Novel Uracil Derivatives: Newly Synthesized Centrally Acting Agents[1)]

Masahiro IMAIZUMI,*,[a] Fumitaka KANO,[b] and Shinji SAKATA[b]

Biology Laboratory[a] and Chemistry Laboratory No. 2,[b] Research & Development Div., Yamasa Shoyu Co., Ltd., 10–1, Araoi-cho 2-chome, Choshi, Chiba 288, Japan. *Received December 27, 1991*

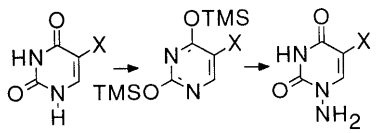
A series of 1-amino-5-substituted uracils and their 4-thio or 2,4-dithio substituted analogues were synthesized and assayed for anti-conflict activity in rats and anesthetic activity in mice. 1-Amino-5-halogenouracils 3b—e, 1-amino-4-thiouracil (9a), and 1-amino-5-halogeno-4-thiouracils 9c, d showed both anti-conflict and anesthetic activities. The most active compound was 1-amino-5-chloro-4-thiouracil (9d) which showed anxiolytic activity at 2 mg/kg of oral administration (*p.o.*) on a modified Geller–Seifter conflict schedule. Its minimum effective dose (MED) was lower than that of diazepam. The 50 percent effective dose (ED_{50}) for anesthetic activity in mice of the compound (9d) was 32.9 mg/kg, *p.o.*

Keywords anxiolytic; anesthetic; anti-conflict; uracil derivative; hypnotic; 1-amino-5-halogenouracil; 1-amino-5-halogeno-4-thiouracil

Inoue *et al.*[2)] reported that uridine showed a sleep promoting effect on nonanesthetized freely moving rats. Yamamoto *et al.*[3)] further reported that analogues of uridine and uracil induced loss of the righting reflex in mice. These facts suggest that a new drug which acts on the central nervous system (CNS) could be developed based on the chemical modification of uracil derivatives. We therefore synthesized a variety of uracil derivatives and evaluated their CNS actions. Loss of the righting reflex and the modified Geller–Seifter conflict test were used as indices of anesthetic and anxiolytic activity, respectively. Among the compounds tested, 1-amino-5-fluorouracil (**3b**) induced the loss of the righting reflex and showed an anti-conflict effect. To our knowledge, there has been no report on the anxiolytic activity of uracil derivatives, although it has been reported that uracil and related compounds depressed the spontaneous activity of mice,[4)] and that some adenine derivatives showed anxiolytic activity.[5)] Thus, we synthesized various 1-aminouracil derivatives and investigated their anesthetic and anxiolytic activity. We report here some structure–activity relationships of new 1-amino-5-substituted uracils.

Chemistry 1-Aminouracil (**3a**)[6)] and 5-substituted 1-aminouracils (**3b—j**) were prepared from uracil (**1a**) and 5-substituted uracils (**1b—j**), respectively, as outlined in Chart 1.

Silylation of **1a—j** with an excess of hexamethyldisilazane in the presence of $(NH_4)_2SO_4$ gave **2a—j**. Amination of trimethylsilyl (TMS) derivatives **2a** and **2b** with *O*-mesitylenesulfonylhydroxylamine (MSH)[7)] in anhydrous CH_2Cl_2 gave **3a** and **3b**[8)] in fair yield, respectively. No evidence of the formation of an N-3 isomer[9)] was observed by high performance liquid chromatography of the crude reaction mixture. Amination of compounds **2c—j** was achieved in the similar fashion.



1a—j **2a—j** (**2h** : X=OTMS) **3a—j**

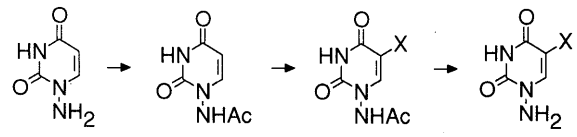
a : X=H **f** : X=CH_3
b : X=F **g** : X=2-(*E*)-bromovinyl
c : X=Br **h** : X=OH
d : X=Cl **i** : X=NO_2
e : X=I **j** : X=SO_2CH_3

Chart 1

The site of amination in compounds **2a—j** was confirmed to be at N-1 on the basis of ultraviolet (UV) absorption spectroscopic studies. It has been previously published that the UV spectra of N-amino purines[10)] and pyrimidines[11)] were similar to their corresponding N-methyl compounds. The λ_{max} observed in the UV spectrum of **3a—j**, except **3h** and **3i**, in alkaline aqueous solution showed no red shift relative to the λ_{max} observed in the UV spectrum of **1a—j**. Structure of **3h** and **3i** was easily assigned by comparison of their UV spectra with reported data for 1-methyl and 3-methyl derivatives of **1h**[12)] and **1i**.[13)]

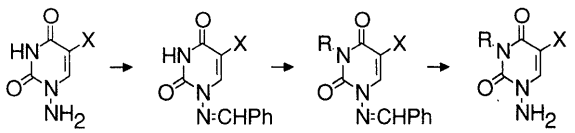
We searched for an alternative procedure for the synthesis of 1-amino-5-halogenouracils **3c—e** as outlined in Chart 2.

Bromination of **3a** with bromine–aq. AcONa or *N*-bromosuccinimide–AcOH failed to provide the desired 5-bromo derivative. However, reaction of the acetyl derivative (**4**) and a 1.1 mol eq of *N*-bromosuccinimide in AcOH at 80 °C gave **5c** in good yield. Deacetylation of **5c**



3a **4** **5c—e** **3c—e**

Chart 2



3c—d **6c—d** **7k—q** **8k—q**

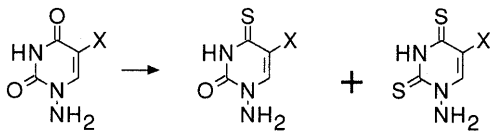
k : X=Br ; R=CH_3
l : X=Br ; R=$PhCH_2$
m : X=Br ; R=*p*-$ClPhCH_2$
n : X=Br ; R=*p*-$BrPhCH_2$
o : X=Br ; R=*p*-CH_3PhCH_2
p : X=Br ; R=2-picolyl
q : X=Cl ; R=*p*-$ClPhCH_2$

Chart 3

with aq. H_2SO_4 at 80 °C provided 1-amino-5-bromouracil (**3c**). In a similar manner, **3d** and **3e** were obtained from **4** in good yield.

Various 3-substituted derivatives (**8k—q**) of **3c**, **d** were prepared in three steps as outlined in Chart 3. Compound **3c** was converted to the benzylidene derivative (**6c**), which was reacted with methyl iodide to give **7k**. In a similar manner, N-3 benzyl derivatives (**7l—q**) were obtained from **6c**, **d** in satisfactory yield. Removal of the benzylidene group from **7k** was achieved by treatment with refluxing aq. H_2SO_4 to give the 3-methyl derivative (**8k**). Compounds **7l—q** were treated similarly to yield compounds **8l—q**.

Thio analogues (**9a**, **c**, **d** and **10a**, **c**, **d**) of **3a**, **c**, **d**, were prepared as outlined in Chart 4. Thiation of **3a** with phosphorus pentasulfide in refluxing dioxane[14] gave 1-amino-4-thiouracil (**9a**) and 1-amino-2,4-dithiouracil (**10a**) in 37 and 17% yields, respectively. The structure of **9a** was established by comparison of its UV spectrum with that of 4-thiouracil and 2-thiouracil,[15] and also by the fact that **9a** could be converted to 4-thiouracil by treatment with isoamyl nitrite in H_2O. Compounds **3c**, **d** were treated similarly to yield compounds **9c**, **d** and **10c**, **d**.



3a,c—d **9a,c—d** **10a,c—d**

Chart 4

Biological Results and Discussion Anesthetic and anti-conflict activities are shown in Table III. Several compounds showed both anesthetic and anti-conflict activities. No compounds showed only one of these activities.

The 5-halogeno compounds (**3b—e**) exhibited the activities, but those compounds (**3f**, **g**) with a hydrocarbon group at the position 5, and those (**3h—j**) with hydrophilic group such as OH, NO_2 and SO_2CH_3 showed neither activity. The halogeno groups and hydrophilic groups have electron-negative properties. Halogeno groups and hydrocarbon groups of **3f**, **g** are hydrophobic substituents, but hydro-carbon groups are electron-donating. From these results, it is thought that the 5-substituents having both electron-negative and hydrophobic properties are very important for anesthetic and anti-conflict activities. As for 4-thio compounds, the 5-non-substituted analogue (**9a**) showed relatively strong activity. The most active compound was 1-amino-5-chloro-4-thiouracil (**9d**), whose minimum effective dose (MED) (2 mg/kg) was more than twofold lower than that of diazepam (5 mg/kg), and **9d** showed a strong anesthetic activity comparable to pentobarbital. It

TABLE I. Physical and Analytical Data for 1-Amino-5-substituted Uracils (**3**) and 1-Amino-4-thiouracil Derivatives (**9**)

Compd.	mp (°C) (Recryst. solv.)	Formula	Analysis (%) Calcd (Found) C	H	N	UV ($\lambda_{max}^{H_2O}$) (ε)	UV ($\lambda_{max}^{0.1\,N\,NaOH}$) ($\varepsilon$)	pK_a	^{1}H-NMR (DMSO-d_6, J=Hz)
3a	247—248[a] (H_2O:EtOH=1:1)	$C_4H_5N_3O_2$	37.80 (37.76	3.97 3.83	33.06 33.02)	267 (8700)	266 (6100)	9.40	5.38 (1H, d, 7.8, H-5), 5.40 (2H, s, 1-NH_2), 7.60 (1H, d, 7.8, H-6), 11.30 (1H, s, NH)
3b	196—199[b] (H_2O)	$C_4H_4FN_3O_2$	33.11 (33.06	2.78 2.85	28.96 28.90)	273 (7700)	274 (5700)	7.53	5.52 (2H, s, 1-NH_2), 8.07 (1H, d, 6.8, H-6), 11.85 (1H, s, NH)
3c	214—215 (dec.) (H_2O)	$C_4H_4BrN_3O_2 \cdot H_2O$	21.45 (21.76	2.70 2.66	18.76 18.71)	285 (8000)	281 (5900)	7.91	5.55 (2H, s, 1-NH_2), 8.14 (1H, s, H-6), 11.84 (1H, s, NH)
3d	225—226 (H_2O)	$C_4H_4ClN_3O_2$	29.74 (29.55	2.50 2.49	26.01 25.73)	281 (7900)	279 (6000)	7.87	5.55 (2H, s, 1-NH_2), 8.09 (1H, s, H-6), 11.86 (1H, s, NH)
3e	195—196 (H_2O)	$C_4H_4IN_3O_2$	18.99 (18.90	1.59 1.61	16.61 16.41)	292 (7600)	284 (5400)	8.27	5.52 (2H, s, 1-NH_2), 8.09 (1H, s, H-6), 11.70 (1H, s, NH)
3f	227—228 (H_2O)	$C_5H_7N_3O_2$	42.55 (42.81	5.00 5.03	29.77 29.97)	272 (8600)	272 (6400)	9.92	1.73 (3H, s, 5-CH_3), 5.38 (2H, s, 1-NH_2), 7.51 (1H, s, H-6), 11.28 (1H, s, NH)
3g	>280 (H_2O)	$C_6H_6BrN_3O_2$	31.06 (31.09	2.61 2.60	18.11 17.90)	299, 252 (9500, 13700)	290, 258 (8600, 13600)	8.23	5.57 (2H, s, 1-NH_2), 6.85 (1H, d, 13.7, vinylic-H), 7.25 (1H, d, 13.7, vinylic-H), 7.97 (1H, s, H-6), 11.60 (1H, s, NH)
3h	281—282 (dec.) (H_2O)	$C_4H_5N_3O_3$	33.57 (33.38	3.52 3.49	29.36 29.22)	286 (7800)	307 (5800)	7.99	5.39 (2H, s, 1-NH_2), 7.07 (1H, s, H-6), 8.55 (1H, s, 5-OH), 11.46 (1H, s, NH)
3i	277—278 (dec.) (H_2O)	$C_4H_4N_4O_4$	27.92 (27.71	2.34 2.25	32.55 32.58)	312 (9100)	329 (10200)	6.59	5.90 (2H, s, 1-NH_2), 9.00 (1H, s, H-6), 12.15 (1H, s, NH)
3j	217—218 (H_2O)	$C_5H_7N_3O_4S$	29.27 (29.36	3.44 3.33	20.48 20.34)	270 (9900)	268 (6500)	7.08	3.17 (3H, s, SO_2CH_3), 5.76 (2H, s, 1-NH_2), 8.09 (1H, s, H-6), 12.05 (1H, s, NH)
9a	209—210 (dec.) (H_2O)	$C_4H_5N_3OS$	33.56 (33.62	3.52 3.54	29.35 29.68)			8.00	5.49 (2H, br s, 1-NH_2), 6.13 (1H, d, 7.3, H-5), 7.55 (1H, d, 7.3, H-6), 12.72 (1H, s, NH)
9c	201—203 (H_2O)	$C_4H_4BrN_3OS$	21.64 (21.61	1.82 1.87	18.92 18.99)			6.87	5.71 (2H, s, 1-NH_2), 8.26 (1H, s, H-6), 13.16 (1H, s, NH)
9d	218—219 (H_2O)	$C_4H_4ClN_3OS$	27.05 (26.81	2.27 2.23	23.66 23.67)			7.00	5.72 (2H, s, 1-NH_2), 8.23 (1H, s, H-6), 13.19 (1H, s, NH)

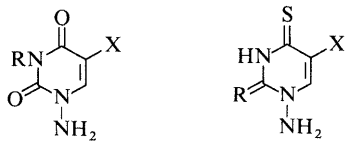
a) Lit.[6] mp 244—245 °C. *b*) Lit.[8] mp 205—207 °C.

TABLE II. Physical and Analytical Data for 1-Aminouracil Derivatives (**8**, **10**) and Intermediates (**4**—**7**)

Compd.	mp (°C) (Recryst. solv.)	Formula	Analysis (%) Calcd (Found) C	H	N	^{1}H-NMR (DMSO-d_6, J=Hz)
4	241—242 (H_2O)	$C_6H_7N_3O_3$	42.61 (42.54	4.17 4.05	24.84 25.10)	1.97 (3H, s, COCH$_3$), 5.55 (1H, dd, 2.4, 7.8, H-5), 7.56 (1H, d, 7.8, H-6), 10.88 (1H, s, NH), 11.50 (1H, s, NH)
5c	236—238 (dec.) (H_2O)	$C_6H_6BrN_3O_3$	29.05 (29.03	2.44 2.40	16.94 17.00)	1.97 (3H, s, COCH$_3$), 8.25 (1H, s, H-6), 11.02 (1H, s, NH), 12.06 (1H, s, NH)
5d	282—284 (dec.) (H_2O)	$C_6H_6ClN_3O_3$	35.40 (35.33	2.97 2.85	20.64 20.58)	1.97 (3H, s, COCH$_3$), 8.35 (1H, s, H-6), 11.03 (1H, s, NH), 12.10 (1H, s, NH)
5e	247—249 (dec.) (H_2O)	$C_6H_6IN_3O_3$	24.43 (24.43	2.05 2.05	14.24 14.03)	1.96 (3H, s, COCH$_3$), 8.16 (1H, s, H-6), 10.97 (1H, s, NH), 11.91 (1H, s, NH)
6c	227—229 (dec.) (H_2O)	$C_{11}H_8BrN_3O_2$	44.92 (44.89	2.74 2.69	14.29 14.42)	7.51—7.59 (3H, m, Ph), 7.82—7.85 (2H, m, Ph), 8.60 (1H, s, H-6), 8.99 (1H, s, N=CHPh), 12.04 (1H, s, NH)
6d	238—239 (dec.) (H_2O)	$C_{11}H_8ClN_3O_2$	52.92 (52.67	3.23 3.11	16.83 16.81)	7.51—7.60 (3H, m, Ph), 7.80—7.85 (2H, m, Ph), 8.56 (1H, s, H-6), 8.99 (1H, s, N=CHPh), 12.09 (1H, s, NH)
7k	153—154	$C_{12}H_{10}BrN_3O_2$	46.78 (46.64	3.27 3.19	13.64 13.53)	3.28 (3H, s, CH$_3$), 7.54—7.59 (3H, m, Ph), 7.84—7.87 (2H, m, Ph), 8.67 (1H, s, H-6), 8.99 (1H, s, N=CHPh)
7l	146—147	$C_{18}H_{14}BrN_3O_2$	56.27 (56.36	3.67 3.56	10.94 10.74)	5.09 (2H, s, CH$_2$Ph), 7.33—7.34 (5H, m, Ph), 7.53—7.58 (3H, m, Ph), 7.84—7.86 (2H, m, Ph), 8.74 (1H, s, H-6), 8.98 (1H, s, N=CHPh)
7m	157—158	$C_{18}H_{13}BrClN_3O_2$	51.64 (51.73	3.13 3.08	10.04 9.91)	5.07 (2H, s, CH$_2$Ph), 7.38 (4H, s, Ph), 7.53—7.59 (3H, m, Ph), 7.83—7.86 (2H, m, Ph), 8.74 (1H, s, H-6), 8.97 (1H, s, N=CHPh)
7n	145—146	$C_{18}H_{13}Br_2N_3O_2$	46.68 (46.77	2.83 2.84	9.07 8.95)	5.05 (2H, s, CH$_2$Ph), 7.31 (2H, d, 8.3, Ph), 7.51—7.57 (5H, m, Ph), 7.83—7.86 (2H, m, Ph), 8.75 (1H, s, H-6), 8.97 (1H, s, N=CHPh)
7o	128—130	$C_{19}H_{16}BrN_3O_2$	57.30 (57.28	4.05 4.02	10.55 10.35)	2.27 (3H, s, CH$_3$), 5.04 (2H, s, CH$_2$Ph), 7.13 (2H, d, 8.0, Ph), 7.24 (2H, d, 8.0, Ph), 7.53—7.59 (3H, m, Ph), 7.83—7.86 (2H, m, Ph), 8.72 (1H, s, H-6), 8.97 (1H, s, N=CHPh)
7p	166—167	$C_{17}H_{13}BrN_4O_2$	53.01 (52.78	3.40 3.35	14.54 14.34)	5.21 (2H, s, CH$_2$Ph), 7.25—7.29 (1H, m, Ph), 7.36 (1H, d, 8.3, Ph), 7.51—7.37 (3H, m, Ph), 7.74—7.78 (1H, m, Ph), 7.84—7.86 (2H, m, Ph), 8.45—8.47 (1H, m, Ph), 8.80 (1H, s, H-6), 9.00 (1H, s, N=CHPh)
7q	159—160	$C_{18}H_{13}Cl_2N_3O_2$	57.77 (57.63	3.50 3.41	11.23 10.97)	5.06 (2H, s, CH$_2$Ph), 7.38 (4H, s, Ph), 7.54—7.59 (3H, m, Ph), 7.84—7.86 (2H, m, Ph), 8.71 (1H, s, H-6), 8.98 (1H, s, N=CHPh)
8k	159—160 (H_2O)	$C_5H_6BrN_3O_2$	27.29 (27.19	2.75 2.62	19.10 19.14)	3.24 (3H, s, CH$_3$), 5.67 (2H, s, 1-NH$_2$), 8.22 (1H, s, H-6)
8l	146—147 (MeOH)	$C_{11}H_{10}BrN_3O_2$	44.62 (44.50	3.40 3.32	14.19 14.23)	5.04 (2H, s, CH$_2$Ph), 5.71 (2H, s, 1-NH$_2$), 7.25—7.34 (5H, m, Ph), 8.26 (1H, s, H-6)
8m	188—189 (MeOH)	$C_{11}H_9BrClN_3O_2$	39.97 (40.06	2.74 2.75	12.71 12.64)	5.02 (2H, s, CH$_2$Ph), 5.70 (2H, s, 1-NH$_2$), 7.31—7.39 (5H, m, Ph), 8.27 (1H, s, H-6)
8n	209—210 (dec.) (MeOH : AcOEt = 2 : 1)	$C_{11}H_9Br_2N_3O_2$	35.23 (35.48	2.42 2.34	11.20 11.01)	5.00 (2H, s, CH$_2$Ph), 5.70 (2H, s, 1-NH$_2$), 7.25—7.27 (2H, m, Ph), 7.50—7.52 (2H, m, Ph), 8.27 (1H, s, H-6)
8o	147—148 (MeOH)	$C_{12}H_{12}BrN_3O_2$	46.47 (46.47	3.90 3.86	13.55 13.51)	2.26 (3H, s, CH$_3$), 4.99 (2H, s, CH$_2$Ph), 5.69 (2H, s, 1-NH$_2$), 7.11 (2H, d, 7.8, Ph), 7.19 (2H, d, 7.8, Ph), 8.25 (1H, s, H-6)
8p	166—167 (MeOH)	$C_{10}H_9BrN_4O_2$	40.43 (40.47	3.05 2.89	18.86 18.95)	5.16 (2H, s, CH$_2$Ph), 5.74 (2H, s, 1-NH$_2$), 7.23—7.30 (2H, m, Ph), 7.72—7.77 (1H, m, Ph), 8.30 (1H, s, H-6), 8.42—8.45 (1H, m, Ph)
8q	189—190 (H_2O)	$C_{11}H_9Cl_2N_3O_2$	46.18 (46.32	3.17 3.16	14.69 14.86)	5.01 (2H, s, CH$_2$Ph), 5.70 (2H, s, 1-NH$_2$), 7.32 (2H, d, 8.3, Ph), 7.38 (2H, d, 8.3, Ph), 8.23 (1H, s, H-6)
10a	199—200 (dec.) (H_2O)	$C_4H_5N_3S_2$	30.17 (30.19	3.16 3.03	26.39 26.32)	6.32 (2H, s, 1-NH$_2$), 6.53 (1H, d, 7.3, H-5), 7.68 (1H, d, 7.3, H-6), 13.95 (1H, s, NH)
10c	192—196 (dec.) (H_2O)	$C_4H_4BrN_3S_2$	20.18 (20.07	1.69 1.60	17.65 17.70)	6.33 (2H, s, 1-NH$_2$), 8.45 (1H, s, H-6), 14.36 (1H, s, NH)
10d	191—195 (dec.) (H_2O)	$C_4H_4ClN_3S_2$	24.81 (25.14	2.08 2.06	21.70 21.33)	6.34 (2H, s, 1-NH$_2$), 8.40 (1H, s, H-6), 14.41 (1H, s, NH)

should be noted that all these biologically active compounds have lower pK_a values owing dissociation of a proton at position 3, as shown in Table I. In fact, a significant correlation (Fig. 1) was shown between pK_a values and logarithms of ED_{50} for anesthetic activitity (F=17.9333; df=4; p<0.014). The association between pK_a values and logarithms of MED for anti-conflict activity also had a tendency to correlate (F=7.4211; df=4; p<0.053). Compounds with OH, NO_2 and SO_2CH_3 at the position 5 also have low pK_a values (Table I) but are inactive biologically (Table III). This result may be rationalized by the poor permeability of these compounds (**3h**—**j**) to the blood brain barrier (BBB) due to the hydrophilic property of the substituents. On the other hand, 4-thio-substitution not only lowers pK_a value more remarkably (Table I) but also may make the compound more hydrophobic, and thus permeable to the BBB. It has already been reported that 4-thio-substitution of pyrimidine nucleoside derivatives increased lipophilicity.[16] Therefore, our synthesized 4-thio compounds are expected to have higher lipophilicity than the corresponding 4-oxo compounds. On the other hand, 3-substituted 5-halogeno compounds **8k**—**q** showed neither activity, although some of them, **8k**, **l**, **o**—**q**, exhibited potentiating activity of thiopental anesthesia (data not

TABLE III. Anesthetic and Anti-conflict Activities of 1-Amino-5-halogenouracil Derivatives



3a—8q **9a—10c**

Compd.	R	X	A: ED_{50} (mg/kg)[a] (anesthetic)			B: MED (mg/kg)[a] (anti-conflict)		A/B[b]	
3a	H	H	>500	i.p.	($n=7$)	>100	*p.o.*		
3b	H	F	<100	*p.o.*[c]		20	*p.o.*	<5	
3c	H	Br	86.4	*p.o.*		10	*p.o.*	8.6	
3d	H	Cl	50.5	*p.o.*		20	*p.o.*	2.5	
3e	H	I	108.1	*p.o.*		>50	*p.o.*[d]		
3f	H	CH_3	>100	i.p.	($n=10$)	NT			
3g	H	2-(*E*)-Bromovinyl	>200	i.p.	($n=5$)	>20	*p.o.*		
3h	H	OH	>400	i.p.	($n=6$)	>50	*p.o.*		
3i	H	NO_2	>100	i.p.	($n=7$)	>20	*p.o.*		
3j	H	SO_2CH_3	>100	i.p.	($n=8$)	>50	*p.o.*		
8k	CH_3	Br	>100	i.p.	($n=10$)	>50	*p.o.*		
8l	$PhCH_2$	Br	>400	*p.o.*	($n=10$)	>50	*p.o.*		
8m	*p*-$ClPhCH_2$	Br	>200	*p.o.*	($n=10$)	>50	*p.o.*		
8n	*p*-$BrPhCH_2$	Br	>200	*p.o.*	($n=6$)	>50	*p.o.*		
8o	*p*-CH_3PhCH_2	Br	>200	*p.o.*	($n=10$)	NT			
8p	2-Picolyl	Br	>200	*p.o.*	($n=6$)	>50	*p.o.*		
8q	*p*-$ClPhCH_2$	Cl	>400	*p.o.*	($n=8$)	>50	*p.o.*		
9a	O	H	118.9	*p.o.*		20	*p.o.*	5.9	
9c	O	Br	34.3	*p.o.*		5	*p.o.*	6.9	
9d	O	Cl	32.9	*p.o.*		2	*p.o.*	16.5	
10a	S	H	>120	i.p.	($n=5$)	>30	*p.o.*		
10c	S	Br	>40	i.p.	($n=5$)	>20	*p.o.*		
Diazepam			46.3	*p.o.*		5	*p.o.*	9.3	
Pentobarbital			28.6	i.p.		5	i.p.	5.7	i.p.

a) See text for methods. *b*) The value of A was divided by the value of B. *c*) Loss of righting reflex was induced in all mice by *p.o.* injection of this compound at a dose of 100 mg/kg ($n=3$). *d*) Anti-conflict activity was shown by i.p. injection of this compound at a dose of 50 mg/kg. NT: not tested.

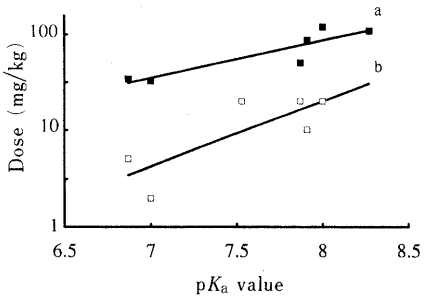


Fig. 1. Linear Correlation between pK_a Value and Effective Dose

Each point represents the effective dose (logarithmic scale) and the pK_a value of active compounds. Two lines represent regression lines. a: ED_{50} for anesthetic activity, $r=0.9042$, b: MED for anti-conflict activity, $r=0.8061$.

shown).

Intraperitoneal (i.p.) administration of 5-bromouracil (**1c**) and oral administration of 1-acetylamino derivative (**5c**) did not show an anti-conflict activity at a dose of 50 mg/kg. 2,4-Dithio compounds (**10a**, **c**) caused neither anesthetic nor anti-conflict activity (Table III).

Benzodiazepines like diazepam have both hypnotic and anxiloytic activity,[17] and pentobarbital, a popular anesthetic barbiturate, also shows anxiolytic activity at a dose lower than that at which anesthesia is induced.[18] Hypnotic or anesthetic activity and anxiolytic activity are usually coexistent, although the relationship between an efficacy of benzodiazepine receptor agonists and their selectivity of pharmacological activities has been argued.[17b,19] Recently anxio-selective anxiolytic compounds which have an affinity for the 5-hydroxytryptamine receptor have been developed.[20] It is interesting that there was a variety of selectivities as to anxiolytic and anesthetic activities in the compounds reported here (see A/B values in Table III). Also, the mechanism(s) of our compounds are interesting. Many anxiolytic non-benzodiazepines which have an affinity for the benzodiazepine receptor were reported.[21] However, our compound, 1-amino-5-bromouracil, did not show an affinity for this receptor (unpublished observation). Further investigations are required for elucidation of the structure–selectivity relationship and mechanism(s).

In conclusion, for anesthetic and anxiolytic activities of our newly synthesized compounds, position 1 and position 3 are important. The 2-oxo group is also involved in the activities because 2,4-dithio compounds showed neither activity. As some of them showed anti-conflict activity comparable to diazepam, 1-amino uracil analogues are expected to be novel, useful, centrally acting agents, especially anxiolytic agents.

Experimental

General Procedure Melting points were determined with a Yamato capillary melting point apparatus MP-21 and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were obtained with a JEOL GSX-400 spectrometer using tetramethylsilane as an internal standard. Electron impact mass spectra (EI-MS) were obtained with a JMS D-300

mass spectrometer. UV absorption spectra were measured on a Shimadzu UV-160A spectrophotometer. pK_a values were determined as previously described[22] by spectrophotometric methods at 20 °C. Column chromatography was performed on Merck Silica gel 60. 5-(*E*)-(2-Bromovinyl)-uracil,[23] 5-methyl-sulfonyluracil[24] and MSH[7] were prepared by a literature procedure.

Silylation The silylations of uracil derivatives were performed according to standard methods. A mixture of uracils (0.1 mol) and ammonium sulfate (340 mg) in hexamethyldisilazane (42 ml) was heated under reflux for 2—24 h. Distillation of the residue after removal of the solvent yielded 2,4-bis(trimethylsilyloxy)-pyrimidines as a colorless oil.

1-Aminouracil (3a) To a solution of freshly prepared **2a** (8.13 g, 31.7 mmol) in CH_2Cl_2 (50 ml) cooled in an ice bath under argon was added 8.12 g (37.7 mmol) of MSH. The suspension was allowed to gradually warm to room temperature and then was stirred for 3 h. The solvent was evaporated *in vacuo* and the residue was partitioned between water (500 ml) and $CHCl_3$ (100 ml). The aqueous layer was adjusted to pH 6.0 by the addition of ion exchange resin (Amberlite IRA-93, OH form). The filtrate was evaporated *in vacuo* and the residue was then recrystallized from 50% EtOH to afford 3.24 g (80%) of **3a**: EI-MS *m/z*: 127 (M^+).

1-Amino-5-bromouracil (3c) Method A: To a solution of freshly prepared **2c** (8.80 g, 26.2 mmol) in CH_2Cl_2 (50 ml) cooled in an ice bath under argon was added MSH (6.71 g, 31.2 mmol). The suspension was allowed to gradually warm to room temperature and then was stirred for 4 h. The solvent was evaporated *in vacuo*. The residue was dissolved in water (200 ml). The pH of the solution was adjusted to 6.0 by the dropwise addition of 2 N NaOH. The solvent was concentrated to a small volume and kept at 4 °C overnight. The precipitate was collected on a filter, washed with water, and recrystallized from water after decolorization with activated charcoal to afford 3.02 g (56%) of **3c**: EI-MS *m/z*: 207,205 (M^+).

Method B: A suspension of compound **5c** (39.7 g, 160 mmol) in 1.2 N H_2SO_4 (800 ml) was brought to 85 °C for 5 h. The reaction mixture was then cooled at 4 °C overnight and the precipitated solid was collected. This solid was suspended in water (300 ml) and the pH was adjusted to 11 by the dropwise addition of conc. NH_4OH. The resultant clear solution was concentrated to a small volume and then allowed to stand at 4 °C for overnight. The precipitate was collected on a filter, washed with water, and recrystallized from water (600 ml) to afford 29.3 g (89%) of **3c**.

1-Acetylamino-5-bromouracil (5c) A suspension of compound **3a** (80.0 g, 629 mmol) in Ac_2O (420 ml) was brought to reflux temperature for 1 h. The solution was then cooled to room temperature and evaporated to dryness *in vacuo*. The residue was suspended in water (400 ml) and the pH was adjusted to 11 by the dropwise addition of conc. NH_4OH. The solvent was concentrated to a small volume and then allowed to stand at 4 °C overnight. The precipitate was collected on a filter, washed with water, and recrystallized from water to afford 64.8 g (61%) of **4**.

N-Bromosuccinimide (33.6 g, 188 mmol) was added to a solution of compound **4** (29.0 g, 171 mmol) in AcOH (270 ml) at 90 °C in small portions, and then the mixture was stirred for 2 h. The solvent was evaporated to dryness *in vacuo*. The residue was suspended in water (100 ml) and kept at 4 °C overnight. The precipitate was collected on a filter, washed with water, and then dried *in vacuo* to yield 39.7 g (94%) of **5c**.

1-Benzylideneamino-5-bromouracil (6c) A mixture of **3c** (2.06 g, 10.0 mmol), benzaldehyde (1.1 ml, 11 mmol), and $TsOH \cdot H_2O$ (190 mg, 1 mmol) in dimethylformamide (DMF, 20 ml) was stirred at room temperature for 1 h. The reaction mixture was quenched by the addition of Et_3N (1 ml) and the solvent was removed *in vacuo*. The solid was suspended in water (20 ml), collected on a filter, washed with EtOH (5 ml), and then dried *in vacuo* to yield 2.75 g (94%) of **6c**.

1-Amino-5-bromo-3-methyluracil (8k) A mixture of **6c** (2.00 g, 6.80 mmol), K_2CO_3 (1.83 g, 13.2 mmol), and methyl iodide (0.47 ml, 7.55 mmol) in DMF (20 ml) was stirred at room temperature for 30 min. The reaction mixture was diluted with AcOEt (20 ml), filtered through Celite, and then the solvent was evaporated to dryness *in vacuo*. The residue was suspended in water (20 ml) and collected on a filter, washed thoroughly with water, and then dried *in vacuo* to yield 2.00 g (95%) of **7k**. A suspension of compound **7k** (1.80 g, 5.84 mmol) in 1.2 N H_2SO_4 (60 ml) was brought to reflux temperature under a Dean–Stark trap for 1 h. The reaction mixture was then cooled to room temperature and the pH of the solution was adjusted to 8 by the dropwise addition of conc. NH_4OH. The solvent was concentrated to a small volume and kept at 4 °C overnight. The precipitated solid was collected and recrystallized from water to afford 1.0 g (78%) of **8k**: EI-MS *m/z*: 221, 219 (M^+).

1-Amino-4-thiouracil (9a) and 1-Amino-2,4-dithiouracil (10a) A suspension of **3a** (1.20 g, 9.44 mmol) and P_2S_5 (5.65 g, 12.7 mmol) in dioxane (120 ml) was brought to reflux temperature and vigorously stirred for 1 h. The solution was then cooled to room temperature and the pH of the solution was adjusted to 11 by the addition of conc. NH_4OH. The precipitated orange gum was collected by filtration and the filtrate was evaporated to dryness *in vacuo*. The yellow solid was placed on the top of the column prepared with silica gel (60 g). Elution of the column with hexane–AcOEt (1 : 1) yielded 440 mg (33%) of **9a** and 140 mg (9%) of **10a**. **9a**: EI-MS *m/z*: 143 (M^+).

Pharmacological Methods Each compound was dissolved or suspended in distilled water containing 0.5% carboxymethyl cellulose (CMC-DW) and physiological saline containing 0.5% carboxymethyl cellulose (CMC-S) for *p.o.* and i.p. administration, respectively. The solution or suspension was administered orally on assay of anti-conflict activity except for pentobarbital. It was administered i.p. and *p.o.* for anesthetic activity.

Anesthetic Activity Male ICR mice (30—40 g) were used for the determination of ED_{50}. Mice were administered at several doses of compounds and observed for loss of the righting reflex. When mice were observed for loss of the righting reflex continuously for more than 1 min, it was considered that a positive reaction was observed. Positive rates were measured with 8 to 15 mice per one dose, and dose response curves were made from positive rates of 5 different doses. The ED_{50} value, which is the dose inducing the loss of the righting reflex in 50% of the mice, was determined by the dose response curve of positive rates. Pentobarbital and compounds **3a**, **f**—**j**, **8k**, and **10a**, **c** were applied by i.p. injection, and the other compounds were applied *p.o.*

Anti-conflict Activity Anti-conflict activity was tested by the method which was based on that of Geller and Seifter.[18b] Male Wistar rats were maintained on a 12/12-h light-dark cycle with light on at 6 a.m. Food was deprived during the dark period and water was supplied all the time except during the experimental period. Afterwards, one week food-deprived schedule rats (7 weeks old) were trained with lever pressing. The animals were trained until they acquired stable rates of reseponse to $FR=10$ (fixed ratio = 10; every tenth response is reinforced with one food pellet.). After that the animals were trained on a schedule comprising two components, one of which is a safety component and the other an alarm component. At 7 min-safety components, animals were reinforced every tenth response without electric foot shocks. At 3 min-alarm components, the animals were signaled by a buzzer and a light which were attached above a lever, and reinforced on $FR=10$ with a 0.5 s electric foot shock. Two components were repeated alternately and 1 session was continued for 1 h. The animals were trained until the number of lever pressing was suppressed less than 19 counts at each alarm component. These animals were used repeatedly for tests at intervals of more than 6 d.

The animals were administered with CMC-DW or drugs and immediately put into an experimental box (1 lever skinner box) and the number of lever pressing was counted. The same animals were used for 3 consecutive days. At day 1 and day 3 the animals were administered with CMC-DW, and with a drug at day 2. Measurement values at days 1 and 3 were used as pre- and post-values, respectively. After we confirmed that post-values returned to pre-values, we averaged the pre- and post-values and used the average as the control values. Six safety components and 6 alarm components at 1 session were divided over the first half (30 min) and the second half (30 min), and the control values at each component were summed up at the first half and at the second half, respectively. Measurement values at day 2, which refer to treatment values, were summed up in the same manner as employed for the control values. At each component and at each half, the statistical significance of difference between the summation of treatment values and the summation of control values was ascertained by the Wilcoxon's signed rank test (2-tailed).[25] The following doses of compounds were administered; 1, 2, 5, 10, 20, 50 mg/kg. A minimum dose, where the summations of treatment values increased significantly from control at one summation or both summations of alarm components, was presented as the MED. CMC-S was used for an assay of pentobarbital instead of CMC-DW.

References and Notes

1) This work was presented in part at the 110th Annual Meeting of Pharmaceutical Society of Japan, Sapporo, Aug. 1989 and the 111th Annual Meeting of Pharmaceutical Society of Japan, Tokyo, March 1990.
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