Novel 4-Phenoxy-2-(1-piperazinyl)quinazolines as Potent Anticonvulsive and Antihypoxic Agents

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A series of 4-phenoxy-2-(1-piperazinyl)quinazolines was synthesized and examined for anticonvulsive and antihypoxic activities. Many of the compounds exhibited potent anticonvulsive activity comparable to that of carbamazepine or phenytoin. Among them, 4-phenoxy-2-(4-propyl-1-piperazinyl)quinazoline (5w) was selected as the most promising candidate antiepileptic drug with few side effects. It seemed that potent anticonvulsive activity was a prerequisite for potent antihypoxic activity.

Keywords quinazoline; anticonvulsive agent; side effect; structure–activity relationship; antihypoxic effect

Quinazoline derivatives are known to be biologically versatile compounds. There are several reports on the biological activities of 2-(1-piperazinyl)quinazolines[1-8]; some of them showed central nervous system (CNS) activities. Compound **1b** showed a weak CNS stimulant activity.[1] Compounds **2** and **3** showed significant CNS depressant and stimulant activities, respectively.[2,3] In the previous paper, we reported the synthesis and antihistaminic activity of **4**,[4] which did not exhibit apparent CNS activity.[9] It seemed that the benzene ring plays an important role in the expression of potent CNS activities. As compound **5a**[5] has a benzene ring and is easily prepared from **1b**, we evaluated the CNS activities of **5a**, and found that it exhibited a potent anticonvulsive activity against maximal electroshock-induced seizure (anti-MES), comparable to that of current antiepileptic agents such as carbamazepine and phenytoin. Compound **5a** was interesting because of the potent anti-MES activity and the unique structure which differs from those of currently used antiepileptic agents.

About 80% of all epileptic patients have benefited from antiepileptic drugs,[10] however, a number of side effects such as ataxia and drowsiness have limited their clinical use.[11] The residual 20% are classified as refractory epileptic patients who do not respond to the current drugs. New drugs that are more effective and/or have fewer side effects are therefore required. We attempted to find such antiepileptic agents among the derivatives of **5a**. This paper deals with the syntheses and biological evaluations (anticonvulsive activity, neurotoxicity, hypnotic effect and acute toxicity) of various 4-phenoxy-2-(1-piperazinyl)quinazolines.

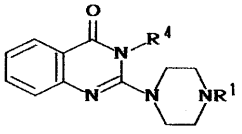
Recently, some antiepileptic agents including phenytoin were revealed to exhibit a significant protective effect against cerebral hypoxia.[12] Thus, the antihypoxic activity of these synthesized compounds was also examined.

Chemistry

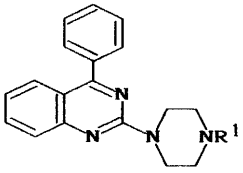
The pathways utilized to obtain compounds for biological evaluations are summarized in Chart 2. Compounds **5a**—**p** and **5u**—**y** were prepared from 4-chloroquinazolines (**6b**—**i**) and substituted phenols in the presence of sodium hydride (NaH) in *N*,*N*-dimethylformamide (DMF). The nitro derivatives (**5o** and **5p**) were reduced to amino derivatives (**5q** and **5r**), then converted to chloro derivatives (**5s** and **5t**) by means of the Sandmeyer reaction. The physicochemical properties of compounds **5** are summarized in Table I.

Starting materials were prepared as shown in Chart 3. The 2-(1-piperazinyl)-4(3*H*)-quinazolinones (**1a**—**i**) were prepared by three methods. In method A, 2-ethylthio-4(3*H*)-quinazolinone (**7a**)[1] was treated with piperazines (R^1=H, CH_3, $CH_2CH=CH_2$ and CH_2Ph) to give the corresponding compounds (**1a**,[6] **1b**, **1e** and **1f**). Compound **1a** was alkylated with alkyl iodide to give the ethyl and propyl derivatives (**1c**—**d**). In method B, compounds **1h** and **1i** were prepared from 2-chloro-4(3*H*)-quinazolinones **7b** and **7c**,[7a] respectively. In method C, **1b** was nitrated with a mixture of nitric acid and sulfuric acid to give a mono nitro derivative, **1g**. Because **1g** was hardly soluble in organic solvents, the structure was determined after chlorination to **6g** (Table II).

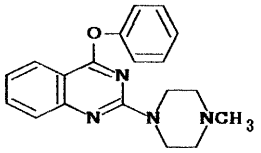
The reaction of 2-(1-piperazinyl)-4(3*H*)-quinazolinones (**1**) with phosphorus oxychloride ($POCl_3$) gave the 4-chloro derivatives (**6**) (Table III). The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **6g** revealed that the nitration of **1g** occurred at the 6- or 7-position of the quinazoline ring. The 7-nitro analogue (**6h**) gave a different ^{1}H-NMR spectrum from that of **6g**, so the structure of **1g** was confirmed to be 2-(4-methyl-1-piperazinyl)-6-nitro-



1b: $R^1=CH_3$, $R^4=H$
2: $R^1=CH_3$, $R^4=Ph$
4: $R^1=CH_3$, $R^4=CH_2CH_2OCH_2CH_3$



3: $R^1=H$, CH_3



5a

Chart 1

TABLE I. 4-Phenoxy-2-(1-piperazinyl)quinazolines (**5**)

Compound	R^1	R^2	R^3	mp (°C) (Recryst. solvent)[a]	Formula	Yield[c] (%)	Analysis (%)[d] Calcd (Found)		
							C	H	N
5a	CH_3	H	H	100.0—104.0 (A)	$C_{19}H_{20}N_4O$	70	71.23 (71.44)	6.29 6.34	17.49 17.24)
5b	CH_3	2-Cl	H	108.5—110.0 (A)	$C_{19}H_{19}ClN_4O$	74	64.31 (64.57)	5.40 5.40	15.79 15.77)
5c	CH_3	3-Cl	H	106.5—108.0 (A)	$C_{19}H_{19}ClN_4O$	71	64.31 (64.38)	5.40 5.33	15.79 15.81)
5d	CH_3	4-Cl	H	111.0—113.0 (A)	$C_{19}H_{19}ClN_4O$	96	64.31 (64.25)	5.40 5.33	15.79 15.63)
5e	CH_3	4-F	H	110.5—112.5 (A)	$C_{19}H_{19}FN_4O$	65	67.44 (67.61)	5.66 5.57	16.56 16.61)
5f[b]	CH_3	3-OCH_3	H	192.5—196.0 (B)	$C_{20}H_{22}N_4O_2 \cdot C_4H_4O_4$	60	61.79 (61.67)	5.62 5.61	12.01 12.04)
5g	CH_3	4-OCH_3	H	99.0—100.0 (C)	$C_{20}H_{22}N_4O_2$	55	68.55 (68.65)	6.33 6.24	15.99 15.98)
5h	CH_3	3-CF_3	H	104.0—106.5 (A)	$C_{20}H_{19}F_3N_4O$	65	61.85 (61.78)	4.93 4.93	14.43 14.50)
5i[b]	CH_3	4-CH_3	H	193.0—194.5 (D)	$C_{20}H_{22}N_4O \cdot C_4H_4O_4$	60	63.99 (63.96)	5.82 5.79	12.44 12.38)
5j	CH_3	4-$COCH_3$	H	128.0—129.5 (C)	$C_{21}H_{22}N_4O_2$	58	69.59 (69.55)	6.12 6.03	15.46 15.47)
5k	CH_3	4-$NHCOCH_3$	H	200.0—202.0 (C)	$C_{21}H_{23}N_5O_2$	61	66.83 (66.83)	6.14 6.17	18.55 18.57)
5l	CH_3	2,4-Cl_2	H	129.0—130.5 (A)	$C_{19}H_{18}Cl_2N_4O$	32	58.62 (58.80)	4.66 4.45	14.39 14.35)
5m	CH_3	3,4-Cl_2	H	149.5—151.5 (A)	$C_{19}H_{18}Cl_2N_4O$	58	58.62 (58.47)	4.66 4.55	14.39 14.23)
5n	CH_3	3-CH_3, 4-Cl	H	107.0—109.0 (A)	$C_{20}H_{21}ClN_4O$	74	65.12 (65.32)	5.74 5.70	15.19 15.12)
5o	CH_3	H	6-NO_2	157.5—159.0 (B)	$C_{19}H_{19}N_5O_3$	57	62.46 (62.25)	5.24 5.16	19.17 19.10)
5p	CH_3	H	7-NO_2	142.0—144.0 (B)	$C_{19}H_{19}N_5O_3$	77	62.46 (62.66)	5.24 5.26	19.17 19.05)
5q	CH_3	H	6-NH_2	87.5— 90.0 (F)	$C_{19}H_{21}N_5O$	92	68.04 (67.78)	6.31 6.30	20.88 21.08)
5r	CH_3	H	7-NH_2	165.0—167.5 (F)	$C_{19}H_{21}N_5O$	72	68.04 (67.95)	6.31 6.36	20.88 20.99)
5s	CH_3	H	6-Cl	108.0—110.0 (A)	$C_{19}H_{18}ClN_4O$	57	64.31 (64.20)	5.40 5.31	15.79 15.75)
5t	CH_3	H	7-Cl	128.5—130.0 (A)	$C_{19}H_{18}ClN_4O$	42	64.31 (64.18)	5.40 5.39	15.79 15.78)
5u	CH_3	H	6,7-$(OCH_3)_2$	171.0—172.5 (C)	$C_{21}H_{24}N_4O_3$	83	66.30 (66.45)	6.36 6.55	14.73 14.47)
5v[b]	CH_2CH_3	H	H	165.5—171.0 (D)	$C_{20}H_{22}N_4O \cdot C_4H_4O_4 \cdot 0.5H_2O$	84	62.73 (62.53)	5.92 5.97	12.19 12.21)
5w[b]	$(CH_2)_2CH_3$	H	H	165.5—167.5 (B)	$C_{21}H_{24}N_4O \cdot C_4H_4O_4$	96	64.64 (64.74)	6.08 6.05	12.06 12.08)
5x[b]	$CH_2CH=CH_2$	H	H	178.0—179.5 (D)	$C_{21}H_{22}N_4O \cdot C_4H_4O_4$	92	64.92 (64.89)	5.67 5.63	12.11 12.30)
5y[b]	CH_2Ph	H	H	187.5—189.5 (D)	$C_{25}H_{24}N_4O \cdot C_4H_4O_4$	61	67.96 (67.94)	5.51 5.50	10.93 10.90)

a) A, hexane; B, EtOH; C, hexane–AcOEt; D, EtOH–AcOEt; E, EtOH–MtOH; F, hexane–$CHCl_3$. *b*) Oily compounds were purified as hydrogen fumarate salts. *c*) Yield of free base. *d*) Analytical results are within ±0.4% of the theoretical values in C, H, N analysis.

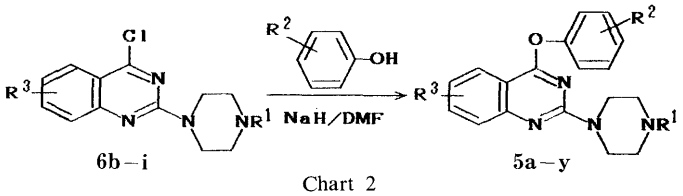


Chart 2

4(3*H*)-quinazolinone.

Biological Results and Discussion

Anti-MES activity was evaluated according to Woodbury and Davenport's method.[13] In order to evaluate the side effects, the neurotoxicity and hypnotic effect were examined. Neurotoxicity was determined by means of the rotarod test,[14] and the hypnotic effect was determined by measuring the potentiating effect on hexobarbital-induced sleep.[15] Antihypoxic activity was determined by means of the gasping test.[16] The results of these evaluations are summarized in Table IV.

Anti-MES Activity First, we will discuss the activity of substituted phenoxy derivatives (**5a—n**). Most of the compounds exhibited significant anti-MES activity, and the

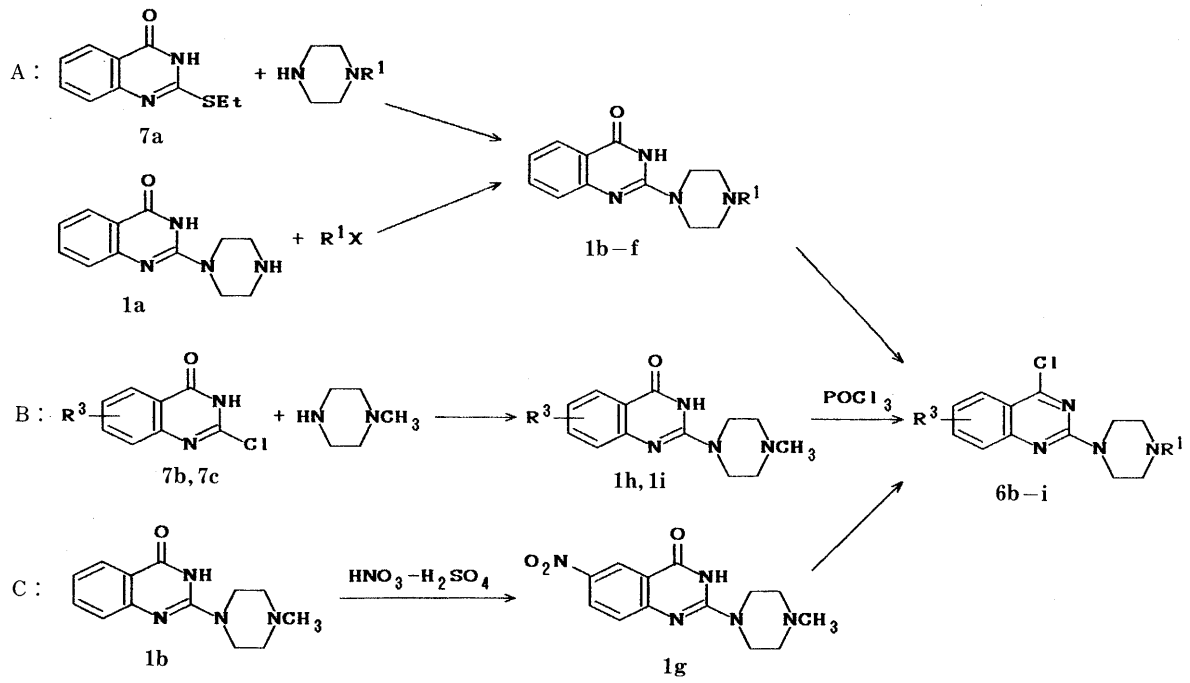


Chart 3

TABLE II. 2-(1-Piperazinyl)-4(3*H*)-quinazolinones (**1**)

Compound	R^1	R^3	mp (°C) (Recryst. solvent)[a]	Formula	Yield[b] (%)	Analysis (%)[c] Calcd (Found)		
						C	H	N
1a	H	H	200.5—211.0 (A)	$C_{12}H_{14}N_4O$	84	62.59 (62.74)	6.13 6.12	24.33 24.09)
1b	CH_3	H	226.5—229.0 (A)	$C_{13}H_{16}N_4O$	81	63.92 (63.95)	6.60 6.60	22.93 22.95)
1c	CH_2CH_3	H	225.5—226.5 (B)	$C_{14}H_{18}N_4O$	47	65.09 (65.26)	7.02 7.12	21.69 21.68)
1d	$(CH_2)_2CH_3$	H	224.0—225.5 (B)	$C_{15}H_{20}N_4O$	50	66.15 (65.90)	7.40 7.34	20.57 20.45)
1e	$CH_2CH=CH_2$	H	196.0—197.5 (A)	$C_{15}H_{18}N_4O$	94	66.65 (66.75)	6.71 6.73	20.73 20.51)
1f	CH_2Ph	H	218.0—219.5 (A)	$C_{19}H_{20}N_4O$	87	71.23 (71.43)	6.29 6.26	17.49 17.41)
1g	CH_3	6-NO_2	270 (dec.)	$C_{13}H_{15}N_5O_3$	85	53.97 (53.78)	5.23 5.37	24.21 24.04)
1h	CH_3	7-NO_2	245 (dec.)	$C_{13}H_{15}N_5O_3 \cdot HCl \cdot H_2O$	83	45.42 (45.04)	5.28 5.10	20.37 20.20)
1i	CH_3	6,7-$(OCH_3)_2$	252.0—253.0 (C)	$C_{15}H_{20}N_4O_3$	85	59.20 (59.17)	6.63 6.60	18.41 18.31)

a) A, AcOEt; B, EtOH–AcOEt; C, $CHCl_3$–AcOEt. *b*) Yields are based on the preceeding isolated intermediates.

potencies were equal to or slightly lower than that of carbamazepine and phenytoin. The potencies of the 2-chloro derivative (**5b**) and 2,4-dichloro derivative (**51**) were weak, so the introduction of substituents at the *ortho* position of the phenoxy moiety seemed to decrease the anti-MES activity.

In order to investigate the effects of substituents on the phenoxy moiety, the quantitative structure–activity relationships (QSAR) were examined. From the analyses using the usual extrathermodynamic parameters,[17,18] Eq. 1 was obtained as the best equation.

$$\log 1/ED_{50} = -0.218(\pm 0.098)(\sum \pi)^2 + 0.126(\pm 0.093)\sum \pi + 4.122(\pm 0.071) \quad (1)$$

$$n=13, \quad r=0.843, \quad s=0.081, \quad F=12.26$$

In Eq. 1, the number in parentheses is the 95% confidence interval, n is the number of data points used in deriving the equation, r is the correlation coefficient, s is the standard deviation and F is the F-ratio between the variances of calculated and observed activities (Table V). The result idicated that anti-MES activity is parabolically related to $\sum \pi$, the summation of the values of hydrophobic parameters of the substituents on the phenoxy moiety. Addition of neither the electronic parameter (σ) nor the steric parameter (E_s) gave a significantly statistically superior equation. From these results, it seemed that the role of the benzene ring of compounds **5** is to increase the lipophilicity

TABLE III. 4-Chloro-2-(1-piperazinyl)quinazolines (**6**)

Compound	R^1	R^3	mp (°C) (Recryst. solvent)[a]	Formula	Yield[c] (%)	Analysis (%) Calcd (Found)		
						C	H	N
6b	CH_3	H	83.5— 85.5 (A)	$C_{13}H_{15}ClN_4$	88	59.43 (59.69	5.75 5.84	21.32 21.13)
6c	CH_2CH_3	H	44.0— 45.5 (A)	$C_{14}H_{17}ClN_4$	92	60.76 (60.90	6.19 6.05	20.24 20.29)
6d[b]	$(CH_2)_2CH_3$	H	145.5 (dec.) (B)	$C_{15}H_{19}ClN_4 \cdot C_4H_4O_4$	94	56.09 (55.98	5.70 5.67	13.77 13.74)
6e	$CH_2CH=CH_2$	H	67.0— 68.0 (A)	$C_{15}H_{17}ClN_4$	71	62.39 (62.50	5.93 5.96	19.40 19.49)
6f	CH_2Ph	H	300 (dec.) (B)	$C_{19}H_{19}ClN_4$[d] $\cdot HCl$	99	60.81 (60.45	5.37 5.44	14.93 14.80)
6g	CH_3	6-NO_2	157.0—159.0 (C)	$C_{13}H_{14}ClN_5O_2$	79	50.74 (50.74	4.59 4.71	22.76 22.74)
6h	CH_3	7-NO_2	114.5—117.0 (C)	$C_{13}H_{14}ClN_5O_2$	60	50.74 (50.91	4.59 4.64	22.76 22.73)
6i	CH_3	6,7-$(OCH_3)_2$	169.5—172.0 (C)	$C_{15}H_{19}ClN_4O_2$	95	55.81 (55.69	5.93 5.94	17.36 17.11)

a) A, hexane; B, EtOH–AcOEt; C, hexane–AcOEt. *b*) Purified as hydrogen fumarate salt. *c*) Yield of free base. *d*) Isolated from reaction mixture as the HCl salt.

TABLE IV. Pharmacological Activities of 4-Phenoxy-2-(1-piperazinyl)quinazolines (**5**)

Compound	Anti-MES[a] ED_{50} (mg/kg, *p.o.*)	Neurotoxicity NTD_{50} (mg/kg. *p.o.*)	P.I.[b]	Hypnotic activity[c]	Antihypoxic activity[d] (% of control)	Acute toxicity LD_{50} (mg/kg, *p.o.*)
5a	20	283	14.2	2/5	132	566
5b	>100		—		125	
5c	31	187	6.0	2/5	147	>800
5d	31	132	4.3	4/5	133	>800
5e	31	200	6.5	2/5	166	>800
5f[e]	20[f]	246[f]	12.3	1/5	147	>1000
5g	31	100	3.2		163	650
5h	35	123	3.5		129	>800
5i[e]	27[f]	115[f]	4.3		157	>800
5j	31	87	2.8	1/5	139	373
5k	71		—		148	
5l	62		—		123	
5m	41		—		113	
5n	35	303	8.7	1/5	119	>1000
5o	>100		—		102	
5p	>100		—		108	
5q	20		—		383	<100
5r	13		—		235	<200
5s	>100		—		124	
5t	>100		—		127	
5u	>100		—		120	
5v[e]	20[f]	303[f]	15.2	0/5	129	876
5w[e]	23[f]	429[f]	18.7	0/10	131	>1000
5x[e]	39[f]	460[f]	11.8	0/5	93	>1000
5y[e]	>100		—		96	
Phenytoin	6.2	79	12.7	7/10[g]	215	373
Carbamazepine	19	81	4.3	8/10[g]	158	>1000

a) Anticonvulsive activity against maximal electroshock-induced seizure. *b*) Protective index. *c*, *d*) See experimental section. *e*) Fumarate. *f*) Calculated as the free base. *g*) Tested at the dose of 100 mg/kg, *p.o.*

of the molecules, which results in potentiation of the anti-MES activity. The optimum π was calculated to be 0.289 and thus the maximum $\log 1/ED_{50}$ was calculated to be 4.14. From this result, it became clear that the most active compounds have already been prepared, and compounds **5a** and **5f** might show nearly maximum potencies.

Next, the effect of substituents on the quinazoline ring on the anti-MES potency was examined. The 6-amino and 7-amino derivatives (**5q** and **5r**) both exhibited potent activity, but simultaneously showed high toxicity. The other 6- or 7-substituted compounds showed negligible activity. Because of the drastic distinction, QSAR analysis was difficult. However, it appeared that 6- and/or 7-substituents are disadvantageous.

Finally, the effect of substituents at the 4-position of the piperazinyl moiety was examined. The order of increasing

TABLE V. Parameters and Results of the QSAR Analysis

Compound	R^2	$\Sigma\pi$[a]	log 1/ED_{50}		$\Sigma\sigma_{(W)}$[c]	$\Sigma\sigma_{(D)}$[c]	log 1/NTD_{50}	
			Obs.[b]	Calcd.			Obs.[b]	Calcd.
5a	H	0	4.20	4.12	0	0	3.05	3.06
5c	3-Cl	0.71	4.06	4.10	0.37	0	3.28	3.29
5d	4-Cl	0.71	4.06	4.10	0.23	0	3.42	3.44
5e	4-F	0.41	4.04	4.14	0.06	0	3.23	3.12
5f	3-OCH_3	−0.02	4.24	4.12	0.12	0	3.15	3.18
5g	4-OCH_3	−0.02	4.05	4.12	0	−0.27	3.54	3.59
5h	3-CF_3	0.88	4.05	4.06	0.43	0	3.50	3.50
5i	4-CH_3	0.56	4.09	4.12	0	−0.17	3.46	3.39
5j	4-$COCH_3$	−0.55	4.07	3.99	0.50	0	3.62	3.57
5k	4-$NHCOCH_3$	−0.97	3.73	3.79				
5l	2,4-Cl_2	1.42	3.80	3.86				
5m	3,4-Cl_2	1.25	3.98	3.94	0.16	0	3.09	3.22
5n	3-CH_3, 4-Cl	1.29	4.02	3.92				

a) Summation of the values of hydrophobic parameters of the substituents. *b*) These values were calculated from ED_{50} and NTD_{50} in Table IV on a molar basis. *c*) Summation of the values of Hammett's electronic parameters of the substituents.

potency of these compounds was as follows: benzyl≪ allyl<propyl, ethyl, methyl. It seemed that the potency was not greatly affected by the substituents of the piperazinyl moiety, except for extremely large substituents (the benzyl group diminished the activity).

From the above results, we concluded that further syntheses aimed at finding more potent derivatives would probably not be successful.

Side Effects and Toxicity The compounds which showed significant anti-MES activity and lower acute toxicity than that of phenytoin were examined for neurotoxicity. Many compounds showed much lower neurotoxicity as well as lower acute toxicity than carbamazepine and phenytoin.

In order to investigate the effects of substituents on the phenoxy moiety, QSAR analyses for neurotoxicity were carried out. The best equation was Eq. 2 (Table V).

$$\log 1/NTD_{50} = 1.035(\pm 0.369)\Sigma\sigma_{(W)} - 1.962(\pm 0.725)\Sigma\sigma_{(D)} + 3.055(\pm 0.106) \quad (2)$$

$$n=10,\quad r=0.943,\quad s=0.076,\quad F=28.8$$

In Eq. 2, σ is Hammett's electronic parameter of the substituent, and $\Sigma\sigma_{(W)}$ is the electron-withdrawing effect calculated from the summation of σ values of all substituents, and $\Sigma\sigma_{(D)}$ is the corresponding electron-donating effect. Because $\Sigma\sigma_{(W)}$ is larger than zero, the positive coefficient indicated a potentiation of neurotoxicity by the introduction of electron-withdrawing substituents. Electron-donating substituents also had the same effect. Therefore, the unsubstituted compound should show minimum neurotoxicity.

The coefficient of $\Sigma\sigma_{(W)}$ is nearly unity, which suggested that a nucleophilic reaction may occur at the 4-position of the quinazoline ring. In contrast, the coefficient of $\Sigma\sigma_{(D)}$ is nearly −2, which suggested that an electrophilic reaction may occur at the oxygen atom. It was reported that 4-phenoxyquinazolines were easily hydrolyzed to 4(3*H*)-quinazolinones.[19] The substituents of the phenoxy moiety would make some contribution to the hydrolysis, and the different coefficients may suggest different mechanisms of hydrolysis. From the above results, the neurotoxicity was assumed to be due to the hydrolyzed compound, **1b**. Gupta and co-workers reported that compound **1b** showed a weak CNS activity.[1] This result is in conflict with our assumption. However, this may be accounted for by the low lipophilicity of compound **1b**, preventing it from passing through the blood-brain barrier.

The usefulness of antiepileptic agents can be evaluated in terms of the protective index (P.I.); the ratio between the ED_{50} and the NTD_{50}. The P.I. values of **5a**, **5v** and **5w** were superior to that of phenytoin.

Carbamazepine and phenytoin potentiated the hexobarbital-induced sleep at the dose of 100 mg/kg. The 4-methyl-1-piperazinyl derivatives (**5a**, **c**, **d**, **e**, **f**, **j** and **n**) showed a potentiating effect at the dose of 200 mg/kg. But the 4-ethyl-, 4-propyl- and 4-allyl-1-piperazinyl derivatives (**5v**, **5w** and **5x**) did not show any potentiating effect even at the dose of 200 mg/kg. It was confirmed that substituents at the 4-position of the piperazinyl moiety have an important effect on the activity to potentiate hexobarbital-induced sleep.

Antihypoxic Activity Compounds **5q** and **5r** exhibited potent antihypoxic activity, comparable to that of phenytoin, at the dose of 50 mg/kg. Other compounds which exhibited potent anti-MES activity showed weak antihypoxic activity. The compounds which exhibited negligible anti-MES activity did not show any antihypoxic activity. It seemed that potent anti-MES activity was a prerequisite for potent antihypoxic activity in this series.

Caillard and co-workers[12] reported that "the oxygen deficiency caused the lethal effect in hypoxic conditions. Anoxia induced convulsions but hypoxemia was aggravated by tonic convulsions which increased muscle metabolism and by apnea during tonic seizures. In preventing seizures, anticonvulsive agents delayed oxygen deficiency and prolonged the life of mice in the face of hypoxia." Recently, Fukuda and co-workers reported that there was a close relation between the anti-MES activity and antihypoxic activity, and that carbamazepine showed potent antihypoxic activity but did not prevent hypoxic convulsion.[20] We can not account for this discrepancy. The precise mechanism of the antihypoxic activity remains to be clarified.

Conclusion

Many 4-phenoxy-2-(1-piperazinyl)quinazolines exhibited potent anti-MES activity comparable to that of carbamazepine or phenytoin. Among them, we selected **5w** as the most promising candidate antiepileptic drug with minimum side effects such as neurotoxicity and hypnosis.

Few compounds which showed potent anti-MES activity also exhibited antihypoxic activity. As already mentioned, there may exist some relationship between the anti-MES activity and antihypoxic activity, and it seemed necessary to find more potent anti-MES compounds to find more potent antihypoxic compounds than phenytoin. It was concluded that potent antihypoxic agents are unlikely to be found in the series of 4-phenoxy-2-(1-piperazinyl)quinazolines.

Experimental

Melting points were measured with a capillary melting point apparatus (Yamato MP-21) and are uncorrected. Infrared (IR) spectra were recorded on a Hitachi 270-50 spectrometer. ^{1}H-NMR spectra were taken on a Hitachi R-24B NMR spectrometer with tetramethylsilane (TMS) as an internal standard. Chemical shifts are given as δ values (ppm): s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet. Elemental analyses were performed by the Analytical Department of Kanebo Research Center. For column chromatography, Silica gel 60 (Merck) was used.

2-(4-Benzyl-1-piperazinyl)-4(3*H*)-quinazolinone (1f) A mixture of 2-ethylthio-4(3*H*)-quinazolinone (**7a**, 5.0 g, 24 mmol) and *N*-benzylpiperazine (8.5 g, 48 mmol) was stirred at 155—160 °C for 3 h. The reaction mixture was triturated with AcOEt and filtered. The resulting solid was recrystallized from AcOEt–EtOH to give **1f** (6.8 g, 87%). ^{1}H-NMR (DMSO-d_6) δ: 2.3—2.6 (4H, t-like), 3.50 (2H, s), 3.5—3.8 (4H, t-like), 7.0—8.1 (10H). Compounds **1a**,[6] **1b**[1] and **1e** were similarly prepared.

2-(4-Ethyl-1-piperazinyl)-4(3*H*)-quinazolinone (1c) A mixture of **1a** (5.0 g, 22 mmol), ethyl iodide (4.0 g, 26 mmol), K_2CO_3 (3.0 g, 22 mmol) and EtOH (50 ml) was stirred at 60 °C for 2 h. The reaction mixture was filtered and the filtrate was concentrated. The residual oil was chromatographed on silica gel with $CHCl_3$–MeOH (20 : 1) to give **1c** (2.6 g, 47%). ^{1}H-NMR (DMSO-d_6) δ: 1.16 (3H, t, J=7 Hz), 2.53 (2H, q, J=7 Hz), 2.61 (4H, t, J=5 Hz), 3.89 (4H, t, J=5 Hz), 7.0—8.2 (4H). Compound **1d** was similarly prepared.

2-(4-Methyl-1-piperazinyl)-6-nitro-4(3*H*)-quinazolinone (1g) To a mixed acid (20 ml of nitric acid and 20 ml of sulfuric acid), **1b** (10 g, 41 mmol) was added portionwise at a rate sufficient to prevent the temperature from exceeding 30 °C. Then, the reaction mixture was stirred at 50 °C for 30 min. The orange reaction mixture was poured onto crushed ice and neutralized with NaOH solution. The resulting precipitate was collected by filtration to give **1g** (10 g, 85%).

2-(4-Methyl-1-piperazinyl)-7-nitro-4(3*H*)-quinazolinone (1h) A mixture of 2,4-dichloro-7-nitroquinazoline[21] (3.6 g, 15 mmol), 2 N NaOH (35 ml) and dioxane (7 ml) was stirred at room temperature for 1 h. The reaction mixture was neutralized with dilute HCl and extracted with AcOEt. The organic layer was washed with water, dried over $MgSO_4$ and concentrated. The resulting solid was recrystallized from hexane–AcOEt to give 2-chloro-7-nitro-4(3*H*)-quinazolinone (**7b**, 2.9 g, 87%). mp 223.0—224.5 °C. IR (KBr): 1690, 1680 cm^{-1}. *Anal.* Calcd for $C_8H_4ClN_4O_3$: C, 42.59; H, 1.79; N, 18.63. Found: C, 42.72; H, 1.72; N, 18.57. Compound **7b** (3.2 g, 14 mmol) was heated with *N*-methylpiperazine (4.5 g, 4.5 mmol) at 130 °C for 30 min, then 2 N NaOH (30 ml) was added to the reaction mixture, and insoluble materials were filtered off. The filtrate was neutralized with dilute HCl and the resulting precipitate was filtered to give **1h** (4.0 g, 83%). Compound **1i** was similarly prepared.

4-Chloro-2-(4-ethyl-1-piperazinyl)quinazoline (6c) A mixture of **1c** (7.7 g, 30 mmol) and $POCl_3$ (8.3 ml, 89 mmol) was heated at 130 °C for 1 h. The reaction mixture was poured onto ice, neutralized with 3 N NaOH and extracted with AcOEt. The organic layer was washed with water, dried over $MgSO_4$ and concentrated. The resulting solid was recrystallized from hexane to give **6c** (7.6 g, 92%). ^{1}H-NMR ($CDCl_3$) δ: 1.12 (3H, t, J=7 Hz), 2.45 (2H, q, J=7 Hz), 2.50 (4H, t, J=5 Hz), 3.94 (4H, t, J=5 Hz), 7.0—8.0 (4H). Other 4-chloro derivatives (**6b**, **6d**—**h**) were similarly prepared. Compound **6f** was obtained as the HCl salt by neutralization of the reaction mixture. The oily compound **6d** was treated with a solution of fumaric acid in EtOH to form the fumarate for elemental analysis. **6g**: ^{1}H-NMR ($CDCl_3$) δ: 2.39 (3H, s), 2.53 (4H, t, J=5 Hz), 4.08 (4H, t, J=5 Hz), 7.60 (1H, d, J=10 Hz), 8.50 (1H, dd, J=10, 3 Hz), 8.97 (1H, d, J=3 Hz). **6h**: ^{1}H-NMR ($CDCl_3$) δ: 2.39 (3H, s), 2.52 (4H, t, J=5 Hz), 4.01 (4H, t, J=5 Hz), 7.91 (1H, dd, J=10, 3 Hz), 8.15 (1H, d, J=10 Hz), 8.39 (1H, d, J=3 Hz).

4-(2-Chlorophenoxy)-2-(4-methyl-1-piperazinyl)quinazoline (5b) NaH (60% in oil) (0.5 g, 12 mmol) was added to a solution of **6b** (2.5 g, 9.5 mmol), *o*-chlorophenol (1.3 g, 10 mmol) and DMF (20 ml) at 0 °C, and the mixture was stirred at 60 °C for 3 h. The reaction mixture was diluted with water and extracted with AcOEt. The organic layer was washed with water, dried over $MgSO_4$ and concentrated. The resulting solid was recrystallized from hexane to give **5b** (2.5 g, 74%). ^{1}H-NMR ($CDCl_3$) δ: 2.26 (3H, s), 2.35 (4H, t, J=5 Hz), 3.70 (4H, t, J=5 Hz), 7.1—8.3 (8H). Compounds **5a**, **5c**—**h** were similarly prepared. The oily compounds **5f**, **5i**, **5v**, **5w**, **5x** and **5y** were purified as the fumarates.

Pharmacological Methods Male ddy strain mice weighing 18—25 g were used in all experiments. All animals were fasted overnight before experiments. Test compounds were dissolved or suspended in 1% acacia solution and administered orally to animals.

Anticonvulsive Activity[13] A group of five mice was used for each dose of test compound. Each animal was electroshocked (60 Hz, 80 mA, 0.2 s) through corneal electrodes 1 h after the administration of test compounds. The prevention of hindlimb tonic extensor reaction was classified as anticonvulsive effect. Median effective dose (ED_{50}) was calculated according to Weil's method.[22]

Neurotoxicity[14] Neurotoxicity was defined as inability of the animals to remain on a horizontal rod (3.4 cm) rotating at 5 rpm. Mice were trained to remain on the rotating rod for at least 60 s. A group of ten mice was used for each dose of test compound. Rotarod performance tests for 60 s were carried out repeatedly at intervals of 1 h for 6 h after the administration of a test compound. Median neurotoxic dose (NTD_{50}) was calculated as the dose capable of exerting neurotoxic activity in 50% of animals according to Weil's method.

Hypnotic Effect[15] Hypnotic effect was examined by measuring the potentiating effect on hexobarbital-induced sleep. A group of five to ten mice was used for each test compound. The animals received a subhypnotic dose (40 mg/kg, i.p.) of hexobarbital 1 h after the administration of a test compound (200 mg/kg, *p.o.*). The hypnotic effect was judged in terms of loss of righting reflex at intervals of 15 min for 90 min. The results were shown as the ratio of the number of animals losing the righting reflex to the number of animals tested.

Acute Toxicity The mortality was observed and recorded for 7 d following the administration of each test compound, and the median lethal dose (LD_{50}) value was calculated according to Weil's method.

Antihypoxic Activity[16] A group of five mice was used for each dose of a test compound. The animals were decapitated 1 h after the oral administration of the test compound. Then, the gasping duration was measured and expressed as percent of the control value.

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