1:10 brei of the B16 melanoma was prepared, and 0.5 mL was inoculated subcutaneously in C57BL/6 mice fortnightly.

For the antitumor testing, 10^6 P388 leukemia cells (or 5×10^6 for P388/DDP, 10^5 for L1210, or 0.5 mL of 1:10 brei for B16) were injected ip into groups of six BDF_1 mice on day 0. On days 1, 5, and 9, Pt compounds in 1% Tween-20/saline (1:9) were administered ip at five concentrations over a 16-fold range incorporating the maximum tolerated dose. Negative control groups were untreated or received only the vehicle, and the positive control group received 5-fluorouracil (50 mg/kg) or cisplatin (6 mg/kg, for B16 and P388/DDP). Animals were observed for 30–60 days. Data are expressed as $\%T/C$, the mean survival time of treated animals vs that of control animals expressed as a percentage.

Acknowledgment. This study was carried out during the tenure of a grant from the Anti-Cancer Council of Victoria, and was assisted by a loan of platinum from Johnson Matthey. Technical assistance from Chris Bingham, Kim Granic, and Virginia Nink is gratefully acknowledged.

Registry No. **1a**, 92181-58-3; **1b**, 92181-60-7; **1c**, 108089-50-5; **1d**, 108089-52-7; **1e**, 108089-48-1; **1f**, 108110-95-8; **2a**, 135389-37-6; **2b**, 135389-39-8; **2c**, 135389-38-7; **3**, 15227-42-6; **4**, 142581-10-0; C_6HF_5, 363-72-4.

Synthesis and Antibronchospastic Activity of 8-Alkoxy- and 8-(Alkylamino)imidazo[1,2-*a*]pyrazines

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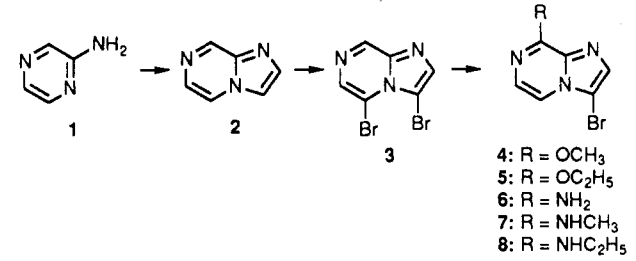
Theophylline still occupies a dominant place in asthma therapy. Unfortunatly its adverse central nervous system (CNS) stimulant effects can dramatically limit its use, and adjustments in the dosage are often needed. We have synthesized a new series of imidazo[1,2-*a*]pyrazine derivatives which are much more potent bronchodilators than theophylline in vivo and do not exhibit the CNS stimulatory profile. In vitro studies on isolated rat uterus and guinea pig trachea confirm the high potentialities of these derivatives. 6-Bromo-8-(methylamino)imidazo[1,2-*a*]-pyrazine-3-carbonitrile (**23**) is identified as the most potent compound of the series. As in the case of theophylline, phosphodiesterase inhibition appears unlikely to be the unique mechanism of action of this series of heterocycles.

Many imidazo[1,2-*a*]pyrazine derivatives have been acclaimed for their pharmacological profile, ranging from antidepressant to inotropic or antiulcer activity.[1] In an earlier paper, several 8-hydrogeno- or 8-bromoimidazo-[1,2-*a*]pyrazines were found to exhibit uterine-relaxing, antibronchospastic, and cardiac-stimulating properties.[2] In the present study, we report the synthesis of new 8-alkoxy- and 8-(alkylamino)imidazo[1,2-*a*]pyrazines (Table I) and investigate their potential as bronchodilator agents. Their pharmacological profile is compared to that of theophylline. 8-(Methylamino)imidazo[1,2-*a*]pyrazine derivatives demonstrate high antibronchospastic activity in vitro and in vivo. Interestingly, these compounds do not exhibit the adverse central nervous system (CNS) stimulant effects of theophylline.

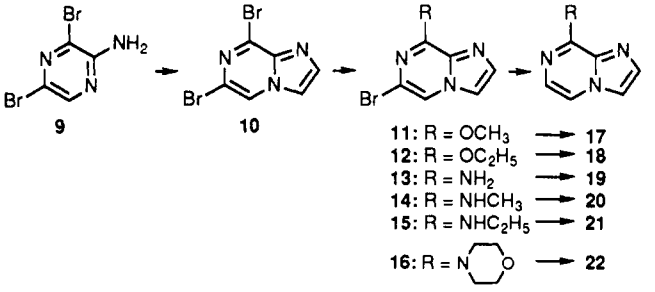
Chemistry

The classical condensation of an α-halogeno carbonyl compound with aminopyrazine (**1**) or 2-amino-3,5-dibromopyrazine (**9**) led to the imidazo[1,2-*a*]pyrazine series. Imidazo[1,2-*a*]pyrazine (**2**) yielded 3,5-dibromoimidazo-[1,2-*a*]pyrazine (**3**) via bromination with bromine in acetic acid.[3,4] The 3-bromo-8-substituted series was obtained via telesubstitution of the bromine atom in position 5 after a nucleophilic attack at the 8-position (Scheme I). This mechanism, which has already been reported for the formation of 5-bromo-8-methoxyimidazo[1,2-*a*]pyrazine[3] (**4**) and extended here to amino or alkylamino derivatives, was first described in the azoloazine series with a bridgehead nitrogen atom by Bradac et al.[4] on the triazolo[4,3-*a*]-

Scheme I. Synthesis of 8-Alkoxy- and 8-(Alkylamino)-3-bromoimidazo[1,2-*a*]pyrazines by Telesubstitution of 3,5-Dibromoimidazo[1,2-*a*]pyrazine



Scheme II



Scheme III

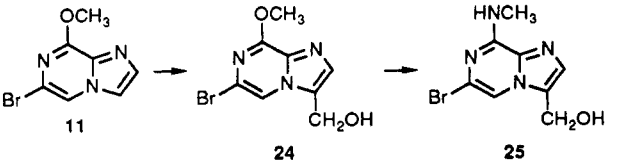


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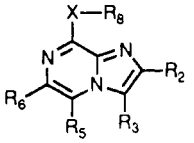
Table I. 8-Alkoxy- and 8-(Alkylamino)imidazo[1,2-*a*]pyrazines



compd	R_2	R_3	R_5	R_6	X	R_8	mp, °C	formula	anal.
4	H	Br	H	H	O	CH_3	136–137	$C_7H_6N_3OBr$	C,H,N
5	H	Br	H	H	O	C_2H_5	125–126	$C_8H_8N_3OBr$	C,H,N
6	H	Br	H	H	NH	H	238–239	$C_6H_5N_4Br$	C,H,N
7	H	Br	H	H	NH	CH_3	143–144	$C_7H_7N_4Br$	C,H,N
8	H	Br	H	H	NH	C_2H_5	82–83	$C_8H_9N_4Br$	C,H,N
11	H	H	H	Br	O	CH_3	208–210	$C_7H_6N_3OBr$	C,H,N
12	H	H	H	Br	O	C_2H_5	164–166	$C_8H_8N_3OBr$	C,H,N
13	H	H	H	Br	NH	H	209–210	$C_6H_5N_4Br$	C,H,N
14	H	H	H	Br	NH	CH_3	161–162	$C_7H_7N_4Br$	C,H,N
15	H	H	H	Br	NH	C_2H_5	98–100	$C_8H_9N_4Br$	C,H,N
16	H	H	H	Br	N		190–192	$C_{10}H_{11}N_4OBr$	C,H,N
17	H	H	H	H	O	CH_3	<50	$C_7H_7N_3O$	C,H,N
18	H	H	H	H	O	C_2H_5	58–59	$C_8H_9N_3O$	C,H,N
19	H	H	H	H	NH	H	220–221	$C_6H_6N_4$	C,H,N
20	H	H	H	H	NH	CH_3	95–96	$C_7H_8N_4$	C,H,N
21	H	H	H	H	NH	C_2H_5	97–98	$C_8H_{10}N_4$	C,H,N
22	H	H	H	H	N		126–128	$C_{10}H_{12}N_4$	C,H,N
23	CN	H	H	Br	NH	CH_3	218–219	$C_8H_6N_5Br$	C,H,N
24	H	CH_2OH	H	Br	O	CH_3	200–201	$C_8H_8N_3O_2Br$	C,H,N
25	H	CH_2OH	H	Br	NH	CH_3	183–184	$C_8H_9N_4OBr$	C,H,N
27	H	CH_2OCH_3	H	Br	NH	CH_3	221–222	$C_9H_{11}N_4OBr$	C,H,N
28	H	$CH_2OC_2H_5$	H	Br	NH	CH_3	133–134	$C_{10}H_{13}N_4OBr$	C,H,N

pyrazine series. Telesubstitution on the 8 position was established from ^1H NMR considerations (Table II). ^1H NMR spectra of compounds **4–8** exhibited one singlet (H_2) and two doublets with a coupling constant of 4.5 Hz, which is the classical J_{56} value.[5] This value is very different from the J_{68} value which has been reported to be very small (~1 Hz) for imidazo[1,2-*a*]pyrazine and 3-bromoimidazo[1,2-*a*]pyrazine[5] or even unobservable in the case of 3,5-dibromoimidazo[1,2-*a*]pyrazine.[4,5] 6,8-Dibromoimidazo[1,2-*a*]pyrazine (**10**) was prepared from 2-amino-3,5-dibromopyrazine (**9**). In the nucleophilic substitution of **10** by an alcohol or an amine, only the bromine atom at position 8 was exchanged to give compounds **11–16** (Scheme II). Further catalytic hydrogenation on Pd/C afforded imidazo[1,2-*a*]pyrazines **17–22** only substituted at position 8. 6-Bromo-8-(methylamino)imidazo[1,2-*a*]-

Table II. ^1H NMR Chemical Shifts of 8-Alkoxy- and 8-(Alkylamino)imidazo[1,2-*a*]pyrazines[a,b]

compd	H_2	H_3	H_5	H_6	R_8
4	7.89		7.64	8.11	3.07
5	7.80		8.02	7.55	4.56–1.42
6	7.64		7.56	7.37	ND
7	7.60		7.46	7.40	2.97[c]
8	7.69		7.50	7.50	4.54, 1.23
11	7.70	8.07	8.63		3.33
12	7.68	8.06	8.58		4.52, 1.23
13	7.51	7.83	8.02		ND
14	7.48	7.79	7.95		2.95[c]
15	7.47	7.79	7.95		4.52, 1.25
16	7.60	7.94	8.22		4.20–3.76
17	7.70	8.12	8.28	7.46	3.47
18	7.66	8.09	8.23	7.41	4.52, 1.40
19	7.51	7.85	7.77	7.20	ND
20	7.55	7.80	7.92	7.32	2.95[c]
21	7.53	7.90	7.78	7.28	3.52, 1.20
22	7.58	7.97	7.98	7.36	4.20–3.77
23		7.63	7.91		3.18[c]
24[d]	8.06		7.53		4.18
25[e]	7.69		7.33		3.15[c]
27[f]	7.64		7.44		3.16[c]
28[g]	7.66		7.47		3.14[c]

[a] Solutions in DMSO-d_6, except for compounds **23–28** ($CDCl_3$). [b] For compounds **4–7** and **18–22**, J_{56} = 4.5 Hz. For compounds **12–22**, J_{23} = 1.0 Hz. J_{56} has not been determined for compound **8**. [c] $J_{NH,CH}$ = 5 Hz. [d] δ 4.95 (s, 2, CH_2). [e] δ 4.88 (s, 2, CH_2). [f] δ 4.65 (s, 2, CH_2), 3.34 (s, 3, OCH_3). [g] δ 4.72 (s, 2, CH_2O), 3.54 (q, 2, OCH_2CH_3), 1.22 (t, 3, CH_3).

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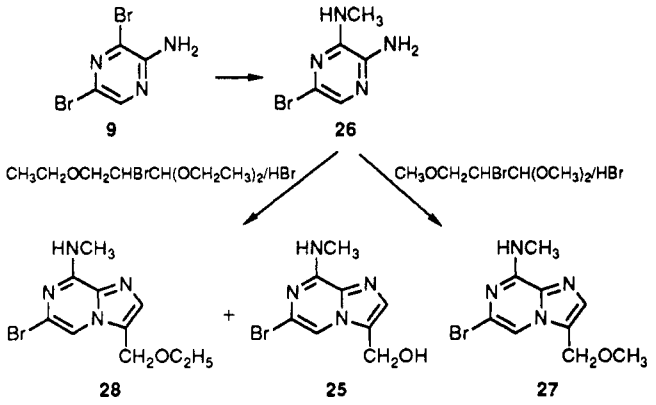
Scheme IV



pyrazine-2-carbonitrile (**23**) was prepared in a similar manner after classical nucleophilic attack of methylamine on the previously described parent 6,8-dibromoimidazo-[1,2-*a*]pyrazine-2-carbonitrile.[2]

As reported in the following pharmacological section, compounds **14** and **23** with a bromine atom and a methylamino group at the 6 and 8 position, respectively, have exhibited potent antibronchospastic activities. In order to evaluate the potential of a substituent at the 3 position on the pharmacological profile of these 8-(alkylamino)-imidazo[1,2-*a*]pyrazines, we focused our interest on the synthesis of 3-(hydroxymethyl)-8-(methylamino) derivatives of this series. Access to 3-substituted imidazo[1,2-*a*]pyrazines has always encountered some difficulties. 3-Amino-2-methyl-8-(2-phenylmethoxy)imidazo[1,2-*a*]-pyrazine and its 8-(2-phenylethyl) homologue have been prepared by Kaminski et al.[6] by direct substitution of the aromatic hydrogen at position 3 using sodium nitrite in acetic acid of *n*-butyl nitrite in THF. In these two cases, reactivity toward electrophilic substitution of position 3 was much enhanced by the simultaneous presence of two activating groups on positions 2 and 8. In fact, all our investigations to fix a nitroso or a nitro group at the 3 position of less activated imidazo[1,2-*a*]pyrazines have failed. 3-Hydroxymethylation of imidazo[1,2-*a*]pyrazine has been obtained by Teulade et al.[7] via an electrophilic attack of formaldehyde after prolonged reflux in a sodium acetate/acetic acid medium. Following the same procedure, 6-bromo-3-(hydroxymethyl)-8-methoxyimidazo[1,2-*a*]pyrazine (**24**) has been obtained by direct reaction of formaldehyde on **11** (Scheme III). Substitution of the methoxy group by the methylamino group took place rapidly under reflux of **24** in an 40% aqueous solution of methylamine to give the desired 6-bromo-3-(hydroxymethyl)-8-(methylamino)imidazo[1,2-*a*]pyrazine (**25**).

In order to obtain directly 3-substituted-8-(methylamino)imidazo[1,2-*a*]pyrazines by classical condensation of a convenient α-halogeno carbonyl compound with 2-amino-5-bromo-3-(methylamino)pyrazine (**26**) (Scheme IV), we have prepared, as previously described,[8] the two following acetals: 2-bromo-1,1,3-trimethoxypropane and 2-bromo-1,1,3-triethoxypropane. These two acetals exhibited different sensitivity to acidic hydrolysis under reflux in a 4.8% HBr aqueous solution. Acidic hydrolysis of the trimethoxy derivative led to only one aldehyde, 2-bromo-3-methoxypropanaldehyde, and its condensation with **26** gave exclusively 6-bromo-8-(methylamino)-3-(methoxymethyl)imidazo[1,2-*a*]pyrazine (**27**). On the other hand, under the same hydrolysis conditions, 2-bromo-1,1,3-triethoxypropane led to a mixture of two aldehydes, 2-bromo-3-ethoxypropanaldehyde and 2-bromo-3-hydroxypropanaldehyde. Their presence was demonstrated by ^{1}H NMR since two doublets of different intensities were observed at 9.63 and 9.28 ppm ($CDCl_3$). Direct condensation of this mixture with **26** gave mainly the 6-bromo-3-(hydroxymethyl)-8-(methylamino)imidazo-[1,2-*a*]pyrazine (**25**) and a low yield of 6-bromo-3-(ethoxymethyl)-8-(methylamino)imidazo[1,2-*a*]pyrazine (**28**).

Table III. Antibronchospastic Activities of Imidazo[1,2-*a*]pyrazine Derivatives and Theophylline

compd	antibronchospastic activity: ED_{50}[a] (μmol/kg)	compd	antibronchospastic activity: ED_{50}[a] (μmol/kg)
4	18 ± 1.4[c]	**18**	>70
5	18 ± 2.0[c]	**19**	>120[b]
6	30.0 ± 1.5	**20**	>100[b]
7	11.0 ± 1.5[c]	**21**	>80[b]
8	32 ± 2.7	**22**	60 ± 11[c]
11	17 ± 1.2[c]	**23**	1.0 ± 0.1[c]
12	9 ● 1.1[c]	**24**	5.5 ± 0.6[c]
13	5.2 ± 0.6[c]	**25**	1.5 ● 0.2[c]
14	2.5 ± 0.4[c]	**27**	2.3 ± 0.3[c]
15	3.7 ● 0.2[c]	**28**	2.2 ± 0.4[c]
16	38 ± 3.2	theophylline	37.0 ● 3.7
17	>70		

[a] Results are given as the mean ± SEM from 4–10 determinations. [b] Highest concentration or dose tested. [c] Statistically different ($p < 0.05$) from theophylline.

Results and Discussion

All newly synthesized 8-substituted imidazo[1,2-*a*]-pyrazines were tested in vivo in an antibronchospastic screen following a modification of a published procedure.[13] ED_{50} were determined for the inhibition of the bronchospasm induced by intravenous doses of histamine on the artificially respired, anesthetized guinea pig. Theophylline was tested for comparison. The results are summarized in Table III. Compounds **17–22** without a bromine atom, exhibited no antibronchospastic activity. All the other derivatives, with a bromine atom on position 3 or 6, demonstrated an in vivo activity which was in all cases, except compound **16**, higher than the activity of theophylline. Monobromation on position 6 led to the

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Table IV. Uterine- and Trachea-Relaxing Activities and Phosphodiesterase Inhibition of Imidazo[1,2-a]pyrazine Derivatives and Theophylline

compd	ED_{50}[a] (mM) uterine-relaxing act.:	ED_{50}[a] (mM) trachea-relaxing act.:	PDE IC_{50}[b] (μM)
7	0.10[c]	0.097[c]	337[c]
13	0.30[c]	0.093[c]	267[c]
14	0.16[c]	0.024[c]	27[c]
15	0.13[c]	0.065[c]	81
23	0.002[c]	0.002[c]	70
theophylline	0.90	0.92	92

[a] Drug concentration (mM) causing 50% decrease of spontaneous contraction of isolated rat uterus or 50% decrease of carbachol-induced trachea contraction. Results are given as the mean from six to eight determinations and the range for all data were within ±10%. [b] Drug concentrations (μM) causing 50% inhibition of phosphodiesterase (PDE). Data represent the mean from three to four determinations and the range for all data were within ±10%. [c] Statistically different ($p < 0.05$) from theophylline.

most active compounds. 3-Bromo derivatives of this series exhibited significantly lower in vivo activity. When the imidazole ring was not substituted, derivatives with an amino or an alkylamino group on position 8 were always more potent than the 8-alkoxy compounds and the highest activities were obtained with a methylamino group on position 8. 6-Bromo-8-(methylamino)imidazo[1,2-a]-pyrazine (14) was, for instance, 15 times more active than theophylline.

Bronchodilator activity of the series is highly enhanced after introduction on the imidazole ring of either a cyano group on position 2 or an hydroxymethyl group on position 3. 6-Bromo-8-(methylamino)imidazo[1,2-a]pyrazine-2-carbonitrile (23) and 6-bromo-3-(hydroxymethyl)-8-(methylamino)imidazo[1,2-a]pyrazine (25) have exhibited the highest in vivo activity of the series. They have been determined to be 37 and 25 times more potent, respectively, than theophylline in the guinea pig histamine-induced bronchospasm test.

In order to perform more extensive studies on the pharmacological properties and the mechanism of action of this new series, we have chosen five derivatives with quite different ED_{50} values which could easily be prepared in sufficient quantity and characterized by good solubility (10^{-2} M) and good stability. For these studies compounds 7, 13–15, and 23 were selected. Imidazo[1,2-a]pyrazine derivatives were tested for their capacity to relax smooth muscles on two experimental models, the isolated rat uterus and the isolated guinea pig trachea. ED_{50} were determined for the inhibition of spontaneous uterine contraction and for the inhibition of tracheal contraction induced by carbachol at 10^{-4} M. Theophylline was tested for comparison. Results are presented in Table IV. In both experimental models the imidazo[1,2-a]pyrazine derivatives demonstrated significantly higher activity than theophylline. The smooth muscle relaxant activities of these derivatives were characterized by similar orders of potency on spontaneously (uterus) and mediator (trachea) contracted tissues. Only compound 14 was much more active in trachea than in uterus preparations. All tested derivatives demonstrated a concentration-dependent smooth muscle relaxant activity and were able to induce an almost total relaxation of the tissues. The much higher in vivo activity of 6-bromo-8-(methylamino)imidazo[1,2-a]pyrazine-2-carbonitrile (23) was confirmed by these two in vitro experiments.

One of the most serious adverse clinical effects of theophylline is its inherent stimulation of the central nervous system. To determine if the imidazo[1,2-a]-pyrazine derivatives presented the same CNS stimulatory profile, we have performed a simple mice motility test (Table V). As expected, theophylline induced a CNS stimulation (130–170% increase of motility in theophylline-treated animals as compared to control). All the imidazo[1,2-a]pyrazine derivatives tested did not exhibit such CNS stimulation. On the contrary, a decrease of mice locomotor activity (13–83% as compared to control) was observed. As LD_{50} values of compounds 7 and 13–15 (Table V) were similar or higher than the LD_{50} determined with theophylline, this effect was not attributed to a direct acute toxicity of these derivatives. Only compound 23 appears more toxic than theophylline.

Table V. Locomotor Activity and Acute Toxicity in Mice

compd	% variation vs control mice activity[a] 55 μmol/kg	% variation vs control mice activity[a] 166 μmol/kg	LD_{50}[b] (mmol/kg)
theophylline	+130	+170	1.9
7	−25	−57	4.2
13	−53	−83	1.3
14	−75	−83	2.2
15	−13	−73	2.9
23	−64	ND	0.5

[a] Compounds and vehicle (control) were given intraperitonealy. Locomotor activity was evaluated by the number of light beam crossings over a 50-min period and are expressed in percentage of control mice activity. [b] Compounds were suspended in a 5% gum arabic solution and were given orally to five male Swiss mice (Iffa Credo) for each dose. Deaths were noted over a 15-day period although murine mortality always occurred within 2 days posttreatment. LD_{50} were evaluated by probit analysis.

Imidazo[1,2-a]pyrazine derivatives were also tested for inhibition of a crude complex of phosphodiesterase 3′,5′-cyclic nucleotide (PDE) from bovine heart, and results are presented in Table IV. The PDE inhibitory potency of these compounds did not correlate with the in vivo and in vitro smooth muscle activities. All the derivatives but 14 exhibited similar or lower potency than theophylline. Although the bronchodilator activity of 6-bromo-8-(methylamino)imidazo[1,2-a]pyrazine-2-carbonitrile (23) was much higher than that of theophylline, its phosphodiesterase inhibition was similar to that of theophylline. A possible explanation for this discrepancy might be the differences in hydrophobicity of these derivatives and consequent variations in their ability to cross cellular membranes.[9] Another possibility is that these compounds, like theophylline, possess additional undefined mechanisms of action. It has been reported that isobutylmethylxanthine (IBMX) can directly stimulate adenylate cyclase activity by blocking the function of the inhibitory guanine regulatory protein Gi.[10] It has also been proposed that some pharmacological properties of methylxanthines are mediated via antagonism of A1 and A2 adenosine receptors.[11] However the absence of CNS stimulation by these derivatives suggests that they have no adenosine receptor antagonist affinity as far as CNS stimulatory activity of theophylline is attributed to adenosine blocking properties.[12] These possibilities are currently under investigation.

Experimental Section

Chemistry. All reactions were followed by TLC with Merck F254 silica gel plates. Melting points were taken on a Kofler hotbench and are uncorrected. ^{1}H NMR spectra were recorded on a Varian EM360 spectrometer. Elemental analysis were performed by the Microanalytical Centre (Montpellier, France). Flash column chromatographies were carried out on Merck silica gel (230–240 mesh). HPLC purity determinations were realized with a Waters 600 solvent delivery system equipped with a Waters 900 photodiode-array detector. 2-Amino-3,5-dibromopyrazine (9),

6,8-dibromoimidazo[1,2-a]pyrazine[4] (10), and 6,8-dibromoimidazo[1,2-a]pyrazine-2-carbonitrile[2] have been synthesized as previously reported.

3-Bromo-8-methoxyimidazo[1,2-a]pyrazine (4) was prepared from 3,5-dibromoimidazo[1,2-a]pyrazine (3) as previously described.[3] 3-Bromo-8-ethoxyimidazo[1,2-a]pyrazine (5) was synthesized in an analogous manner. No detectable trace of the 5-ethoxy isomer was observed from the synthesis of 5.

6-Bromo-8-methoxyimidazo[1,2-a]pyrazine (11) was synthesized as described by Bradac et al.[4] Compound 12 was prepared in an analogous manner.

8-Amino-6-bromoimidazo[1,2-a]pyrazine (13). A solution of 1 g (3.6 mmol) of 6,8-dibromoimidazo[1,2-a]pyrazine (10) in 50 mL of ammoniacal ethanol was heated to 120 °C for 5 h in a 250-mL autoclave. Evaporation of the solvent followed by crystallization from ethanol gave 0.74 g (96%) of the desired product.

8-Amino-3-bromoimidazo[1,2-a]pyrazine (6) was prepared in an analogous manner.

General Procedure for the Preparation of 8-(Alkylamino)imidazo[1,2-a]pyrazines. 6-Bromo-8-(methylamino)imidazo[1,2-a]pyrazine-2-carbonitrile (23). A mixture of 1 g (3.3 mmol) of 6,8-dibromoimidazo[1,2-a]pyrazine-2-carbonitrile[2] in 9 mL of a 40% strength aqueous methylamine solution was stirred for 12 h. Extraction with CH_2Cl_2 followed by chromatography on a silica gel column eluted with ether provided 0.67 g (80%) of the desired compound.

Compounds 7, 8, and 14–16 were similarly prepared by using the procedure described above.

8-Methoxyimidazo[1,2-a]pyrazine (17) was prepared as previously described by Bradac et al.[4] Compounds 18–22 were prepared in an analogous manner.

6-Bromo-3-(hydroxymethyl)-8-methoxyimidazo[1,2-a]pyrazine (24). A mixture of 6-bromo-8-methoxyimidazo[1,2-a]pyrazine (11) (2 g, 8.77 mmol), sodium acetate (2.7 g, 33 mmol), acetic acid (1.9 mL, 33 mmol), and 17 mL (220 mmol) of a 37% solution of formaldehyde in water was heated in an autoclave at 120 °C for 2 h. Upon cooling, 30 mL of water was added to the reaction. After alkalinization with Na_2CO_3 and extraction with methylene chloride, the organic phase was dried and evaporated. The residue was chromatographed on a silica gel column eluted with 3% methanol–methylene chloride to give 0.6 g of the unreacted product 11. Further elution with 10% methanol–methylene chloride afforded 1 g (3.9 mmol, 44.5%) of 24.

6-Bromo-3-(hydroxymethyl)-8-(methylamino)imidazo[1,2-a]pyrazine (25). A mixture of 1 g (3.9 mmol) of 6-bromo-3-(hydroxymethyl)-8-methoxyimidazo[1,2-a]pyrazine (24) in 9 mL of a 40% aqueous methylamine solution was heated under reflux for 3 h. Extraction with methylene chloride and chromatography on a silica gel column eluted with 5% methanol–methylene chloride provided a small amount of unreacted product 24 and 0.72 g (2.8 mmol, 72%) of the desired compound.

2-Amino-5-bromo-3-(methylamino)pyrazine (26). A mixture of 3 g (11.8 mmol) of 2-amino-3,5-dibromopyrazine (9) in 30 mL of a 40% strength aqueous methylamine solution was heated to 130 °C for 17 h in a 125-mL autoclave. Extraction with CH_2Cl_2 and chromatography on a silica gel column eluted with 5% methanol–methylene chloride afforded 1.75 g (73%) of 26.

6-Bromo-3-(ethoxymethyl)-8-(methylamino)imidazo[1,2-a]pyrazine (28). A mixture of 3 g (11.8 mmol) of 2-bromo-1,1,3-triethoxypropane,[5] concentrated hydrobromic acid (1 mL), and water (10 mL) was heated under reflux for 1 h. The mixture was then cooled, ether was added, and the layers were separated. The organic phase was dried and poured into a solution of 2 g (10 mL) of 2-amino-5-bromo-3-(methylamino)pyrazine (26) in DMF (5 mL). The reaction was dissolved in water (20 mL), neutralized with sodium carbonate, and extracted with chloroform. The organic layer was dried and evaporated and chromatography of the residue on a silica gel column eluted with 3% methanol–methylene chloride furnished 0.5 g (1.8 mmol, 18%) of 28. Further elution with 10% methanol–methylene chloride yielded 0.9 g (3.5 mmol, 35%) of 6-bromo-3-(hydroxymethyl)-8-(methylamino)-imidazo[1,2-a]pyrazine (25).

6-Bromo-3-(methoxymethyl)-8-(methylamino)imidazo[1,2-a]pyrazine (27) was prepared in an analogous manner from 2-bromo-1,1,3-trimethoxypropane and 26. No detectable trace of 25 was observed from the synthesis of 27.

Pharmacology. Bronchospasm Induced in Anesthetized Guinea Pigs. The method was based on the technique described by Konzett and Rossler.[13] Adult male Dunkin–Hartley guinea pigs (Iffa Credo, France), weighing 400–600 g, were anesthetized (ethyl carbamate, 1.20 g/kg ip). The trachea was cannulated after a tracheotomy was performed, and a respirator for small animals (Palmer A.C., Palmer Ltd, England) delivered a constant air flow (1 mL/100 g × 60/min). A tube was connected from the trachea cannula to a Marey capsule fitted to a lever in order to record the variation of the air excess at each insufflation on a Kymograph. The jugular vein was cannulated to allow intravenous administration of drugs. Bronchospasms were induced by intravenous injections of histamine. The dose of histamine (8–12 μg/kg) was determined for each animal as the dose that doubled the variation of air excess. The test drugs were injected iv and followed 30 s later by another histamine administration. The ED_{50} of the tested drug, calculated from the regression line, was represented as the dose that reduced by 50% the air excess induced by histamine. The results presented in Table III are means ± SEM of 4–10 determinations.

Guinea Pig Trachea Preparation. Adult male Dunkin–Hartley guinea pigs (Iffa Credo, France), weighing 400–500 g, were killed by a blow to the head. Contractility of guinea pig tracheal segments (four tracheal rings in all cases) was measured by adapting the method previously described by Hooker.[14] The organs were connected to an isometric recorder under a basal tension of 0.5 g in an oxygenated (95% O_2, 5% CO_2) organ bath (37 °C) with a myograph transducer connected to a Physiograph Narco Bio-system. The bronchoconstrictor agent (10^{-4} M carbachol) induced a contraction which reached a stable maximum within 1–5 min. Cumulative test compound dose–response curves were determined, taking the intensity of the initial contraction as 100%. The IC_{50} were obtained from the regression line. The results presented in Table IV are means ± SEM of six to eight determinations.

Rat Uterus Preparation. Female Wistar rats (190–210 g) were decapited 24 h after intraperitoneal injection of estradiol benzoate (0.1 mg/kg). The external third of the uterine horn was dissected out and mounted vertically in an oxygenated organ bath (37 °C) with De Jalon solution of the following composition (mM): NaCl, 153.8; KCl, 5.6; $CaCl_2$, 2.16; $NaHCO_3$, 1.8; glucose, 5.5. Spontaneous uterine contractions were recorded isometrically with a myograph transducer connected to a Physiograph Narco Bio-system. The preparation was left for 30 min and the liquid was changed three times before any administration of drugs. The activities of drugs were measured as the dose required to reduce the amplitude of spontaneous contractions by 50% (ED_{50}). The results presented in Table IV are means ● SEM of six to eight determinations.

Effects on the Exploratory Motor Activity in Mice. Locomotor activity of the compounds was studied in groups of 10 male Swiss mice (20–22 g) by means of an automatic Apelab activity meter (France). Compounds were suspended in a 5% gum arabic solution. Solutions or vehicule (control) were given intraperitonealy. Locomotor activity has been determined by the measurement of the number of light beam crossings carried out for a 50-min period. Results were expressed in percentage of control mice motor activity.

Phosphodiesterase (PDE) Inhibition. PDE assay was carried out according to the procedure of Levallois et al.[15] Bovine heart phosphodiesterase (Sigma Co., ref. P0134) was used in this assay: This PDE is a crude complex from bovine heart lyophilized preparation containing near saturation levels of protein activator (calmodulin) and Ca^{2+}. A mixture of 1 μM [8-^{3}H]adenosine cyclic monophosphate in 10 mM Tris HCl, pH 7.5, and 1 mM $MgCl_2$ was incubated (10 min at 30 °C) with 5×10^{-4} units of PDE. The enzymatic reaction was stopped by heating 3 min in boiling water. The resulting [^{3}H]AMP was separated from excess substrate by

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thin-layer chromatography on silica gel plates (Merck, ref. 5567, elution with propanol-methanol-34% ammonia-water 45:15:20:20) and measured by radioactivity counting. As cyclic AMP and adenosine have identical R_f values under these conditions, control experiments were carried out and showed that further degradation of [^{3}H]AMP into [^{3}H]adenosine was negligible: No difference was observed in the amount of [^{3}H]AMP in the presence or in the absence of 5'-nucleotidase inhibitors. This result is consistent with the low 5'-nucleotidase activity of PDE (ref. P0134 from Sigma). Inhibition assays were carried out by adding various inhibitor concentrations (or no inhibitor for the blank) in DMSO to the incubation mixture. IC_{50} values were determined by plotting uninhibited velocity/inhibited velocity (V_0/V) vs the inhibitor concentration.

Synthesis and Chemical Characterization of N-Substituted Phenoxazines Directed toward Reversing Vinca Alkaloid Resistance in Multidrug-Resistant Cancer Cells

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Received November 18, 1991

A series of 21 N-substituted phenoxazines has been synthesized in an effort to find more specific and less toxic modulators of multidrug resistance (MDR) in cancer chemotherapy. Thus, *N*-(ω-chloroalkyl)- and *N*-(chloroacyl)phenoxazines were found to undergo iodide-catalyzed nucleophilic substitution on reaction with various secondary amines, including *N*,*N*-diethylamine, *N*,*N*-diethanolamine, morpholine, piperidine, pyrrolidine and (β-hydroxyethyl)piperazine. Products were characterized by UV, IR, ^{1}H-, and ^{13}C-NMR, mass spectral data, and elemental analyses. All of the compounds were examined for cytotoxicity and for their ability to increase the accumulation of the vinca alkaloids, vincristine (VCR) and vinblastine (VLB) in multidrug-resistant GC_3/Cl (human colon adenocarcinoma) and KBCh^R-8-5 (HeLa variant) cell lines. Compounds were compared to the standard modulator verapamil (VRP). Substitutions on the phenoxazine ring at position 10 were associated with an increase in antiproliferative and anti-MDR activities. Modification of the length of the alkyl bridge and the type of amino side chain also influenced the potency of these effects. From among the compounds examined, 10 derivatives were found to increase the accumulation of VCR and VLB in GC_3/Cl and KBCh^R-8-5 cells relative to the effect of VRP, suggesting that with the exception of pyrrolidinyl, the tertiary amine attachments to the phenoxazine nucleus linked through a three- or four-carbon alkyl chain resulted in enhanced anti-MDR activity. On the basis of their 50% growth inhibitory (IC_{50}) values, five of the ten compounds, namely, 10-(3'-chloropropyl)phenoxazine, 10-[3'-[*N*-bis(hydroxyethyl)-amino]propyl]phenoxazine, 10-(3'-*N*-morpholinopropyl)phenoxazine, 10-(4'-*N*-morpholinobutyl)phenoxazine and 10-(*N*-piperidinoacetyl)phenoxazine were selected as relatively nontoxic chemosensitizers. These modulators, at nontoxic concentrations, potentiated the cytotoxicity of VCR and VLB in GC_3/Cl and KBCh^R-8-5 cells. Further, two compounds 10-(3'-*N*-morpholinopropyl)phenoxazine, and the butyl derivative, enhanced accumulation of VLB in GC_3/Cl, KBCh^{R}8-5 and highly resistant KB-V1 cells to a level significantly greater than the maximal level achieved with VRP. Additional experiments to understand the mechanism of action of these agents in modulating MDR are in progress.

Introduction

A major reason for failure of treatment of cancer patients is resistance to conventional chemotherapeutic agents. One type of drug resistance, called multidrug resistance (MDR) is characterized by cross-resistance to functionally and structurally unrelated drugs, typically doxorubicin, vincristine (VCR), vinblastine (VLB), colchicine, and actinomycin D. The gene responsible for this form of MDR has been cloned[1] and encodes a glycoprotein called p-glycoprotein (P-gp), which shows homology to a number of bacterial transport proteins.[2-4] Biochemical studies suggest that P-gp may act as an energy-dependent drug efflux pump. The capacity of P-gp to bind cytotoxic drugs has been demonstrated.[5] A number of drugs from various therapeutic and chemical classes appear to be active in modifying MDR in model systems, including the calcium channel blocker, verapamil[6] (VRP), the calmodulin inhibitor, trifluoperazine,[7] the antiarrhythmic drug, quinidine,[8] reserpine,[9] cyclosporin A,[10] vinca alkaloid analogs,[11] dihydropyridines,[12-14] and pyridine analogs.[15] Agents that reverse MDR apparently did not seem to have common features, but putatively each inhibits the pump activity of P-gp.[16,17] When the activity of the pump is inhibited anticancer agents accumulate in MDR cells with resulting

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0022-2623/92/1835-3358$03.00/0