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Structure-Activity Study of Diuretic Azanaphthalene Derivatives

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Regression analysis by the Free-Wilson technique was applied to estimate the contribution of substituents on azanaphthalene skeletons to diuretic activity of pyrimido[4,5-*d*]-pyridazine and pyrido[3,4-*d*]pyridazine derivatives. In the former group, one of the most preferable compounds was 2-phenyl-5,8-dimorpholinopyrimido[4,5-*d*]pyridazine and that in the latter group proved to be 1,4-dimorpholine-7-phenylpyrido[3,4-*d*]pyridazine. Subsequently, electron density distributions on a variety of azanaphthalene skeletons were calculated by extended Hückel Theory calculation. Linear multiple regression analysis to find a correlation between diuretic activity and electron density at some positions of azanaphthalene skeletons suggested that the diuretic activity might be influenced largely by electronic structures at the ring junction.

Recently Yurugi and his coworkers have synthesized an enormous number of azanaphthalene derivatives,[2] which can be classified into eleven groups based on their fundamental skeletons, namely, pyrimido[4,5-*d*]pyridazine, pyrazino[2,3-*d*]pyridazine, pyridazino[4,5-*c*]-pyridazine, pyrido[2,3-*d*]pyridazine, pyrido[3,4-*d*]pyridazine, pyrimido[4,5-*d*]pyrimidine, pyrimido[5,4-*d*]pyrimidine, pyrido[2,3-*d*]pyrimidine, pyrido[2,3-*d*]pyrimidine, pyrido[4,5-*d*]-pyrimidine and phthalazine. The screening tests performed by Kikuchi, *et al.*[3] have revealed that many of the derivatives exhibit potent diuretic activity and the active compounds are particularly abundant among the derivatives with two skeletons, pyrimido[4,5-*d*]pyridazine and pyrido[3,4-*d*]pyridazine, represented by 5,8-dimorpholino-2-phenylpyrimido[4,5-*d*]pyridazine (DS-210) and 1,4-dimorpholino-7-phenylpyrido[3,4-*d*]pyridazine (DS-511) respectively.

In the present paper, attempts were made to analyze structure-activity relationships of those derivatives expecting to find a lead for a more excellent compound. The analyses were conducted by the following two methods: 1) Regression analyses by the Free-Wilson technique[4] were applied to estimate the contribution of substituents on the above two azanaphthalene rings to diuretic activity. 2) Electron density distributions on the azanaphthalene skeletons were calculated by the extended Hückel Theory (EHT) calculation,[5] and linear multiple regression model was applied to find correlations between diuretic activity and electron density at some positions of azanaphthalene rings.

1) Location: *Jusohonmachi, Yodogawa-ku, Osaka, 532, Japan.*

2) *a*) S. Yurugi, M. Hieda, T. Fushimi, Y. Kawamatsu, H. Sugihara, and M. Tomimoto, *Chem. Pharm. Bull.* (Tokyo), **20**, 1513 (1972); *b*) S. Yurugi and M. Hieda, *ibid.*, **20**, 1522 (1972); *c*) S. Yurugi, M. Hieda, T. Fushimi, Y. Kawamatsu, H. Sugihara, and M. Tomimoto, *ibid.*, **20**, 1528 (1972); *d*) S. Yurugi, T. Fushimi, H. Sugihara, and M. Hieda, *Yakugaku Zasshi*, **92**, 1333 (1972); *e*) A. Miyake, K. Itoh, N. Tada, Y. Oka, and S. Yurugi, *Chem. Pharm. Bull.* (Tokyo), **23**, 1488 (1975); *f*) A. Miyake, Y. Oka, and S. Yurugi, *ibid.*, **23**, 1500 (1975); *g*) A. Miyake, K. Itoh, N. Tada, Y. Oka, and S. Yurugi, *ibid.*, **23**, 1505 (1975); *h*) Y. Oka, K. Omura, K. Itoh, A. Miyake, M. Tomimoto, N. Tada, and S. Yurugi, *ibid.*, **23**, 2239 (1975); *i*) Y. Oka, K. Itoh, A. Miyake, N. Tada, K. Omura, M. Tomimoto, and S. Yurugi, *ibid.*, **23**, 2306 (1975); *j*) K. Omura, N. Tada, M. Tomimoto, Y. Usui, Y. Oka, and S. Yurugi, *ibid.*, "in press."

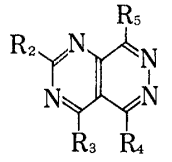
3) K. Nishikawa, H. Shimakawa, Y. Inada, Y. Shibouta, S. Kikuchi, S. Yurugi, and Y. Oka, *Chem. Pharm. Bull.* (Tokyo), **24**, 2057 (1976).

4) *a*) S.H. Free, Jr. and J.W. Wilson, *J. Med. Chem.*, **7**, 315 (1964); *b*) T. Fujita and T. Ban, *ibid.*, **14**, 148 (1971); *c*) E. Mizuta, N. Suzuki, Y. Miyake, M. Nishikawa, and T. Fujita. *Chem. Pharm. Bull.* (Tokyo), **23**, 5 (1975).

5) R. Hoffman, *J. Chem. Phys.*, **39**, 1397 (1963).

1) Regression Analyses Using the Free-Wilson Technique

I. Pyrimido[4,5-d]pyridazine Group——Diuretic activities of 44 pyrimido[4,5-*d*]pyridazine derivatives (I), have been reported in Table III of the previous paper.[3] In these derivatives were involved 8, 5, 22, and 22 substituents for R_2, R_3, R_4 and R_5, respectively. In this case,



I

Chart 1

the Free-Wilson method produces 48 equations involving 4 restriction equations. This simultaneous equations are unable to be solved, because of a larger number of variables (8+5+22+22+1=58). However, in 37 derivatives out of these 44, the substituent R_4 is the same with the substituent R_5 and more than 22 variables are consequently eliminated. We attempted to analyze the structure-activity relationships using these 37 derivatives, which produced 40 equations involving 3 restriction equations with 34 variables. As parameters for the diuretic activity, urine volume (V), amount of sodium and potassium ions in the urine ($U_{Na}V$ and U_KV), and $U_{Na}V/U_KV$ ratio (U_{Na}/U_K) at the dose of 30 mg/kg were used for the regression analyses. For the compounds which reached the maximal diuresis below this dose, the data were estimated by extrapolating the linear portion of the log dose-response plot to 30 mg/kg, as shown in Fig. 1.

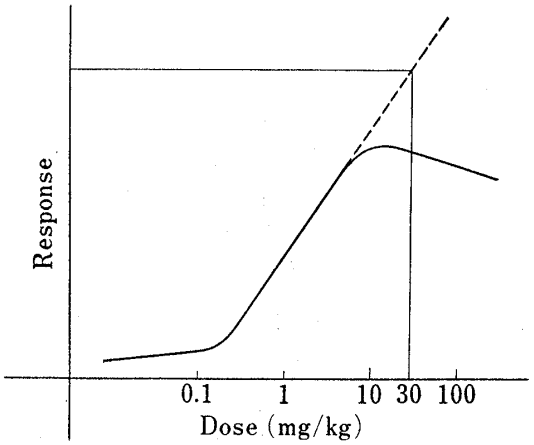


Fig. 1. Estimation of a Biological Response at the Dose of 40 mg/kg by the Extrapolation of the Linear log Dose-response Curve when the Response Reaches Maximum with a Lower Dose

———: log dose-response curve
- - - - - -: extrapolation

In the Free-Wilson method it is assumed that biological activity of a compound is the mathematical sum of the contributions of the substituents and parent skeleton, as represented by Eq. 1,

$$\log Y = \sum G_i X_i + c \qquad \text{Eq. 1}$$

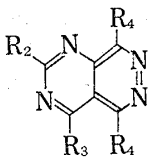
where Y represents magnitude of the biological activity, G_i is the log activity contribution or the log activity enhancement factor of the i-th substituent, and X_i takes the value of 1 or 0, depending on the presence or absence of the i-th substituent at each position. The overall average of the log activity values in the set compounds is employed for the term c. Table I gives the results of the regression analyses of pyrimido[4,5-*d*]pyrimidine groups by the use of Eq. 1.

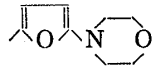
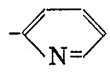
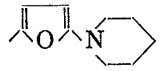
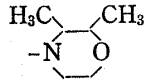
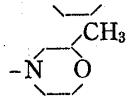
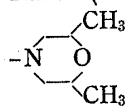
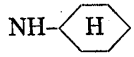
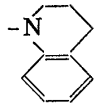
Table I. Statistical Results of the Regression Analyses Using Eq. 1 for Pyrimido[4,5-*d*]pyridazine Group

Activity parameter	$n^{a)}$	$r^{b)}$	$s^{c)}$	$F_6^{30d)}$
V	37	0.987	0.057	7.60
$U_{Na}V$	37	0.964	0.103	2.66
U_KV	37	0.938	0.096	1.46
U_{Na}/U_K	37	0.979	0.045	4.67

a) Number of compounds included in the analysis.
b) multiple correlation coefficient
c) standard deviation
d) *F* value

TABLE II. Calculated Group Contributions, G Values, of Pyrimido[4,5-*d*]pyridazine Derivatives

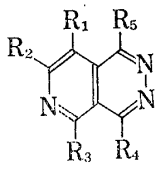


	R	$n^{a)}$	V	$U_{Na}V$	U_KV	U_{Na}/U_K
R_2	C_6H_5	24	0.086	0.098	0.016	0.080
	p-Cl-C_6H_4	2	−0.021	−0.103	0.062	−0.160
	m-CH_3-C_6H_4	3	−0.133	−0.116	−0.017	−0.090
		1	−0.154	−0.131	0.030	−0.192
	p-NO_2-C_6H_5	2	−0.166	−0.153	0.077	−0.219
		2	−0.168	−0.171	−0.068	−0.103
		2	−0.218	−0.294	−0.150	−0.137
		1	−0.363	−0.439	−0.206	−0.210
R_3	H	33	0.047	0.048	0.012	0.035
	C_4H_9	1	−0.320	−0.358	−0.106	−0.237
	$CH_2C_6H_5$	1	−0.346	−0.362	−0.064	−0.305
	CH_3	1	−0.407	−0.390	−0.067	−0.300
	C_6H_5	1	−0.473	−0.471	−0.160	−0.310
R_4		11	0.257	0.251	0.125	0.120
		7	0.080	0.084	−0.019	0.097
		1	0.034	0.035	0.033	0.030
		1	0.027	0.033	0.102	−0.066
		1	0.027	0.040	−0.007	0.047
	$NHCH(CH_3)_2$	2	−0.133	−0.145	−0.122	−0.031
		1	−0.156	−0.080	−0.003	−0.083
		1	−0.170	−0.129	0.059	−0.185
	NH-$C_6H_4Cl(m)$	1	−0.174	−0.238	−0.123	−0.118
	NH-C_6H_5	1	−0.178	−0.222	−0.055	−0.140
	$NHC_2H_4OCH_3$	1	−0.185	−0.177	0.019	−0.172
		1	−0.188	−0.119	−0.051	−0.073
	$NHCH_2C_6H_5$	1	−0.208	−0.291	−0.118	−0.176
		1	−0.212	−0.255	−0.077	−0.140
	$NHC_2H_4OC_2H_5$	1	−0.220	−0.184	−0.068	−0.129
	OCH_3	1	−0.241	−0.139	−0.144	−0.003
	SCH_3	1	−0.281	−0.384	−0.133	−0.239
	$N(CH_3)C_2H_4OH$	1	−0.281	−0.390	−0.144	−0.234
	OH	1	−0.290	−0.234	−0.095	−0.144
		1	−0.369	−0.320	−0.193	−0.110
	c		0.117	0.113	0.040	0.079

a) number of compounds with the substituents

For these analyses, F values at 95% level of significance are calculated to be 3.81, which means that the levels of significance for the analyses of V and U_{Na}/U_K are greater than 95%. The log activity contributions, G_i values, for the V, $U_{Na}V$, U_KV and U_{Na}/U_K are listed in Table II.

II. Pyrido[3,4-*d*]pyridazine Group——Structure-activity relationships were analyzed for 46 pyrido[3,4-*d*]pyridazin ederivatives(II), in which R_4 is the same with R_5, out of the 50 derivatives described in Table IV of the preceding paper.[3] The regression analyses of this group performed using Eq. 1 gave the statistics shown in Table III. Since the critical value F_{10}^{35} (0.05) is 2.68 and F_{10}^{35} (0.01) is 4.21, correlation to V, $U_{Na}V$ or U_{Na}/U_K, was significant at the 99% level and that to U_KV was significant at the 95% level. G_i values for V, $U_{Na}V$, U_KV and U_{Na}/U_K are listed in Table IV.



II
Chart 2

Table III. Statistical Results of the Regression Analyses Using Eq. 1 for Pyrido[3,4-*d*]pyridazine Group

Activity parameter	n	r	s	F_{10}^{35}
V	46	0.979	0.085	6.55
$U_{Na}V$	46	0.979	0.081	6.63
U_KV	46	0.955	0.075	2.99
U_{Na}/U_K	46	0.979	0.048	6.51

Results and Discussion

As is clear from G_i values for R_1 in Table IV, hydrogen, methyl, methoxy and ethoxy groups at R_1 enhance urine and sodium excretion, and hydrogen, methyl and methoxy groups improve U_{Na}/U_K ratio. On the other hand ethyl, propyl, benzyl, phenyl, butyloxy and chloro groups are unfavorable for the activity. These results suggests that a lipophilic substituent at R_1 is undesirable to diuretic activity. Comparison of G_i values in Tables II and IV shows that substituent contributions of R_2, R_3 and R_4 in pyrimido[4,5-*d*]pyridazine derivatives are respectively similar to those of R_2, R_3 and R_4 in pyrido[3,4-*d*]pyridazine derivatives. This similarity probably comes from the structural resemblance of the both groups. As for the substituent R_2, unsubstituted phenyl is more favorable than substituted phenyl or heteroaromatic groups in the both skeletons. The relatively large G_i values of ethyl group at R_2 of pyrido[3,4-*d*]pyridazine series are rather unreliable, since only one compound having ethyl group at R_2 is included in the analysis. The most preferable substituents for R_3 and R_4 are calculated to be hydrogen and morpholino groups respectively in the both skeletons.

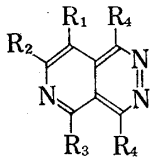
From the above results, the desirable compounds having the most preferable substituents at each position are deduced as depicted in Chart 3.[6] The compound Ia is just DS-210 and IIa is DS-511, both of which have already been synthesized and evaluated to have potent diuretic activity.[7] Compounds IIb and IIc have also been proved to show potent activity by the screening tests.[3] Therefore, no new potent compounds could be predicted by this analysis.

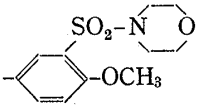
2) Relationships between Diuretic Activity and Electronic Structure of Azanaphthalene Rings

The analysis of relationships between diuretic activity reported in the preceding paper[3] and electronic structure of the azanaphthalene rings was conducted on eleven representative

6) In this report, the numbering of skeletons is conveniently determined as shown in Chart 3.
7) *a*) K. Nishikawa and S. Kikuchi, *Jap. J. Pharmacol.*, Suppl., **22**, 103 (1972); *b*) Y. Inada, K. Nishikawa, and S. Kikuchi, *ibid.*, Suppl., **23**, 120 (1973); *c*) K. Nishikawa, Y. Inada, H. Shimakawa, I. Kuramoto, M. Isono, and S. Kikuchi, *J. Takeda Res. Lab.*, **32**, 539 (1973).

Table IV. Calculated Group Contributions, G Values, fo Pyrido[3,4-*d*]pyridazine Derivatives

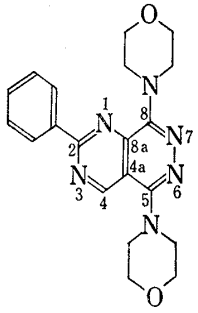


	R	$n^{a)}$	V	$U_{Na}V$	U_KV	U_{Na}/U_K
R_1	CH_3	3	0.084	0.060	−0.045	0.097
	H	23	0.055	0.069	0.022	0.045
	OCH_3	10	0.045	0.028	0.001	0.016
	OC_2H_5	2	0.036	0.035	0.107	−0.046
	Cl	2	−0.095	−0.129	0.043	−0.156
	C_2H_5	1	−0.219	−0.201	−0.464	−0.156
	C_6H_5	2	−0.282	−0.322	−0.024	−0.249
	$CH_2C_6H_5$	1	−0.293	−0.272	−0.147	−0.122
	OC_4H_9	1	−0.321	−0.277	−0.186	−0.088
	C_3H_7	1	−0.456	−0.471	−0.250	−0.215
R_2	C_6H_5	16	0.210	0.203	0.135	0.069
	C_2H_5	1	0.174	0.369	−0.009	0.369
	p-HOC_6H_4	3	0.104	0.085	0.089	−0.007
	p-$CH_3OC_6H_4$	1	0.054	−0.020	0.064	−0.084
	p-ClC_6H_4	1	0.008	−0.057	0.018	−0.098
	$CH_2CH(CH_3)_2$	2	0.001	0.050	0.053	0.027
		1	−0.050	−0.121	−0.066	−0.047
	CH_3	8	−0.081	−0.061	−0.103	0.020
	$CH(CH_3)C_2H_5$	1	−0.174	−0.154	−0.139	−0.005
	C_3H_7	1	−0.190	−0.131	−0.152	0.033
	p-$OCH_2C_6H_5C_6H_4$	3	−0.216	−0.243	−0.013	−0.201
	$CH(CH_3)_2$	1	−0.216	−0.175	−0.206	0.013
	C_4H_9	1	−0.238	−0.217	−0.196	−0.005
		1	−0.251	−0.259	−0.103	−0.152
	o-OHC_6H_4	1	−0.270	−0.313	−0.191	−0.124
	m-$OCH_2C_6H_5C_6H_4$	1	−0.270	−0.309	−0.178	−0.124
	o-$OCH_2C_6H_5C_6H_4$	1	−0.285	−0.317	−0.178	−0.132
	m-OHC_6H_4	1	−0.293	−0.325	−0.178	−0.140
	$CH_2C_6H_5$	1	−0.372	−0.361	−0.156	−0.199
R_3	H	43	0.031	0.035	0.014	0.020
		1	−0.332	−0.398	−0.084	−0.312
	Cl	1	−0.429	−0.482	−0.281	−0.214
	$CH_2C_6H_5$	1	−0.581	−0.616	−0.258	−0.318
R_4		32	0.041	0.055	0.030	0.028
		4	0.040	0.000	−0.016	0.022
		6	−0.073	−0.101	−0.049	−0.051
	OH	2	−0.178	−0.246	−0.039	−0.212
		1	−0.211	−0.192	−0.262	0.015
	$NHCH_2C_6H_5$	1	−0.478	−0.469	−0.258	−0.274
	c		0.199	0.207	0.120	0.085

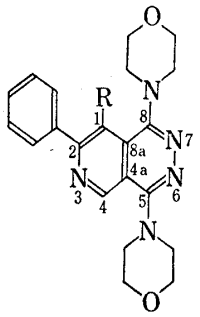
a) number of compounds with the substituents

compounds chosen from the aforementioned eleven skeletons. Based on the results described in the previous section, compounds such as Ia and IIa, in which a phenyl group is substituted at the 2 position,[6] no functional group is substituted at the 1, 3 and 4 positions and morpholino groups are substituted at 5, 6, 7 and 8 positions when atoms at these positions are carbon, were chosen as the representative derivatives of each skeleton.

Electronic parameters used for the analysis were calculated[8] by the EHT–MO calculation. The electronic structure of each azanaphthalene skeleton would be little influenced by the phenyl group, since the group is located at the same position in the every representative compound. Although the electron-donating morpholino groups seemed to have considerable influence on the electronic structure, it has been proved by the argument given below that the influence of the molpholino groups is also negligible. Accordingly, the calculation was performed on each unsubstituted azanaphthalene skeleton. Coordinates of each atom on the skeleton were calculated by assuming that aromatic ring was a regular hexagon. The σ and π electrons on the aromatic ring are usually orthogonal to each other, so that they can be dealt with separately. In the case of EHT–MO calculation, the lowest unoccupied molecular orbital (LUMO) is generally occupied by π electrons, but the highest occupied molecular orbital (HOMO) is not necessarily π orbital. In the present calculations for the azanaphthalene skeletons, HOMO was proved to be σ orbital in each skeleton. The reactivity of atoms on the skeleton seems to be influenced not only by MO energy levels but also by the direction of orbitals. In the present study we assume that the skeletons interact with a binding area on the receptor site in the direction of π atomic orbitals which are perpendicular to the azanaphthalene planes. Therefore π electrons were mainly used for the analysis.

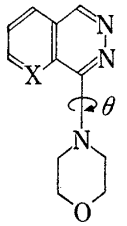


Ia

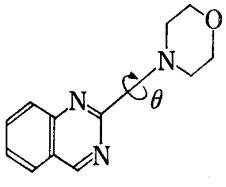


IIa : R=H
IIb : R=OCH_3
IIc : R=CH_3

Chart 3



IIIa : X=CH
IIIb : X=N



IV

Chart 4

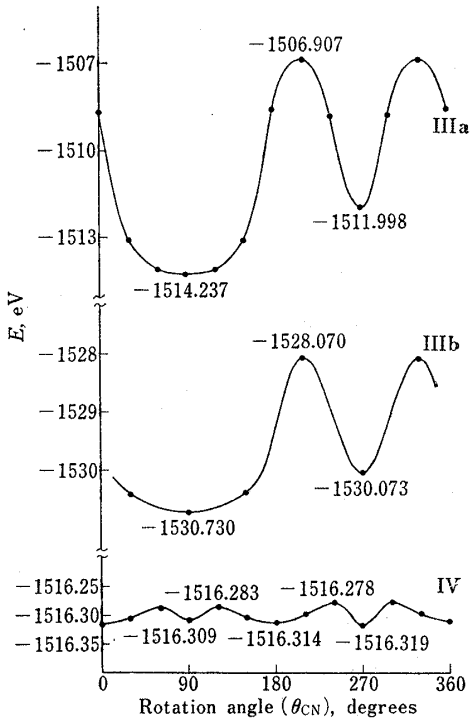


Fig. 2. Rotation Angle of Morpholino Group for IIIa, IIIb and IV, and Calculated Total Energies

8) The calculations were carried out on the IBM 370—158 at the data processing section of Takeda Chemical Industries, Ltd. using the program made by Prof. T. Yonezawa and his collaborators at Kyoto University.

In an attempt to examine the influence of morpholino substituent to the electronic structure of azanaphthalene skeletons, investigations were undertaken on three model compounds, namely 1-morpholinophthalazine (IIIa), 8-morpholinopyrido[2,3-*d*]pyridazine (IIIb) and 2-morpholinoquinazoline (IV). The EHT–MO calculations of these compounds were carried out by estimating the coordinates of each atom in the morpholino group from a Deriding stereomodel. The total energy *vs.* rotation of morpholino group was calculated in order to find the preferred comformation of each compound. The results are given in Fig. 2.[9] In the cases of IIIa and IIIb, the energy is found to be minimum at $\theta_{CN}=90°$, indicating that the compounds are most stable when the lone pair on nitrogen atom of the morpholino group lies on a plane common with the naphthalene ring and perpendicular to the π-electrons of the aromatic ring. In such a situation, participation of the lone pair in the resonance with the π-electrons would be inhibited. The influence of morpholino group, therefore, can be neglected in the derivatives with skeletons **1**—**7** in Table VI. On the other hand in the case of IV, which is related with skeletons **8**—**21** in Table VI, relatively stable conformers are found at $\theta_{CN}=90°$, 180° (or 0°) and 270°, although energy barriers and the difference of total energies between each conformers are very small. Among the three conformers, which are presumed to be present with nearly equal probability, conformers at $\theta_{CN}=90°$ and 270° are left out of consideration for the same reason with that for IIIa and IIIb. In the conformer at $\theta_{CN}=180°$ (or 0°), the pair electrons of morpholino nitrogen are expected to have some influence on the electronic structure of the azanaphthalene ring by virtue of resonance effect. The calculated π atomic orbital (AO) populations and atomic bond populations between 4a and 8a-position on unsubstituted quinazoline and IV (Table V) have revealed that the difference between values of both compounds are negligibly small in comparison to the differences between skeletons (see Table VI). As for the derivatives in which two molpholino groups are substituted at the 2 and 3 positions, skeletons **22**—**28** in Table VI, it is obvious from the standpoint of steric factor that the two morpholino groups can not take the position favorable for the resonance with the π-electrons in the ring. From the above argument it is pertinent to

Table V. AO Populations and Atomic Bond Populations between 4a and 8a-Position on Quinazoline and IV

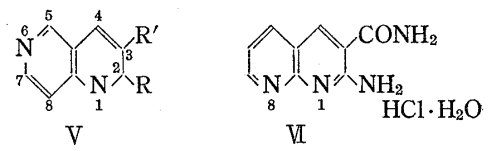
Position[a]	Quinazoline		2-Morpholino quinazoline (IV)	
	$N_r^{\pi ho}$[b]	$N_r^{\pi nho}$[c]	$N_r^{\pi ho}$[b]	$N_r^{\pi nho}$[c]
1	0.544	0.229	0.492	0.262
2	0.005	0.575	0.000	0.504
3	0.472	0.148	0.486	0.077
4	0.421	0.108	0.338	0.166
4a	0.016	0.571	0.045	0.529
5	0.046	0.182	0.051	0.131
6	0.004	0.086	0.000	0.117
7	0.085	0.055	0.045	0.018
8	0.093	0.000	0.137	0.000
8a	0.314	0.047	0.313	0.021
$M_{4a,8a}$[d]	1.025		1.027	

a) refer to Chart 3
b) atomic orbital population of HOMO in the π system
c) atomic orbital population of NHOMO in the π system
d) atomic bond population between 4a and 8a-position

9) A conformer in which lone pair of nitrogen in morpholino group is directed perpendicularly downward against the plane of azanaphthalene ring was determined as $\theta_{CN}=0$. The morpholino group was rotated clockwise and the deviation from the initial position was expressed by θ_{CN} value.

conclude that, as far as the azanaphthalene derivatives in the present study are concerned, the influence of substituents on the skeletons can be neglected.

Recently, Hawes, *et al.*[10] investigated the diuretic activity of a series of 2,3-disubstituted 1,6-naphthyridines (V) and 2-amino-1,8-naphthyridine-3-carboxamide hydrochloride monohydrate (VI). Their results suggested that N-1 was essential in binding to receptor site and N-8 enhanced drug-receptor interaction. In the present pyrido[3,4-*d*]pyridazine series, however, many compounds without nitrogen atoms at positions corresponding to 1 and 8-positions showed excellent diuretic activity. Although the receptor site for the both groups might be entirely different, the drug-receptor interaction will be influenced not only by the position of nitrogen atom of the skeleton but also by the electronic features of some particular positions. Atomic population and/or atomic orbital population is considered to be inappropriate as the parameter of the structure-activity relationship, since those of each atom in the skeleton are greatly influenced by the number of nitrogen atoms involved.



V VI

Chart 5

On the other hand, atomic orbital population of HOMO in the π system ($N_r^{\pi ho}$) is influenced by the position, rather than the number, of nitrogen atoms. Calculations of $N_r^{\pi ho}$ values for every position of all the eleven skeletons and careful inspection of the results revealed an interesting tendency that the skeletons having high $N_r^{\pi ho}$ at the ring junction, *i.e.* 4a and/or 8a position (Chart 3), possess strong diuretic activity. Moreover, in some skeletons in which the difference between HOMO energy and the next HOMO (NHOMO) energy is very small, the influence of atomic orbital population of NHOMO ($N_r^{\pi nho}$) would not be negligible. Therefore the sum (N^*_r) of $N_r^{\pi ho}$ and $N_r^{\pi nho}$ was employed as a parameter for the present analysis of structure-activity relationship. Atomic bond population ($M_{x,y}$) was also used as a parameter. N^*_r at the 4a, 8a and 4-position, and atomic bond population ($M_{4a,8a}$) between 4a and 8a-position, which were used for the regression analyses, were shown in Table V. The data at the dose of 10 mg/kg[3] were used for the analyses of V and $U_{Na}V$, and the data at the dose of 30 mg/kg[3] for the analyses of U_KV. In the case that maximal diuresis was reached below those doses, the data were estimated by extrapolation as described above (Fig. 1).

Results and Discussion

$$\log V = 0.637(\pm 0.478)N^*_{4a} - 0.178$$
$$n = 11,\ r = 0.708,\ s = 0.136,\ F^1_9 = 9.07 \qquad \text{Eq. 2}$$
$$\log V = 0.619(\pm 0.333)N^*_{4a} - 5.01(\pm 3.43)M_{4a,8a} + 4.978$$
$$n = 11,\ r = 0.891,\ s = 0.093,\ F^1_8 = 11.3 \qquad \text{Eq. 3}$$
$$\log V = 0.807(\pm 0.577)N^*_{4a} - 5.24(\pm 3.59)M_{4a,8a} - 0.225(\pm 0.554)N^*_{8a} + 5.012$$
$$n = 11,\ r = 0.904,\ s = 0.093 \qquad \text{Eq. 4}$$

Equations 2, 3 and 4 were obtained from the analyses of V data. The figures in parentheses are 95% confidence intervals, n is the number of data points, r is the correlation coefficient, and s is the standard deviation. In equation 2, the F ratio between the variances of the calculated and observed activities was significant at 95% level (F^1_9 (0.05)=5.12). The combination of N^*_{4a} and $M_{4a,8a}$ (Eq. 3) resulted in a statistically significant improvement in correlation (F^1_8(0.05)=5.32), but addition of N^*_{8a} term (Eq. 4) did not significantly improve (compare values of s). Since a similar tendency to V was observed in the found values of $U_{Na}V$ (Table VI), the regression analysis for $U_{Na}V$ was calculated by using N^*_{4a} and $M_{4a,8a}$,

10) E.M. Hawes, D.K.J. Goreki, and D.D. Johnson, *J. Med. Chem.*, **16**, 849 (1973).

affording Eq. 5 with a significant correlation ($F_8^2(0.05)=4.46$). Eq. 6 obtained by combining N_{4a}^*, $M_{4a,8a}$ and N_{8a}^* was not so significantly improved as in Eq. 5 ($F_7^1(0.05)=5.59$).

$$\log U_{Na}V = 0.524(\pm 0.433)N_{4a}^* - 6.13(\pm 4.46)M_{4a,8a} + 6.162$$
$$n = 11,\ r = 0.836,\ s = 0.120,\ F_8^2 = 9.25 \qquad \text{Eq. 5}$$
$$\log U_{Na}V = 0.847(\pm 0.712)N_{4a}^* - 6.53(\pm 4.42)M_{4a,8a} + 0.386(\pm 0.684)N_{8a}^* + 6.222$$
$$n = 11,\ r = 0.871,\ s = 0.115,\ F_7^1 = 1.78 \qquad \text{Eq. 6}$$

The U_KV data showed a somewhat different tendency from V and $U_{Na}V$ data, and the found value in the skeleton 9 was characteristic. Since the large value seemed to be attributable to high value of N_4^*, the linear combination of N_{4a}^* and N_4^* was used in the regression analysis for U_KV and a correlation was obtained as shown in Eq. 7.

$$\log U_KV = 0.203(\pm 0.528)N_4^* + 0.511(\pm 0.539)N_{4a}^* - 0.211$$
$$n = 11,\ r = 0.631,\ s = 0.149,\ F_8^2 = 2.65 \qquad \text{Eq. 7}$$

N_4^* term was not statistically significant because of the presence of skeleton **16** with fairly small found value compared to the high value of N_4^*. However, this small value for U_KV seems to have resulted from the small V value. The regression analysis excluding this data led to Eq. 8 with a significant correlation. Although in this equation the absolute value of

Table VI. Parameter Values Used in the Regression Analyses of Azanaphthalene Skeletons, and Found and Calculated Values of Diuretic Activities

No.	Positions of nitrogen in the ring[c]	N_{4a}^{*} [a]	N_{8a}^{*} [a]	N_4^{*} [a]	$M_{4a,8a}$ [b]	V Found	V Calcd.	$U_{Na}V$ Found	$U_{Na}V$ Calcd.	U_KV Found	U_KV Calcd.
1	6, 7	0.433	0.433	0.588	1.004	1.02	1.66	0.97	1.72	1.39	1.77
2	1, 6, 7	0.415	0.444	0.411	1.038	1.10	1.09	0.99	1.05	1.58	1.41
3	3, 6, 7	0.730	0.132	0.393	1.000	3.13	2.66	3.18	2.62	2.20	1.78
4	4, 6, 7	0.444	0.415	0.255	1.038		1.14		1.08		1.19
5	1, 3, 6, 7	0.826	0.317	0.218	1.032	2.29	2.11	2.28	1.87	1.26	1.56
6	1, 4, 6, 7	0.460	0.460	0.060	1.042	1.21	1.12	1.00	1.04	1.04	0.95
7	3, 4, 6, 7	0.372	0.719	0.361	0.998	2.07	1.63	2.46	1.74	1.69	1.28
8	6, 8	0.587	0.362	0.529	1.025		1.61		1.52		1.87
9	1, 6, 8	0.862	0.239	0.708	1.038	1.91	2.07	1.53	1.79	3.34	2.93
10	3, 6, 8	0.867	0.351	0.375	1.010		2.89		2.67		1.95
11	4, 6, 8	0.560	0.408	0.274	1.050		1.18		1.05		1.34
12	1, 3, 6, 8	0.886	0.170	0.299	1.022	2.41	2.58	2.03	2.32	1.81	1.80
13	1, 4, 6, 8	0.545	0.267	0.129	1.035		1.35		1.26		1.11
14	3, 4, 6, 8	0.574	0.362	0.581	1.004		2.04		2.04		1.98
15	5, 7	0.362	0.587	0.773	1.025		1.17		1.16		2.10
16	1, 5, 7	0.408	0.560	0.748	1.050	0.97	0.94	0.85	0.88	0.77	2.10
17	3, 5, 7	0.458	0.801	0.481	1.030	1.42	1.28	1.42	1.24	1.34	1.59
18	4, 5, 7	0.239	0.862	0.338	1.038		0.85		0.84		1.11
19	1, 3, 5, 7	0.518	0.518	0.292	1.054	0.98	1.05	1.08	0.95	1.08	1.32
20	1, 4, 5, 7	0.267	0.545	0.402	1.035		0.91		0.90		1.23
21	3, 4, 5, 7	0.240	0.788	0.725	1.000		1.33		1.44		1.79
22	5, 8	0.413	0.413	0.666	1.025		1.29		1.27		1.92
23	1, 5, 8	0.497	0.313	0.743	1.037		1.26		1.18		2.26
24	3, 5, 8	0.536	0.456	0.432	1.027		1.50		1.43		1.60
25	4, 5, 8	0.313	0.497	0.277	1.037		0.97		0.95		1.10
26	1, 3, 5, 8	0.545	0.267	0.346	1.035		1.35		1.26		1.45
27	1, 4, 5, 8	0.306	0.306	0.450	1.022		1.13		1.15		1.35
28	3, 4, 5, 8	0.316	0.448	0.698	1.002		1.44		1.54		1.85

a) The sum of atomic orbital population of HOMO and NHOMO in the π system at the 4a, 8a and 4-position.
b) Atomic bond population between 4a and 8a-position.
c) refer to Chart 3

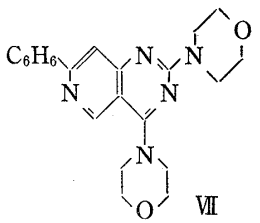
the coefficient of N^*_{4a} is less than 95% confidence interval, the contribution of N^*_{4a} is significant at 90% level.

$$\log U_K V = 0.533(\pm 0.409)N^*_f + 0.356(\pm 0.367)N^*_{4a} - 0.218$$
$$n = 10,\ r = 0.835,\ s = 0.095,\ F^2_7 = 8.05 \qquad \text{Eq. 8}$$

The calculated values for the representatives of the eleven groups using Eq. 3, 5 and 8 are given in Table VI, together with those of several other azanaphthalene skeletons.

For the V and $U_{Na}V$ data, fairly good agreement was observed between the calculated and the found values, except for those of skeletons **1** and **7** (Table VI). In the skeleton **1** the found value is considerably smaller than the calculated. Thus, some discrepancy between the calculated and the found value would be expected in the absence of nitrogen atoms at the 1, 3 and 4 position. Therefore, the values for the derivatives of **8**, **15** and **22** are predicted to be smaller than the calculated values. As for the $U_K V$, the result shown in Table VI is in fair agreement with the found value.

It was suggested from the above considerations that, among the compounds which had not been synthesized before these analyses, a compound with skeleton **10** would possess potent diuretic activity, since active electrons are concentrated at the 4a position and delocalization of electrons to the bond between 4a and 8a is relatively small. The derivative of **14** also seemed to be promising. Based on these speculations, 2,4-dimorpholino-7-phenylpyrido[4,3-*d*]-pyrimidine (VII), a representative of the skeleton **10** was synthesized. The diuretic data of the compound turned out to be V=2.03 and $U_{Na}V$=1.62 at the dose of 10 mg/kg, and $U_K V$=1.57 at the dose of 30 mg/kg.[3] Although each observed value was somewhat lower than had been expected from the calculation, it was proved that this compound belonged to a class with relatively potent diuretic activity.



VII

Chart 6

The revised regression analyses including the data of compound VII gave Eq. 9, 10 and 11 in place of Eq. 3, 5 and 8. The level of

$$\log V = 0.543(\pm 0.319)N^*_{4a} - 4.52(\pm 3.44)M_{4a,8a} + 4.512$$
$$n = 12,\ r = 0.867,\ s = 0.097,\ F^2_9 = 13.7 \qquad \text{Eq. 9}$$
$$\log U_{Na}V = 0.415(\pm 0.423)N^*_{4a} - 5.42(\pm 4.58)M_{4a,8a} + 5.488$$
$$n = 12,\ r = 0.780,\ s = 0.129,\ F^2_9 = 7.00 \qquad \text{Eq. 10}$$
$$\log U_K V = 0.539(\pm 0.392)N^*_f + 0.303(\pm 0.325)N^*_{4a} - 0.196$$
$$n = 11,\ r = 0.815,\ s = 0.094,\ F^2_8 = 7.91 \qquad \text{Eq. 11}$$

significance of the F ratio for each regression analysis reached as high as 97.5%. Similarly to Eq. 8, the contributions of N^*_{4a} in Eq. 10 and 11 are significant at 90% level. There were little differences between the coefficients of the parameters of Eq. 9, 10 and 11 and those of Eq. 3, 5 and 8, and the calculated values of the diuretic activity using Eq. 9, 10 and 11 agreed closely with those using Eq. 3, 5 and 8, respectively. These results indicate that the structure-activity relationships for the diuretic activity of the azanaphthalene compounds are well represented by Eq. 3, 5 and 8 or Eq. 9, 10 and 11. Since these equations were drived by using the data of the compounds with phenyl and morpholino groups which were estimated as the most suitable substituents from the analysis using the Free-Wilson technique, they are not necessarily applicable to the compounds with other substituents.

Experimental

Synthesis of 2,4-Dimorpholino-7-phenylpyrido[4,3-*d*]pyrimidine (VII)——A mixture of 4-amino-6-phenylpyridine-3-carboxylic acid[11] (3.0 g) and 8.6 g of urea was melted by heating at 190—200°. To the cooled

11) Merck & Co., Inc., U.S. Patent 3655679 (1972) [*C. A.*, **74**, 12544*e* (1972)].

reaction mixture was added 30 ml of water. The insoluble substance was collected by filtration and dried to give 3.0 g (90%) of 7-phenylpyrido[4,3-*d*]pyrimidine-2,4(1H, 3H)-dione as colorless crystals, mp>300°. *Anal.* Calcd. for $C_{13}H_9O_2N_3$: C, 65.26; H, 3.79; N, 17.57. Found: C, 65.30; H, 3.75; N, 17.54.

A mixture of this compound (3.0 g), $POCl_3$ (30 ml) and α-picoline (3 ml) was heated at 100—120° for 3 hr. Excess $POCl_3$ was removed by evaporation *in vacuo*, and to the residue was added ice-water. Filtration of the resulting crystals gave crude 2,4-dichloro-7-phenylpyrido[4,3-*d*]pyrimidine, which was refluxed with morpholine (30 ml) for 2 hr. Excess morpholine was evaporated *in vacuo*, and to the residue was added water (30 ml). Resulting crystals were filtered, chromatographed on a silica gel column (Eluent, acetone: benzene = 1: 3), and recrystallized from EtOH to give 200 mg (4%) of V, mp 180—181°. *Anal.* Calcd. for $C_{21}H_{23}O_2N_5$: C, 66.82; H, 6.14; N, 18.56. Found: C, 66.60; H, 6.24; N, 18.50.

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