4-Amino-6-chloro-2-piperazinopyrimidines with Selective Affinity for α_2-Adrenoceptors

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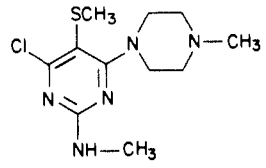
A series of 4-amino-6-chloro-2-piperazinopyrimidines were synthesized and evaluated for their ability to interact with α_1- and α_2-adrenoceptors in vitro in binding assays using [^{3}H]WB-4101, [^{3}H]clonidine, and [^{3}H]idazoxan as radioligands. Some compounds were also tested as inhibitors of [^{3}H]spiroperidol binding. Several members of this series showed high and selective affinity for α_2-adrenoceptors. The nature of the 4-amino substituent seems to be the most critical factor in determining the potency at these receptors.

A previous communication[1] described 2-amino-6-chloro-4-piperazinopyrimidines as inhibitors of spiroperidol binding. One of these derivatives, mezilamine, first reported by Mattioda et al.[2] as an antiemetic compound, was shown to be an atypical dopamine (DA) antagonist, having, in addition, agonistic properties at postsynaptic α_1- and antagonistic properties at presynaptic α_2-adrenoceptors.[3]



This result prompted us to synthesize compounds structurally related to mezilamine, in order to search for α_2-antagonists devoid of substantial affinity for α_1 or DA receptors.

In this paper we describe the synthesis and preliminary evaluation of the 4-amino-6-chloro-2-piperazinopyrimidines **1–27** (Table I).

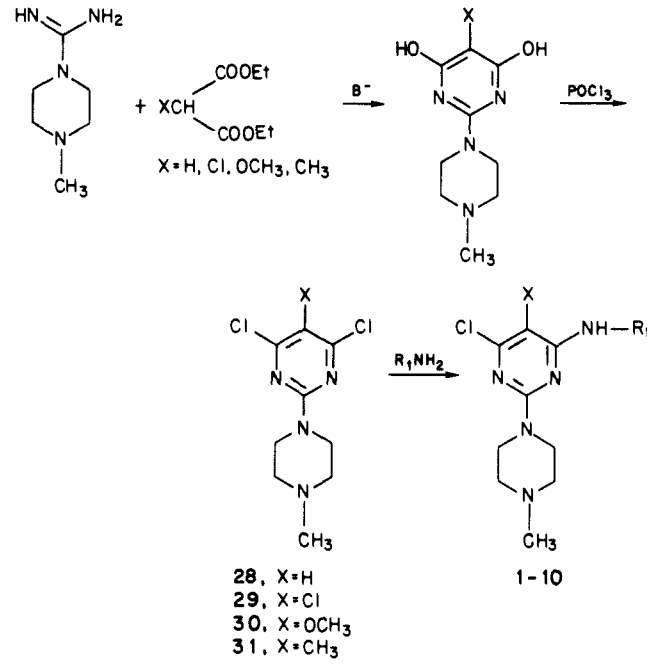
Chemistry. Scheme I shows the classical synthetic route[4] used for the preparation of 2-(*N*-methylpiperazino)pyrimidines **1–10**, bearing the H, Cl, OCH_3, or CH_3 group as a substituent in position 5 (method A). 1-Methyl-4-piperazinecarboxamidine was condensed with the appropriate diethyl malonate derivative to give the corresponding 4,6-dihydroxy-2-(*N*-methylpiperazino)pyrimidine. The crude products were treated directly with $POCl_3$, and the resulting dichloro derivatives, **28–31**, were converted to the desired compounds, **1–10**, by treatment with a primary amine, R_1–NH_2.

The pyrimidines **11–20**, bearing the methylthio group in position 5, were prepared as outlined in Scheme II (method B).[5] Reaction of 1,4-dimethylpiperazine with 5-(methylthio)-2,4,6-trichloropyrimidine led to the formation of chloromethane and **32**.

The assigned structure for **32** was based upon ^{13}C NMR data, C(4) and C(6) being found to be equivalent. The intermediate **32** was then reacted with the appropriate amine, R_1-NH_2, to give **11–20**. The N-unsubstituted piperazines, **25** and **26**, were obtained by the same method (Scheme II), starting from 5-(methylthio)-2,4,6-trichloropyrimidine or 5-methyl-2,4,6-trichloropyrimidine but using 1-methyl-4-formylpiperazine instead of 1,4-dimethylpiperazine. Condensation of the resulting dichloro derivatives, **33** and **34**, with isopropylamine, followed by cleavage of the formyl group in an acidic medium, gave the desired products, **25** and **26**.

The bromo derivatives, **21–24**, were obtained from their methylthio analogues, **11**, **12**, **14** and **16**, by treatment with BrONa in aqueous acetic acid, as previously described[6]

Scheme I. Method A



(method C). In a similar way **27** was prepared from 6-chloro-2-(*N*-formylpiperazino)-4-(isopropylamino)-5-(methylthio)pyrimidine, **36**, by treatment with BrONa, followed by hydrolytic cleavage of the formyl group.

Receptor Binding Studies in Vitro. Compounds **1–27** were tested in vitro for α_2-receptor affinity using a [^{3}H]-clonidine binding assay on rat cortical membranes. These compounds were evaluated also for their ability to interact with α_1-receptors by measuring the inhibition of [^{3}H]W-B-4101 binding to rat cortical membranes in vitro. We also examined the ability of some of the compounds to interact

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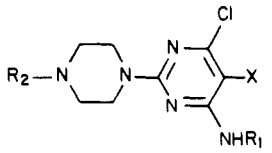
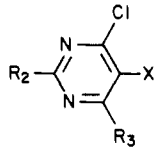
Table I. Physicochemical Data for 4-Amino-6-chloro-2-piperazinopyrimidines



compd	X	R_1	R_2	method	formula	mp, °C	recrystd solvent
1	H	CH_3	CH_3	A	$C_{10}H_{16}ClN_5 \cdot 2HCl$	>260	EtOH
2	H	i-C_3H_7	CH_3	A	$C_{12}H_{20}ClN_5 \cdot HCl \cdot 0.5H_2O$	225 dec	H_2O
3	Cl	H	CH_3	A	$C_9H_{13}Cl_2N_5 \cdot HCl$	260	
4	Cl	CH_3	CH_3	A	$C_{10}H_{15}Cl_2N_5 \cdot HCl$	>260	EtOH
5	Cl	i-C_3H_7	CH_3	A	$C_{12}H_{19}Cl_2N_5 \cdot HCl$	252	
6	Cl	c-C_5H_9	CH_3	A	$C_{14}H_{21}Cl_2N_5 \cdot HCl$[a]	258	H_2O
7	OCH_3	CH_3	CH_3	A	$C_{11}H_{18}ClN_5O \cdot HBr$	not det	H_2O
8	OCH_3	i-C_3H_7	CH_3	A	$C_{13}H_{22}ClN_5O \cdot HCl$[b]	142	EtOH–H_2O (1:1)
9	OCH_3	c-C_5H_9	CH_3	A	$C_{15}H_{24}ClN_5O \cdot HCl$	>260	H_2O
10	CH_3	i-C_3H_7	CH_3	A	$C_{13}H_{22}ClN_5 \cdot 2HCl$	258	acetone
11	SCH_3	H	CH_3	B	$C_{10}H_{16}ClN_5S$[c]	131	EtOH–H_2O (1:1)
12	SCH_3	CH_3	CH_3	B	$C_{11}H_{18}ClN_5S \cdot HCl$	222	EtOH
13	SCH_3	C_2H_5	CH_3	B	$C_{12}H_{20}ClN_5S \cdot HCl$	223	AcOEt–EtOH (4:1)
14	SCH_3	i-C_3H_7	CH_3	B	$C_{13}H_{22}ClN_5S$	68	EtOH–H_2O (1:1)
15	SCH_3	t-C_4H_9	CH_3	B	$C_{14}H_{24}ClN_5S \cdot HCl$	246	H_2O
16	SCH_3	c-C_5H_9	CH_3	B	$C_{15}H_{24}ClN_5S \cdot HCl$[d]	202	H_2O
17	SCH_3	c-C_6H_{11}	CH_3	B	$C_{16}H_{26}ClN_5S \cdot HCl$	202	H_2O
18	SCH_3	C_6H_5	CH_3	B	$C_{16}H_{20}ClN_5S \cdot HCl$	263	H_2O
19	SCH_3	CH_2–C_6H_5	CH_3	B	$C_{17}H_{22}ClN_5S \cdot HCl$[e]	210	AcOEt–EtOH (5:1)
20	SCH_3	CH_2–CH_2–C_6H_5	CH_3	B	$C_{18}H_{24}ClN_5S \cdot HCl$	214	EtOH
21	Br	H	CH_3	B	$C_9H_{13}BrClN_5 \cdot HCl$	>260	EtOH
22	Br	CH_3	CH_3	B	$C_{10}H_{15}BrClN_5 \cdot HCl$	265 dec	EtOH–MeOH (2:1)
23	Br	i-C_3H_7	CH_3	B	$C_{12}H_{19}BrClN_5 \cdot HCl \cdot 0.5H_2O$	229	Me_2CO–H_2O (1:1)
24	Br	c-C_5H_9	CH_3	B	$C_{14}H_{21}BrClN_5 \cdot HCl$[f]	238	H_2O
25	CH_3	i-C_3H_7	H	C	$C_{12}H_{20}ClN_5 \cdot HCl$	200	
26	SCH_3	i-C_3H_7	H	C	$C_{12}H_{20}ClN_5S \cdot HCl$	147	H_2O
27	Br	i-C_3H_7	H	C	$C_{11}H_{17}BrN_5 \cdot HCl$	250 dec	EtOH

[a] H: calcd, 6.05; found, 5.42. [b] O: calcd, 4.76; found, 4.33. [c] C: calcd, 43.87; found, 44.21. [d] H: calcd, 6.66; found 6.16. [e] C: calcd, 51.00; dound, 51.46. [f] Br: calcd, 19.43; found 18.75.

Table II. Physicochemical Data for Pyrimidine Intermediates



compd	X	R_2	R_3	formula	mp, °C	recrystd solvent
28	H	*N*-methylpiperazino	Cl	$C_9H_{12}Cl_2N_4$	80	cyclohexane
29	Cl	*N*-methylpiperazino	Cl	$C_9H_{11}Cl_3N_4$	115	cyclohexane
30	OCH_3	*N*-methylpiperazino	Cl	$C_{10}H_{14}Cl_2N_4O$[a]	101	cyclohexane
31	CH_3	*N*-methylpiperazino	Cl	$C_{10}H_{14}Cl_2N_4$	68	cyclohexane
32	SCH_3	*N*-methylpiperazino	Cl	$C_{10}H_{14}Cl_2N_4S$	82	EtOH–H_2O (3:1)
33	CH_3	*N*-formylpiperazino	Cl	$C_{10}H_{12}Cl_2N_4O$	216	toluene
34	SCH_3	*N*-formylpiperazino	Cl	$C_{10}H_{12}Cl_2N_4OS$	136	EtOH
35	CH_3	*N*-formylpiperazino	NH-i-C_3H_7	$C_{13}H_{20}ClN_5O$	169	
36	SCH_3	*N*-formylpiperazino	NH-i-C_3H_7	$C_{13}H_{20}ClN_5OS$	92	EtOH–H_2O (1:1)

[a] C: calcd, 43.32; found, 42.82.

with D_2 DA receptors by measuring the inhibition of [^{3}H]spiroperidol binding to rat striatal membranes in vitro. Displacement by 10 compounds of [^{3}H]idazoxan (RX-781094, 2-[2-(1,4-benzodioxanyl)]-2-imidazoline), a new selective α_2-antagonist,[7] is also reported. These receptor binding data for compounds **1–27** and for reference compounds are listed in Table III. Mezilamine[3] and yohimbine[8] were used as the reference α_2-antagonists and phenoxybenzamine[9] as the reference α_1-antagonist.

Results and Discussion

The most potent inhibitors of [^{3}H]clonidine binding were the 4-isopropylamino derivatives **5**, **10**, **14**, **23**, **25**, **26**, and **27** and the 4-cyclopentylamino derivative **16**, all being more potent than yohimbine. These results suggest that steric hindrance in the vicinity of the nitrogen atom of the 4-amino group could be a critical factor for α_2-receptor affinity. The importance of this factor was studied in the 5-methylthio series (compounds **11–20**). Introduction of large substituents in the 4-amino group such as *tert*-butyl, cyclohexyl, phenyl, benzyl, or phenethyl significantly reduced the affinity. Moreover, the affinity was also lowered by even small substituents: i-$C_3H_7 > C_2H_5 > CH_3 > H$. N-Methylation of the piperazine nitrogen did not substantially affect the affinity.

The results obtained from the [^{3}H]clonidine binding assay were confirmed for 10 of the compounds (Table III) by using [^{3}H]idazoxan as the radioligand. There was a good correlation between the K_i values for displacement of these two α_2 ligands, according to the following equation:

$$\log K_i \text{ (idazoxan)} = 0.426 + 0.859 \log K_i \text{ (clonidine)}$$

$$n = 10,\ r = 0.93,\ F_{1,8} = 55.8\ (F_{1,8}/0.01 = 11.26)$$

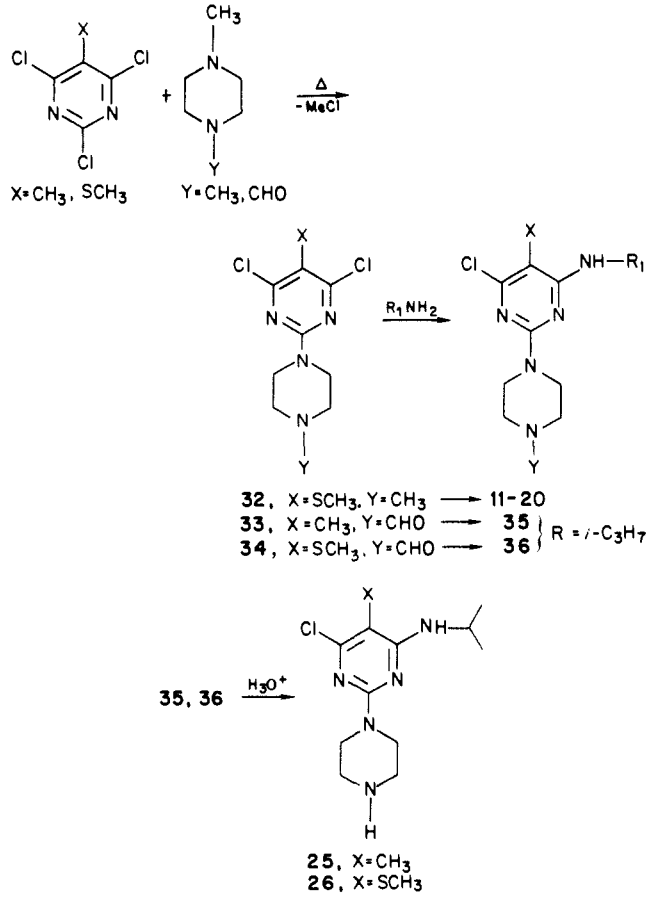
Within this series, most of the compounds were found to

Table III. Receptor Binding Data for 4-Amino-6-chloro-2-piperazinopyrimidines

compd	X	R_1	R_2	inhibition of binding: K_i, nM[a] spiroperidol	WB-4101	clonidine	idazoxan
1	H	CH_3	CH_3	630	280	50	100
2	H	i-C_3H_7	CH_3		>180	130	200
3	Cl	H	CH_3		67	50	
4	Cl	CH_3	CH_3	1200	4	44	
5	Cl	i-C_3H_7	CH_3	2400	42	5	14
6	Cl	c-C_5H_9	CH_3		240	120	97
7	OCH_3	CH_3	CH_3		1700	150	
8	OCH_3	i-C_3H_7	CH_3		310	50	
9	OCH_3	c-C_5H_9	CH_3	670	1700	50	29
10	CH_3	i-C_3H_7	CH_3	880	200	16	
11	SCH_3	H	CH_3	1000	820	620	
12	SCH_3	CH_3	CH_3		1800	360	
13	SCH_3	C_2H_5	CH_3		1800	90	
14	SCH_3	i-C_3H_7	CH_3	640	360	26	
15	SCH_3	t-C_4H_9	CH_3	920	450	180	
16	SCH_3	c-C_5H_9	CH_3	1200	76	22	
17	SCH_3	c-C_6H_{11}	CH_3		240	290	
18	SCH_3	C_6H_5	CH_3		140	240	500
19	SCH_3	CH_2-C_6H_5	CH_3	14	100	1500	
20	SCH_3	CH_2-CH_2-C_6H_5	CH_3	1400	180	910	
21	Br	H	CH_3	10000	820	1600	
22	Br	CH_3	CH_3	200	180	150	
23	Br	i-C_3H_7	CH_3	480	290	7	
24	Br	c-C_5H_9	CH_3		250	140	230
25	CH_3	i-C_3H_7	H	2900	860	6	13
26	SCH_3	i-C_3H_7	H	3000	500	36	62
27	Br	i-C_3H_7	H	880	200	16	28
clonidine				>4000	310	1.5	
yohimbine				2000	360	45	48
phenoxybenzamine				120	1.8		
mezilamine				40	91	9	12

[a] K_i values are the mean of triplicate determinations using four different membrane preparations. Standard deviations were always smaller than 15%.

Scheme II. Method B



have weak affinity for α_1-adrenoceptors compared to phenoxybenzamine, but no obvious structure–activity relationship emerges from the binding data concerning the α_1/α_2 selectivity.

Among the compounds tested in the [^{3}H]spiroperidol assay, only the 4-benzylamino derivative, **19**, was found to have high affinity for D_2 receptors. It is interesting to note that in the 2-amino-4-(*N*-methylpiperazino)pyrimidine series,[1] the position isomer of **19** also exhibited a high degree of affinity for the DA receptor.

In conclusion, the results obtained in the present study demonstrate that structural modification of mezilamine, a DA antagonist having agonist properties at α_1- and antagonist properties at α_2-receptors, can lead to compounds with selective affinity for α_2-adrenoceptors.

The most selective compound was **25** (α_2/α_1 = 143). This compound has been selected for in-depth pharmacological studies; it has been found to be a more potent and more selective α_2-adrenoceptor antagonist than yohimbine in vivo.[10] Details of the pharmacology of **25** will be published elsewhere.

Experimental Section

Chemistry. Melting points were determined on a Kofler hot stage. NMR spectra were recorded on a Varian T-60 or JEOL-90 spectrometer; 70–230-mesh silica gel was used for column chromatography. The purity of compounds was checked by TLC analysis on silica gel GF plates, and components were visualized by UV fluorescence properties. All compounds exhibited proper spectral characteristics and were homogeneous by TLC analysis. Microanalysis results on new compounds are within ±0.4% of the theoretical values unless otherwise indicated.

Method A. 6-Chloro-4-(methylamino)-2-(*N*-methylpiperazino)pyrimidine Dihydrochloride (1). Step A. 4,6-Dichloro-2-(*N*-methylpiperazino)pyrimidine (28). 1-Methyl-4-piperazinecarboxamidine hydrogen sulfate[11] (47.7 g, 0.25 mol) and a solution of MeONa obtained by dissolving Na (11.5 g, 0.5 mol) in MeOH (270 mL) were refluxed for 0.5 h. Diethyl malonate (40 g, 0.25 mol) was added and reflux maintained for 5 h. The mixture was cooled, poured into H_2O (500 mL), made

neutral with AcOH (45 mL), and evaporated in vacuo. The residue was stirred with warm $CHCl_3$ (1 L), and the resultant solid was filtered off: crude 4,6-dihydroxy-2-(*N*-methylpiperazino)pyrimidine (70 g) was thus obtained, which was converted into its dichloro derivative by refluxing with $POCl_3$ (180 mL) for 3 h. The mixture was poured into ice (1 kg), made alkaline with 10 N NaOH (700 mL), extracted with $CHCl_3$ (3 × 600 mL), and evaporated under reduced pressure. The residue was chromatographed on silica gel by eluting with toluene–diethylamine (9:1) to give **28** (8.4 g, 13.6%). After crystallization from cyclohexane an analytical sample melted at 80 °C. Anal. ($C_9H_{12}Cl_2N_4$) C, H, Cl, N.

Step B. 6-Chloro-4-(methylamino)-2-(*N*-methylpiperazino)pyrimidine Dihydrochloride (1). The product from step A (**28**) (7 g, 0.028 mol), toluene (140 mL), pulverized K_2CO_3 (2.1 g, 0.015 mol), and a 33% solution of methylamine in EtOH (30 mL, 0.035 mol) were refluxed for 32 h. The mixture was filtered and evaporated in vacuo. The residue was purified by chromatography on silica gel by eluting with toluene–diethylamine (9:1). It was converted into the dihydrochloride and recrystallization from EtOH gave 1 (4.85 g, 55%), mp >260 °C. Anal. ($C_{10}H_{16}ClN_3 \cdot 2HCl$) C, H, Cl, N.

Method B. 4-Amino-6-chloro-2-(*N*-methylpiperazino)-5-(methylthio)pyrimidine Hydrochloride (11). Step A. 4,6-Dichloro-2-(*N*-methylpiperazino)-5-(methylthio)pyrimidine (32). To a solution of 1,4-dimethylpiperazine (38 g, 0.33 mol) in toluene (300 mL) was added dropwise, at ca. 100 °C, 5-(methylthio)-2,4,6-trichloropyrimidine[12] (69 g, 0.30 mol) in toluene (300 mL), and the mixture was stirred at 90 °C for 2 h. After the mixture was cooled to room temperature, it was filtered and concentrated under reduced pressure. Chromatography on silica gel by eluting with toluene–MeOH (87:13) afforded **32** (69 g, 78.5%). After crystallization from EtOH–H_2O (3:1) an analytical sample melted at 82 °C. ^{13}C NMR δ 158.5 (C_2), 166.6 (C_4, C_6). Anal. ($C_{10}H_{14}Cl_2N_4S$) C, H, Cl, N, S.

Step B. 4-Amino-6-chloro-2-(*N*-methylpiperazino)-5-(methylthio)pyrimidine (11). The product from step A (**32**) (30 g, 0.102 mol) and MeOH (330 mL) containing 60 g of NH_3 were heated at 130 °C in an autoclave for 3 h. The mixture was evaporated in vacuo, and the residue was purified by chromatography on silica gel by eluting with $CHCl_3$–diethylamine (98:2) to give 11 (10 g, 35.8%). After crystallization from EtOH–H_2O (1:1) an analytical sample melted at 131 °C. Anal. ($C_{10}H_{16}ClN_5S$) C, H, N.

Method B. 6-Chloro-4-(isopropylamino)-5-(methylthio)-2-piperazinopyrimidine Hydrochloride (26). Step A. 4,6-Dichloro-2-(*N*-formylpiperazino)-5-(methylthio)pyrimidine (34). To a solution of 1-formyl-4-methylpiperazine (42.3 g, 0.33 mol) in toluene (300 mL) was added dropwise, at ca. 100 °C, 5-(methylthio)-2,4,6-trichloropyrimidine (69 g, 0.30 mol) in toluene (300 mL), and the mixture was stirred at 90 °C for 2 h. After the mixture was cooled to room temperature, it was filtered and evaporated in vacuo to dryness to give **34** (89.1 g, 96.7%), mp 136 °C. ^{13}C NMR δ 158.4 (C_2), 166.8 (C_4, C_6). Anal. ($C_{10}H_{12}Cl_2N_4OS$) C, H, Cl, N.

Step B. 6-Chloro-2-(*N*-formylpiperazino)-4-(isopropylamino)-5-(methylthio)pyrimidine (36). Compound **34** (77 g, 0.25 mol), toluene (500 mL), triethylamine (31 g, 0.3 mol), and isopropylamine (17.7 g, 0.3 mol) were heated at 100 °C for 4 h. The warm mixture was filtered, and the filtrate was washed with H_2O and concentrated under reduced pressure. Chromatography of the residue on silica gel by eluting with toluene–diethylamine (95:5) afforded a product, two crystallizations of which from EtOH–H_2O (1:1) yielded **36** (34.2 g, 41.5%), mp 92 °C. Anal. ($C_{13}H_{20}ClN_5OS$) C, H, Cl, N, O, S.

Step C. 6-Chloro-4-(isopropylamino)-5-(methylthio)-2-piperazinopyrimidine Hydrochloride (26). Compound **36** (32.95 g, 0.10 mol), H_2O (600 mL), and concentrated HCl (60 mL) were refluxed for 2 h, then 5 g of charcoal was added and the cooled mixture was filtered. The filtrate was made alkaline with 10 N NaOH (100 mL) and extracted with $CHCl_3$ (2 × 350 mL). The organic layer was evaporated in vacuo. The residue was dissolved in EtOH and converted to the hydrochloride. Three crystallizations from H_2O gave **26** (12.55 g, 37.1%), mp 147 °C. Anal. ($C_{12}H_{20}ClN_5S \cdot HCl$) C, H, Cl, N, S.

Method C. 4-Amino-5-bromo-6-chloro-2-(*N*-methylpiperazino)pyrimidine Hydrochloride (21). To a solution of 11 (8.1 g, 0.0296 mol) in AcOH (35 mL) was added over 1 h a 1.2 N solution of sodium hypobromite (240 mL). After the mixture stood for 1 h at room temperature, it was cooled at 10 °C, made alkaline with 10 N NaOH (50 mL), and extracted with $CHCl_3$ (2 × 100 mL); the organic layer was washed with H_2O and concentrated under reduced pressure. Chromatography on silica gel by eluting with $CHCl_3$–diethylamine (92:8) yielded 2.8 g, which was converted into the hydrochloride in EtOH. Recrystallization from EtOH afforded **21** (2.1 g, 20.7%), mp >260 °C. Anal. ($C_9H_{13}BrClN_5 \cdot HCl$) C, H, Br, Cl, N.

Method C. 5-Bromo-6-chloro-4-(isopropylamino)-2-piperazinopyrimidine Hydrochloride (27). This compound was prepared as **21** but starting from **36** instead of 11. The resulting product was then hydrolyzed according to the method described for **26**. From **36** (33 g, 0.1 mol) 5-bromo-6-chloro-4-(isopropylamino)-2-(*N*-formylpiperazino)pyrimidine (21.4 g) was obtained. Hydrolysis gave **27** after two recrystallizations from H_2O (5.35 g, 14.4%), mp 250 °C dec. Anal. ($C_{11}H_{17}BrClN_5 \cdot HCl$) C, H, Br, Cl, N.

Biological Methods. The affinities of selected compounds for central α-adrenergic (α_1 and α_2) and dopaminergic (D_2) receptors were determined by using methods described previously.[13-15] Rat brain cortex (α_1 or α_2) or rat brain striatum (D_2) was dispersed, using an ultraturax homogenizer, and the membranes were suspended in 20 vol of ice-cold 50 nM Tris·HCl buffer (pH 7.4). The homogenates were centrifuged at 50 000*g* for 10 min, and the pellets were washed twice with the same volume of buffer. The final pellets were resuspended either in the homogenization buffer (α_1 or α_2) or in supplemented 50 nM Tris·HCl buffer[16] (D_2) at a final protein concentration of 2 ng/mL, for the binding assays.

The radioligands [^{3}H]spiroperidol (29.5 Ci/mmol), [^{3}H]clonidine (66.8 Ci/mmol), and [^{3}H]WB-4101 (19 Ci/mmol) were obtained from New England Nuclear and [^{3}H]idazoxan (40 Ci/mmol) from Commissariat à l'Energie Atomique. Radioligand concentrations were 0.3 nM [^{3}H]spiroperidol (D_2), 1 nM [^{3}H]WB-4101 (α_1), 1 nM [^{3}H]clonidine, or 3 nM [^{3}H]idazoxan (α_2). Nonspecific binding was determined as that binding occurring in the presence of 10^{-6} M butaclamol (D_2), 10^{-4} M noradrenaline (α_1), and 2.10^{-6} M yohimbine (α_2). The reaction was started by addition of the membrane suspension (1 ng of protein for α_1 and α_2, 0.2 ng for D_2). The final incubation volume was 5 mL.

After incubation for 15 min at 25 °C, the membranes were harvested by vacuum filtration through GF/B glass fiber filters, which were then washed twice with 5 mL of buffer. Radioactivity was measured by liquid scintillation spectrometry.

IC_{50} for these compounds were obtained from five-point displacement curves. K_i values were then calculated by using the formula

$$K_i = \frac{IC_{50}}{1 + [C]/K_D}$$

where [C] was the concentration of the radioligand. The equilibrium dissociation constant, K_D, had been determined previously from Scatchard plots as 0.3 nM for [^{3}H]spiroperidol, 0.22 nM for [^{3}H]WB-4101, 0.57 nM for [^{3}H]clonidine, and 4.9 nM for [^{3}H]-idazoxan.

Acknowledgment. We are indebted to Drs. Barieux, Mattioda, Obellianne, and Rocquet for their initial contribution to the research on 6-chloro-2,4-diaminopyrimidines. We thank Dr. Villatte for spectral and analytical data and Mrs. Thibaut for her secretarial assistance. Expert technical assistance was provided by Ms. Do-Huu and Gontier and Mrs. Dupre and Doerflinger.

Registry No. 1, 84727-45-7; 1·2HCl, 84727-46-8; 2, 102396-27-0; 2·HCl, 84727-47-9; 3, 84727-50-4; 3·HCl, 84727-51-5; 4, 84727-52-6; 4·HCl, 84727-53-7; 5, 84727-54-8; 5·HCl, 84727-55-9; 6, 84727-56-0; 6·HCl, 84727-57-1; 7, 102396-28-1; 7·HBr, 102396-40-7; 8, 84727-

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58-2; 8·HCl, 84727-59-3; 9, 84727-62-8; 9·HCl, 84727-63-9; 10, 84727-70-8; 10·2HCl, 84727-71-9; 11, 84727-84-4; 12, 102396-30-5; 12·HCl, 102396-41-8; 13, 102396-31-6; 13·HCl, 102396-42-9; 14, 61973-58-8; 15, 84727-88-8; 15·HCl, 84707-98-2; 16, 84708-05-4; 16·HCl, 84708-06-5; 17, 84708-07-6; 17·HCl, 84708-08-7; 18, 83199-77-3; 18·HCl, 83199-78-4; 19, 102396-32-7; 19·HCl, 102396-43-0; 20, 102396-33-8; 20·HCl, 102396-44-1; 21, 102396-34-9; 21·HCl, 102396-45-2; 22, 102396-35-0; 22·HCl, 102396-46-3; 23, 102396-36-1; 23·HCl, 61973-50-0; 24, 102396-37-2; 24·HCl, 102396-47-4; 25, 102396-29-2; 25·HCl, 84727-77-5; 26, 84708-09-8; 26·HCl, 84727-80-0; 27, 102396-39-4; 27·HCl, 102396-48-5; 28, 84727-44-6; 29, 84727-49-1; 30, 84727-61-7; 31, 84727-69-5; 32, 84727-83-3; 33, 84727-78-6; 34, 84727-81-1; 35, 84727-79-7; 36, 84727-82-2; 36 (X = Br), 102396-38-3; 5-methyl-2,4,6-trichloropyrimidine, 1780-36-5; 5-methylthio-2,4,6-trichloropyrimidine, 24795-76-4; 1,4-dimethylpiperazine, 106-58-1; 4-methyl-1-piperazinecarboxaldehyde, 7556-55-0; 4,6-dihydroxy-2-(*N*-methylpiperazino)pyrimidine, 81746-24-9; 5-chloro-4,6-dihydroxy-2-(*N*-methylpiperazino)pyrimidine, 84727-48-0; 4,6-dihydroxy-5-methoxy-2-(*N*-methylpiperazino)pyrimidine, 84727-60-6; 4,6-dihydroxy-5-methyl-2-(*N*-methylpiperazino)pyrimidine, 84727-68-4; 1-methyl-4-piperazinecarboxamidine, 45798-01-4.

Synthesis and Class III Antiarrhythmic Activity of (Phenylbut-2-enyl)ammonium Salts. Effect of Conformation on Activity

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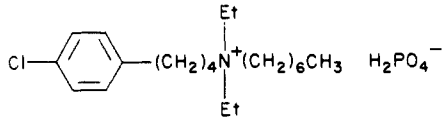
The syntheses of seven 4-(substituted phenyl)but-2-en(*or* yn)yl quaternary ammonium salts and four related tertiary amines are described. The Meerwein arylation reaction was the preferred synthetic method for the required intermediate 1-aryl-4-halo-2-butenes (**15a–c**, **18**). In the case of **18**, the trans stereochemistry of the Meerwein adduct of 2,3-dimethylbutadiene was established unambiguously by 2D NMR and X-ray studies. The title compounds represent conformationally restricted analogues of the class III antiarrhythmic agent clofilium (**1**) and exhibit comparable potency and efficacy in the in vitro evaluation using isolated canine Purkinje fibers. These results suggest that the alkylene chain in **1** is extended in the active conformation. Computer-aided conformational analysis (MM2) supports this conclusion. Selective catalytic hydrogen conditions were developed for the conversion of the unsaturated analogue **2** to clofilium (**1**) with minimal hydrogenolysis of the allylic quaternary ammonium moiety, thus completing a novel and efficient synthesis of this substance.

Sudden cardiac death is a major public health problem in the United States today.[1] Ventricular arrhythmias that progress to ventricular tachycardia and fibrillation are the most common cause of sudden cardiac death. We have been interested in the development of pure class III antiarrhythmic agents that are expected to be effective in ventricular arrhythmias caused by reentry mechanisms. According to the designation of antiarrhythmic agents defined by Vaughan Williams, a class III compound prolongs the action potential duration of the cardiac cell without depressing conduction in cardiac tissue.[2] Relatively few compounds of this type have been reported to date, and most, such as sotalol and amiodarone, possess other activities as well.[3]

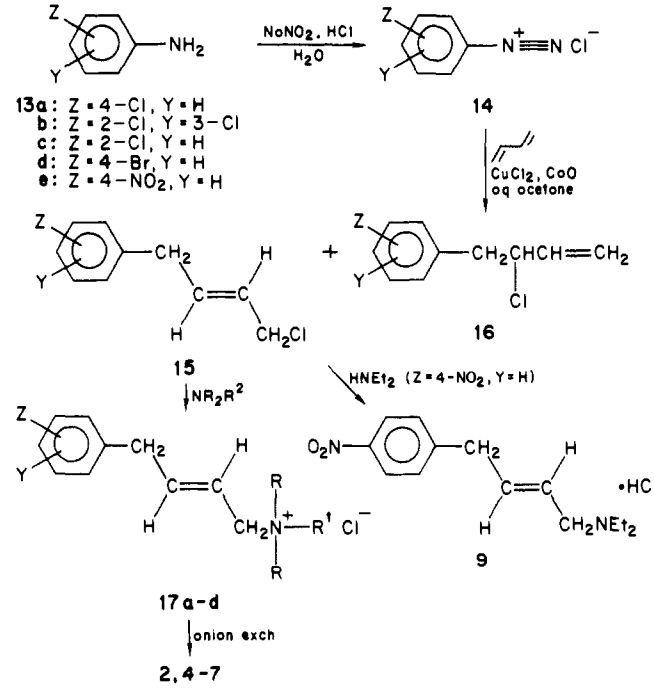
One compound that appears to exhibit pure class III activity is clofilium (**1**).[4] In this study the effect on class



1

III activity of introducing conformational restrictions between the phenyl ring and the quaternary nitrogen atom of the clofilium molecule was examined. The fully saturated four-carbon connecting chain of **1** allows the molecule to assume a variety of spatial orientations of the aromatic ring and the ammonium group including, in principle, a folded conformation in which the quaternary nitrogen interacts with the electron-rich π cloud of the aromatic

Scheme I. Synthesis of Ammonium Salts and Amines Employing the Meerwein Arylation Reaction of Butadiene



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