1.000	0.952	0.926	0.814	0.693	0.736	0.755	0.802	0.886
2		1.000	0.891	0.773	0.646	0.673	0.707	0.740	0.898
3			1.000	0.816	0.696	0.715	0.724	0.744	0.858
4				1.000	0.599	0.656	0.704	0.755	0.736
5					1.000	0.931	0.573	0.545	0.711
6						1.000	0.694	0.687	0.741
7							1.000	0.965	0.788
8								1.000	0.784
9									1.000

TABLE XX. Spearman Rank Correlation Coefficients Matrix Showing Degree of Collinearity ($\rho^{a)}$) between the Nine Test Data

Test No.	1	2	3	4	5	6	7	8	9
1	1.000	0.943	0.974	0.780	0.688	0.702	0.730	0.780	0.886
2		1.000	0.922	0.761	0.625	0.640	0.681	0.719	0.909
3			1.000	0.803	0.673	0.680	0.764	0.800	0.879
4				1.000	0.582	0.581	0.625	0.668	0.708
5					1.000	0.877	0.537	0.546	0.614
6						1.000	0.680	0.662	0.669
7							1.000	0.958	0.766
8								1.000	0.784
9									1.000

a) $\rho=1-\frac{6\Sigma D^2}{N(N^2-1)}$: D is the difference of the ranks, N is number of data.

(I–2=1.0) and R^{10}=H (I–3=0.0) are preferable for the maximization of the activities.

In order to assess the pharmacological screenings the intercorrelation data of biological activities could be helpful. Both a cardinal and an ordinal correlation matrices were exhibited (Table XIX and XX); in the former the simple correlation coefficients r and in the latter Spearman rank correlation coefficients ρ crossing over nine tests were calculated. Common 29 compounds in all tests were used for computing the correlation coefficients. Looking over both tables one can appreciate the relative characters of all pharmacological tests easier and definitely. The characteristics of test 1, 2, 3, and 9, those of test 7 and 8 and those of test 5 and 6 are closely related, while test 4 is not similar to any other tests. Considering the

essential characters of both correlations the rank correlation is assumed to be more important than the simple correlation for the pharmacological assessment based on statistics, but in this example no significant difference has not been found. The practical evidence for the importance of rank correlations remains to be found in the future.

In drug design studies there is another important purpose to be considered, which is the isolation of one specific or selective activity. Generally speaking, one drug has several biological activities, while in many cases only one selective activity is desirable for clinical use and other activities are assumed to be related to the side effects. We have to devise the drugs with the undesirable side effects as little as possible. Let's consider one case in which we are required to develop a sleep-inducer without muscle-relaxant and muscle-uncoordinating effects from the clinical purpose. In order to solve this problem the maximization of anesthesia-potentiating effect (test 9) which is one of the indices of a sleep inducer and the minimization of inclined plane test effect (eq 5), rotating rod test effect (eq 6), traction effect (eq 7) and balance test (eq 8) could be essential. The effective doses (50%) of test 5 and 6 are much larger than those of test 7, 8 and 9, and are not necessary to be accounted for. The comparison of eq (9) with eq (7) and (8) suggests that R^7 (π–7) is not related to the selectivity, $R^9=CH_3$ (I–2=1.0) and $R^{10}=CH_3$ (I–3=1.0) are the most important factors of the selectivity and strong electron-attracting function in R^3 and R^4 are slightly better for the isolation of sleep-inducing activity.

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