Synthesis and Vasodilator Activity of New Piperazine Derivatives

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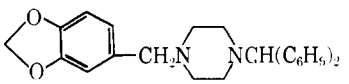
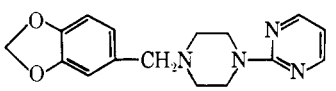
Thirty-seven 1,4-disubstituted piperazines have been prepared in which the 1 substituents are benzyl or phenylalkyl or its mono- or polyalkoxy, alkylenedioxy, or alkoxyhydroxy derivatives, and the 4 substituents are pyrimidyl and its substituted derivatives, quinazolinyl or triazinyl. Some derivatives were found to have potent vasodilating properties in anesthetized rabbits. Furthermore some of them exhibit analgetic and antiinflammatory properties. A structure–activity relationship study was carried out.

A considerable body of literature is recorded on the biological effects of compounds containing the piperazine moiety. In the field of the 4-substituted 1-aralkylpiperazines, hypotensive and vasodilator effects have been reported.[1] More recently, we have described[2] the potent coronary vasodilator properties of a piperonylpiperazine derivative (I). This observation stim-



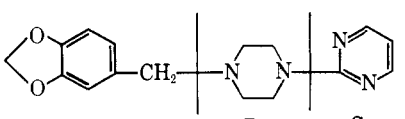
I

ulated our interest in the preparation and testing of other piperonylpiperazine derivatives and led us to synthesize **1**, which was found to have significant peripheral vasodilator properties.[3]



1

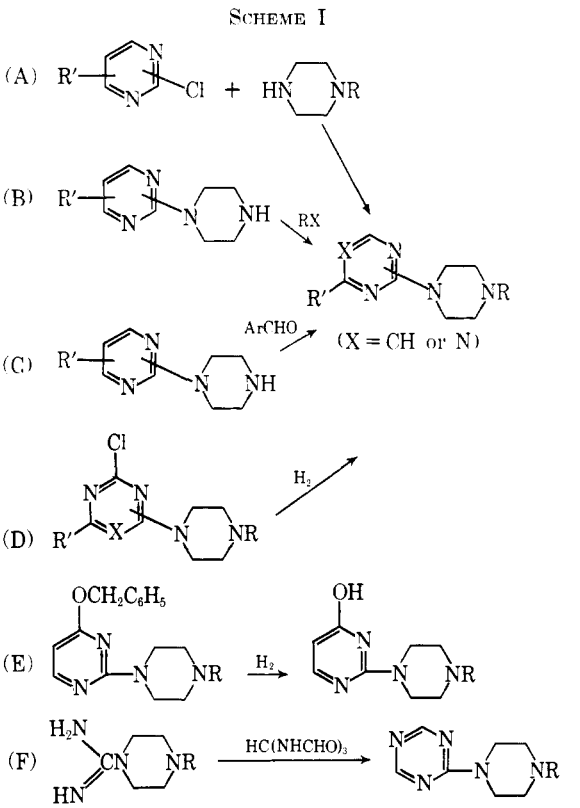
In a study of this class of compounds in depth, the effect on activity with appropriate changes was determined, the structure of **1** being divided into three



A B C

portions. Each parameter was varied selectively. (a) For the modifications of portion A, we replaced the methylenedioxy by polyalkoxy, alkoxyhydroxy, or dihydroxy groups. We studied also the influence of lengthening the distance between the piperazine N and the benzylic C, or the deletion of the methylene group in the A moiety. (b) For the modifications of portion B, we altered piperazine to 2-methylpiperazine, homopiperazine, or N,N′-dimethylethylenediamine. In portion C the pyrimidine group was replaced by alkoxy, alkyl, hydroxy, chloro, N-substituted aminopyrimidine groups, and substituted *s*-triazine or quinazoline groups.

SCHEME I



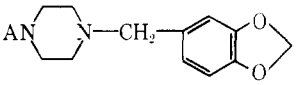
Chemistry.—The piperazine derivatives (Tables I–III) were obtained by six general methods according to Scheme I. In method A, substituted 2- or 4-halogenopyrimidine or its derivatives were generally condensed with the appropriate N-monosubstituted piperazine in DMF in the presence of K_2CO_3. In method B, aralkyl halides were condensed with the appropriate N-monosubstituted piperazine or its derivatives. In method C, condensation of an aromatic aldehyde with an N-monosubstituted piperazine and catalytic hydrogenation under pressure was performed in one step. In method D, the chlorine atom of a 1-substituted 4-(2-chloro-4-pyrimidyl or -*s*-triazinyl)-piperazine was hydrogenolyzed under pressure over Pd–C to yield the 1-substituted 4-(substituted 4-pyrimidyl or 2-*s*-triazinyl)piperazine. In method E, the benzyl group of 1-substituted 4-(4-benzyloxy-2-pyrimidyl)piperazine was hydrogenolyzed under pressure to give the 1-substituted 4-(4-hydroxy-2-pyrimidyl)piperazine, and in method F, a substituted 4-guanidinopiperazine sulfate was treated with triformami-

(1) (a) J. R. Boissier, C. Dumont, R. Ratouis, and J. Pagny, *Arch. Intern. Pharmacodyn.*, **133**, 29 (1961); (b) G. Quesnel, R. Chalaust, H. Schmitt, G. Kroneberg, and H. Schmitt, *ibid.*, **128**, 17 (1960); (c) J. R. Boissier, R. Ratouis, and C. Dumont, *J. Med. Chem.*, **6**, 541 (1963); (d) R. L. Moffitt and R. K. S. Lim, *Federation Proc.*, **15**, 461 (1956).

(2) M. Laubie, J. C. Le Douarec, and H. Schmitt, *Arch. Intern. Pharmacodyn.*, **151**, 313 (1964).

(3) M. Laubie and J. Peltier, Abstracts of Papers, IIIrd International Pharmacological Congress, Sao Paulo, July 24–30, 1966.

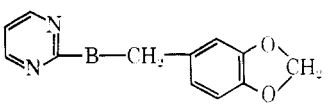
TABLE I



No.	A	Method	Yield crysld, %	Crysln solvent	Mp, °C[b] of amine or salt	Formula[c]
1	2-$C_4H_3N_2$[h]	A	89.5	AE	98	$C_{16}H_{18}N_4O_2$
2	2-Cl-4-$C_4H_2N_2$	A	36	E 98	94	$C_{16}H_{17}ClN_4O_2$
3	4-$C_4H_3N_2$	D	65	AE	192–198	$C_{16}H_{18}N_4O_2 \cdot 2HCl$
4	4-CH_3O-2-$C_4H_2N_2$	A	90	AP	89–90	$C_{17}H_{20}N_4O_3$
5	4-C_2H_5O-2-$C_4H_2N_2$	A	68	AE	225	$C_{18}H_{22}N_4O_3 \cdot 2HCl$
6	4-BzO-2-$C_4H_2N_2$	A	87	AP	104	$C_{23}H_{24}N_4O_3$
7	4-OH-2-$C_4H_2N_2$	E	69.5	DMF	214	$C_{16}H_{18}N_4O_3$
8	4-$OCH_2CO_2C_2H_5$-2-$C_4H_2N_2$	A	25	AE	195–200	$C_{20}H_{24}N_4O_5 \cdot 2HCl \cdot H_2O$
9	5-Cl-2-$C_4H_2N_2$	A	62	E 98	99–101	$C_{16}H_{17}ClN_4O_2$
10	4-CH_3-2-$C_4H_2N_2$	A	61	AE	212–215	$C_{17}H_{20}N_4O_2 \cdot 2HCl$
11	4,6-$(CH_3)_2$-2-C_4HN_2	A	50	AE	256	$C_{18}H_{22}N_4O_2 \cdot 2HCl$
12	4,5-$(CH_3)_2$-2-C_4HN_2	A	44	AE	245	$C_{18}H_{22}N_4O_2 \cdot HCl$
13	4-NH_2-2-$C_4H_2N_2$	A	35	AP	151–152	$C_{16}H_{19}N_5O_2$
14	2-NH_2-4-$C_4H_2N_2$	A	30	AP	171	$C_{16}H_{19}N_5O_2$[f]
15	4-$NHCH_3$-2-$C_4H_2N_2$	A	35	AP	234	$C_{17}H_{21}N_5O_2 \cdot 2CH_4O_3S \cdot H_2O$[e]
16	4-$N(CH_3)_2$-2-$C_4H_2N_2$	A	40	AP	102–103	$C_{18}H_{23}N_5O_2$
17	4-$N(CH_2CH_2OH)_2$-2-$C_4H_2N_2$	A	32	M 98	220–225	$C_{20}H_{27}N_5O_4 \cdot 2HCl$
18	2-$C_3H_2N_3$[i]	F[d]	33.5	M 98	207–211	$C_{15}H_{17}N_5O_2 \cdot 2HCl$
19	4-Cl-6-CH_3O-2-C_3N_3	A	62	M 98	146	$C_{16}H_{18}ClN_5O_3$
20	6-CH_3O-2-C_3HN_3	D	29	AE	230 dec	$C_{16}H_{19}N_5O_3 \cdot HCl$
21	4,6-$(CH_3O)_2$-2-C_3N_3	A	36	AE	250 dec	$C_{17}H_{21}N_5O_4 \cdot HCl$
22	2-$C_8H_5N_2$[j]	A	44	AC	141	$C_{20}H_{20}N_4O_2$
23	4-$C_8H_5N_2$	A	50	AM	230–233	$C_{20}H_{20}N_4O_2 \cdot 2HCl \cdot H_2O$

[a] AE, absolute EtOH; AM, absolute MeOH; AP, absolute *i*-PrOH; E 98, 98% EtOH; M 98, 98% MeOH; AC, EtOAc. [b] Uncorrected melting points, Kofler hot stage microscope. [c] Compound also prepared by acid hydrolysis from **6**. [d] Compound prepared according to ref 4 (see the Experimental Section). [e] All compounds have been analyzed for C, H with results for those elements lying within a ±0.4% limit. [f] The structure of this compound was confirmed by treating **2** with ammoniacal EtOH at 110° (mp 171°). [g] Bis(methane sulfonate). [h] $C_4H_3N_2$ = pyrimidyl ($C_4H_2N_2$ and C_4NH_2 = mono- and disubstituted pyrimidyl, respectively). [i] $C_3H_2N_3$ = *s*-triazinyl (C_3HN_3 and C_3N_3 = mono- and disubstituted *s*-triazinyl, respectively). [j] $C_8H_5N_2$ = quinazolinyl.

TABLE II



No.	B	Method	Yield crysld, %	Crysln solvent[a]	Mp, °C[b] of amine or salt	Formula[d]
24	—$NCH(CH_3)CH_2N(CH_2)_2$—	B	26	AE	195–197	$C_{17}H_{20}N_4O_2 \cdot 2HCl$
25	—$NCH_2CH(CH_3)N(CH_2)_2$—	A	54	AM	225–228	$C_{17}H_{20}N_4O_2 \cdot 2HCl$
26	—$N(CH_2)_2N(CH_2)_3$—	B	59	AE	104	$C_{15}H_{20}N_4O_2$
27	—$N(CH_2)_2N$(CH$_3$)(CH$_3$)—	B	36	AP	180–185	$C_{16}H_{20}N_4O_2 \cdot 2HCl \cdot 0.5H_2O$
28	—$N(CH_2)_2N^+(CH_2)_2$—	c	55	AM	251	$C_{17}H_{21}IN_4O_2$

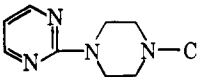
[a,b] See corresponding footnotes in Table I. [c] Compound prepared from **1** by quaternization with MeI. [d] See footnote *e* in Table I.

domethane according to the method of Bredereck, *et al.*[4] The 1-substituted 4-(2-*s*-triazinyl)piperazine was then obtained.

Structure–Activity Relationships. Vasodilating Action (Table IV).—Half of the compounds were inactive as vasodilators; only **29** was analgetic and anti-inflammatory. The active structure seems to be strictly related to the starting compound (**1**). To retain a high activity, the following changes could be made. (a) The methylenedioxy group could only be replaced by ethylenedioxy (**29**), OH- or OCH_3-substituted benzyl derivatives being far less active (**30–34**). Likewise the lengthening of the distance between the piperazine nitrogen and the phenyl ring, or the deletion of methylene groups, decreased the activity (**35–40**). (b) The piperazine moiety could be substituted by CH_3, the most favorable position being 2 (**24**). Quaternization or exchange of the piperazine moiety into homopiperazine or N,N′-dimethylethylenediamine abolished the activity (**26–28**). (c) Among the substituents of the pyrimidine moiety, only 4-CH_3 and 4-NH_2 (**10**, **13**) were the most favorable. If the pyrimidine ring is linked in the 4 position to the piperazine N-atom (**3**), the activity is

(4) H. Bredereck, F. Effenberger, A. Hofmann, and M. Hajek, *Angew. Chem. Intern. Ed. Engl.*, **2**, 655 (1963).

TABLE III



No.	C	Method	Yield crystd, %	Crystn[a] solvent	Mp, °C[b] of amine or salt	Formula[c]
29	3,4-[O(CH$_2$)$_2$O]C$_6$H$_3$CH$_2$	A	45	AE	220–226	C$_{17}$H$_{20}$N$_4$O$_2$·HCl
30	3,4-(CH$_3$O)$_2$C$_6$H$_3$CH$_2$	A	75.5	AP	101	C$_{17}$H$_{22}$N$_4$O$_2$
31	2,4-(CH$_3$O)$_2$C$_6$H$_3$CH$_2$	C	37.5	AE	210–214	C$_{17}$H$_{22}$N$_4$O$_2$·2HCl
32	2,3,4-(CH$_3$O)$_3$C$_6$H$_2$CH$_2$	A	60	AP	105	C$_{18}$H$_{24}$N$_4$O$_3$
33	3-(CH$_3$O)-4-OHC$_6$H$_3$CH$_2$	C	55	AM	185–188	C$_{16}$H$_{20}$N$_4$O$_2$·2HCl·0.5H$_2$O
34	3,4-(OH)$_2$C$_6$H$_3$CH$_2$	C	49	M 98	207–212	C$_{15}$H$_{18}$N$_4$O$_2$·2HCl·0.5H$_2$O
35	2-CH$_3$OC$_6$H$_4$	A	39	AE	73	C$_{15}$H$_{18}$N$_4$O
36	3-CH$_3$OC$_6$H$_4$	A	65	AM	77–78	C$_{15}$H$_{18}$N$_4$O
37	4-CH$_3$OC$_6$H$_4$	A	76	AE	108–110	C$_{15}$H$_{18}$N$_4$O
38	3,4-(OCH$_2$O)C$_6$H$_3$(CH$_2$)$_2$	B	31	AE	233–235	C$_{17}$H$_{20}$N$_4$O$_2$·2HCl·H$_2$O
39	3,4-(OCH$_2$O)C$_6$H$_3$O(CH$_2$)$_2$	B	58	AE	102	C$_{17}$H$_{20}$N$_4$O$_3$
40	(C$_6$H$_5$)$_2$CH	A	26	E 98	170	C$_{21}$H$_{22}$N$_4$

[a-c] See footnotes *a*, *b*, and *e*, respectively, in Table I.

TABLE IV

No.	Toxicity, LD$_{50}$, mg/kg ip (mice)	Vasodilating action[a]	No.	Toxicity, LD$_{50}$, mg/kg ip (mice)	Vasodilating action[a]
1	690.3	++++ d	23	~50	++++ d
3	244	+	24	830	+++
4	622	++	25	~600	++
5	498	0	26	~300	+
7	201	++	27	~75	0
8	>1000	0	28	~125	0
9	393	++	29	720	++++ d
10	~400	+++ d	30	406	+
11	~800	0	31	207	0
12	576	0	32	210	0
13	195	++++	33	~400	0
14	51.6	++++	34	~400	++
15	~150	++ d	35	482	+
16	~150	0	36	178	0
17	~750	0	37	248	+
18	432	0	38	≥400	0
20	~200	Vasoconstrictive effect	39	~1000 *po*	Insoluble in water
21	~400	+++	40	269	++
22	>500	++++ d			

[a] Per cent increase in blood flow of the femoral artery relative to control values: ++++ = 100, +++ = 75–100, ++ = 50–75, + = 25–50. d = duration of action ≥10 min.

lost, unless this ring is 4-substituted by NH_2 (**14**). Likewise, replacement of the pyrimidine by a triazine group led to an inactive substance (**18**). The activity was unexpectedly almost recovered by two additional OCH_3 substituents in the 4 and 6 positions (**21**). At best, the pyrimidine ring could be replaced by quinazoline linked in the 2 or 4 position without losing activity (**22**, **23**). Other cardiovascular tests showed that all these vasodilating substances are inactive in the nictitating membrane test in the cat and reserpine hypertension test in pithed rats. The lack of such properties indicates that these products do not interfere with sympathetic transmission and catecholamine stores. The most active compound, 1-(2-pyrimidyl)-4-piperonylpiperazine (**1**), was tested in anesthetized dogs. Slow intravenous perfusion (10 μg/kg/min during 10 min) did not affect arterial pressure nor cardiac frequency and output. The venous and arterial femoral output were raised considerably (>100%) and this increase lasted longer than 60 min. The same effects were observed by intraduodenal administration of 0.5–1 mg/kg of **1**. This compound, as all the others, had no action on the coronary, cerebral, and renal circulations.

Analgetic and Antiinflammatory Actions.—Two compounds (**29**, **38**) were as active as aminopyrine at 100 mg/kg and 50 mg/kg ip, respectively, in the modified hot plate test. Only three compounds (**13**, **38**, **39**) were equipotent to phenylbutazone and four (**1**, **7**, **12**, **30**) exhibited a mild activity in the kaolin paw edema test.

Experimental Section[5]

Pharmacological Methods. (a) Vasodilating Action (Table IV).—Drugs were studied for possible vasodilating activity in anesthetized rabbits. The femoral artery was perfused at constant pressure. The substances were administered by the intravenous route at a dosage range of 1–5 mg/kg. The changes in flow of the femoral artery reflected the changes in the vascular

(5) All melting and boiling points are not corrected. Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for those elements or functions were within ±0.4% of the theoretical values.

resistance in the limb. The scale of activities was determined by the per cent increase in blood flow of the femoral artery relative to control values.

(b) Circulatory Action in the Dog.—The circulatory effects of the most active substance (**1**) were tested in dogs:[3] cardiac output by Fick's method,[6a] cutaneous and muscular circulation by determination of femoral artery flow, cerebral circulation by Bovet's method,[6b] andrenal circulation by PAH and creatinine method.[7]

(c) Analgetic and Antiinflammatory Actions.—The analgetic action was studied in mice by the modified hot plate test and the antiinflammatory action in rats, by the kaolin paw edema method.[8,9]

Substituted Halogenopyrimidines and Derivatives.—The following compounds were prepared according to the literature methods: 2-chloropyrimidine,[10] 4-methoxy-2-chloropyrimidine and 4-ethoxy-2-chloropyrimidine,[11] 4-amino-2-chloropyrimidine and 2-amino-4-chloropyrimidine,[12] 4-dimethylamino-2-chloropyrimidine,[13] 4-methyl-2-chloropyrimidine,[14] 4,5-dimethyl-2-chloropyrimidine,[15] 4,6-dimethyl-2-chloropyrimidine,[16] 2,4-dichloropyrimidine,[17a] 2,5-dichloropyrimidine,[17b] 4,6-dimethoxy-2-chloro-*s*-triazine,[18] 6-methoxy-2,4-dichloro-*s*-triazine,[18] 2-chloroquinazoline,[19] 4-chloroquinazoline.[20]

The following compounds were prepared in our laboratory: 4-methylamino-2-chloropyrimidine (mp 132°) and 4-bis(hydroxyethyl)amino-2-chloropyrimidine (mp 110–112°) according to ref 13 and 4-benzyloxy-2-chloropyrimidine (mp 80°) and 4-carbethoxymethoxy-2-chloropyrimidine [bp 105–108° (0.7 mm)] according to ref 11 from 2,4-dichloropyrimidine.

Substituted Aralkyl Halides.—1-(3,4-Methylenedioxyphenyl)-2-chloroethane was prepared from alcohol,[21] by chlorination with $SOCl_2$ [bp 121–125° (0.9 mm)]. 1-(3,4-Methylenedioxyphenoxy)-2-bromoethane was prepared from safrole and 1,2-dibromoethane in the presence of NaOEt (mp 65–67°).

Substituted piperazines used as intermediates were obtained from commercial sources, while others were prepared according to the literature methods: 1-(3,4-dimethoxybenzyl)piperazine,[16] 1-(2,3,4-trimethoxybenzyl)piperazine,[22] 1-(3,4-ethylenedioxybenzyl)piperazine,[23] 1-(3,4-methylenedioxybenzyl)piperazine,[23] and 1-benzhydrylpiperazine.[24]

1-(3,4-Methylenedioxybenzyl)-2-methylpiperazine.—A solution of 70 g (0.408 mole) of 1-carbethoxy-3-methylpiperazine,[25] 70.6 g (0.414 mole) of piperonyl chloride in 500 ml of DMF, with 90 g of K_2CO_3, was stirred under reflux for 4 hr. After cooling, the salt was filtered off, and the solvent was evaporated to dryness *in vacuo*. The oily residue was dissolved in 250 ml of ether and the solution was extracted several times into 2 *N* HCl. The acid solution was then rendered alkaline with excess K_2CO_3 and extracted with ether. After removal of the solvent *in vacuo*, an oily residue weighing 125 g was obtained. This was dissolved in 1750 ml of 95% EtOH and stirred under reflux with 475 g of KOH pellets for 27 hr. After removal of the solvent *in vacuo*, the residue was treated with H_2O (1 l.) and extracted ($CHCl_3$, 500 ml). The extract was dried (K_2CO_3) and the solvent was evaporated *in vacuo*. The oily residue was distilled and gave 63 g (66%) of pure product, bp 155–160° (1 mm). *Anal.* ($C_{13}H_{18}N_2O_2$) C, H, N.

1-(2-Pyrimidyl)-2-methylpiperazine was prepared in the same manner as mentioned above from 2-chloropyrimidine and 1-carbethoxy-3-methylpiperazine; yield 24%, bp 130–135° (1.5 mm). *Anal.* ($C_9H_{14}N_4$) C, H, N.

1-(2-Pyrimidyl)homopiperazine.—A solution of 50 g (0.48 mole) of homopiperazine and 28.5 g (0.25 mole) of 2-chloropyrimidine in 300 ml of 95% MeOH was stirred under reflux for 90 min. After removal of the solvent *in vacuo*, the pasty residue was treated with 100 ml of 4 *N* NaOH and 25 g of NaOH pellets and was extracted several times ($CHCl_3$). After drying (K_2CO_3) and removal of the solvent *in vacuo*, the oily residue was distilled and gave 30 g (67.4%) of pure product, bp 149–152° (9 mm); **dihydrochloride,** mp 235–236°. *Anal.* ($C_9H_{14}N_4 \cdot 2HCl$) C, H, Cl, N.

N-(2-Pyrimidyl)-N-methyl-N′-methylethylenediamine was prepared in the same manner as above from N,N′-dimethylethylenediamine and 2-chloropyrimidine; yield 55%, bp 92–93° (1.25 mm), n^{28}D 1.5380; **dihydrochloride,** mp 158–160°. *Anal.* ($C_9H_{14}N_4 \cdot 2HCl$) C, H, Cl, N.

General Methods for the Preparation of 1,4-Disubstituted Piperazines. Method A. 1-(3,4-Methylenedioxybenzyl)-4-(2-pyrimidyl)piperazine (15).—A solution of 12.5 g (0.109 mole) of 2-chloropyrimidine and 23.2 g (0.105 mole) of 1-piperonylpiperazine, in 150 ml of DMF, was stirred at 130° for 8.3 hr in the presence of 31 g of anhydrous K_2CO_3. After cooling to 25°, the salt was filtered off, and the solvent was evaporated to dryness *in vacuo*. The pasty residue was treated with H_2O (100 ml) and filtered off. After drying in air overnight, it was recrystallized from 60 ml of anhydrous EtOH by cooling to 0°; yield 30 g (89.5%), mp 98°.

Method B. 1-(3,4-Methylenedioxyphenoxyethyl)-4-(2-pyrimidyl)piperazine (39).—Worked up as in A, a solution of 7.55 g (0.046 mole) of 1-(2-pyrimidyl)piperazine and 11.87 g (0.0484 mole) of 1-(3,4-methylenedioxyphenoxy)-2-bromoethane, in 150 ml of DMF, was stirred at 130° for 6 hr in presence of 13.8 g of anhydrous K_2CO_3 to give 8.7 g (59%) of material, mp 102°.

Method C. 1-(3-Methoxy-4-hydroxybenzyl)-4-(2-pyrimidyl)-piperazine Hydrochloride (33).—A solution of 10 g (0.065 mole) of vanillin and 7.2 g (0.0438 mole) of 1-(2-pyrimidyl)piperazine in 200 ml of 98% EtOH was stirred and heated at 70°, under 7 kg/cm² of H_2 over 2 g of 5% Pd-C. After 2 hr the theoretical quantity of H_2 was absorbed. The catalyst was removed and the alcoholic solution was concentrated. The oily residue was treated with 50 ml of ether and 60 ml of 4 *N* HCl and the acidic layer was decanted. After making the acidic solution alkaline with excess K_2CO_3, the base was extracted several times with $CHCl_3$. After drying (K_2CO_3) and removal of the solvent *in vacuo*, the residue (14 g) was dissolved in 60 ml of hot EtOH and clarified with Darco and the solution was saturated with HCl gas without cooling. On cooling to 0°, the product crystallized. It was filtered, washed with cold EtOH, and recrystallized from 175 ml of MeOH with cooling overnight at 0°; yield 9.05 g (55%) of colorless crystals, mp 185–188°.

Method D. 1-(3-4-Methylenedioxybenzyl)-4-(4-pyrimidyl)-piperazine Hydrochloride (3).—A solution of 9.9 g (0.03 mole) of (3,4-methylenedioxybenzyl)-4-(2-chloro-4-pyrimidyl)piperazine and 30 ml of 1 *N* NaOH in 250 ml of MeOH was stirred under 7 kg/cm² of H_2 over 2 g of 10% Pd-C. After 2 hr, the theoretical quantity of H_2 was absorbed. The catalyst was removed and the MeOH was concentrated *in vacuo*. The residue was taken up in H_2O (100 ml) and $CHCl_3$ (50 ml) and the chloroform layer was poured off. After drying (K_2CO_3) and removal of the solvent, the residual oil was dissolved in 30 ml of 90% EtOH and the solution was saturated with HCl gas without cooling. On cooling to 0°, the crystals were filtered off, washed (cold MeOH), and dried in a vacuum desiccator, yielding 8.1 g (73%) of salt, mp 192–198°.

Method E. 1-(3,4-Methylenedioxybenzyl)-4-(4-hydroxy-2-pyrimidyl)piperazine (7).—A solution of 36.5 g (0.09 mole) of **6** in 900 ml of the anhydrous EtOH was stirred under 17 kg/cm² of H_2 over 9 g of 10% Pd-C for 5 hr at 25°. Then, the solution was acidified with 100 ml of 2 *N* HCl and the catalyst was removed and washed several times with H_2O. The acidic solution was concentrated *in vacuo*. The crystalline residue was dissolved

(6) (a) A. Fick, *Sitzber. Physik. Med. Ges. Wurzburg*, 36 (1870); (b) D. Bovet, M. Virno, G. L. Gatti, and A. Carpi, *Arch. Intern. Pharmacodyn.*, **110**, 380 (1957).

(7) L. G. Wesson, Jr., *Methods Med. Res.*, **5**, 181 (1952).

(8) A. Adami and E. Marazzi, *Arch. Intecc. Pharmacodyn.*, **107**, 322 (1956).

(9) J. Hiltebrecht, *Arzneimittel-Forsch.*, **4**, 507 (1954).

(10) I. C. Kogon, R. Minin, and C. G. Overberger, "Organic Syntheses," Coll. Vol. IV, John Wiley and Sons, Inc., New York, N. Y., 1963, p 182.

(11) G. W. Kenner, C. B. Reese, and A. R. Todd, *J. Chem. Soc.*, 855 (1955).

(12) G. Hilbert and T. B. Johnson, *J. Am. Chem. Soc.*, **52**, 1152 (1930).

(13) Winthrop Chemical Co., U. S. Patent 2,219,858 (1940).

(14) Badische Anilin und Soda Fabrik, British Patent 913,910 (1962).

(15) S. Sugasawa, S. Yamada, and M. Narahashi, *J. Pharm. Soc. Japan*, **71**, 1345 (1951).

(16) T. Matsukawa and B. Ohta, *ibid.*, **69**, 489 (1940).

(17) (a) N. Whittaker, *J. Chem. Soc.*, 1568 (1951); (b) S. P. English, J. H. Clark, R. G. Shepherd, H. W. Mason, J. Krapcho, and R. O. Roblin, *J. Am. Chem. Soc.*, **68**, 1039 (1946).

(18) J. R. Dudley, J. T. Thurston, F. C. Schaeffer, D. Holm-Hansen, C. J. Hull, and P. Adams, *ibid.*, **73**, 2986 (1951).

(19) R. Gabriel and R. Stelzner, *Ber.*, **29**, 1300 (1896).

(20) A. B. Sen and R. R. Singh, *J. Indian Chem. Soc.*, **36**, 787 (1959).

(21) F. W. Semmler and F. Bartelt, *Ber.*, **41**, 2752 (1908).

(22) G. L. Regnier and R. Canevari, French Patent 1,302,958 (1962).

(23) G. L. Regnier, M. Grajzon, and J. C. Le Douarec, U. S. Patent 3,119,826 (1964).

(24) Abbott Laboratories, British Patent 752,332 (1956).

(25) H. W. Stewart, R. J. Turner, J. J. Denton, S. Kushner, L. M. Brancone, W. L. McEwen, R. I. Hewitt, and Y. Subbarow, *J. Org. Chem.*, **13**, 134 (1948).

in 75 ml of boiling H_2O and the solution was filtered and neutralized at pH 8 with K_2CO_3. The crystalline base was collected on a filter, washed (H_2O), and recrystallized from 60 ml of DMF. On cooling to 0°, the crystals were filtered off, washed (cold MeOH), and dried in a vacuum desiccator, yielding 9.8 g (69.5%) of product, mp 214°.

Method F. 1-(3,4-Methylenedioxybenzyl)-4-guanidinopiperazine sulfate.—A mixture of 55 g (0.25 mole) of 1-piperonylpiperazine and 35.3 g (0.254 mole) of S-methylisothiuronium sulfate in 250 ml of H_2O was heated to boiling for 6 hr. On cooling to room temperature overnight, the crystallized product was collected on a filter and recrystallized from 60% *i*-PrOH yielding 40 g (63%) of neutral sulfate, mp 260–262°. *Anal.* ($C_{13}H_{18}N_4O_2 \cdot 0.5H_2SO_4$) C, H, N, S.

1-(3,4-Methylenedioxybenzyl)-4-(2-*o*-triazinyl)piperazine Hydrochloride (1).—A mixture of 35.6 g (0.114 mole) of the above sulfate and 150 ml of DMF was treated with 3.2 ml (0.114 mole) of concentrated H_2SO_4 and 22 g (0.154 mole) of triformamidomethane.[26] The mixture was then heated for 5 hr at 150°. There was incomplete dissolution. After cooling at 10°, the insoluble crystals were filtered off and the solution was concentrated *in vacuo*. The oily residue was taken up in 50 ml of 4 *N* NaOH and extracted several times into 150 ml of $CHCl_3$. After washing and drying (K_2CO_3), the solvent was evaporated to dryness and the residue weighing 29 g was dissolved in 75 ml of anhydrous EtOH. The solution was saturated with HCl gas and after cooling 19.5 g of crystals of salt was obtained; they were recrystallized from 325 ml of 98% MeOH to give 14.2 g (33.5%) of white product, mp 207–211°.

(26) H. Bredereck, R. Gompper, H. Rempfer, K. Kleem, and H. Heck, *Ber.*, **92**, 329 (1959).

Acetylenic Carbamates. III. The N-Cycloaliphatic Derivatives

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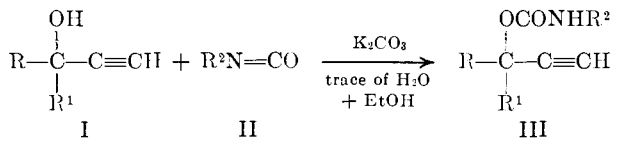
The series of acetylenic carbamates was extended with emphasis on the N-cycloaliphatic derivatives. The N-cycloaliphatics had much less cell culture cytotoxicity and neurotoxicity as determined by intrathecal studies in dogs than the NH_2 compounds, even though their acute toxicities were about the same. Most of the N-cycloaliphatic compounds exhibited antitumor activity.

It has recently been reported[1] that a series of acetylenic carbamates possessed potent antitumor activity against several experimental neoplasms in animals. From the initial structure–activity relationship study, it was apparent that the N-cycloaliphatic groups were very beneficial in promoting antitumor activity. This paper presents the investigation of the relationship of certain toxicological effects to substitution on the nitrogen and an extensive structure–activity study of the carbamates with the N-cycloaliphatic moiety, using the X5563 and C1498 tumor systems.

Chemistry.—Since the compounds in this series all involve monosubstitution on N, use was made of the reaction of the 2-propyn-1-ols with a cycloaliphatic isocyanate as previously described.[1] An improvement in this method was utilized that greatly accelerated the reaction, and yields up to 80% were obtained. This involved the use of CH_2Cl_2 or MeCN as solvent with addition of a catalytic amount of K_2CO_3 and a trace of H_2O and EtOH. All products are listed in Tables I



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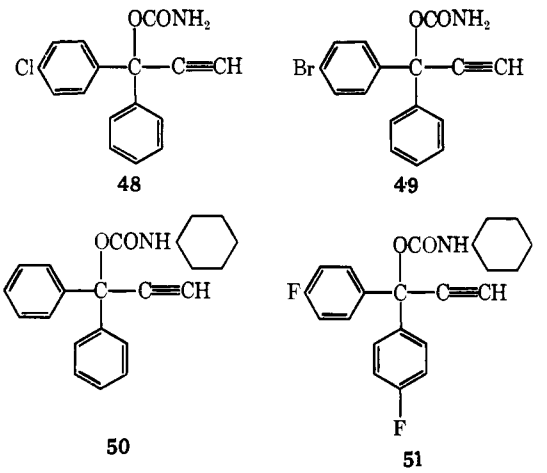
and II; their purity was determined by the usual physical methods (nmr and ir spectra and elemental analyses).

Pharmacology. Toxicity Studies.—The influence of structural changes on certain toxicological effects were investigated with particular emphasis on the NH_2 and N-cycloaliphatic carbamates. The acute toxicities[2] in mice of six of the carbamates are listed in Table III. There appear to be no significant differences in acute toxicities of the NH_2 and N-cycloaliphatic compounds (**48** and **50**, see Chart I); however, a comparison of the phenyl with *p*-fluorophenyl shows that the fluoro compound is more toxic (**50** and **51**).

CHART I[a]



48 **49** **50** **51**

Studied in diverse *in vitro* cell systems, dramatic differences in activity were observed (Table III). The NH_2 carbamates **48** and **49** showed inhibition against the nonparasitic protozoa *Tetrahymena pyriformis*, *Euglena gracilis*, and *Ochromonas malhamensis*, the algae *Chlorella vulgaris* and *Scenedesmus basiliensis*, and the human cell HeLa, previously used to detect potential antitumor agents. For the N-cycloaliphatics **50**, **51**, **29**, and **31** a complete lack of cytotoxicity was demonstrated (although these do show potent antitumor effects). This difference in cytotoxicity has been demonstrated against other tissue culture and bacterial cell systems.

The *in vitro* cell studies were extended to all of the

(1) R. D. Dillard, G. Poore, D. R. Cassady, and N. R. Easton, *J. Med. Chem.*, **10**, 40 (1967).

(2) Long-term toxicity studies with two of the carbamates, **48** and **50**, will be published elsewhere.