ditional amount (324 mg, 1.72 mmol) of the reagent at 0 °C for 0.5 h. The resulting mixture was poured into ice–water and separated. The methylene chloride solution was dried with magnesium sulfate, and the solvent was evaporated under reduced pressure to give a trichloroacetylcarbamoyl derivative. It was dissolved in a 20:1 mixture of chloroform and methanol (1 mL) and applied to a silica gel (10 g) packed in a column. After this had stood for 2 h, it was eluted with the same solvent mixture to give **20** (230 mg, 95%) as a foam: NMR (a 5:1 mixture of $CDCl_3$ and CD_3OD) 1.13 (t, 3 H, J = 7 Hz, CH_3), 3.2–4.4 (m, 10 H, 5 CH_2), 3.77 (s, 3 H, CH_3), 5.5–6.0 (m, 3 H, C_6 H and C_7 H, NCHCO), 6.95 (s, 1 H, $CHPh_2$), 7.05 (d, 2 H, J = 8 Hz), 7.2–7.6 (m, 12 H, aromatic protons), 10.00 (d, 1 H, J = 7 Hz, CONH), carbamoylamino protons were deuterated; IR ($CHCl_3$) 3500, 1780, 1712, 1683 cm^{-1}.

A solution of **20** (220 mg, 0.26 mmol) dissolved in methylene chloride (2 mL) was treated with anisole (0.4 mL) and trifluoroacetic acid (0.4 mL) at 0 °C for 1 h and, after dilution of the reaction mixture with benzene (5 mL), the solvent was removed under reduced pressure to yield a crude product, which was triturated successively with petroleum ether and ethyl acetate to produce **8a** (140 mg, 78%) as an amorphous powder: IR (KBr) 3300, 1785, 1720, 1680 cm^{-1}.

Biochemistry. Bacterial Stains. *E. cloacae* 214, *E. cloacae* 53, and *E. coli* W3110 RTEM were kindly supplied by M. H. Richmond (University of Bristol, England). Other strains were from Shionogi Research Laboratories stocks.

Preparation of Crude Extracts. Constitutive β-lactamase producers, *E. coli* 6, *E. cloacae* 214, *E. cloacae* 53, *E. coli* W3110 RTEM, and *Klebsiella* sp. 363, were grown statically in nutrient phosphate broth (Nissui, Japan) at 37 °C for 16 h. Cultures were harvested by centrifugation at 3000*g* for 15 min at 4 °C. The pellets were washed once with 0.1 M potassium phosphate buffer (pH 7.0) and then suspended in the same buffer for ultrasonic disruption.

Overnight culture of the inducible β-lactamase producer, *Proteus vulgaris* 31, in nutrient phosphate broth was diluted tenfold with the same broth and grown for 2 h in a shaker at 37 °C. Penicillin G was added to the culture to a final concentration of 100 μg/mL, and bacteria were grown for another 2 h. The culture was harvested by centrifugation, washed once with the phosphate buffer, and suspended in the same buffer for ultrasonic disruption.

Bacteria were disintegrated by ultrasonication (Sonicator-150, Ohtake, Japan) for 2 min in an ice–water bath. After centrifugation at 33000*g* for 30 min at 4 °C to remove cell debris, the supernatant fluids were passed through a millipore membrane filter, 0.22 μm (Millipore Corp.) and stocked at −78 °C until used.

Purification of β-Lactamases. β-Lactamases produced from *E. coli* W3110 RTEM and *Klebsiella* sp. 363 were partially purified using DEAE-Sephadex A-25. Crude extracts dialyzed against 0.01 M Na_2HPO_4–KH_2PO_4 (pH 8.0) were loaded onto a DEAE-Sephadex A-25 column equilibrated against the same buffer. A gradient was constructed from 180 mL of 0.01 M (pH 8.0) and 0.5 M (pH 6.2) phosphate buffer. β-Lactamases from *E. coli* 6, *E. cloacae* 214, *P. vulgaris* 31, and *E. cloacae* 53 were partially purified using CM-Sephadex C-50 as described previously.[23]

Active fractions were pooled, dialyzed against 0.1 M potassium phosphate buffer (pH 7.0), and stocked at −78 °C until used.

Assay of β-Lactamase. Potassium phosphate buffer of 0.1 M (pH 7.0) was used in the assay. β-Lactamase activity was determined at 30 °C by spectrophotometric assay, using the change in optical density at a definite wavelength in the ultraviolet region as described previously.[24] The difference of the absorption coefficient of the β-lactam upon hydrolysis was obtained from the spectrum of the β-lactam compound before and after the complete hydrolysis by β-lactamase. The enzyme was diluted with the buffer containing 0.001% gelatin. A 0.2-mL portion of the enzyme solution was added to 2 mL of substrate solution (final concentration of substrate was 100 μM), and the reaction mixture was incubated in a 1-cm cuvette in a Hitachi (Japan) spectrophotometer Model 200-20, through which water of 30 °C was circulated. The decrease of optical density was recorded with a Hitachi recorder 200. The hydrolysis rate was calculated from the slope of the recorded line.

Susceptibility Tests. Antibacterial activity was determined by the agar dilution method using sensitivity test agar (Eiken, Japan). An overnight culture of bacteria in tryptosoy broth (Eiken, Japan) was diluted to about 10^6 cells/mL with the same broth and inoculated with an inoculating device onto agar containing serial twofold dilutions of an antibiotic. Organisms were incubated at 37 °C for 18–20 h. The minimum inhibitory concentration (MIC) of an antibiotic was defined as the lowest concentration that inhibited visible growth.

Acknowledgment. We are grateful to M. Narisada, Y. Hamashima, H. Matsumura, and W. Nagata for the synthesis of the compounds and their helpful discussions.

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Piperazinylquinoxalines with Central Serotoninmimetic Activity

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Regioselective syntheses of substituted 2-chloroquinoxalines and derived 2-(1-piperazinyl)quinoxalines are described. Selectivity in regards to serotonin reuptake blocking and serotoninmimetic activities of the piperazinylquinoxalines is reported. In general, introduction of a 6-substituent into the piperazinylquinoxaline enhanced serotonin reuptake blocking activity and diminished serotoninmimetic activity. Unsubstituted and 3-hydroxypiperazinylquinoxalines had primarily serotoninmimetic activity.

Recognition that serotonin plays an important role in the physiology of the normal mammalian central nervous system (CNS) as well as in certain pathological states has stimulated the search for novel serotonin-like agents. A previous publication[1] described a series of piperazinylpyrazine derivatives having potent and selective central serotoninmimetic activity. In this work we report the syntheses of some piperazinylquinoxalines[2] and their evaluation as serotonin agonists and neuronal serotonin-reuptake inhibitors.

(1) Lumma, W. C., Jr.; Hartman, R. D.; Saari, W. S.; Engelhardt, E. L.; Hirschmann, R.; Clineschmidt, B. V.; Torchiana, M. L.; Stone, C. A. *J. Med. Chem.* **1978**, *21*, 536.

(2) Some of these compounds have been disclosed in the patent literature: German Offen. 2433397 (1975); *Chem. Abstr.* **1975**, *82*, 156377*g*.

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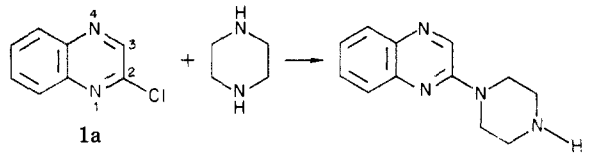
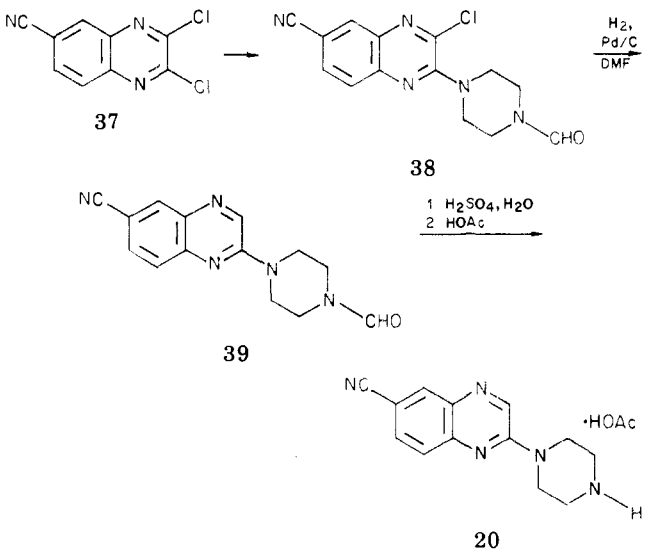
Chemistry. Displacement of halogen by piperazine, or its substituted derivatives, from a 2-haloquinoxaline served as a preparative method for most of the 2-piperazinylquinoxalines **1–36** (methods A–C), which are summarized in Tables I and II.



1a 1

In the case of the 6-cyano compound (**20**), selective

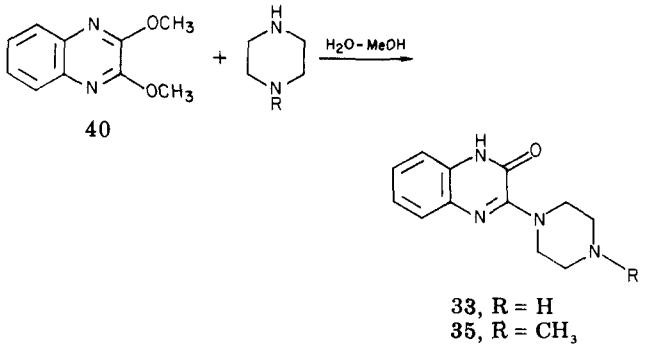


37 38 39 20

displacement of the 2-chlorine of 2,3-dichloro-6-cyanoquinoxaline (**37**) with *N*-formylpiperazine, followed by reductive dehalogenation of **38**[3] and hydrolysis of **39**, served as a regioselective synthesis (method D). Selective halogen displacement[4] from 2,3-dichloroquinoxalines was also employed in the synthesis of compounds **5–7**. In these reactions, it was assumed that an electron (resonance) withdrawing substituent (CN, SO_2NH_2) in the 6 position activates the 2-chlorine, whereas an inductively electron-withdrawing substituent in the 6 position activates the 3-chlorine (cf. method O). This assumption is justified by the work of Illuminati et al.,[5] who demonstrated a rate enhancement of 1164, 174.3, 5.78, and 11.13, respectively, for 6-NO_2, 7-NO_2, 6-Cl, and 7-Cl substituents for the methanolysis of 2-chloroquinoxaline. The factor of two in the solvolysis rate enhancement for a 7-Cl vs. a 6-Cl substituent explains the exclusive ammonolysis of the 3-chlorine in 2,3,6-trichloroquinoxaline.[3]

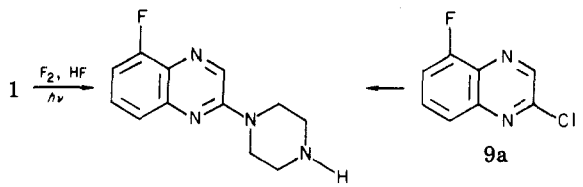
Synthesis of 3-keto analogues of 1 (**33–36**) was accomplished by hydrolytic displacement with piperazines (in the presence of H_2O) on 2,3-dimethoxyquinoxaline for **33** and **35** (method E) and by halogen displacement from the novel 3-keto-2-chloro intermediates for **34** and **36** (method F).

The 6-amino derivative (**18**) was obtained from the 6-nitro derivative (**16**) by catalytic reduction (method G). The 6-hydroxy analogue (**17**) was prepared from 6-methoxy-2-chloroquinoxaline (**22a**) by $AlCl_3$ demethylation in C_6H_6, followed by halogen displacement with piperazine (method H).



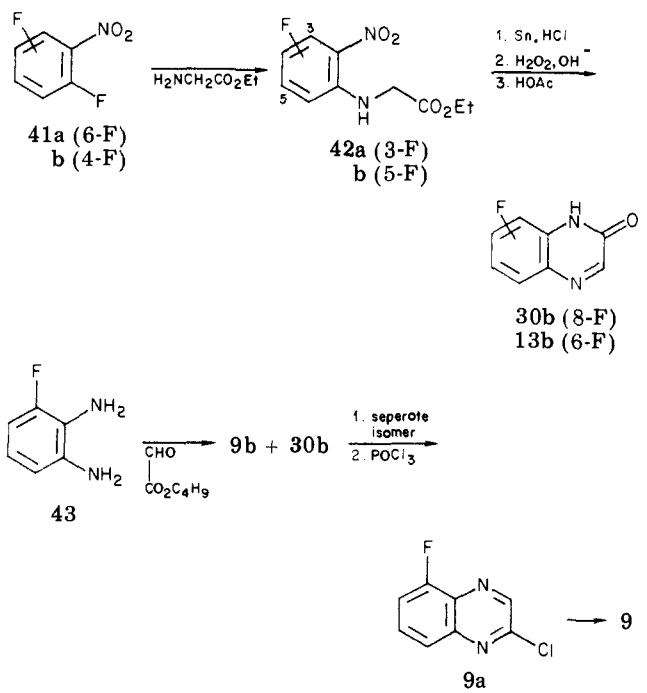
40 33, R = H 35, R = CH_3

The 5-fluoro derivative (**9**) was synthesized by a novel, regioselective photofluorination[6] (method I) of **1** and also unambiguously from 2-chloro-5-fluoroquinoxaline (**9a**).



9 9a

Many of the required 2-chloroquinoxaline intermediates have been described in the literature[7a] and were obtained by reaction of 2(1*H*)-quinoxalinones[7a,8] with $POCl_3$. These intermediates are listed in Tables III and IV. Syntheses of new 2(1*H*)-quinoxalinones were patterned after the methods of Crowther et al.[3] and are illustrated for the 6- and 8-fluoro derivatives (**13b** and **30b**) and the 6-SO_2CH_3

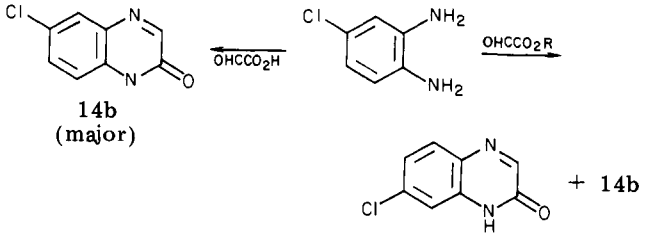


41a (6-F) b (4-F) 42a (3-F) b (5-F) 30b (8-F) 13b (6-F) 43 9a

(3) A similar synthetic strategy was employed for the synthesis of 2-amino-7-haloquinoxalines: Crowther, A. F.; Curd, F. H. S.; Davey, D. G.; Stacey, G. J. *J. Chem. Soc.* **1949**, 1260.
(4) Haworth, R. D.; Robinson, S. *J. Chem. Soc.* **1948**, 777.
(5) Illuminati, G.; Linda, P.; Marino, G.; Zinato, E. *Ric. Sci. Rend. Ser. A.* **1963**, *3*, 535; *Chem. Abstr.* **1963**, *59*, 9753*b*.
(6) (a) Kollonitsch, J. U.S. Patent 4004996, 1977; *Isr. J. Chem.* **1978**, *17*, 53. (b) The equipment used has been described: Kollonitsch, J.; Barash, L. *J. Am. Chem. Soc.* **1976**, *98*, 5591.
(7) (a) Wolf, F. J.; Pfister, K., 3rd; Beutel, R. H.; Wilson, R. M., Jr.; Robinson, C. A.; Stevens, J. R. *J. Am. Chem. Soc.* **1949**, *71*, 6; (b) For proof of structure of nitration products of **1b** see: Otomasu, H.; Yoshida, K. *Chem. Pharm. Bull.* **1960**, *8*, 475.
(8) For synthesis and structure of both *N*-oxides of **1a**, see: Landquist, J. K. *J. Chem. Soc.* **1953**, 2876.

derivative (methods J and K). Condensation of 3-fluoro-1,2-benzenediamine (**43**) with *n*-butyl glyoxylate (method L) gave a 1:1 mixture of 5- and 8-fluoro-2(1*H*)-quinoxalinones (**9b** and **30b**). One of these isomers (the 8-isomer) was identical with the product **30b** from cyclization of *N*-(3-fluoro-2-nitrophenyl)glycine ethyl ester (**42a**). The other isomer was converted to the piperazine derivative **9** whose hydrogen fumarate was identical with that of the photofluorination product of **1**.

We have observed that the regioselectivity of condensation of certain 4-substituted 1,2-diaminobenzenes (e.g., 4-chloro and 4-oxo) with esters of glyoxylic acid can be altered from ca. a 1:1 mixture of the 6- to 7-isomers to a preponderance (>5:1) of the higher melting, less soluble 6-isomer of the 2(1*H*)-quinoxalinone (e.g., **14b**) by con-

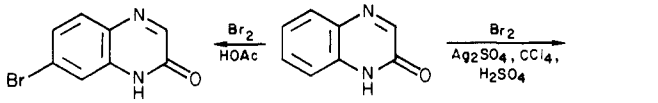


14b (major)

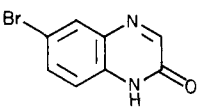
27b ca. 1:1

ducting the condensation in the presence of 1 equiv of a strong acid and/or by employing glyoxylic acid as the condensing reagent. This fact was employed in a synthesis of pure 6-chloro-2(1*H*)-quinoxalinone-2,3-$^{14}C_2$[10] and in the synthesis of 6-(trifluoromethyl)-2(1*H*)-quinoxalinone (**19b**). The effect of acid in these cases may be to protonate the more basic 4-NH_2 group of the starting diamine so that condensation occurs faster between the 3-NH_2 and aldehyde function of the glyoxylic acid derivative.

Direct bromination of 2(1*H*)-quinoxalinone (**1b**) by the



44 1b



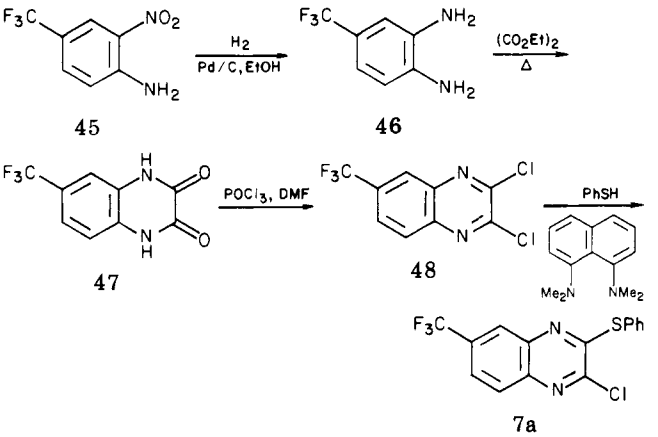
15b

procedure of Cheeseman[11a] for quinoxaline-2,3(1*H*,4*H*)-dione gave predominantly the 6-isomer (**15b**, method N). This result is in contrast to bromination of **1b** in acetic acid,[11b] which we have verified to give exclusively the 7-isomer **44**.

As discussed earlier, selective displacement of one halogen from a 6-substituted 2,3-dichloroquinoxaline depends on the nature of the 6-substituent. In the case of inductively electron-withdrawing substituents, the 3-chlorine can be displaced preferentially,[3] as illustrated for the synthesis of **7a** (method O) from **45**.

Biological Results and Discussion

The piperazinylquinoxalines of Tables I and II were examined for their ability to elicit head twitches in rats,



45 46 47 48

7a

a response characteristic of a serotoninmimetic action in the central nervous system.[1,12] Serotonin neuronal reuptake blocking activity was determined by the antagonism of the head twitch response induced by treatment of rats with *p*-chloromethamphetamine (PCMA).[13] PCMA has previously been shown to release bound serotonin from brain storage sites.[14] This serotonin releasing action of PCMA is dependent on uptake of PCMA into serotonin neurons and is antagonized by known serotonin neuronal reuptake blocking agents.[15]

Several piperazinylquinoxalines were found to have potent and differential actions on central serotoninergic systems. Variation of nuclear substituents yielded derivatives which differentially inhibited the neuronal reuptake of serotonin, exhibited only serotoninmimetic activity, or had both of these actions.

The unsubstituted piperazinylquinoxaline (**1**) and its 5-fluoro (**9**), 6-fluoro (**13**), and 4-*N*-oxide (**8**) derivatives were found to be the most effective members of the series in producing head twitches. The 8-fluoro (**30**), 3(4*H*)-keto (**33**), and 1-*N*-oxide derivatives were less potent in this action.

Introduction of other substituents (Cl, NO_2, CF_3, OCH_3) into the 6 position of the piperazinylquinoxaline nucleus suppressed serotoninmimetic activity but resulted in potent inhibitors of serotonin neuronal reuptake. The most potent and selective reuptake inhibitor proved to be the 6-chloro derivative (**14**) (ED_{50} = 0.5 mg kg^{-1}; Table I). In the rat assay for reuptake blocking activity, **14** was more potent than chlorimipramine (ED_{50} = 2.0 mg kg^{-1}). Other studies have demonstrated that **14** is a serotonin reuptake inhibitor having little interaction with the adrenergic and dopaminergic systems.[13]

The 6-CN derivative (**20**) appeared to have both serotoninmimetic and serotonin reuptake blocking actions (Table I). The 6-Br (**15**), 6-CH_3 (**21**), 6-SO_2CH_3 (**23**), 6-OH (**17**), and 6-NH_2 (**18**) analogues were comparatively inactive as agonists or reuptake blockers. The facts that the 5-F (**9**) and 6-F (**13**) analogues were predominately apparent agonists rather than reuptake blockers, whereas compounds with larger[16] 6-substituents were reuptake blockers, suggest that the presynaptic reuptake receptor is less

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(11) (a) Cheeseman, G. W. H. *J. Chem. Soc.* **1962**, 1170. (b) Linda, P.; Marino, G. *Ric. Sci. Rend., Ser. A.* **1963**, *3*, 225; *Chem. Abstr.* **1963**, *59*, 7523*d*.
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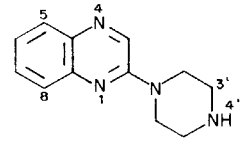
(15) Meek, J. L.; Fuxe, K.; Carlsson, A. *Biochem. Pharmocol.* **1971**, *20*, 707.
(16) For a comparison of substituent parameters, see: Hansch, C.; Leo A.; Unger, S. H.; Kim, K. H.; Nikaitani, D.; Sien, E. J. *J. Med. Chem.* **1973**, *16*, 1207.

Table 1. Chemical Properties and Head-Twitch Activity of 2-(1-Piperazinyl)quinoxalines

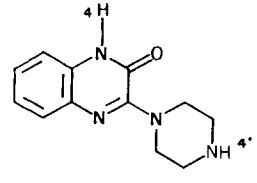


compd	substituents	formula	mp, °C	recrystn solvent	method	% yield[a]	anal.	rat head twitches;[b] dose, mg/kg ip			PCMA antagonism;[c] dose, mg/kg ip		
								40	4	0.4	40	4	0.4
chlorimipramine											ED_{50} = 2.0 (0.6-6.7)		
1		$C_{12}H_{14}N_4 \cdot HCl$	287-288.0 dec	MeOH-EtOH	A		C, H, N, Cl	*e*	12/12	9/12	*e*	2/12	2/12
2	*N*'-oxide	$C_{12}H_{14}N_4O \cdot HCl \cdot H_2O$	264-266 dec		A	29	C, N, Cl; H[f]	4[g]	2[g]	2[g]			
3	3-Cl	$C_{12}H_{13}N_4Cl \cdot HCl$	264-267	MeOH-EtOH	A			1[h]	3[h]	2[h]			
4	3-NH_2	$C_{12}H_{15}N_5 \cdot 2HCl$	296-298 dec	MeOH-EtOH	B	50	C, H, N, Cl	6	3	2	0	1	1
5	3-Cl, 6-SO_2NH_2, 4'-Me	$C_{13}H_{15}N_5ClO_2S$	202.5-204	EtOH	A	12	C, H, N	4	1	3	2	2	2
6	3-SC_6H_5, 6-Cl	$C_{18}H_{17}N_4ClS \cdot HCl$	276-277.5	EtOH	A[i]	16	C, H, N	0	1	1	1	2	1
7	3-SC_6H_5, 6-CF_3	$C_{19}H_{17}N_4F_3S \cdot HCl$	262-263	*i*-PrOH	A[i,j]	68	C, H, N	0	2	2	0	0	1
8	N^4-oxide	$C_{12}H_{14}N_4O \cdot HCl$	242-243 dec				C, H, N, Cl	*e*	6	6			
9	5-F	$C_{12}H_{13}N_4F \cdot C_4H_4O_4 \cdot 0.75H_2O$[k]	209.5-210.5 dec	H_2O	C	71	C, H, N, F	*e*	6	6			
10	5-Cl	$C_{12}H_{13}N_4Cl \cdot HCl$	323-325 dec	H_2O	A	52	C, H, N	0	2	0	2/4	0	0
11	5-Me	$C_{13}H_{16}N_4 \cdot HCl$	314-315.5 dec	EtOH-H_2O	A	73	C, H, N	5[g]	4[g]	0[g]	1[g]	1[g]	0[g]
12	5-OMe	$C_{13}H_{16}N_4O \cdot HCl \cdot 0.5H_2O$	251-252	EtOH	A	56	C, H, N, Cl	3	2	1	2	1	0
13	6-F	$C_{12}H_{13}N_4F \cdot 2HCl$	310-311	MeOH	C	24	C, H, N,	5[l]	4[l]	4[l]	4	2	2
14	6-Cl	$C_{12}H_{13}N_4Cl \cdot HCl$	320-321 dec	MeOH	C	75	C, H, N, Cl	2	0	1	6	6	4[d]
15	6-Br	$C_{12}H_{13}N_4Br \cdot HCl$	312-314 dec	EtOH-H_2O	A	52	C, H, N	2[l]	4[l]	0[l]	2[l]	3[l]	2[l]
16	6-NO_2	$C_{12}H_{13}N_5O_2 \cdot HCl$	335-336 dec	dil HCl	A[i,j]	27	C, H, N, Cl	2	2	4	5	6	5
17	6-OH	$C_{12}H_{14}N_4O \cdot AcOH$	185-187	*i*-PrOH	H	12	C, H, N	1	0	0	2	0	
18	6-NH_2	$C_{12}H_{15}N_5 \cdot C_4H_4O_4$[k]	*m*	EtOH-H_2O	G	6	C, H, N[k]	0	2	1	3	4	2
19	6-CF_3	$C_{13}H_{13}N_4F_3 \cdot HCl$	312-313 dec	EtOH-H_2O	C	47	C, H, N	3/10	2/10	0/10	9/10	7/10	6/10
20	6-CN	$C_{13}H_{13}N_5 \cdot AcOH$	174-176	ethanol	D	67	C, H, N		11/36[g]	5/36[g]	24/36[g]	13/36[g]	5/36[g]
21	6-Me	$C_{13}H_{16}N_4 \cdot HCl$	280-283 dec	EtOH-H_2O	A	12	C, H, N	0/12	4/12	0/12	8/12	2/12	1/12
22	6-OMe	$C_{13}H_{16}N_4O \cdot HCl$	300-301 dec	MeOH	C	24	C, H, N, Cl	*e*	1	1		5	3
23	6-SO_2Me	$C_{13}H_{16}N_4O_2S \cdot C_4H_4O_4$[k]	205-206 dec	EtOH	A[i,j]	32	C, H, N	2[l]	1[l]	0[l]	0[l]	0[l]	2[l]

24	6-Cl, 4'-Me	$C_{13}H_{15}N_4Cl\cdot HCl$	292–293	EtOH–H_2O	A	11	C, H, N	1[g]	1[g]	0[g]	4[g]	2[g]	2[g]
25	6-Cl, 4'-Ac	$C_{14}H_{15}N_4ClO$	170–172	*n*-BuCl		52		1	2	2	0	1	0
26	6,7-Cl_2	$C_{12}H_{12}N_4Cl_2\cdot HCl$	347 dec	H_2O	A[j]	83	C, H, N	1	2	0	3	4	1
27	7-Cl	$C_{12}H_{13}N_4Cl\cdot C_4H_4O_4$	216 dec				C, H, N	3[g]	1[g]	0[g]	5[g]	3[g]	1[g]
28	7-NO_2	$C_{12}H_{13}N_5O_2\cdot HCl$	305–306 dec	H_2O	A[i,j]	26	C, H, N	0[l]	0[l]	1[l]	4[l]	5[l]	2[l]
29	7-OMe	$C_{13}H_{16}N_4O\cdot 2HCl$	258–259	MeOH–*i*-PrOH	A	11	C, H, N, Cl	6[l]	5[l]	1[l]			
30	8-F	$C_{12}H_{13}N_4F\cdot C_4H_4O_4$[k]	215–218 dec	EtOH–H_2O	C	(13)	C, H, N, F	*e*	6	1	*e*	1	0
31	3'-one	$C_{12}N_{12}N_4O$	216–217	MeOH	A	53	C, H, N	3	0	0			
32	3'-CO_2H	$C_{13}H_{14}N_4O_2$	241–243 dec[n]	AcOH	A	(4)	C, H, N	0	1	2	0	0	0

[a] Yields are for analytical samples, except crude yields which are in parentheses. [b] Results are expressed as the number of rats exhibiting at least one head twitch/number of rats tested during a 2-min observation period beginning 30 min after ip administration of compound. Unless otherwise noted, six rats were used at each dose level. Doses expressed as base weight. [c] *p*-Chloromethamphetamine (6.0 mg/kg) was administered ip 1 h after ip administration of compound, and the rats were observed as above for head movements 30 min later. Unless otherwise noted, six rats were used at each dose level. The results are expressed as the number of rats *not* exhibiting head twitches after PCMA (PCMA antagonism). [d] ED_{50} = 0.5 (0.3–1.0) mg/kg. Calculated as the base (95% confidence limits). [e] Animals succumbed. [f] H: calcd, 6.02; found, 5.59. [g] Tested at 9.0, 3.0, and 1.0 mg/kg ip. [h] Tested at 20.0, 5.0, and 1.2 mg/kg ip. [i] *i*-PrOH in place of *n*-BuOH. [j] Reaction carried out at room temperature. [k] Hydrogen fumarate. [l] Tested at 6.0, 2.0, and 0.67 mg/kg ip. [m] No definite melting point or decomposition point. [n] AcOH solvate.

Table II. Chemical Properties and Head-Twitch Activity of 2-(1-Piperazinyl)-3(4*H*)-quinoxalinone Derivatives



compd	substituents	formula	mp, °C	recrystn solvent	method	% yield[a]	anal.	rat head twitches:[b] dose, mg/kg ip 40	4	0.4	PCMA antagonism:[c] dose, mg/kg ip 40	4	0.4
33	H	$C_{12}H_{14}N_4O\cdot AcOH$	190–194 dec	2-propanol	E	37	C, H, N	0	6	2	1		
34	4-Me	$C_{13}H_{16}N_4O$	80–82	hexane–CH_2Cl_2	A	(100)	C, H, N	5[d]	3[d]	1[d]	0[d]	2[d]	1[d]
35	4'-Me	$C_{13}H_{16}N_4O$	204–204.5	CH_3CN	E	29	H, N; C[e]	6	0	0	0	0	1
36	4-$CH_2CH_2NMe_2$	$C_{16}H_{23}N_5\cdot 2HCl\cdot H_2O$	202–204	2-propanol	F	(26)	C, H, N	1	0	1	0	0	2

[a] Yields are for analytical samples, except crude yields which are in parentheses. [b] Results are expressed as the number of rats exhibiting at least one head twitch/number of rats tested during a 2-min observation period beginning 30 min after ip administration of compound. Unless otherwise noted, six rats were used at each dose level. Doses expressed as base weight. [c] *p*-Chloromethamphetamine (6.0 mg/kg) was administered ip 1 h after ip administration of compound, and the rats were observed for head movements 30 min later. Unless otherwise noted, six rats were used at each dose level. [d] Tested at 9.0, 3.0, and 1.0 mg/kg ip. [e] C: calcd, 63.91; found, 64.48.

Table III. 2-(1*H*)-Quinoxalinones

compd	substituents	method	% yield	mp, °C	solvent	spectra
9b	5-F	L	16	289.5–291	THF	δ F 144.4 (q of d, J = 5, 8, and <1 Hz) Me_2SO-d_6/$FCCl_3$
10b	5-Cl	M		313–315		
11b	5-CH_3	M		286–288	HOAc	δ H_3 8.67, δ CH_3 2.84 (CF_3CO_2D)
12b	5-OCH_3	M		226–228		
13b	6-F	K	35	300–302	THF	
15b	6-Br	N		298–300	HOAc	
19b	6-CF_3	M		252–253	EtOH	
21b	6-CH_3	K[a]		266–268	(crude)	
23b	6-SO_2CH_3	K		347–350	(crude)	δ $H_{3,5}$ 8.33 (br s), δ $H_{7,8}$ 7.82 (AB)
30b	8-F	J	22	208–210	i-C_3H_7OH	δ F 145.4 (d of d, J = 5 and 8 Hz)
34b	4-CH_3, 3-oxo	F		283–285	(crude)	
36b	4-$CH_2CH_2N(CH_3)_2$, 3-oxo	F		not isolated		

[a] Analogous method; from 3-fluoro-4-nitrotoluene.

Table IV. 2-Chloroquinoxaline Intermediates

compd	substituents	method	% yield	mp, °C	solvent	spectra
6a	3-SC_6H_5, 6-Cl	O		92–98	n-C_6H_{14}	δ H_8 7.83 (J_{AB} = 9 Hz)
7a	3-SC_6H_5, 6-CF_3	O[a]	24	105.5–106.5	n-C_6H_{14}	
9a	5-F	O-B[a]	89	88–89[b]		M^+ 182.0042[d] (182.00469)
10a	5-Cl	O-B[a]		132–135[b,e]		
11a	5-CH_3	O-B[a]		93–94[b,e]		
12a	5-OCH_3	O-B[a]		111–113[b,e]		δ H_3 8.76, δ $H_{6,7,8}$ 7.67 (d), 7.12 (m), δ OCH_3 4.04
13a	6-F	O-B[a]		129–132[b]		δ H_3 8.94; M^+ 182.0040[d]
15a	6-Br	O-B[a]		150–152.5[b]		δ H_3 8.86, δ H_5 8.37 (br s), δ $H_{7,8}$ 7.95 (sharp s)
17a	6-OH	O-A	88			
19a	6-CF_3	O-B[a]		118–120[b]		δ H_3 8.25 (Me_2SO-d_6)
21a	6-CH_3	O-B[a]		102–104[b,e]		
23a	6-SO_2CH_3	O-B[a]		not isolated		
30a	8-F	O-B[a]		112–118[b,c]		M^+ 182.0038[d]
4	4-CH_3, 3-oxo	F[a]		129–130[b]		
36a	4-$CH_2CH_2N(CH_3)_2$, 3-oxo	F				

[a] Analogous method. [b] Sublimed. [c] Contained 1–5% 5-isomer by NMR. [d] Molecular ion and empirical formula obtained by high-resolution mass spectroscopy match calculated values. [e] See ref 7a.

sensitive than the agonist rceptor sites to steric effects. The relative inactivity of the 5-substituted series, other than 5-F, is consistent with this view and suggests that subtle electronic factors may also play a role in determining activity. Inactivity of the *N*-methyl and *N*-acetyl analogues of **14** (**24** and **25**, respectively) suggests that an NH function is necessary for reuptake blocking action.

Experimental Section

All melting points were obtained on a Thomas-Hoover Unimelt capillary melting point apparatus using open capillaries and are uncorrected. Microanalytical results on new compounds are indicated by atomic symbols and are within ±0.4% of theoretical values unless otherwise noted. NMR spectra were recorded on Varian T-60 or EM-390 spectrometers in $CDCl_3$–Me_4Si unless otherwise specified.

2-(1-Piperazinyl)quinoxalines. Method A. 2-(1-Piperazinyl)quinoxaline Hydrochloride (1). A mixture of 164 g (1.00 mol) of 2-chloroquinoxaline and 172 g (2.00 mol) of anhydrous piperazine in 1 L of 2-butanol was stirred at reflux under N_2 for 16 h. After concentration under reduced pressure, the residue was partitioned between 1 L of 2 N Na_2CO_3 solution and 1.5 L of $CHCl_3$. The combined $CHCl_3$ extract was washed with dilute NaOH until free of piperazine and then dried (K_2CO_3), filtered, and concentrated under reduced pressure to give the crude base, which was treated with EtOH–HCl in EtOH to give the hydrochloride. Recrystallization from EtOH gave the analytical sample; see Table I for salts, reaction conditions, and physical data for this and other analogues.

Method B. 3-Amino-2-(1-piperazinyl)quinoxaline Dihydrochloride (4). 3-Amino-2-chloroquinoxaline (179 g, 1.00 mol) and anhydrous piperazine (172 g, 2.00 mol) were heated in 1 L of DMF at 100 °C with stirring under N_2 for 2.5 h. The cooled mixture was diluted with 3 L of H_2O and filtered. The filtrate was concentrated to 300 mL under vacuum, diluted with 200 mL of saturated Na_2CO_3, and extracted with $CHCl_3$. The combined $CHCl_3$ extract was washed with K_2CO_3 solution, dried (K_2CO_3), filtered, and concentrated under vacuum to give the base, which was converted to the dihydrochloride in EtOH–HCl.

Method C. 5-Fluoro-2-(1-piperazinyl)quinoxaline Hydrogen Fumarate (9). A mixture of 1.4 g (7.7 mmol) of 2-chloro-5-fluoroquinoxaline and 1.3 g (15 mmol) of anhydrous piperazine was stirred at 125–130 °C under N_2 for 1.5 h. The cooled mixture was partitioned between 100 mL of $CHCl_3$ and 100 mL of 1 N K_2CO_3. The combined $CHCl_3$ extract was dried (K_2CO_3), filtered, and concentrated under vacuum to an orange semisolid, which was converted to the salt with fumaric acid in EtOH–H_2O.

Method D. 6-Cyano-2-(1-piperazinyl)quinoxaline Acetate (20). Step A. A mixture of 2,3-dichloro-6-cyanoquinoxaline (94 g, 0.42 mol) and *N*-formylpiperazine (121 g) in 600 mL of MeCN was stirred 3 h at room temperature and diluted with 600 mL 1.2 N HCl to give a precipitate of the crude product, which was leached with boiling $CHCl_3$. The $CHCl_3$-soluble solid was recrystallized from *n*-butyl chloride to give 54.1 g (43%) of 3-chloro-6-cyano-2-(4-formyl-1-piperazinyl)quinoxaline (**38**), mp 198–200 °C.

Step B. The purified product from step A was hydrogenated in 250 mL of DMF and 35 mL of Et_3N in the presence of 3.5 g of 10% Pd/C at an initial pressure of 26 psi for 5.5 h at room temperature. The mixture was filtered through diatomaceous earth, and the cake was washed with DMF (25 mL) and H_2O (750 mL). The cake was extracted with four 150-mL portions of boiling

$CHCl_3$, and the $CHCl_3$ used to extract the filtrate. The $CHCl_3$ extracts were washed with H_2O, dried (Na_2SO_4), filtered, and concentrated in vacuo to give 23.1 g (47%) of 6-cyano-2-(4-formyl-1-piperazinyl)quinoxaline (**39**), mp 208–211 °C.

Step C. The product from step B was hydrolyzed in 250 mL of dioxane and 50 mL of 1.8 M H_2SO_4 at 80 °C for 3 h and the mixture cooled to give 23.8 g of crude 6-cyano-2-(1-piperazinyl)quinoxaline bisulfate, which was partitioned between aqueous Na_2CO_3 and $CHCl_3$. The base, isolated from the dried and filtered $CHCl_3$ extract by evaporation, was converted to the acetate salt in absolute EtOH containing HOAc. The light yellow crystalline 6-cyano-2-(1-piperazinyl)quinoxaline acetate (**20**), 16.8 g (67%), was collected by suction and dried, mp 174–176 °C.

Method E. 2-(1-Piperazinyl)quinoxalin-3(4*H*)-one Acetate (33). Anhydrous piperazine (313 g, 3.64 mol) and 2,3-dimethoxyquinoxaline (281 g, 1.48 mol) were mixed under N_2 with an efficient mechanical stirrer and heated to 135 °C (internal temperature). Water (26 mL) was added dropwise during 3 h and an exotherm was noted. The mixture was stirred and refluxed for 1 h, after which the distillate was collected up to an internal temperature of 130 °C. After stirring overnight at room temperature, the mixture was diluted with H_2O (350 mL) and filtered, and the crude base (238 g) was washed with H_2O and air-dried. The crude product was boiled with 2.5 L of 2-propanol and filtered, and the filtrate was treated with glacial HOAc (80 mL). The stirred solution was cooled to 40 °C and filtered to give the pure acetate salt (**33**; 158 g, 37%), mp 190–194 °C dec.

Method F. 3-(1-Piperazinyl)-1-[2-(dimethylamino)ethyl]-2(1*H*)-quinoxalinone (36). Step A. A solution of 2-chloronitrobenzene (15.8 g, 0.10 mol) and 2-(dimethylamino)ethylamine (8.8 g, 0.10 mol) in 100 mL of MeCN was refluxed for 18 h, the solvent was replaced with Me_2SO (100 mL), and heating continued for 3 h at 80–90 °C. The mixture was basified with dilute Na_2CO_3 solution and extracted with CH_2Cl_2. The CH_2Cl_2 extract was extracted with dilute HCl, and the aqueous acid extracts were basified with Na_2CO_3 solution and extracted with hexane. The hexane extracts were concentrated in vacuo to an oil, which was treated with EtOH–HCl in 2-propanol to give the crude hydrochloride of 2-[[2-(dimethylamino)ethyl]amino]-nitrobenzene: yield, 5.1 g; mp 179–181 °C.

Step B. Hydrogenation of 4.91 g (20 mmol) of the hydrochloride from step A in 50 mL of absolute EtOH with 0.4 g of 10% Pd/C for 15 min resulted in the theoretical H_2 uptake. The solution was filtered through diatomaceous earth, and the filtrate was treated with 10 mL of diethyl oxalate at reflux for 3 h with removal of EtOH. The reaction residue was filtered to give 4.2 g of green solid, which was recrystallized from EtOH–H_2O to give 2.37 g of crude 1-[2-(dimethylamino)ethyl]-2,3(1*H*,4*H*)-quinoxalinedione (**36b**), which was added to a cooled mixture of 2.5 mL of DMF and 3 mL of $POCl_3$. The resulting mixture was heated at 95 °C for 30 min under N_2. The mixture was cooled, diluted with CH_2Cl_2, and filtered to give 2.35 g of crude 1-[2-(dimethylamino)ethyl]-3-chloro-2(1*H*)-quinoxalinone hydrochloride. The crude hydrochloride was treated with a solution of piperazine (5 g) in 15 mL of MeCN with stirring for 4.5 h at room temperature. The mixture was concentrated in vacuo and diluted with 40 mL of H_2O containing 1 g of KOH, and the solution was filtered and extracted with CH_2Cl_2. The CH_2Cl_2 extracts were washed with H_2O, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was taken up in 2-propanol, the solution was filtered and treated with EtOH-HCl, and the crude hydrochloride was collected on cooling (2.05 g, 26%). Recrystallization from 2-propanol gave pure 3-(1-piperazinyl)-1-[[2-(dimethylamino)ethyl]amino]-2(1*H*)-quinoxalinone dihydrochloride hydrate, mp 202–204 °C.

Method G. 6-Amino-2-(1-piperazinyl)quinoxaline Hydrogen Fumarate (18). 6-Nitro-2-(1-piperazinyl)quinoxaline hydrochloride was neutralized with a solution of 1.1 g of Na_2CO_3 in 50 mL of H_2O, and the base was extracted with EtOAc. The EtOAc solution of the base (1.4 g, 5.4 mmol) was hydrogenated at atmospheric pressure over a 10% Pd/C catalyst until 3 equiv of H_2 had been taken up. After filtering, the filtrate was concentrated under reduced pressure and the residue was converted to a crude hydrogen fumarate salt in EtOH. The impure salt was chromatographed on 25 g of polymethacrylic acid resin and the product was isolated by elution with 1 M HOAc. Fractions containing product were combined and lyophilized to a yellow solid, which was treated with 8 mL of 0.4 M fumaric acid in 95% ethanol to give 0.22 g of the pure salt, which crystallized slowly.

Method H. 6-Hydroxy-2-(1-piperazinyl)quinoxaline Acetate (17). Step A. 6-Methoxy-2-chloroquinoxaline (3 g, 0.015 mol) was added to a suspension of 4.8 g (0.036 mol) of $AlCl_3$ in 100 mL of C_6H_6. The mixture was refluxed for 3 h and concentrated to dryness, and the residue was slurried with 50 mL of ice–H_2O and filtered to give 2.4 g (88%) of 6-hydroxy-2-chloroquinoxaline as a tan solid, mp 246 °C dec.

Step B. The crude product of step A (2.4 g, 14 mmol) and piperazine (4.9 g, 57 mmol) were combined neat, and the mixture was heated under N_2 to 140 °C (bath temperature) for 3 h. Excess piperazine was sublimed in vacuo, and the cooled residue was triturated with H_2O (20 mL), filtered, washed with H_2O, and taken up in MeOH. The filtered MeOH solution was treated with 40 mL of 0.4 M fumaric acid in 95% EtOH–H_2O. The precipitated hydrogen fumarate was dissolved in H_2O. The solution was fed to an IRA 120 (H^+ form) ion-exchange resin column, and the free base was eluted with 4 N NH_4OH. Product containing fractions were fed to an IRC 50 (H^+ form) resin column, which was eluted with 1 N aqueous HOAc. Piperazine was eluted first, followed by fractions containing product which were combined and lyophilized. The crude product was recrystallized from 20 mL of 2-propanol to give pure product (0.5 g, 12%), mp 185–187 °C.

Method I. Photofluorination of 2-(1-Piperazinyl)-quinoxaline. Employing a method previously described,[6] 1.07 g (5.00 mmol) of 2-(1-piperazinyl)quinoxaline in 20 mL of HF was treated with a stream of F_2–He at dry ice–Me_2CO temperature under irradiation by an unfiltered 2500-W external Hanovia high-pressure mercury lamp. After 10 mmol of F_2 (calibrated gauge) had passed through the solution, the mixture was purged with He and brought to room temperature while HF evaporated, and the crude residue was partitioned between aqueous Na_2CO_3 and $CHCl_3$. The $CHCl_3$ extracts were dried (Na_2SO_4), filtered, and concentrated under vacuum to give 0.62 g of solid residue, mp 69–79 °C. F microanalysis indicated that the crude product contained 50–60% monofluoro derivative and ^{19}F NMR indicated a single monofluorinated product (multiplet at +151.3 ppm relative to $FCCl_3$).

The crude base was treated with 10 mL of 0.4 M fumaric acid in 95% aqueous EtOH and 25 mL of absolute EtOH to give 0.25 g of 5-fluoro-2-(1-piperazinyl)quinoxaline hydrogen fumarate (**9**), mp 208–208.5 °C, not depressed by mixture with the product from **9a** (see Table I).

Quinoxaline Intermediates. Method J. 8-Fluoroquinoxalin-2(1*H*)-one (30b). Step A. Glycine ethyl ester hydrochloride (15 g, 0.107 mol) was slurried in C_6H_6 and treated with 11 mL of 10 N NaOH with stirring at 5–10 °C. The mixture was treated with K_2CO_3 (anhydrous) until the aqueous phase was a thick paste and the C_6H_6 layer was decanted.

The decanted C_6H_6 layer was further dried with K_2CO_3 (anhydrous) and treated with 7.0 g (44 mmol) of 2,6-difluoronitrobenzene at reflux for 20 h under N_2. The mixture was cooled, washed with H_2O, dried ($MgSO_4$), filtered, and concentrated under vacuum to give a red oil which was chromatographed on activity III alumina. Elution with C_6H_6 gave fractions containing 2.30 g of *N*-(3-fluoro-2-nitrophenyl)glycine ethyl ester (**42a**), mp 61–63 °C.

Step B. To a stirred suspension of 2.02 g (8.35 mmol) of *N*-(3-fluoro-2-nitrophenyl)glycine ethyl ester and 4.0 g of Sn (33.6 g-atoms) in 13 mL of absolute EtOH was added 8.3 mL of concentrated HCl. The mixture was stirred for 45 min until the mild exotherm subsided and then heated for 45 min on the steam bath. The clear, colorless solution was saturated with H_2S and filtered hot through diatomaceous earth. The filtrate was adjusted to pH 10 with Na_2CO_3, cooled, and filtered to give crude 8-fluoro-3,4-dihydro-2(1*H*)-ketoquinoxaline, which was slurried in 19 mL of 8% NaOH solution. The resulting suspension was treated with 1.7 mL of 30% H_2O_2 and heated for 15 min on the steam bath until a clear solution formed. The mixture was filtered and acidified with glacial HOAc to give the crude product, which was recrystallized from boiling 2-propanol (filtered to remove insolubles) to give 8-fluoro-2(1*H*)-quinoxalinone (**30b**): yield 0.30 g (22%); mp 208–210 °C.

Method K. 6-Fluoroquinoxalin-2(1*H*)-one (13b). Step A. In a sequence similar to method J, 14 g (58%) of *N*-(5-fluoro-2-nitrophenyl)glycine ethyl ester, mp 116–117 °C (*n*-butyl chloride), was prepared from 15.9 g (0.10 mol) of 2,4-difluoronitrobenzene: ^{1}H NMR δ 8.52 (1 H, s), 8.24 (1 H, d of d, J = 9 and 6 Hz), 6.42 (2 H, complex) (inter alia).

Step B. Reduction of 12.1 g (50 mmol) of *N*-(5-fluoro-2-nitrophenyl)glycine ethyl ester with 45.4 g of mossy Sn (0.381 g-atom) in HCl, followed by oxidation of the crude dihydro intermediate with H_2O_2–NaOH, gave 2.83 g (35%) of crude 6-fluoro-2(1*H*)-quinoxalinone (**13b**), mp 274–287 °C dec. Recrystallation from THF gave the pure product: mp 300–302 °C; for NMR, see Table III.

***N*-[5-(Methylthio)-2-nitrophenyl]glycine Ethyl Ester.** MeSH was bubbled through a solution of 1.2 g (0.052 g-atom) of Na in 100 mL of absolute EtOH (under N_2) until saturated. To the resulting stirred solution was added 12.11 g (50 mmol) of *N*-(5-fluoro-2-nitrophenyl)glycine ethyl ester (method K, step A) at room temperature. After 1 h at room temperature, the thick suspension was filtered, and the cake was washed with EtOH and recrystallized from *n*-butyl chloride to give 12.6 g (82%) of deep yellow prisms: mp 80–81 °C; ^{1}H NMR δ 8.63 (1 H, br, NH), 8.17 (1 H, d, J = 9.5 Hz), 6.62 (1 H, d of d, J = 9.5 and 1.5 Hz), 6.44 (1 H, d, J = 1.5 Hz), 4.37 (2 H, q, J = 7.5 Hz), 4.12 (2 H, d, J = 5 Hz), 2.5 (3 H, s), 1.35 (3 H, t, J = 7.5 Hz).

***N*-[5-(Methylsulfonyl)-2-nitrophenyl]glycine Ethyl Ester.** The ester **42** (12.2 g, 45.2 mmol) was added to a stirred solution of 4.0 mL of 30% aqueous H_2O_2 in 200 mL of glacial HOAc. After 1.5 h, 1 mL of additional 30% H_2O_2 was added at 30–35 °C, and the mixture was maintained at this temperature for 3 h longer and treated with 5 mL of additional 30% H_2O_2. The mixture was warmed gently on the steam bath 2 h, diluted with 200 mL of ice–H_2O, cooled, and filtered to give 17.0 g of crude product which was recrystallized from C_6H_6 to give 11.0 g (80%) of yellow orange prisms: mp 156–157 °C; ^{1}H NMR ($CDCl_3$) δ 6.69 (1 H, d, J = 8.5 Hz), 3.08 (3 H, s) (inter alia).

6-(Methylsulfonyl)-2-(1-piperazinyl)quinoxaline (23). A suspension of 10.8 g (33.3 mmol) of the ester **43** and 1.0 g of 10% Pd/C in 200 mL of absolute EtOH was hydrogenated in a Parr shaker until theoretical H_2 had been absorbed (1 h) and filtered through diatomaceous earth, and the filtrate was concentrated under vacuum. The tan solid residue was treated directly with a solution of 12.3 g (72.5 mmol) of $AgNO_3$ in 100 mL of H_2O, to which had been added sufficient concentrated NH_4OH to dissolve the initial Ag_2O precipitate. The resulting mixture was refluxed vigorously for 1 h and filtered hot through diatomaceous earth, and the filtrate was made strongly basic with NaOH and refiltered. The filtrate was then acidified with glacial HOAc to give 3.75 g of tan precipitate, mp 347–350 °C, identified as crude 6-(methylsulfonyl)quinoxalin-2(1*H*)-one (**23b**) by its NMR spectrum (Table III). The crude quinoxalinone was dried in vacuo and converted to the title compound by reaction with $POCl_3$ (7.5 mL) and *N*,*N*-dimethylformamide (0.1 mL) for 1 h at 100 °C. The crude 6-(methylsulfonyl)-2-chloroquinoxaline was isolated by removal of $POCl_3$ under high vacuum and reacted directly with piperazine in 2-propanol at room temperature to give 6-(methylsulfonyl)-2-(1-piperazinyl)quinoxaline (**23**), isolated as the hydrogen fumarate (**23**; Table I).

Method L. 5-Fluoro- and 8-Fluoroquinoxalin-2(1*H*)-ones (9b and 30b). To a solution of 3-fluoro-1,2-diaminobenzene (prepared from 2-fluoro-6-nitroaniline, 40.4 mmol, according to Kirk and Cohen[9]) in 50 mL of 95% aqueous ethanol was added 6.5 g (50 mmol) of *n*-butyl glyoxylate. The mixture was refluxed for 3 h under N_2 and cooled to 0 °C overnight. Filtration gave 8.8 g of crude product, which was heated with 4.8 g of Na_2CO_3 in 50 mL of H_2O, and the solution was filtered. The dark filtrate was treated with 2.6 mL of glacial HOAc, heated to steam-bath temperature, and filtered to give 1.8 g of crude 5-isomer (**5a**), mp 268–282 °C. The filtrate was acidified to pH 6 and filtered to give the crude 8-isomer, which was recrystallized from 2-propanol to give 1.5 g (23%) of 8-isomer (**30b**), mp 206–208 °C, mmp with product from method J undepressed.

The crude 5-isomer was heated with a solution of 1.7 g of $NaHCO_3$ in 30 mL of H_2O, and the suspension was filtered hot to give a cake which was recrystallized from THF to give 1.09 g (16%) of 5-fluoroquinoxalin-2(1*H*)-one (9b), mp 289.5–291 °C.

Method M. Condensation of 4-(Trifluoromethyl)-1,2-benzenediamine with Glyoxylic Acid. 2-Nitro-4-(trifluoromethyl)benzenamine (176 g, 0.85 mol) was hydrogenated in 2 L of EtOH in the presence of 4.4 g of 10% Pd/C at 40 psi of H_2 for 3.5 h at 25–60 °C in a Parr apparatus. The resulting suspension was treated with 400 mL of 2 N HCl and filtered through diatomaceous earth. The filtrate was treated with 100 g of glyoxylic acid·H_2O and stirred for 3 h at room temperature. The thick precipitate was collected and washed with H_2O to give crude product, mp 175–202 °C. Recrystallization from 650 mL of 1,2-dimethoxyethane gave 18.6 g, mp 249–252 °C, which was further recrystallized from absolute EtOH to give pure 6-(trifluoromethyl)-2(1*H*)-quinoxalinone (**19b**), mp 252–253 °C.

Method N. Bromination of Quinoxalin-2(1*H*)-one. To a stirred solution of quinoxalin-2(1*H*)-one (14.6 g, 0.100 mol) and Ag_2SO_4 (15.6 g, 0.050 mol) in concentrated H_2SO_4 (100 mL) at 20 °C was added Br_2 (5.2 mL, 0.10 mol). The mixture was stirred with an efficient mechanical stirrer for 24 h, diluted with CCl_4 (100 mL), heated to 50 °C, and filtered. The deep red filtrate was poured onto crushed ice, and the precipitate was collected and recrystallized twice from HOAc to give 9.0 g of tan plates of 6-bromoquinoxalin-2(1*H*)-one (**15b**): mp 298–300 °C; ^{1}H NMR (NaOD, D_2O) δ 8.14 (1 H, br s), 7.61 (1 H, d of m), 7.30 (1 H, d of m), 7.25 (1 H, m).

Bromination of 1.46 g (10.0 mmol) of quinoxalin-2(1*H*)-one in HOAc[11b] gave 1.18 g of russet crystals (dec 210–220 °C without melting) of 6-bromoquinoxalin-2(1*H*)-one (**15b**): ^{1}H NMR (Me_2SO-d_6) δ 12.5 (1 H, br), 8.23 (1 H, s), 7.75 (1 H, m), 7.46 (2 H, m). Addition of a sample of the 6-isomer gave new signals, including δ 8.00 (1 H, d, $J \approx 2$ Hz) which was clearly resolved for the 6-isomer, indicating that little, if any, 6-isomer is formed under these conditions.

2-Chloroquinoxaline Intermediates. Method O. 6-(Trifluoromethyl)-3-(phenylthio)-2-chloroquinoxaline. Step A. A solution of 4-amino-3-nitrobenzotrifluoride (20.6 g, 0.100 mol) in 250 mL of absolute EtOH was hydrogenated with 0.50 g of 10% Pd/C catalyst in a Parr apparatus. After 1.5 h, the theoretical amount of H_2 had been absorbed, and the mixture was vented to N_2 and filtered through diatomaceous earth into a flask containing 150 mL of diethyl oxalate. The filtrate was refluxed with removal of EtOH until an exotherm occurred and a tan solid separated. The mixture was cooled and filtered to give 20.94 g of crude 6-(trifluoromethyl)quinoxaline-2,3(1*H*,4*H*)-dione, mp 342–343 °C dec.

Step B. The crude product from step A (18.4 g, 0.080 mol) was suspended in 150 mL of $POCl_3$, and the mixture was treated with 2 mL of DMF and refluxed for 1.5 h under N_2. The mixture was cooled and concentrated under vacuum, and the residue was carefully quenched on crushed ice to give a buff solid, which was recrystallized from *n*-C_4H_9Cl to give pink plates of 6-(trifluoromethyl)-2,3-dichloroquinoxaline, mp 81.5–82.5 °C (17.8 g).

Step C. The product from step B (2.67 g, 10.0 mmol) was combined with 2.15 g (10.1 mmol) of 1,8-bis(dimethylamino)-naphthalene in 100 mL of C_6H_6, and the stirred solution was cooled under N_2 to 0–5 °C and treated dropwise with a solution of 1.04 mL (1.11 g, 10.1 mmol) of freshly distilled thiophenol in 30 mL of C_6H_6. The mixture, containing a precipitate, was stirred for 1 h, warmed to room temperature, and filtered to remove the hydrochloride of the starting diamine. The filtrate was concentrated under vacuum and the residue was leached with boiling hexane, which was filtered and cooled to give 0.86 g of crude product, mp 101–105 °C. Recrystallization from hexane gave pure 6-(trifluoromethyl)-3-(phenylthio)-2-chloroquinoxaline (**7a**): mp 105.5–106.5 °C; ^{1}H NMR δ 8.2 (1 H, br m), 7.71 (2 H, AB), 7.5 (5 H, m).

Biological Assay for Central Serotoninmimetic Activity. Central serotonin activation was assessed in female Sprague–Dawley rats (150–250 g) on a quantal basis as previously described by Corne, Pickering, and Warner.[12] Results are expressed as the number of rats exhibiting at least one head twitch/number of rats tested during a 2-min observation period beginning 30 min after ip administration of compound. With control animals receiving vehicle (1% methylcellulose) alone, 30 of 260 (12%) exhibited head twitches.

Serotonin neuronal reuptake blocking activity was determined by the ability of the compounds to antagonize the head twitch

response induced by *p*-chloromethamphetamine (PCMA). PCMA, 6.0 mg/kg, was administered ip 1 h after ip administration of the test compound, and the rats were observed for head movements 30 min later. Of 232 control animals treated with drug vehicle (1% methylcellulose) prior to PCMA, 215 (93%) exhibited head twitches. ED_{50} values are defined as the dose of test compound necessary to antagonize the head twitch response induced by PCMA in 50% of the animals.

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Fluorinated Retinoic Acids and Their Analogues. 2. Synthesis and Biological Activity of Aromatic 4-Fluoro Analogues[1]

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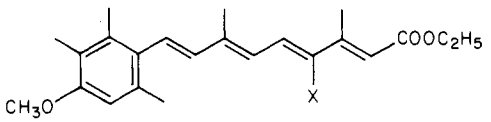
Ethyl (*E,Z,E,E*)-3,7-dimethyl-4-fluoro-9-(4-methoxy-2,3,6-trimethylphenyl)nonatetraenoate (**10a**) has been found to cause a marked regression of chemically induced skin papillomas in mice. A new synthesis of this compound was achieved by condensation of 4-fluoro aldehyde **7** or **8** with the aromatic phosphonium salt **9a**. Several analogues (**10a–e**) having different substituted aromatic moieties were also prepared and tested for their antipapilloma effect. The monochloro analogue **10b** was shown to have comparable activity to the parent compound **10a**.

The prophylactic and therapeutic effect of an aromatic retinoic acid analogue, **1**, on chemically induced benign and

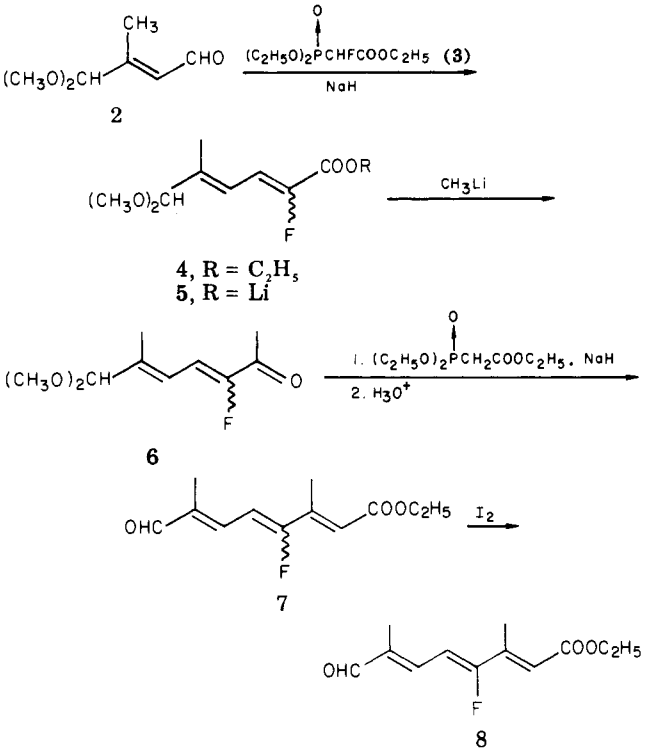


1, X = H
10a, X = F

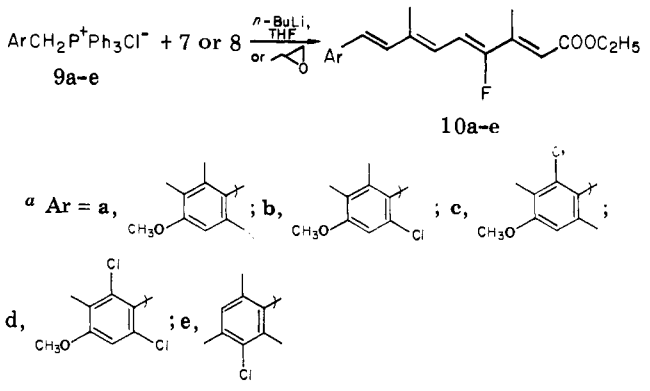
malignant epithelial tumors in mice has been well demonstrated.[2-4] In an effort to search for more effective compounds, we synthesized and studied the biological properties of various fluorinated analogues of **1** having one or two fluorine atoms or a trifluoromethyl group at different positions of the side chain.[1b] Among these analogues, the 4-fluoro derivative **10a** appeared to possess higher biological activity than compound **1** in the mouse skin antipapilloma test.[1b] Preparation of other substituted aromatic analogues of **10a** therefore was of interest. In this paper we describe the synthesis and biological activity of the aromatic 4-fluororetinoic acid analogues **10a–e** (Scheme II).

Chemistry. Preparation of analogues of **10a** having different substituted aromatic groups first required a practical synthesis of the fluoro side-chain aldehyde **7** or **8** (Scheme I). Treatment of the readily available 4,4-dimethoxy-3-methyl-2-butenal[6] (**2**) with the anion of triethyl fluorophosphonoacetate[7] afforded the 2-fluoro ester

Scheme I



Scheme II[a]



[a] Ar = a, ; b, ; c, ; d, ; e,

4 (58% yield) as a mixture of 2*Z* and 2*E* isomers. The lithium salt **5**, prepared from **4** with LiOH, was converted

(1) (a) This paper has been presented, see K.-K. Chan, "Abstracts of Papers", 178th National Meeting of the American Chemical Society, Washington, D.C., Sept 9–14, 1979; American Chemical Society: Washington, D.C., 1979; Abstr MEDI 62. (b) For paper 1 in this series, see Pawson, B. A.; Chan, K.-K.; DeNoble, J.; Han, R. J.; Piermattie, V.; Specian, A. C.; Srisethnil, S.; Trown, P. W.; Bohoslawec, O.; Machlin, L. J.; Gabriel, E. *J. Med. Chem.* **1979**, *22*, 1059.
(2) Bollag, W. *Experientia* **1974**, *30*, 1198.
(3) Bollag, W. *Chemotherapy (Basel)* **1975**, *21*, 236.
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(6) Isler, O.; Ed. "Carotenoids"; Birkhäuser Verlag: Basel, 1971; pp 389–397. We thank Dr. Surmatis of Hoffman-La Roche Inc., Nutley, N.J., for supplying us a large amount of compound **2**.

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