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Structure-Activity Relationships of the Diuretic Activity of Triaza- and Tetraaza-naphthalene Compounds[1]

Kohei Nishikawa, Hisao Shimakawa, Yoshiyuki Inada, Yumiko Shibouta, Shintaro Kikuchi, Shojiro Yurugi, and Yoshikazu Oka

Medicinal Research Laboratories, Central Research Division, Takeda Chemical Industries, Ltd.[2]

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The diuretic activity of 219 nitrogen containing heterocyclic compounds, classified into 13 groups based on the structural features, was studied on the saline loaded rats. One hundred and four out of the 219 compounds were active at the oral doses of 10 to 30 mg/kg. Several of pyrimidopyridazines, pyridazinopyridazines and pyridopyridazines produced as potent diuresis and natriuresis as hydrochlorothiazide at the oral dose of 0.1 mg/kg, in which 2-phenyl-5,8-dimorpholinopyrimido[4,5-*d*]-pyridazine (DS-210) and 1,4-dimorpholino-7-phenylpyrido[3,4-*d*]pyridazine (DS-511) were selected for more extensive evaluation as diuretic agents. Structure-activity relationships of the tested compounds are discussed.

Yurugi, *et al.* have synthesized a series of pyrimido- and pyrido-pyridazines,[3] some of which have been found to show diuretic activity in diuretic assay using rats. Although it is known that in various nitrogen-containing heterocyclic compounds pteridine exemplified by triamterene and a few pyrimido- or pyrido-pyrimidines[4,5] possess a diuretic activity, there has been no report on the diuretic activity of pyrimido- or pyrido-pyridazine derivatives. We have already reported the diuretic properties of 2-phenyl-5,8-dimorpholinopyrimido[4,5-*d*]-pyridazine (DS-210)[6,7] and 1,4-dimorpholino-7-phenylpyrido[3,4-*d*]pyridazine (DS-511),[8] and the data indicated that they possessed an excellent diuretic activity and some diuretic characteristics different from any of the known diuretics such as thiazides, carbonic anhydrase inhibitors or furosemide. In view of these interesting results the synthetic works have been extended to other triaza- and tetraaza-naphthalene derivatives such as pyrazino-pyridazines,[9] pyridazino-pyridazines,[10] pyrimido-pyrimidines and pyrido-pyrimidines, and pyridazines and phthalazines.

In this paper the screening results for the diuretic activity of 219 derivatives from above-mentioned series are presented and their structure-activity relationships are discussed.

1) A part of this report was presented at the 3rd Symposium on the Drug-Activity, Gifu, November, 1974.
2) Location: *Juso-honmachi, Yodogawa-ku, Osaka, 532, Japan.*
3) *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. Yuguri, *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."
4) H. Graboyes, C.E. Jaffe, I.J. Pachter, J.P. Rosenbloom, and J. Weistock, *J. Med. Chem.*, **11**, 568 (1968).
5) Parke-Davis Co., U.S. Patent 3639401, 3657264 (1972).
6) K. Nishikawa and S. Kikuchi, *Japan J. Pharmacol.* Suppl., **22**, 103 (1972).
7) Y. Inada, K. Nishikawa, and S. Kikuchi, *ibid.* Suppl., **23**, 120 (1973).
8) K. Nishikawa, Y. Inada, H. Shimakawa, I. Kuramoto, M. Isono, and S. Kikuchi, *J. Takeda Res. Lab.*, **32**, 539 (1973).
9) S. Yurugi and M. Hieda, *Yakugaku Zasshi*, **92**, 1322 (1972).
10) M. Hieda, K. Omura, and S. Yurugi, *ibid.*, **92**, 1327 (1972).

Methods

1. Assay of Diuretic Activity——The diuretic activity of test agents was assayed according to the method described by Lipschitz, *et al.*[11] Groups of 6 male Sprague-Dawley rats weighing about 200 g were withdrawn of diet and water 18 hr prior to study. One group was used as untreated control and rats were given 25 ml/kg *p.o.* of 0.9% NaCl and rats in other test groups were treated likewise with 25 ml/kg of 0.9% NaCl containing 0.1 to 30 mg/kg of test agents suspended in a small amount of gummi arabicum. Immediately thereafter, by keeping the rats in metabolism cages (1 rat/cage) spontaneous urine was collected for 5 hr. The parameters used for diuretic evaluation were urine volume (V) and amounts of sodium and potassium in the urine ($U_{Na}V$ and U_KV), which were calculated as the values per 100 g body weight per 5 hr. The relative potency of test compounds was expressed as the ratio of the response evoked by test animals to those evoked by control ones. Data obtained were analyzed statistically with the Student's t-test between the control and test groups, and the probability levels of significant difference were described as $*0.01<p<0.05$, $**0.001<p<0.01$ and

Table I. Classification of Test Compounds and Diuretic Activity

Group	Structure	No. of Compd.	Diuretic (mg/kg)			Anti-diuretic (mg/kg)	
			30	1	0.1	30	10
I	pyrimido[4,5-*d*]pyridazine						
Ia	2,4,5,8-tetrasubstituted-pyrimido[4,5-*d*]pyridazine	44	20	4	1		
Ib	8-morpholino-5-oxo-2-phenyl-6-substituted-5,6-dihydroxypyrimido[4,5-*d*]pyridazine	10	3				
Ic	2,3,4,5,8-pentasubstituted-3,4-dihydropyrimido[4,5-*d*]pyridazine	48	27	4		3	
Id	5,8-dimorpholino-2-phenyl-1,4-disubstituted-1,4-dihydropyrimido[4,5-*d*]pyridazine	6	3				
II	pyrazino[2,3-*d*]pyridazine						
	2-phenyl-5,8-disubstituted-pyrazino[2,3-*d*]pyridazine	3					
III	pyridazino[4,5-*c*]pyridazine						
	3-phenyl-5,8-disubstituted-pyridazino[4,5-*c*]pyridazine	2	2	2	1		
IV	pyrido[2,3-*d*]pyridazine						
	2-phenyl-5,8-disubstituted-pyrido[2,3-*d*]pyridazine	6	2				
V	pyrido[3,4-*d*]pyridazine						
Va	1,4,5,7,8-pentasubstituted-pyrido[3,4-*d*]pyridazine	50	31	11	5	1	
Vb	1,4-dimorpholino-7-phenyl-5,5-disubstituted-5,6-dihydropyrido[3,4-*d*]pyridazine	5	4	3	1		
VI	phthalazine						
	1,4,6,7-tetrasubstituted-phthalazine	6				2	
VII	pyridazine						
	1,4,5,6-tetrasubstituted-pyridazine	4					
VIII	pyrimido[4,5-*d*]pyrimidine						
VIIIa	7-phenyl-2,4-disubstituted-pyrimido[4,5-*d*]pyrimidine	11	7	1			
VIIIb	5,7-dimorpholino-2-phenyl-4-substituted-3,4-dihydro-pyrimido[4,5-*d*]pyrimidine	5	1			1	
IX	pyrimido[5,4-*d*]pyrimidine						
	2,4-dimorpholino-6-phenylpyrimido[5,4-*d*]pyrimidine	2				1	
X	pyrido[2,3-*d*]pyrimidine						
	2,4,5,6,7-pentasubstituted-pyrido[2,3-*d*]pyrimidine	8	2				
XI	pyrido[3,2-*d*]pyrimidine						
	2,4-dimorpholino-6-phenylpyrido[3,2-*d*]pyrimidine	1					
XII	pyrido[3,4-*d*]pyrimidine						
	6-phenyl-2,4,8-trisubstituted-pyrido[3,4-*d*]pyrimidine	7	1				
XIII	pyrido[4,3-*d*]pyrimidine						
	2,4-dimorpholino-7-phenylpyrido[4,3-*d*]pyrimidine	1	1				
	Total	219	104	26	8	8	0

11) W.L. Lipschitz, Z. Hadidian, and A. Kerpscar, *J. Pharmacol. Exp. Ther.*, **79**, 97 (1943).

***$p<0.001$. The reference compound used was hydrochlorothiazide (Esidrex, CIBA) (10 mg/kg, *p.o.*) Urinary sodium and potassium were measured with a flame photometer (Hitachi, 205 D).

2. Test Compounds——As shown in Table I, 219 compounds tested were classified into the groups of I to XIII based on the structural features. These are pyrimido[4,5-*d*]pyridazines (I), pyrazino[2,3-*d*]pyridazines (II), pyridazino[4,5-*d*]pyridazines (III), pyrido[2,3-*d*]pyridazines (IV), pyrido[3,4-*d*]pyridazines (V), phthalazines (VI), pyridazines (VII), pyrimido[4,5-*d*]pyrimidines (VIII), pyrimido[5,4-*d*]pyrimidines (IX), pyrido[2,3-*d*]pyrimidines (X), pyrido[3,2-*d*]pyrimidines (XI), pyrido[3,4-*d*]pyrimidines (XII) and pyrido[4,3-*d*]pyrimidines (XIII).

Results and Discussions

1. Diuretic Activity of Hydrochlorothiazide

Table II shows diuretic activity of hydrochlorothiazide (10 mg/kg) obtained in 6 experiments with groups of 5 or 6 rats. Hydrochlorothiazide at the dose of 10 mg/kg *p.o.* was previously confirmed to produce the maximum diuresis in the rats.[8] When values were expressed as the ratio of the response evoked by hydrochlorothiazide-treated rats to that evoked by control rats, the mean increases in V, $U_{Na}V$, U_KV and U_{Na}/U_K caused by hydrochlorothiazide were 1.82±0.14, 1.97+0.24, 1.62±0.09 and 1.23±0.11 times, described by means ±S.D., respectively, over the control values.

TABLE II. Diuretic Activity of Hydrochlorothiazide (HC)

Group	Dose mg/kg *p.o.*	No. of rats	V ml/5 hr/100 g, B.W.	$U_{Na}V$ μeq/5 hr/100 g, B.W.	U_KV μeq/5 hr/100 g, B.W.	U_{Na}/U_K
Control	—	6	2.66±0.40[a]	323±59	91±15	3.58±0.70
HC	10	6	4.34±0.74***(1.63)	606±63***(1.87)	146±11***(1.60)	4.15±0.27 (1.16)
Control	—	6	1.94±0.43	294±40	82±13	3.66±0.61
HC	10	6	3.69±0.81** (1.90)	517±85***(1.76)	129±32** (1.59)	4.10±0.64 (1.12)
Control	—	6	1.94±0.81	263±99	71±22	3.67±0.41
HC	10	6	3.95±0.47***(2.04)	526±40***(2.00)	120±24** (1.71)	4.47±0.67*(1.22)
Control	—	5	1.98±0.42	214±54	78± 9	2.83±0.96
HC	10	5	3.90±0.26***(1.97)	526±39***(2.46)	132±20***(1.69)	4.08±0.60*(1.44)
Control	—	5	2.42±0.35	340±43	79±13	4.36±0.86
HC	10	5	4.03±0.46***(1.66)	584±49***(1.72)	115±29* (1.46)	5.37±1.65 (1.23)
Control	—	6	2.53±0.59	276±52	107±22	2.60±0.79
HC	10	6	4.38±0.38***(1.73)	557±35***(2.02)	177±34** (1.66)	3.22±0.45 (1.20)

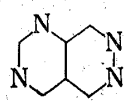
a) means±S.D., significant difference: *$0.01<p<0.05$, **$0.001<p<0.01$ and *** $p<0.001$, numbers in the parentheses: the ratio of the response evoked by hydrochlorothiazide(HC)-treated group to that evoked by control group

2. Diuretic Activity of Test Agents

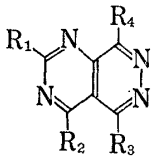
a) The First Screening——Diuretic data on 219 compounds screened at the dose of 30 mg/kg *p.o.* are shown in Tables I and III. In Table I, 104 of 219 compounds showed significant diuretic action, in which diuretic evaluation was performed for V and/or $U_{Na}V$. Eight of 219 compounds caused antidiuresis. Chemical structures and diuretic data of all compounds tested are shown in Table III.

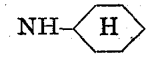
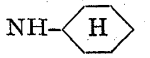
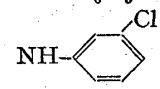
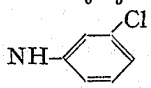
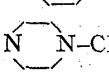
b) The Second Screening——As shown in Table IV, 87 compounds which significantly enhanced both water and sodium excretion in the first screening were further tested at the dose of 0.1 to 10 mg/kg *p.o.* At the dose of 1 mg/kg *p.o.* 24 compounds were effective in promoting water and sodium excretion. Eight derivatives shown in Table V, 2-phenyl-5,8-dimorpholinopyrimido[4,5-*d*]pyridazine (DS-210), 3-phenyl-5,8-dimorpholinopyridazino[3,4-*d*]pyridazine (DS-515), 1,4-dimorpholino-7-phenylpyrido[3,4-*d*]pyridazine (DS-511), 1,4-dimorpholino-7-(*p*-hydroxyphenyl)pyrido[3,4-*d*]pyridazine (DS-984), 1,4-bis-(2-methylmorpholino)-8-methylpyrido[3,4-*d*]pyridazine (DS-1058), 1,4-dimorpholino-7-phenyl-8-methylpyrido[3,4-*d*]pyri-

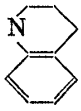
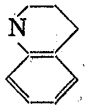
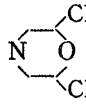
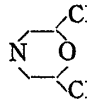
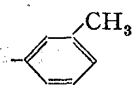
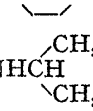
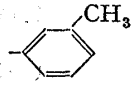
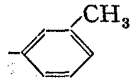
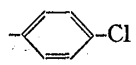
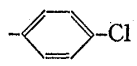
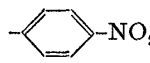
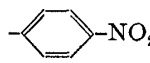
Table III. The First Screening on Diuretic Activity

I. Pyrido[4,5-*d*]pyridazine 

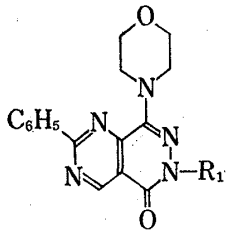
Ia. 2,4,5,8-Tetrasubstituted-pyrimido[4,5-*d*]pyridazine



DS No.	R_1	R_2	R_3	R_4	V	$U_{Na}V$	U_KV	U_{Na}/U_K
315	C_6H_5	H	OH	OH	0.91	1.06	0.94	1.12
248	C_6H_5	H	OH	$N(C_2H_5)(C_2H_4OH)$	1.18	1.28	1.08	1.17
428	C_6H_5	H	OH		1.32	1.45*	1.14	1.12
211	C_6H_5	H	OCH_3	OCH_3	1.02	1.32*	0.84	1.55**
408	C_6H_5	H	SCH_3	SCH_3	0.93	0.74	0.84	0.91
407	C_6H_5	H		SCH_3	1.10	0.83	0.83	1.01
239	C_6H_5	H	Cl	$NHCH(CH_3)_2$	1.15	1.23	1.11	1.10
238	C_6H_5	H	$NHCH(CH_3)_2$	Cl	1.05	1.19	1.00	1.31
240	C_6H_5	H	$N(CH_2C_6H_5)(C_2H_4OH)$	Cl	1.11	1.26	1.11	1.00
233	C_6H_5	H	Cl		1.14	0.96	1.04	0.92
235	C_6H_5	H	$NHCH(CH_3)_2$	$NHCH(CH_3)_2$	1.29	1.15	0.82	1.36
271	C_6H_5	H	NHC_2H_4–OCH_3	NHC_2H_4–OCH_3	1.16	1.21	1.22	1.05
272	C_6H_5	H	NHC_2H_4–OC_2H_5	NHC_2H_4–OC_2H_5	1.07	1.19	1.00	1.16
412	C_6H_5	H	$N(CH_3)(C_2H_4OH)$	$N(CH_3)(C_2H_4OH)$	0.93	0.75	0.86	0.90
234	C_6H_5	H	$NHCH_2C_6H_5$	$NHCH_2C_6H_5$	1.10	0.93	0.89	1.04
510	C_6H_5	H			1.20	1.35**	1.34*	1.02
373	C_6H_5	H	NHC_6H_5	NHC_6H_5	1.18	1.09	1.03	1.13
374	C_6H_5	H			1.19	1.05	0.88	1.19
209	C_6H_5	H			1.67***	1.99***	1.15***	1.74***
208	C_6H_5	H			1.56***	1.85***	0.64***	2.99***
462	C_6H_5	H			0.76	0.87	0.75	1.21

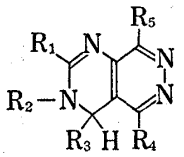
DS No.	R_1	R_2	R_3	R_4	V	$U_{Na}V$	U_KV	$U_{Na}U_K$
463	C_6H_5	H			1.09	1.01	0.98	1.13
210	C_6H_5	H			1.93***	2.15***	1.26**	1.71***
470	C_6H_5	H			1.74***	1.96***	1.48*	1.34
488	C_6H_5	H			1.74***	1.97***	1.26	1.67**
493	C_6H_5	H			1.24*	1.38**	1.16	1.29
489	C_6H_5	H			1.15	1.38**	1.04	1.32**
250		H			0.80	0.90	0.88	1.05
245		H			1.23	1.21*	0.89	1.39*
244		H			1.49***	1.66***	1.21	1.40
260		H			1.71**	1.41*	1.19	1.21
259		H			2.01***	1.68**	1.81*	0.97
262		H			1.15	1.36*	1.51*	0.92
261		H			1.87***	1.65**	1.53*	1.10
417		H			1.07	0.91	0.78	1.16
405		H			2.38***	2.86***	1.52**	1.71**
419		H			1.03	0.85	0.70	1.19
416		H			1.64**	1.38*	1.16	1.24
418		H			0.76	0.64	0.67	1.00
406		H			1.85**	1.91**	1.61*	1.10
854	C_6H_5	CH_3			1.13	1.18	1.30	0.95
860	C_6H_5	C_4H_9			1.38	1.26	1.31*	0.94
855	C_6H_5	$CH_2C_6H_5$			1.30*	1.27*	1.19	1.10
861	C_6H_5	C_6H_5			0.97	0.98	1.05	0.93

Ib. 8-Morpholino-5-oxo-2-phenyl-6-substituted-5,6-dihydropyrimido[4,5-*d*]pyridazine

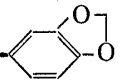


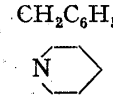
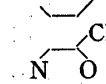
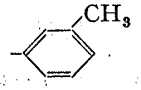
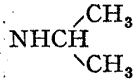
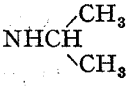
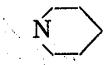
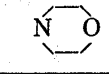
DS-No.	R_1	V	$U_{Na}V$	U_KV	U_{Na}/U_K
513	$CH(CH_3)_2$	1.18	1.16	1.06	0.95
534	CH_2–$COOC_2H_5$	0.93	1.05	1.06	1.01
514	$CH_2C_6H_5$	1.07	1.01	1.11	0.76
717	$C_2H_4C_6H_5$	1.04	0.90	1.01	0.85
718	C_2H_4–$CH(C_6H_5)_2$	0.98	0.95	1.00	0.89
775	C_2H_4–N(piperazine)–N–(2-Cl-phenyl)	1.41**	1.48***	0.95	1.57**
852	C_2H_4–N(piperazine)–N–C_2H_4–$CH(C_6H_5)_2$	0.88	1.07	0.95	1.14
536	C_2H_4–N(morpholine)	1.61**	1.71**	0.89	1.94**
774	C_2H_4–N(2-CH$_3$-morpholine)	0.88	0.98	0.80	1.19
851	C_3H_6–N(morpholine)	1.50*	1.79**	1.04	1.79***

Ic. 2,3,4,5,8-Pentasubstituted-3,4-dihydropyrimido[4,5-*d*]pyridazine

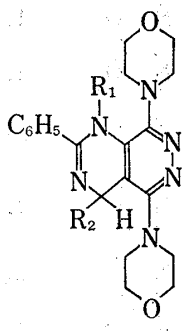


DS No.	R_1	R_2	R_3	R_4=R_5	V	$U_{Na}V$	U_KV	U_{Na}/U_K
381	C_6H_5	H	H	morpholine	2.03***	1.96**	2.22***	0.83
533	C_6H_5	CH_3	H	morpholine	0.55***	0.60**	0.94	0.65*
643	C_6H_5	C_2H_5	H	morpholine	0.72**	0.71	0.68	1.01
683	C_6H_5	C_3H_7	H	morpholine	0.98	1.09	0.97	1.14
682	C_6H_5	CH_2–CH_2=CH_2	H	morpholine	0.72	0.77	0.66*	1.20
736	C_6H_5	C_4H_9	H	morpholine	0.76	0.82	0.91	0.91
715	C_6H_5	$CH_2C_6H_5$	H	morpholine	1.36**	1.51**	1.04	1.38
697	C_6H_5	C_3H_6Cl	H	morpholine	1.01	0.99	1.41	0.68
716	C_6H_5	C_4H_8Br	H	morpholine	1.06	1.00	1.00	0.97

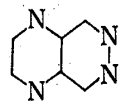
DS No.	R_1	R_2	R_3	$R_4=R_5$	V	$U_{Na}V$	U_KV	U_{Na}/U_K
561	C_6H_5	$COCH_3$	H		1.78***	1.60**	1.34	1.13
562	C_6H_5	$COCH(CH_3)_2$	H		1.97***	1.92***	1.10	2.06***
563	C_6H_5	COC_6H_5	H		1.69**	1.59*	1.17	1.24
564	C_6H_5	$COOC_2H_5$	H		1.42	1.46*	0.97	1.43
769	C_6H_5	H	CH_3		1.02	1.06	1.24	0.80
770	C_6H_5	H	C_2H_5		1.11	1.25	0.71	1.61*
806	C_6H_5	H	$CH(CH_3)_2$		1.28	1.34	1.09	1.23
737	C_6H_5	H	CH_2–CH=CH_2		1.90***	2.10***	1.14	1.82**
771	C_6H_5	H	C_4H_9		0.88	1.04	0.44*	2.40*
853	C_6H_5	H	t-C_4H_9		2.65***	2.45***	2.90***	0.85
807	C_6H_5	H	$C_2H_4CH(CH_3)_2$		1.44*	1.40**	1.14	1.26*
808	C_6H_5	H	C_6H_{13}		1.06	1.14	1.00	1.18
644	C_6H_5	H	$CH_2C_6H_5$		2.94***	3.03***	1.56**	1.87***
773	C_6H_5	H	CH_2–C_6H_4–CH_3		1.91***	2.27***	1.29	1.56*
810	C_6H_5	H	CH_2–C_6H_4–Cl		1.84***	2.25***	1.12	2.02*
811	C_6H_5	H	CH_2–C_6H_4–OCH_3		2.05***	2.26***	1.81***	1.12
812	C_6H_5	H	CH_2– 		1.63*	1.86***	1.16	1.44*
856	C_6H_5	H	CH_2– 		2.28***	2.38***	1.52**	1.64**
815	C_6H_5	H	$C_2H_4C_6H_5$		1.03	1.03	0.84	1.33
809	C_6H_5	H			1.41**	1.40**	1.31**	1.09
772	C_6H_5	H	C_6H_5		0.77	0.96	0.61	1.44
804	C_6H_5	H	CH_2OH		1.88***	1.87***	1.78***	1.05
805	C_6H_5	H	$CHCH_3$(OH)		1.67**	1.76***	1.68***	1.05
813	C_6H_5	H	CHC_6H_5(OH)		1.98***	1.94***	1.64***	1.18
857	C_6H_5	H			1.83**	2.00**	1.73*	1.24
814	C_6H_5	H			1.74***	1.77***	1.31*	1.35
816	C_6H_5	H			0.91	0.93	0.86	1.13
738	C_6H_5	$CH_2C_6H_5$	$CH_2C_6H_5$		1.02	1.41*	0.62**	2.29***

DS No.	R_1	R_2	R_3	R_4	R_5	V	$U_{Na}V$	U_KV	U_{Na}/U_K
956	C_6H_5	H	H	NHC_6H_5	NHC_6H_5	1.06	1.09	1.15	0.96
817	C_6H_5	H	C_2H_5		C_2H_5	1.18	1.24	0.80	1.53
818	C_6H_5	H	$CH(CH_3)_2$		$CH(CH_3)_2$	1.11	1.23	0.89	1.29
819	C_6H_5	H	C_4H_9		C_4H_9	1.35***	1.49**	1.04	1.33
820	C_6H_5	H	C_6H_{13}		C_6H_{13}	1.00	1.06	1.33	0.75
821	C_6H_5	H	$CH_2C_6H_5$		$CH_2C_6H_5$	0.95	1.07	1.49	0.65
802	C_6H_5	H	$CH_2C_6H_5$			1.62**	1.57**	1.16	1.37**
803	C_6H_5	H	$CH_2C_6H_5$			1.72***	1.74***	1.51*	1.21
955		H	H			0.94	1.24	0.86	1.50
954		H	H			1.29	1.45*	0.84	1.77*
427		H	H			1.14	1.36*	1.47	0.92

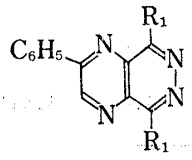
Id. 5,8-Dimorpholino-2-phenyl-1,4-disubstituted-1,4-dihydroxypyrimido[4,5-*d*]pyridazine



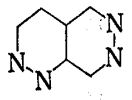
DS-No.	R_1	R_2	V	$U_{Na}V$	U_KV	U_{Na}/U_K
702	H	H	2.04***	1.76**	3.58***	0.47**
701	$CH_2C_6H_5$	H	1.47*	1.48**	1.36**	1.14
700	C_3H_7	C_3H_7	1.20	1.38	0.88	1.46*
699	$CH_2CH{=}CH_2$	$CH_2CH{=}CH_2$	1.18	1.25	0.98	1.24
735	C_4H_9	C_4H_9	1.34*	1.65**	0.91	1.80**
685	$CH_2C_6H_5$	$CH_2C_6H_5$	1.22	1.26	1.16	1.04

II. Pyrazino[2,3-*d*]pyridazine 

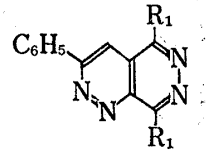
2-Phenyl-5,8-disubstituted-pyrazino[2,3-*d*]pyridazine



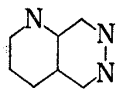
DS-No.	R_1	V	$U_{Na}V$	U_KV	U_{Na}/U_K
207	NHC_6H_5	1.18	1.23	0.86	1.50*
206		1.24	1.04	0.99	1.15
205		1.21	1.00	1.04	0.98

III. Pyridazino[4,5-*c*]pyridazine 

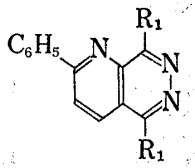
3-Phenyl-5,8-disubstituted-pyridazine[4,5-*c*]pyridazine



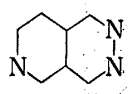
DS-No.	R_1	V	$U_{Na}V$	U_KV	U_{Na}/U_K
516		1.80**	1.96***	1.02	1.85***
515		1.90***	1.94***	1.37	1.21

IV. Pyrido[2,3-*d*]pyridazine 

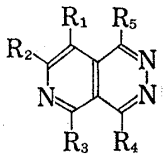
2-Phenyl-5,8-disubstituted-pyrido[2,3-*d*]pyridazine

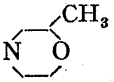
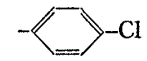
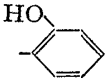
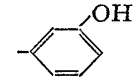
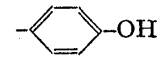
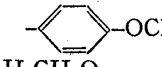
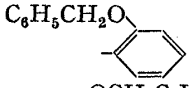
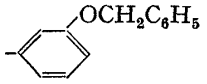
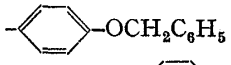
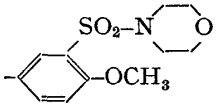
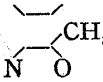
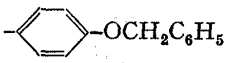
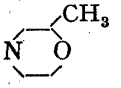
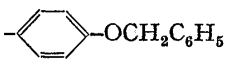
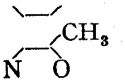


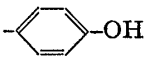
DS-No.	R_1	V	$U_{Na}V$	U_KV	U_{Na}/U_K
491	$NHCH(CH_3)_2$	1.47*	1.51***	0.79	1.95***
492	$NHCH_2C_6H_5$	0.92	1.00	0.89	1.13
545	NH– 	1.26	1.24	0.94	1.28
546	NHC_6H_5	1.28	1.15	1.02	1.05
544		1.22	0.95	0.82	1.15
490		1.21*	0.97	1.58	0.63

V. Pyrido[3,4-*d*]pyridazine 

Va. 1,4,5,7,8-Pentasubstituted-pyrido[3,4-*d*]pyridazine

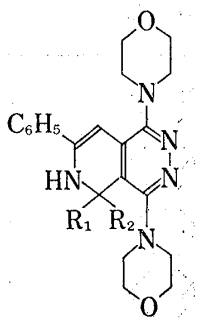


DS No.	R_1	R_2	R_3	$R_4=R_5$	V	$U_{Na}V$	U_KV	U_{Na}/U_K
512	H	C_6H_5	H	$NHCH_2C_6H_5$	1.04	1.11	1.08	0.88
950	H	C_6H_5	H		1.92***	2.10***	1.07	1.71**
908	H	C_6H_5	H		1.79***	1.95***	0.91	2.13**
511	H	C_6H_5	H		2.42***	2.44***	1.54**	1.56*
936	H	C_6H_5	H		2.44***	2.36***	1.39*	1.72*
938	H		H		2.16***	2.04***	1.60**	1.20
986	H		H		1.14	1.13	0.99	1.13
985	H		H		1.08	1.10	1.02	1.09
984	H		H		1.84***	1.93***	1.59**	1.22
962	H		H		2.40***	2.22***	1.78**	1.24
983	H		H		1.10	1.12	1.02	1.11
982	H		H		1.14	1.14	1.02	1.13
981	H		H		1.38*	1.48**	1.52**	0.99
964	H		H		1.19	1.28	1.21	1.06
963	H		H		1.89***	1.76***	1.32	1.35
1045	H		H		1.66***	1.85***	1.58**	1.08
1047	H		H		1.23	1.16	1.47	0.86
1046	H		H		0.97	0.84	1.11	0.82
883	H	C_6H_5	$CH_2C_6H_5$		0.84	0.83	1.12	0.81
957	H	C_6H_5	Cl		1.19	1.13	1.06	1.03
958	H	C_6H_5			1.49*	1.37*	1.67*	0.82
845	Cl	CH_3	H		1.05	1.09	1.26	0.89
1061	Cl	C_6H_5	H		2.54***	2.51***	1.81***	1.36*
1043	CH_3	C_6H_5	H		2.03***	2.38***	1.46	1.52*
1058	CH_3	C_6H_5	H		2.41***	2.78***	1.69***	1.55**

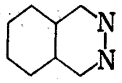
DS No.	R_1	R_2	R_3	$R_4=R_5$	V	$U_{Na}V$	U_KV	U_{Na}/U_K
1055	CH_3		H		2.43***	2.58***	1.90**	1.30
1054	C_2H_5	C_6H_5	H		1.83***	1.80***	1.52**	1.23
1060	C_3H_7	C_6H_5	H		1.06	1.07	1.12	0.97
600	OCH_3	CH_3	H		1.21	1.22	0.95	1.16
599	OCH_3	CH_3	H		2.12***	2.24***	1.32	1.56*
866	OCH_3	C_3H_7	H		1.34	1.56**	1.03	1.52
865	OCH_3	$CH(CH_3)_2$	H		1.26	1.41*	0.91	1.45*
867	OCH_3	C_4H_9	H		1.20	1.28*	0.93	1.39
840	OCH_3	$CH_2CH(CH_3)_2$	H		1.58**	1.51***	1.24*	1.22
839	OCH_3	$CH_2CH(CH_3)_2$	H		1.67***	2.01***	1.40*	1.53*
868	OCH_3	$CHCH_2CH_3$ | CH_3	H		1.39**	1.48**	1.06	1.39
1048	OCH_3	C_6H_5	H		1.46**	1.62***	1.07	1.51*
841	OCH_3	$CH_2C_6H_5$	H		0.88	0.92	1.02	0.89
844	OC_2H_5	CH_3	H		1.05	1.07	1.05	1.03
843	OC_2H_5	CH_3	H		1.69**	1.73***	1.72**	1.04
869	OC_4H_9	CH_3	H		0.74*	0.91	0.75	1.16
1049	C_6H_5	CH_3	H		0.81	0.82	1.09	0.80
1057	C_2H_5	C_6H_5	H		1.12	1.54**	1.13	1.49
1056	$CH_2C_6H_5$	C_6H_5	H		1.54*	1.69**	1.42	1.20

DS No.	R_1	R_2	R_3	R_4	R_5	V	$U_{Na}V$	U_KV	U_{Na}/U_K
598	H	CH_3	H	OH	OH	1.12	1.02	0.92	1.01
959	H	C_6H_5	H	OH	OH	1.97***	1.84***	2.01***	0.91
960	H	C_6H_5	H		OH	1.62*	1.82**	1.22	1.48
961	H	C_6H_5	H	OH		1.63***	1.74***	1.33	1.29
1011	H	C_6H_5	H		OCH_3	0.98	0.92	0.91	0.93
1012	H	C_6H_5	H	OCH_3		1.39*	1.28	1.72*	0.67

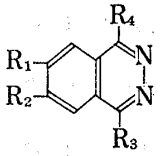
Vb. 1,4-Dimorpholino-7-phenyl-5,5-disubstituted-5,6-dihydropyrido[3,4-*d*]pyridazine



DS-No.	R_1	R_2	V	$U_{Na}V$	U_KV	U_{Na}/U_K
935	H	H	2.20***	2.24***	2.27**	0.99
913	$C(CH_3)_3$	H	2.51***	2.40***	1.81**	1.30
862	$CH_2C_6H_5$	H	2.45***	2.28***	1.50*	1.56*
863	C_6H_5	H	1.47**	1.39*	1.10	1.34
884	$CH_2C_6H_5$	CH_3	1.18	1.24	1.35*	0.92

VI. Phthalazine 

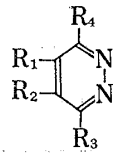
1,4,6,7-Tetrasubstituted-phthalazine



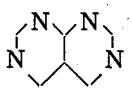
DS-No.	R_1	R_2	R_3	V	$U_{Na}V$	U_KV	U_{Na}/U_K
471	H	H		0.96	1.01	0.90	1.13
465	H	H		1.23	1.09	1.23	0.91
464	H	H		1.04	0.99	1.13	0.90
916	C_6H_5	H		1.19	1.25	0.58**	2.26*
907	C_6H_5	H		1.06	0.76*	1.39*	0.53**
918	C_6H_5	OCH_3		0.68	0.65*	0.81	0.81

VII. Pyridazine 

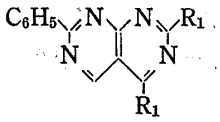
1,4,5,6-Tetrasubstituted-pyridazine

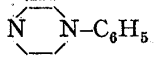
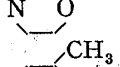
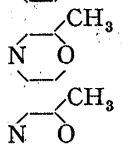


DS-No.	R_1	R_2	R_3	R_4	V	$U_{Na}V$	U_KV	U_{Na}/U_K
687	Br	H	C_6H_5	CH_3	1.06	1.04	0.91	1.08
686	COOH	H	C_6H_5	CH_3	0.90	0.92	0.94	0.94
688	CONH	H	C_6H_5	CH_3	1.20	1.10	1.11	1.06
535	CHO	NH_2			0.98	0.97	1.06	0.88

VIII. Pyrimido[4,5-*d*]pyrimidine 

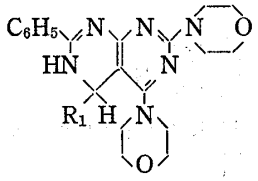
VIIIa. 7-Phenyl-2,4-disubstituted-pyrimido[4,5-*d*]pyrimidine



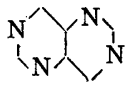
DS-No.	R_1	V	$U_{Na}V$	U_KV	U_{Na}/U_K
663[a)]	NH_2	1.58***	1.71***	1.60*	1.05
750	$N(C_2H_5)_2$	1.36**	1.43**	1.17	1.24
753	$NHCH(CH_3)_2$	1.22**	1.21	1.23	1.04
748	$NHCH_2C_6H_5$	1.20	1.19	1.25	0.95
749	NHC_2H_4OH	1.03	1.10	0.96	1.24
664		1.53***	1.64**	0.78	2.04**
752		0.82	0.81	0.97	0.82
565		2.25***	2.17***	1.81*	1.03
923		1.71*	1.76**	1.33	1.23
751		2.12***	2.06***	1.32	1.67*
922		0.81	1.02	0.71	1.30

a) This compound was screened at the dose of 10 mg/kg.

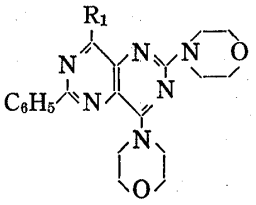
VIIIb. 5,7-Dimorpholino-2-phenyl-4-substituted-3,4-dihydropyrimido-[4,5-*d*]pyrimidine



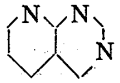
DS-No.	R_1	V	$U_{Na}V$	U_KV	U_{Na}/U_K
886	CH_3	0.65*	0.97	1.47***	0.65*
887	C_2H_5	0.87	1.08	1.10	1.00
888	C_4H_9	0.91	0.97	0.95	1.08
858	$CH_2C_6H_5$	2.01**	1.92***	1.50*	1.32*
889	C_6H_5	1.17	0.99	1.15	0.83

IX. Pyrimido[5,4-*d*]pyrimidine 

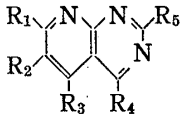
2,4-Dimorpholino-6-phenylpyrimido[5,4-*d*]pyrimidine

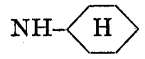
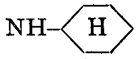
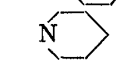
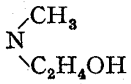
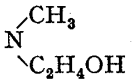


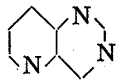
DS-No.	R_1	*V*	$U_{Na}V$	U_KV	U_{Na}/U_K
566	H	0.98	1.08	1.08	1.41
924		0.38*	0.50	0.78	0.60

X. Pyrido[2,3-*d*]pyrimidine 

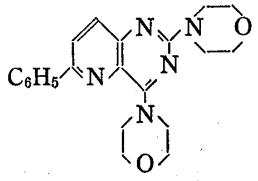
2,4,5,6,7-Pentasubstituted-pyrido[2,3-*d*]pyrimidine



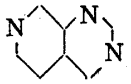
DS-No.	R_1	R_2	R_3	R_4	R_5	*V*	$U_{Na}V$	U_KV	U_{Na}/U_K
915	C_6H_5	H	H			1.23	1.04	1.05	1.04
912	C_6H_5	H	H			0.79	0.90	0.97	1.06
914	C_6H_5	H	H			1.19	1.30	1.28	1.15
910	C_6H_5	H	H			2.17***	2.23***	3.34***	0.64***
893	C_6H_5	H	CH_3			0.74	0.91	0.87	1.06
911	C_6H_5	Br	H			1.56**	1.40*	1.58	0.94
902	OH	H	H		C_6H_5	1.02	0.90	0.90	1.03
901	OH	H	H		C_6H_5	1.12	1.11	0.90	1.17

XI. Pyrido[3,2-*d*]pyrimidine 

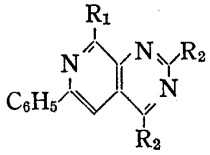
2,4-Dimorpholino-6-phenylpyrido[3,2-*d*]pyrimidine

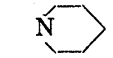
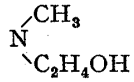
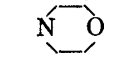
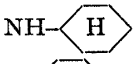
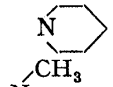
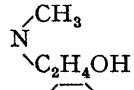
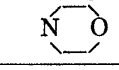


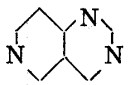
DS-No.	*V*	$U_{Na}V$	U_KV	U_{Na}/U_K
909	0.97	0.85	0.77	1.14

XII. Pyrido[3,4-*d*]pyrimidine 

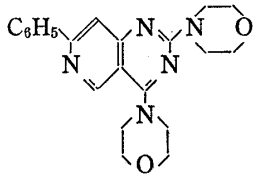
6-Phenyl-2,4,8-trisubstituted-pyrido[3,4-*d*]pyrimidine



DS-No.	R_1	R_2	V	$U_{Na}V$	U_KV	U_{Na}/U_K
899	H		0.90	1.07	0.98	1.04
900	H		1.08	1.10	1.03	1.06
896	H		1.71**	1.66**	1.34	1.29
897	CH_3		0.86	1.00	0.96	1.07
895	CH_3		0.83	0.91	0.55**	1.69**
898	CH_3		0.88	0.96	0.92	1.03
894	CH_3		1.02	0.99	0.87	1.35

XIII. Pyrido[4,3-*d*]pyrimidine 

2,4-Dimorpholino-7-phenylpyrido[4,3-*d*]pyrimidine



DS-No.	V	$U_{Na}V$	U_KV	U_{Na}/U_K
1062	2.01**	1.70***	1.57*	1.12

significant difference: * $0.01<p<0.05$, ** $0.001<p<0.01$ and *** $p<0.001$

dazine (DS-1043), 1,4-dimorpholino-7-phenyl-8-methoxypyrido[3,4-*d*]pyridazine (DS-1048) and 1,4-dimorpholino-7-phenyl-5-benzyl-5,6-dihydropyrido[3,4-*d*]pyridazine (DS-862) were significantly effective in promoting the excretion of water and sodium at the dose of 0.1mg/kg.

3. Mode of Diuresis

a) Time Course of Diuretic Activity——Figure 1 shows the time course of diuresis caused at oral dose of 1 mg/kg of DS-210, DS-515, DS-511, DS-1043, DS-1048 and DS-862. Hydrochlorothiazide (10 mg/kg *p.o.*) was used as a reference. Each of 6 compounds caused diuretic effects similar to those of hydrochlorothiazide, but was more rapid in onset of the action than hydrochlorothiazide.

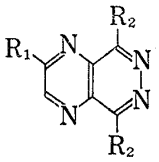
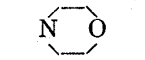
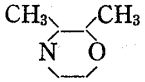
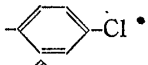
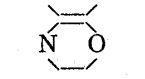
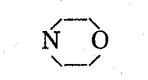
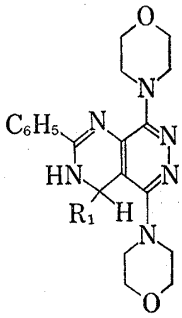
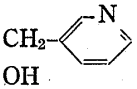
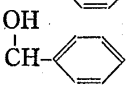
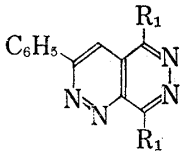
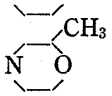
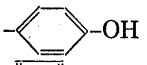
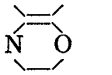
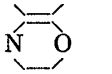
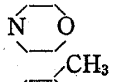
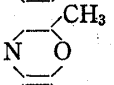
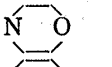
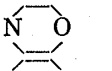
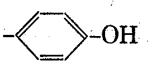
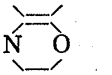
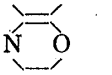
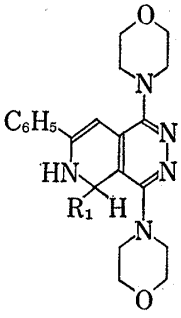
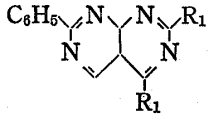
b) Diuresis by the Combined Use of DS-Compounds——Table VI shows the diuretic activity by single dose of four DS-compounds, DS-210, DS-515, DS-511 and DS-862, at the maximum diuretic dose (10 mg/kg *p.o.*) and by combined use of DS-210 with either of DS-515, DS-511 or DS-862. The diuretic activity between DS-210 alone and the combined use was not different each other.

TABLE IV. The Second Screening on Diuretic Activity

Group	DS-No.	Dose mg/kg, *p.o.*	*V*	$U_{Na}V$	U_KV	U_{Na}/U_K
Ia	209	1	1.16	1.28	1.07	1.23
	208	10	2.08***	2.51***	1.43	1.51
		5	1.69**	2.20***	1.90***	1.02
		1	1.00	1.10	1.30	1.12
	210	10	2.29***	2.28***	1.16	1.93***
		1	1.50**	1.43*	1.11	1.26
		0.1	1.48**	1.40**	1.18	1.15
	470	10	1.64**	1.72***	1.36	1.29
		1	1.11	1.19	1.03	1.12
	488	10	1.72**	1.80***	1.15	1.58**
		1	1.30*	1.34*	1.00	1.33
		0.1	0.92	0.97	1.10	0.85
	493	10	1.27	1.45*	0.93	1.64**
		1	1.33	1.32	1.14	1.18
	244	10	1.75*	1.70*	1.38	1.31*
		1	1.20	1.19	1.01	1.20*
	260	5	1.20	1.07	0.96	1.17
	259	5	1.88***	1.68**	1.38	1.18
		1	1.37**	1.39**	1.34	1.08
		0.1	0.70	0.69	0.85	0.84
	261	1	1.07	1.12	1.18	0.97
	405	10	1.77***	2.02***	1.24*	1.48*
		1	1.30	1.49	1.32	1.13
	416	10	1.64**	1.38*	1.16	1.24
		1	1.57*	1.59**	1.25	1.30**
		0.1	0.63	0.89	0.82	1.10
	406	10	1.24*	1.19	1.17	1.03
		1	1.21	1.20	1.24	1.01
	855	10	1.09	0.98	0.83	1.22
Ib	775	10	1.24	1.32	0.97	1.43*
	536	10	1.09	1.25	1.49**	0.97
		1	0.98	1.14	1.18	1.09
	851	10	1.76**	1.69**	1.06	1.56***
		1	1.39	1.32	1.19	1.11
Ic	381	10	2.03***	1.96**	2.22***	0.83
		1	1.38	1.41*	1.26	1.08
		0.1	1.17	1.01	1.01	1.05
	533	10	1.35	1.16	1.20	0.95
		1	1.30	1.13	1.18	0.93
	643	10	0.87	0.90	0.85	1.01
	682	10	1.18	1.15	1.11	1.04
	715	10	1.56*	1.63***	1.17	1.47*
		1	1.15	1.09	1.06	1.03
	561	10	1.39*	1.37*	1.27	1.12
		1	1.26	1.12	1.06	1.03
	562	10	1.56*	1.39*	1.04	1.53
		1	1.14	1.15	1.01	1.12
	563	10	0.94	0.96	1.04	1.00
	737	10	1.85**	2.09***	1.17	1.64*
		1	1.21	1.37	1.01	1.34
	853	1	1.07	1.26	1.04	1.32
	807	10	1.45*	1.42*	1.06	1.47
		1	0.88	0.90	0.84	1.09
	644	10	1.88***	1.92***	0.78	2.40**
		1	1.40*	1.37*	1.22	1.07
		0.1	0.75	0.98	0.69	1.53
	773	10	2.23***	2.30***	1.48*	1.56**
		1	1.39	1.30	1.07	1.20
	810	10	1.92***	1.85***	0.68**	2.71***
		1	1.15	1.29	1.20	1.03
	811	10	1.85***	1.71***	0.90	1.85***
		1	1.28	1.36	1.10	1.22
	812	10	1.73*	1.43*	1.02	1.38*
		1	1.35	1.50	1.07	1.37
	856	1	1.33*	1.30*	1.13	1.04
		0.1	0.99	1.13	1.23	0.90
	809	10	1.78*	1.79***	1.25	1.43*
		1	1.35	1.14	0.91	1.19
	804	10	2.17***	2.02***	1.58**	1.25
		1	0.82	0.88	0.96	0.93
	805	10	2.47***	2.28***	1.56**	1.50*
		1	1.15	1.27	1.08	1.21
	813	10	2.24***	1.74***	1.05	1.64*
		1	1.79*	1.95***	1.57**	1.25**
		0.1	0.85	0.95	1.24	0.77
	857	10	1.78**	1.36*	0.81	1.91*
		1	0.94	1.09	1.22	0.89
	814	10	1.62*	1.50**	0.85	1.80**
		1	0.76	0.93	0.99	0.97
	819	1	0.85	0.83	0.91	0.94
	802	1	1.28	1.26	0.99	1.20
	803	1	1.24	1.17	1.13	1.04
Id	702	10	1.96***	1.60**	1.68**	0.95
		1	1.30	1.19	1.02	1.13
	701	10	1.48*	1.36*	1.43	1.00
		1	0.93	0.86	0.98	0.83
	735	10	0.91	0.97	0.70	1.46
III	516	10	1.80**	1.96***	1.02	1.85***
III	516	1	1.42*	1.41**	1.16	1.17
		0.1	1.07	1.30	1.00	1.32
	515	10	2.07***	2.46***	1.61**	1.51**
		1	1.59***	1.77**	1.44*	1.19
		0.1	1.70**	1.69**	1.09	1.55***
IV	491	10	1.31	1.24	0.84	1.51***
Va	950	1	1.51*	1.23	1.11	1.12
		0.1	1.03	0.99	1.15	0.83
	908	10	2.51***	2.63***	1.23	2.11***
		1	1.64**	1.65***	1.25	1.40
		0.1	0.90	0.89	0.77*	1.12
	511	10	2.37***	2.17***	1.36	1.62*
		1	2.43***	2.48***	1.81***	1.36*
		0.1	1.73***	1.77***	1.27	1.38*
	936	1	2.39***	1.89***	1.30	1.49*
		0.1	1.00	1.06	0.99	1.10
	938	1	1.07	1.09	1.08	0.94
	984	10	2.05***	2.45***	1.54**	1.59*
		1	1.40*	1.53**	1.17	1.31
		0.1	1.61***	1.70***	1.05	1.59**
	962	1	1.25	1.23	1.08	1.09
	981	10	1.51*	1.54**	1.13	1.36*
		1	1.03	1.18	1.04	1.13
	963	1	1.43**	1.46**	1.13	1.24
		0.1	1.07	1.21	1.33	0.91
	1045	10	2.18***	2.02***	0.99	2.42*
		1	0.99	1.08	0.95	1.17
	958	1	1.12	1.15	0.71	1.28
	1061	1	1.91***	1.89***	1.72**	1.11
		0.1	1.24	1.30	1.38	0.99
	1058	1	2.90***	2.19**	1.08	2.17*
		0.1	1.44*	1.36**	0.76	1.97*
	1043	10	2.87***	2.81***	1.41*	2.02*
		1	2.04***	1.93***	1.39*	1.36*
		0.1	1.60***	1.86***	1.32*	1.13
	1055	1	2.32***	1.88***	0.78	2.47***
		0.1	1.18	1.12	0.75	1.48*
	1054	10	1.71***	1.86***	1.58*	1.15
		1	1.36**	1.59**	1.14	1.47
		0.1	1.06	0.96	1.04	0.95
	599	10	1.45	1.65**	1.12	1.32
		1	1.08	1.21	1.03	1.22
	840	10	1.79*	1.75**	1.45*	1.25
		1	1.38	1.34	1.10	1.28
	839	10	1.58**	1.89***	1.15	1.69**
		1	1.32	1.29	1.19	1.03
	868	10	1.13	0.98	1.26	0.81
	1048	10	2.74***	2.68***	1.71***	1.47*
		1	2.29***	2.30***	1.62***	1.33
		0.1	1.55***	1.63***	1.42**	1.10
	843	10	1.81***	1.97***	1.15	1.67***
		1	1.25	1.35	1.17	1.23
	869	1	1.01	0.87	1.08	0.83
	1056	1	1.06	0.94	0.69	1.34
	959	10	1.14	1.14	1.03	1.06
	960	10	0.93	1.00	0.90	1.11
	961	10	0.78	0.84	0.68	1.22
Vb	935	1	2.04*	1.71**	1.27	1.36
		0.1	1.10	1.34	1.34	0.97
	913	10	2.67***	2.71***	2.57***	1.01
		1	2.01***	2.04***	1.80***	1.18
		0.1	1.13	1.13	1.06	1.08
	862	10	2.02***	2.06***	1.60*	1.26
		1	1.81***	1.72***	1.08	1.59***
		0.1	1.25*	1.52**	1.31	1.15
	863	10	1.38	1.23	1.23	1.02
VI	907	1	0.94	0.97	0.94	0.98
	918	1	0.85	0.88	0.87	0.97
VIIIa	663	10	1.58*	1.71***	1.60*	1.05
		1	1.03	1.10	1.50	0.80
	750	1	0.92	0.97	0.95	0.97
	664	10	1.05	1.04	0.83	1.18
	565	10	2.41***	2.03***	1.63*	1.28
		1	1.38*	1.44**	1.64*	0.85
		0.1	0.80	0.74	0.66	1.24
	923	1	1.10	0.97	1.03	0.93
	751	10	1.55**	1.57*	1.43*	1.03
		1	1.03	0.94	0.74	1.21
VIIIb	886	1	0.86	0.97	0.98	0.98
	858	10	1.80**	1.80**	1.37	1.34*
		1	0.96	1.04	0.98	1.06
IX	924	1	1.06	0.96	0.93	1.01
X	910	10	1.91**	1.53	2.43***	0.63**
		1	1.03	0.95	1.12	0.85
	911	1	1.14	1.11	1.03	1.10
XII	896	1	0.82	0.91	0.79	1.21
XIII	1062	10	2.03**	1.62	1.09	1.51*
		1	0.78*	0.84*	0.97	0.91

significant difference: * $0.01<p<0.05$, ** $0.001<p<0.01$ and *** $p<0.001$

TABLE V. Active Substances at the Oral Dose of 1 mg/kg

Group	Structure	DS-No.	R_1	R_2	R_3
Ia		210[a]	C_6H_5		
		488	C_6H_5		
		259			
		416			
Ic		381	H		
		644	$CH_2C_6H_5$		
		856			
		813			
III		516			
		515[a]			
Va		908	C_6H_5		
		511[a]	C_6H_5		
		936	C_6H_5		
		984[a]			
		963			
		1061	C_6H_5		Cl
		1058[a]	C_6H_5		CH_3
		1043[a]	C_6H_5		CH_3
		1048[a]	C_6H_5		OCH_3
		1055			CH_3
		1054	C_6H_5		C_2H_5
Vb		935	H		
		913	$C(CH_3)_3$		
		862[a]	$CH_2C_6H_5$		
VIIIa		565			

a) Diuretic at the dose of 0.1 mg/kg.

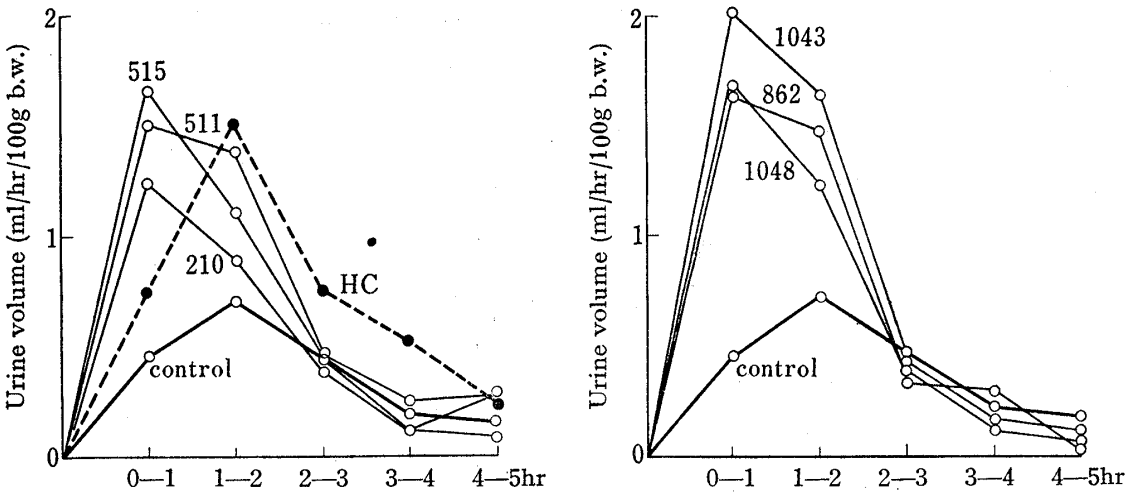


Fig. 1. Time Course of Urine Excretion of DS-210, 511, 515, 862, 1043, 1049 and Hydrochlorothiazide (HC)

DS-210, 515, 511, 862, 1043 or 1048: 1 mg/kg, *p.o.* hydrochlorothiazide: 10 mg/kg, *p.o.*

TABLE VI. Diuretic Action of DS-210 in Combined Use with DS-515, 511 or 862

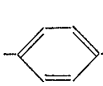
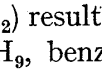
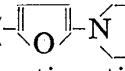
Group	Dose mg/kg *p.o.*	No. of rats	*V* ml/5 hr/100 g B.W.	$U_{Na}V$ ueq/5 hr/100 g B.W.	U_KV	U_{Na}/U_K
Control	—	13	1.76±0.39[a)]	240±44	70±19	3.57±0.96
DS-210	10	13	3.92±0.65***	560±78***	102±18***	5.52±0.68***
DS-515	10	6	4.45±0.17***	672±38***	115±21***	6.09±1.59***
DS-511	10	12	4.26±0.50***	585±61***	108±23***	5.59±1.10***
DS-862	10	7	4.10±0.81***	582±86***	80±24	7.83±2.71***
DS-210+ DS-515	10+10	7	4.14±0.33***	573±39***	100±10**	5.79±0.78***
DS-210+ DS-511	10+10	7	4.13±0.43***	611±93***	92±29	6.97±1.28***[b)]
DS-210+ DS-862	10+10	7	3.97±0.52***	572±86***	92±11*	6.37±1.44***

a) means±S.D., significant difference between control and experimental groups, * $0.01 < p < 0.05$, ** $0.001 < p < 0.01$ and *** $p < 0.001$, and between DS-210 treated group and the other, *b*) $0.001 < p < 0.01$

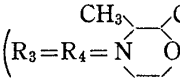
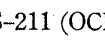
4. Structure-Activity Relationships

Among the structural features of I to XIII classified in Table I, pyrimido[4,5-*d*]pyrimidines (I), pyridazino[3,4-*d*]pyridazines (III), pyrido[3,4-*d*]pyridazines (V) and pyrimido[4,5-*d*]-pyrimidines (VIII) gave potent diuretic compounds in the diuretic assay at the dose of 1 mg/kg *p.o.* The remaining structures were, in general, ineffective or weakly active.

The effect of modifications of the substituent on diuretic activity was mainly studied on the groups of I and V.

In the series of I-a the most potent compound was DS-210 ($R_1=C_6H_5$, $R_2=H$, $R_3=R_4=$ ). The diuretic activity caused by modifying the substituent at the 2-position (R_1) decreased in the order: DS-210>DS-259 ($R_1=$ ), DS-416 ()>DS-244(), DS-405 ()>DS-261 (), DS-406(). Introduction of a substituent at the 4-position (R_2) resulted in loss of diuretic activity (DS-854, DS-860, DS-855 and DS-861, in which CH_3, C_4H_9, benzyl and phenyl groups are substituted at the 4-position,

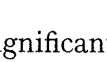
respectively). In comparison with DS-210 the substances which have various substituents at R_3 and R_4 showed weaker diuretic activity in the following order:

DS-210>DS-488 ($R_3=R_4=$ )>DS-209 (), DS-208 (), DS-470 (),

DS-493 ()>DS-211 (OCH_3), DS-428 ($R_3=OH$, $R_4=$ ), DS-510 (),

DS-489 ().

Mono- or disubstitution of R_3 and R_4 by SCH_3 or alkylamino group resulted in loss of activity.

In the I-b series DS-775 (C_2H_4- ), DS-536 (C_2H_4-) and DS-851 ($R_1=$ ) possessed significant diuretic activity with little effect on potassium excretion, but these were ineffective or weakly active in the lower doses such as 1 and 10 mg/kg *p.o.*

In the series of I-c, 3,4-dihydro derivatives of I-a, DS-381 ($R_1=C_6H_5$, $R_2=R_3=H$, $R_4=R_5=$ ) exhibited a potent diuretic activity, but enhanced potassium excretion more than DS-210. The effects of introduction of substituents at the 2-,5- and 8-position (R_1, R_4 and R_5, respectively) on the diuretic activity were almost parallel to those of the I-a series. The substituents other than H at R_2 of DS-381 reduced the diuretic activity. The substitution by $CH_2C_6H_5$ (DS-715), $COCH_3$ (DS-561), $COCH(CH_3)_2$ (DS-562) and COC_6H_5 (DS-563) caused less kaliuresis than DS-381, and the lower alkyl substituents such as CH_3 (DS-533), C_2H_5 (DS-643), $CH_2CH=CH_2$ (DS-682) and C_4H_9 (DS-736) rather caused antidiuresis. The substitution of R_3 by a saturated straight alkyl group such as CH_3 (DS-769), C_2H_5 (DS-770), C_4H_9 (DS-771) and C_6H_{13} (DS-808) resulted in loss of activity, but the substitution by a branched or unsaturated alkyl group such as $CH_2-CH=CH_2$ (DS-737), t-C_4H_9 (DS-853) and $C_2H_4CH(CH_3)_2$ (DS-807) gave active diuretics. The substitution of R_3 by a group containing α-hydroxy group such as CH_2OH (DS-804), $\underset{OH}{CHCH_3}$ (DS-805) and $\underset{OH}{CHC_6H_5}$ (DS-813) also caused significant diuresis. Thus, among the group I-c potent compounds were DS-644 ($R_3=CH_2-C_6H_5$), DS-856 ($R_3=CH_2-$ ) and DS-813, which were effective even at the dose of 1 mg/kg. Substitution of para position of $CH_2C_6H_5$ group in DS-644 by CH_3 (DS-773), Cl (DS-810) and OCH_3 (DS-811), and substitution of R_3 by  (DS-809),  (DS-857) and  (DS-814) slightly decreased the diuretic activity, while the substitution by $C_2H_4C_6H_5$ (DS-815), C_6H_5 (DS-772) and  (DS-816) resulted in loss of activity. DS-738, a 3,4-dibenzyl derivative of DS-381, showed far less diuretic activity than DS-381, and rather potassium sparing activity.

In the I-d series DS-702 ($R_1=R_2=H$), a tautomer of DS-381, possessed a similar diuretic activity to DS-381. Introduction of groups other than H to R_1 or to both R_1 and R_2 markedly reduced diuretic activity.

Of the two compounds in the III series, DS-515 ($R_1=$ ) was active at a small dose as 0.1 mg/kg *p.o.*, which proved to be one of the most potent compounds among 219 derivatives screened in the present work, while the piperidino derivative (DS-516) was less active.

In the IV series, DS-491 ($R_1=NHCH(CH_3)_2$) showed a weak diuretic activity. The

other five compounds including DS-490 (R_1= ), an analogue of DS-511, were inactive.

In the V-a series DS-511 (R_1=R_3=H, R_2=C_6H_5, R_4=R_5= ), which has the same substituents with those in DS-210 (I-a), emerged as one of the most potent compounds. Introduction of Cl (DS-938), OH (DS-984), OCH_3 (DS-962) and $OCH_2C_6H_5$ (DS-981) to the para position of the 7-phenyl group in DS-511 sustained the activity, where DS-984 was as potent as DS-511. On the other hand, substitution of *ortho*- or *meta*-position of the 7-phenyl group by *o*-OH (DS-986), *m*-OH (DS-985), *o*-$OCH_2C_6H_5$ (DS-983) and *m*-$OCH_2C_6H_5$ (DS-982) afforded compounds inactive at the dose of 30 mg/kg *p.o.* Introduction of substituent such as $CH_2C_6H_5$, Cl and  to the 5-position (R_3) markedly reduced the diuretic activity. The diuretic activity of compounds having various substituents of R_4 and R_5 decreased in the order: DS-511>DS-936 (R_4=R_5= )>DS-908 ()>DS-950 (), and DS-512 ($NHCH_2C_6H_5$) was inactive. Compounds in which one of the two morpholino groups in DS-511 was substituted by a hydroxyl group (DS-960 and DS-961) were fairly active at the dose of 30 mg/kg *p.o.*, in contrast to the case of the I-a series. Other potent compounds in the V-a series were DS-1043 (R_1=CH_3, R_2=C_6H_5, R_3=H, R_4=R_5= ), DS-1048 (R_1=OCH_3, R_2=C_6H_5, R_3=H, R_4=R_5= ) and DS-1058 (R_1=CH_3, R_2=C_6H_5, R_3=H, R_4=R_5= ), all of which possessed diuretic activity comparable to DS-511. Substitution of R_1 by ethyl or *n*-propyl group (DS-1054 and DS-1060) decreased the activity. Introduction of Cl or an alkoxy group at the 8-position (R_1) further made possible the synthesis of a series of derivatives bearing aliphatic substituents at R_2, where several of them (DS-599, DS-840, DS-868 and DS-843) were active, although less potent than DS-511.

In the V-b series, 5,6-dihydro derivatives of DS-511, DS-935 (R_1=R_2=H) was active, but less potent than DS-511. Substitution of R_1 by $C(CH_3)_3$ (DS-913) or $CH_2C_6H_5$ (DS-862) showed a potent diuretic activity, in which DS-862, an analogue of DS-644 in the I-c series, was active even at the dose of 0.1 mg/kg *p.o.* On the other hand, DS-863 (R_1=C_6H_5) was weakly active and DS-884 (R_1=$CH_2C_6H_5$, R_2=CH_3), a 5,5-disubstituted derivative, was inactive.

No diuretic compounds were found among the six phthalazine and four pyridazine derivatives in the series VI and VII.

In the series of VIII-a, DS-565 (R_1= ) was more potent than DS-663 (R_1=NH_2) which has already been reported as a diuretic compound by Graboyes, *et al.*:[4] DS-565 was active at the dose of 1 mg/kg *p.o.*, whereas DS-663 was inactive at the same dose. Modifications of the substituent R_1 resulted in a decrease in diuretic activity in the order: DS-565>DS-663, DS-751 ()>DS-750 ($N(C_2H_5)_2$), DS-664 (), DS-923 ()>DS-753 ($NHCH(CH_3)_2$), while DS-748 ($NHCH_2C_6H_5$), DS-749 $NH(CH_2)_2OH$), DS-752 () and DS-922 () were inactive.

The VIII-b series are 3,4-dihydro derivative of VIII-a. Effect of modifications of the substituent R_1 was similar to that of I-c. Namely, DS-858 ($CH_2C_6H_5$) was active at the dose

of 10 mg/kg *p.o.* DS-888 (C_4H_9) and DS-889 (C_6H_5) were inactive, and DS-886 (CH_3) rather antidiuretic.

In the remaining series, DS-910 (series X), DS-896 (series XII) and DS-1062 (series XIII), which were analogues of DS-511 and DS-565, possessed a diuretic activity. However, the other analogues, DS-566 (series IX) and DS-909 (series XI) showed no effect.

Based on the screening results at the doses of 0.1 and 1 mg/kg *p.o.*, the following conclusions on structure-activity relationships were obtained (Table V).

1) Structural features giving potent diuretic compounds are pyrimido[4,5-*d*]pyridazine (I-a) and its 3,4-dihydro derivatives (I-c), pyridazino[3,4-*d*]pyridazine (III), pyrido[3,4-*d*]-pyridazine (V-a) and its 5,6-dihydro derivatives (V-b), and pyrimido[4,5-*d*]pyrimidine (VIII-a).

2) Among the amino groups di-substituted on the pyridazine ring (I-a, III and V-a) and pyrimidine ring (VIII-a), morpholino group is the most preferable for a diuretic activity.

3) The introduction of a phenyl group to R_1 of pyrimido (I-a, b and VIII-a), pyridazino (III) and pyrido ring (V) seems desirable to potentiate the activity.

4) In the series V-a, the introduction of Cl, alkyl or alkoxy groups to the 8-position leads to potent diuretic compounds.

5) Substituents other than H at the 4-position of the I-a series markedly reduce the diuretic activity.

6) In the series of I-c and V-b, 4-benzyl and 5-benzyl derivatives respectively give the potent diuresis with less effect on potassium excretion.