Cyclic Nucleotide Phosphodiesterase Inhibition by Imidazopyridines: Analogues of Sulmazole and Isomazole as Inhibitors of the cGMP Specific Phosphodiesterase

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The synthesis and phosphodiesterase (PDE) inhibitory profile of a series of imidazopyridines, including sulmazole and isomazole, on separated PDE isoenzymes are described. The results show that both sulmazole and isomazole are weak inhibitors of PDE III, and their inotropic activity is unlikely to be due to PDE III inhibition alone. Surprisingly, both compounds were found to be significant inhibitors of the cGMP specific isoenzyme, PDE V, and a series of simple 2-substituted phenylimidazo[4,5-*b*]pyridines have been made to investigate the SAR of PDE activity. This has been shown to be sensitive to chain length, polarity, and the nature of the heteroatom linking group. Potent PDE V inhibitors, many of which are also significant inhibitors of PDE IV, have been identified.

Introduction

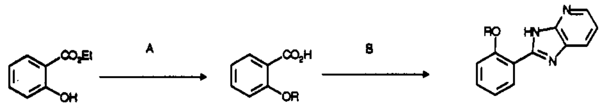
In recent years there has been a resurgence of interest in inhibitors of cyclic nucleotide phosphodiesterases (PDEs),[1-6] enzymes responsible for the intracellular hydrolysis of the second messengers cAMP and cGMP.[7] Renewed interest has been encouraged by evidence of multiple molecular forms of PDEs with a range of substrate specificities and nonuniform tissue distribution.[9] Specific inhibitors of PDE isoenzymes, therefore, have the potential for more selective pharmacological action[10,11] than earlier nonselective inhibitors such as isobutylmethylxanthine (IBMX). Thus, while PDE IV[12] is specific for cAMP and selective inhibitors such as rolipram and denbufylline have shown potential for CNS activity,[13-15] PDE V is specific for cGMP and selective inhibitors such as zaprinast lead to smooth muscle relaxation,[16] even though this last compound was originally developed as a prophylactic antiallergic agent.[17] The PDE III isoenzyme shows only a slight preference for cAMP as a substrate, and selective inhibitors such as milrinone, imazodan, and cilostamide are well-known[18] for their cardiovascular, particularly positive inotropic, activity. These last examples, and structurally related compounds, form a large group within the general class of new, non-catecholamine, nonglycosidic positive inotropic agents, from which the sulmazole and isomazole series stand out as a structurally distinct group of compounds whose positive inotropic actions have been variously ascribed to a number of mechanisms including calcium sensitization[19] and PDE inhibition.[20] As part of our interest in selective PDE inhibitors we assayed both sulmazole and isomazole but found that while neither compound is a potent inhibitor of PDE III, both, in particular sulmazole, inhibited PDE V. These compounds show some similarity to the PDE V inhibitor zaprinast in having an *o*-alkoxyphenyl function adjacent to a heterocyclic ring-NH. We now report the synthesis and evaluation of a series of *o*-alkoxy derivatives of 2-phenylimidazo[4,5-*b*]pyridines related to sulmazole as inhibitors of the separated PDE isoenzymes.

Chemistry

The substituted 2-phenylimidazo[4,5-*b*]pyridines were synthesized by one of three methods.

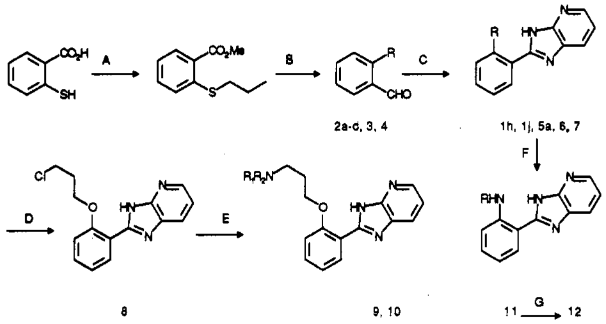
The simple 2-(2-alkoxyphenyl)imidazopyridines (**1a-g**) were prepared using method A (Scheme I) starting with ethyl salicylate. Alkylation gave the O-substituted salicylates which were saponified to the corresponding salicylic acids with methanolic sodium hydroxide. Subsequent condensation with 2,3-diaminopyridine and cyclodehydration was effected using phosphorus oxychloride in a one-pot operation to produce the required imidazo[4,5-*b*]pyridines.

Scheme I [a]



[a] Reagents: (A) 1. RBr, K_2CO_3; 2. NaOH, MeOH; 3. HCl; (B) 2,3-diaminopyridine, $POCl_3$, Δ.

Scheme II [a]



[a] Reagents: (A) 1. MeOH, HCl, Δ; 2. PrBr, K_2CO_3, Δ; (B) 1. $LiAlH_4$; 2. $(COCl)_2$, DMSO; (C) 2,3-diaminopyridine, S_8, Δ; (D) PPh_3, CCl_4; (E) R_1R_2NH, EtOH; (F) H_2, 10% Pd/C; (G) 1. propanal, 4A sieves; 2. $NaBH_4$.

In method B (Scheme II), salicyl aldehydes **2a**,[21] **2b**,[22] **2c**,**d**,[23] **3**, and **4** were heated with elemental sulfur and 2,3-diaminopyridine[24] to provide imidazo[4,5-*b*]pyridines **1h**,**j**, **5a**,**b**, **6**, and **7**; the 2-(propylthio)benzaldehdye **3** was prepared from thiosalicylic acid via esterification, alkylation, $LiAlH_4$ reduction, and Swern oxidation. Functional group modification of the primary hydroxy group of **5a** gave the chloro and alkylamino derivatives **8**–**10**. The aniline **11**[25] was obtained by reduction of **7**[26] and subsequent reductive alkylation gave the propylamino derivative **12**.

In method C (Scheme III) the appropriate salicylic acids (from method A) were converted to their acid chlorides and treated with 2,3-diaminopyridine to give the corre-

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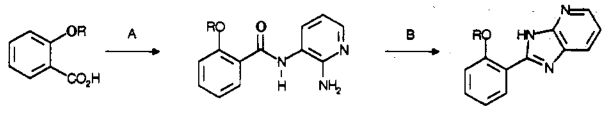
Scheme III[a]



13, 14

[a] Reagents: (A) 1. $(COCl)_2$, DMF, 2. 2,3-diaminopyridine; (B) $POCl_3$, Δ.

sponding amides, which were isolated and then cyclodehydrated by heating with phosphorus oxychloride to give 13 and 14.

Literature procedures were used to prepare the hydroxyphenyl compound 15[27] (readily acetylated to give the acetate 16) and the imidazo[1,2-*a*]pyridine 17.[28] The imidazo[4,5-*c*]pyridines 18 and 19 were obtained by appropriate modification of method A.

Table I summarizes the physicochemical data of the imidazopyridines prepared by the above methods.

Discussion

The inhibitory activities of the imidazo[4,5-*b*]pyridines are summarised in Table II. While isomazole is a more potent inhibitor of PDE III than is sulmazole, qualitatively in line with the greater inotropic activity of isomazole over sulmazole,[29] they are both relatively weak inhibitors when compared with inotropic PDE III inhibitors such as imazodan (Chart I).[30] Comparison of the positive inotropic and PDE III inhibitory activities of sulmazole and isomazole with those of selective PDE III inhibitors (Table III) indicates greater inotropic activity for sulmazole and isomazole relative to PDE III activity, which is consistent with PDE III inhibition being only a contributary mechanism to the positive inotropic activity of sulmazole and isomazole. Neither compound showed significant inhibition of PDE IV, but surprisingly both, in particular sulmazole, inhibited PDE V. Comparison of the activities of sulmazole and isomazole with the simple methoxy analogues **1a** and **19** indicates that while the methylsulfinyl group is not required for PDE V inhibition, its effect on PDE III inhibition is unclear. The hydroxy and acetoxy compounds **15** and **16** had similar activity to the methoxy compound **1a**, but increasing the chain length of the alkoxy group resulted in a progressive increase in PDE V activity from methoxy to pentyloxy (**1a–e**) with a subsequent decline seen with the hexyloxy analogue **1f**. Although branching within the chain was tolerated at a position β to the alkoxy oxygen, similar branching at the α position led to a significant diminution in activity. Thus the isobutoxy, benzyloxy, and cyclopropylmethoxy derivatives **1g**, **1j**, and **14** were active, but the *tert*-butoxy and cyclopentyloxy compounds **1h** and **13** were less active. These results suggest that there is only a limited degree of steric tolerance at the binding site of the alkoxy chain with the position α to the alkoxy oxygen being particularly sensitive toward substitution. The more potent inhibitors of PDE V also showed significant inhibition of PDE IV (**1c–g**,**j**, **8**), but in general PDE III inhibition was not seen. An exception to this is the isobutoxy compound **1g** with a PDE III IC_{50} value of 6.6 μM. We do not have an explanation for the unexpected and high level of PDE III inhibition observed for this single compound in the current series of imidazol[4,5-*b*]pyridines. In the imidazo[4,5-*c*]pyridine series, a similar increase in PDE V activity was observed with increasing alkoxy chain length,[31] and for example, the pentyloxy compound **18** is equiactive with the imidazo[4,5-*b*]pyridine analogue **1e**.

In order to define further the structural features of the side chain necessary for PDE V activity, the effects of additional side-chain substituents were investigated. The presence of a terminal hydroxy (**5a**,**b**) or alkylamino (**9**, **10**) function caused a marked reduction in activity when compared to the corresponding unsubstituted alkoxy compound or a homologue of equivalent chain length. These results indicate that the alkyl chain occupies an essentially lipophilic pocket at the active site in which polar (and particularly protonated cationic) species are not accommodated. Consistent with this hypothesis, the chloropropoxy derivative **8** had similar activity to that of the butoxy compound **1d**.

Finally, the effect of replacing the alkoxy oxygen moiety with other linking groups was also investigated. Both the propylthio and propylamino derivatives (**6**, **12**) were of reduced activity when compared to the corresponding oxygen analogue **1c**, which would be expected if the electron-donor ability or charge density of the heteroatom is important for binding at the active site. Electron donation could be important for the maintainence of coplanarity by intramolecular H-bonding to the imidazole NH. Interestingly, the imidazo[1,2-*a*]pyrimidine **17**, which lacks the NH for intramolecular hydrogen bonding,[32] has only one fifth the activity of the isomeric imidazo[1,2-*b*]pyridine **1c**.

Conclusion

The present studies have shown that both sulmazole and isomazole are relatively weak inhibitors of PDE III when compared to other compounds whose inotropic activity has been attributed to PDE III inhibition. Thus it seems likely that other mechanisms, in addition to PDE III inhibition, are responsible for the inotropic activity of sulmazole and isomazole.

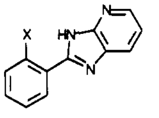
In a series of *o*-alkoxy derivatives of 2-phenylimidazo[4,5-*b*]pyridines related to sulmazole and isomazole, the PDE V inhibitory activity has been shown to be sensitive to both the chain length and polarity. Optimal activity was observed with a linear four- or five-carbon chain, but introduction of polar groups within the binding locus of this chain abolished its activity enhancing effect. The nature of the heteroatom linking group also affected the activity with oxygen linked compounds showing highest activity.

Experimental Section

Chemistry. Melting points were determined on a Buchi 510 melting point apparatus and are uncorrected. Analytical data were provided by the Analytical Sciences department at SB. NMR spectra were obtained for all compounds as $CDCl_3$ or d_6-DMSO solutions on a Bruker AM250 spectrometer, and chemical shifts are quoted in parts per million (δ) relative to tetramethylsilane. Mass spectral determinations were carried out on a VG analytical 7070F mass spectrometer. All final compounds were analyzed for C, H, and N and gave results within ±0.4% of the theoretical value. Analytical and preparative chromatography was carried out on Merck Kieselgel 60 grade silica. All starting materials were obtained from commercial sources and were used as received unless otherwise stated.

Preparation of Substituted Imidazo[4,5-*b*]pyridines: Method A. 2-(2-Butoxyphenyl)-1*H*-imidazo[4,5-*b*]pyridine (1d). A stirred mixture of methyl salicylate (45.6 g, 0.3 mol), bromobutane (41.1 g, 0.3 mol), KI (7.0 g, 0.04 mol), and K_2CO_3 (48.3 g, 0.35 mol) in 400 mL of dry acetone was heated under

Table I. Physical Properties and Methods of Preparation of Fused Imidazoles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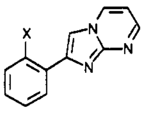
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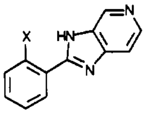
18, 19

no.	X	method	mp, °C	recryst solvent	formula[a]
1a	OCH_3	A	200	MeCN	$C_{13}H_{11}N_3O{\cdot}0.5HCl{\cdot}0.3H_2O$
1b	OC_2H_5	A	146	MeCN	$C_{14}H_{13}N_3O$
1c	$O(CH_2)_2CH_3$	A	152	MeCN	$C_{15}H_{15}N_3O$
1d	$O(CH_2)_3CH_3$	A	146–148	MeCN	$C_{16}H_{17}N_3O$
1e	$O(CH_2)_4CH_3$	A	97	MeCN	$C_{17}H_{19}N_3O$
1f	$O(CH_2)_5CH_3$	A	76	MeCN	$C_{18}H_{21}N_3O$
1g	$OCH_2CH(CH_3)_2$	A	138	MeCN	$C_{16}H_{17}N_3O$
1h	$OC(CH_3)_3$	B	195–196	EtOAc	$C_{16}H_{17}N_3O$
1j	OCH_2Ph	B	136–137	C_6H_{12}/PhMe	$C_{19}H_{15}N_3O$
5a	$O(CH_2)_2OH$	B	158	acetone	$C_{14}H_{13}N_3O_2$
5b	$O(CH_2)_3OH$	B	148	CH_2Cl_2/Et_2O	$C_{15}H_{15}N_3O_2{\cdot}0.1H_2O$
6	$S(CH_2)_2CH_3$	B	170	EtOH	$C_{15}H_{15}N_3S{\cdot}0.2H_2O$
7	NO_2	B	252	EtOH	$C_{12}H_8N_4O_2$
8	$O(CH_2)_3Cl$		153	EtOAc	$C_{15}H_{14}ClN_3O$
9	$O(CH_2)_3NHCH_3$		169	EtOH	$C_{16}H_{14}N_4O{\cdot}HCl$
10	$O(CH_2)_3N(CH_3)_2$		215	EtOH	$C_{17}H_{20}N_4O{\cdot}HCl$
11	NH_2		267	EtOH/Et_2O	$C_{12}H_{10}N_4{\cdot}2.1HCl{\cdot}0.5H_2O$
12	$NH(CH_2)_2CH_3$	B	240	EtOH/Et_2O	$C_{15}H_{16}N_4O{\cdot}0.5H_2O$
13	Oc-C_5H_9	C	163–164	EtOH	$C_{17}H_{17}N_3O$
14	OCH_2c-C_3H_5	C	140	EtOH	$C_{16}H_{15}N_3O$
15	OH		>300	EtOH	$C_{12}H_9N_3O{\cdot}0.1H_2O$
16	$OCOCH_3$		202	$CHCl_3/Et_2O$	$C_{14}H_{11}N_3O_2$
17	$O(CH_2)_2CH_3$		133	EtOH	$C_{15}H_{15}N_3O$
18	$O(CH_2)_4CH_3$		195.5–196.5	MeCN/MeOH	$C_{17}H_{19}N_3O{\cdot}HCl$
19	OCH_3		175–176	MeCN/MeOH	$C_{13}H_{11}N_3O{\cdot}1.5HCl{\cdot}1.0H_2O$

[a] Microanalysis (C, H, N), ±0.4% for the formula given.

Table II. PDE Inhibitory Activity of Imidazopyridines

		PDE IC_{50}, μM[a,b]		
no.	X	V	III	IV
1a	OCH_3	34 ± 7	13%	1%
1b	OC_2H_5	18 ± 4	15%	23%
1c	$O(CH_2)_2CH_3$	6 ± 1	0%	11 ± 2
1d	$O(CH_2)_3CH_3$	1 ± 0.1	0%	6 ± 2
1e	$O(CH_2)_4CH_3$	1 ± 0.1	0%	6 ± 1
1f	$O(CH_2)_5CH_3$	4 ± 1	0%	16 ± 3
1g	$OCH_2CH(CH_3)_2$	3 ± 0.2	2 ± 0.2	3 ± 0.4
1h	$OC(CH_3)_3$	29% (52%)	0%	0%
1j	OCH_2Ph	4 ± 0.8	0%	8 ± 2
5a	$O(CH_2)_2OH$	64 ± 8	2%	14%
5b	$O(CH_2)_3OH$	24 ± 8	0%	30%
6	$S(CH_2)_2CH_3$	24 ± 2	0%	21%
7	NO_2	13% (37%)	0%	4%
8	$O(CH_2)_3Cl$	2 ± 0.1	7%	9 ± 2
9	$O(CH_2)_3NHCH_3$	12% (43%)	0%	5%
10	$O(CH_2)_3N(CH_3)_2$	14% (47%)	8%	7%
11	NH_2	nd	nd	nd
12	$NH(CH_2)_2CH_3$	26 ± 4	0%	11%
13	Oc-C_5H_9	15 ± 4	12%	19 ± 2
14	OCH_2c-C_3H_5	6 ± 1	15%	37 ± 6
15	OH	37 ± 10	15%	0%
16	$OCOCH_3$	40 ± 14	2%	0%
17	$O(CH_2)_2CH_3$	33 ± 3	2%	16%
18	$O(CH_2)_4CH_3$	1 ± 0.2	20%	50%
19	OCH_3	42 ± 5	6%	20%
sulmazole		21 ± 3	2% (35%)	0% (31%)
isomazole		58 ± 5	23% (65%)	2% (26%)
zaprinast		1 ± 0.1	14%	13%

[a] nd denotes not determined. [b] Percent inhibition at 10 μM concentration of test compound. Figures in parentheses are at 100 μM.

Chart I

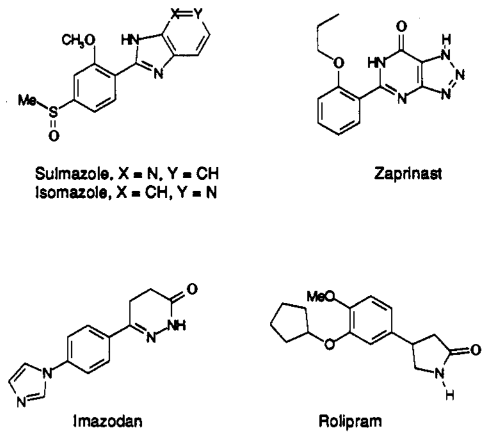

Table III. Comparison of Positive Inotropic and PDE III Inhibitory Activities of Sulmazole, Isomazole, and Selective PDE III Inhibitors

compound	ED_{50}, mg/kg[a]	IC_{50}, μM	$IC_{50}/(ED_{50} \times 10^3)$
sulmazole	0.3[b]	150	0.5
isomazole	0.03[b]	42	1.4
imazodan	0.05[c]	6[d]	0.12
indolidan	0.007[c]	0.08[d]	0.01
milrinone	0.037[c]	0.3[d]	0.008

[a] Effective dose for 50% increase in contractility following iv administration to anaesthetised dogs. [b] Data from ref 29. [c] Data from ref 30. [d] Data from ref 12.

reflux for 48 h, allowed to cool to room temperature, and poured into water (1 L). The resulting mixture was extracted into Et_2O (3 × 250 mL), the extracts were washed with 2 N NaOH (2 × 250 mL), water, and brine, dried ($MgSO_4$), and evaporated under reduced pressure. Distillation in vacuo gave methyl 2-butoxybenzoate as a clear liquid, bp 102–105 °C (1 mmHg) (31.6 g, 50%).

NaOH (2 N, 165 mL) was added to the ester (31.0 g, 0.15 mol), the reaction mixture was refluxed for 2 h and cooled to room temperature, and the solution was acidified to pH 3 with 12 N HCl. The resulting oil was extracted with CH_2Cl_2 (3 × 200 mL), and the organic extracts were washed with water and brine. Drying ($MgSO_4$) followed by filtration and evaporation under reduced pressure gave 2-butoxybenzoic acid as a pale yellow liquid (24.6 g, 87%) which was used without further purification. A stirred mixture of the acid (2.9 g, 0.015 mol) and 2,3-diaminopyridine (1.6 g, 0.015 mol) in $POCl_3$ (35 mL) was heated under reflux for 7 h. The resulting dark reaction mixture was allowed to stand at room temperature for 16 h and evaporated to dryness under reduced pressure. The residue was treated with water, and the pH was adjusted to 6 with 2 N NaOH solution. The mixture was extracted with CH_2Cl_2 (2 × 25 mL), and the combined extracts were washed with water and brine and dried ($MgSO_4$). Evaporation under reduced pressure gave a dark oil which was purified by chromatography on silica gel using $CHCl_3$ as the eluting solvent. Evaporation of the appropriate fractions gave a solid which was recrystallized from MeCN to give the title compound as cream-colored crystals (1.2 g, 31%): mp 146–148 °C. Anal. ($C_{16}H_{17}N_3O$) C, H, N.

2-(2-Methoxyphenyl)-1*H*-imidazo[4,5-*b*]pyridine (1a) and 2-(2-methoxyphenyl)-1*H*-imidazo[4,5-*c*]pyridine (18) were prepared in an analogous manner. *o*-Anisic acid was converted into **1a** in 38% yield, after recrystallization from MeCN/water, mp 200 °C. Anal. ($C_{13}H_{11}N_3O{\cdot}0.5HCl{\cdot}0.3H_2O$) C, H, N. Similarly, but using 3,4-diaminopyridine, **19** was obtained in 14% yield, after recrystallization from MeCN/MeOH, mp 175–176 °C. Anal. ($C_{13}H_{11}N_3O{\cdot}1.5HCl{\cdot}1.0H_2O$) C, H, N.

2-(2-Ethoxyphenyl)-1*H*-imidazo[4,5-*b*]pyridine (1b). 2-Ethoxybenzoic acid was converted into the title compound in 35% yield, after recrystallization from MeCN, mp 146 °C. Anal. ($C_{14}H_{13}N_3O$) C, H, N.

2-(2-Propoxyphenyl)-1*H*-imidazo[4,5-*b*]pyridine (1c). Methyl salicylate was converted into methyl 2-propoxybenzoate and the ester saponified to 2-propoxybenzoic acid in 43% overall yield. The acid was converted into the title compound in 69% yield, after recrystallization from MeCN, mp 152 °C. Anal. ($C_{15}H_{15}N_3O$) C, H, N.

2-(2-(Pentyloxy)phenyl)-1*H*-imidazo[4,5-*b*]pyridine (1e) and 2-(2-(Pentyloxy)phenyl)-1*H*-imidazo[4,5-*c*]pyridine (18). Methyl salicylate was converted into methyl 2-(pentyloxy)benzoate and the ester saponified to 2-(pentyloxy)benzoic acid in 51% overall yield. The acid was converted into **1e** in 12% yield, after recrystallization from MeCN, mp 97 °C. Anal. ($C_{17}H_{19}N_3O$) C, H, N.

In an analogous manner, but using 3,4-diaminopyridine, **18** was obtained in 47% yield after recrystallization from MeCN/MeOH, mp 195.5–196.5 °C. Anal. ($C_{17}H_{19}N_3O{\cdot}HCl$) C, H, N.

2-(2-(Hexyloxy)phenyl)-1*H*-imidazo[4,5-*b*]pyridine (1f). Methyl salicylate was converted into methyl 2-(hexyloxy)benzoate and the ester saponified to 2-(hexyloxy)benzoic acid in 66% overall yield. The acid was converted into the title compound in 15% yield, after recrystallization from MeCN, mp 76 °C. Anal. ($C_{18}H_{21}N_3O$) C, H, N.

2-(2-(Isobutyloxy)phenyl)-1*H*-imidazo[4,5-*b*]pyridine (1g). Methyl salicylate was converted into methyl 2-(isobutyloxy)benzoate and the ester saponified to 2-(isobutyloxy)benzoic acid in 51% overall yield. The acid was converted into the title compound in 29% yield, after recrystallization from MeCN, mp 138 °C. Anal. ($C_{16}H_{17}N_3O$) C, H, N.

2-(2-Propylthio)benzaldehyde (3). Thiosalicylic acid (15.4 g, 0.1 mol) was refluxed for 16 h with methanolic hydrogen chloride to give the methyl ester as an oil (12.6 g, 75%). The ester (5.0 g, 30 mmol) was dissolved in dry THF (50 mL) and added dropwise to a stirred suspension of NaH (1.4 g, 60 mmol) in dry THF (50 mL). When effervescence ceased, 3-bromopropane (4.4 g, 36 mmol) was added and the reaction stirred for a further 60 min. The mixture was diluted with Et_2O and carefully poured into water. The organic layer was separated, dried ($MgSO_4$), and evaporated to give methyl (2-thiopropoxy)benzoate (6.0 g, 95%) as a mobile liquid. A solution of this material (4.0 g, 19 mmol) in dry THF (50 mL) was added to a stirred suspension of $LiAlH_4$ (0.5 g, 13 mmol) in dry THF (50 mL) at 0 °C. The reaction was allowed to warm to room temperature over 30 min and kept for a further 16 h before the addition of excess EtOAc. The mixture was poured into water, and the precipitated solids were removed by filtration. Extraction with Et_2O and evaporation of the dried ($MgSO_4$) extract gave 2-(propylthio)benzyl alcohol (3.2 g, 93%) which was dissolved in dry CH_2Cl_2 (30 mL) and added to a previously prepared mixture of DMSO (3.8 g, 42 mmol) and oxalyl chloride (2.5 g, 20 mmol) in dry CH_2Cl_2 (60 mL) at −78 °C. After 15 min, Et_3N (8.9 g, 88 mmol) was added and the mixture allowed to warm to room temperature. Water was added, and the organic layer was separated, washed with 1 N HCl, water, and saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and evaporated. The residue was filtered through silica gel using 9:1 Et_2O–petroleum ether as the eluting solvent. Evaporation of the solvents gave the title compound as a mobile liquid (3.0 g, 95%).

Preparation of Substituted Imidazo[4,5-*b*]pyridines: Method B. 2-(2-(3-Hydroxypropyl)phenyl)-1*H*-imidazo[4,5-*b*]pyridine (5b). An intimate mixture of 2-(3-hydroxypropoxy)benzaldehyde (**2d**) (R = $O(CH_2)_3OH$, 5.0 g, 28 mmol), 2,3-diaminopyridine (3.0 g, 28 mmol), and sulfur (1.8 g, 56 mmol) was heated at 120 °C for 3 h. After the mixture was cooled to room temperature, EtOH was added and insoluble materials were removed by filtration. The filtrate was evaporated onto silica gel and purified by flash chromatography using 95:5 CH_2Cl_2–MeOH as the eluting solvent. Evaporation of the appropriate fractions gave a solid which was treated with activated charcoal and recrystallized from CH_2Cl_2/Et_2O to give the title compound (2.7 g, 36%), mp 148 °C. Anal. ($C_{15}H_{15}N_3O_2{\cdot}0.1H_2O$) C, H, N.

The following compounds were prepared in an analogous manner:

2-(2-(2-Hydroxyethoxy)phenyl)-1*H*-imidazo[4,5-*b*]pyridine (5a). 2-(2-Hydroxyethoxy)benzaldehyde (**2c**) (R = OCH_2CH_2OH) was converted into the title compound in 40% yield after recrystallization from acetone, mp 158 °C. Anal. ($C_{14}H_{13}N_3O_2$) C, H, N.

2-(2-(Propylthio)phenyl)-1*H*-imidazo[4,5-*b*]pyridine (6). 2-(Propylthio)benzaldehyde (**3**) was converted into the title compound in 42% yield after recrystallization from aqueous EtOH, mp 170 °C. Anal. ($C_{15}H_{15}N_3S{\cdot}0.2H_2O$) C, H, N.

2-(2-Nitrophenyl)-1*H*-imidazo[4,5-*b*]pyridine (7). 2-Nitrobenzaldehyde (**4**) was converted into the title compound in 73% yield after recrystallization from EtOH, mp 252 °C. Anal. ($C_{12}H_8N_4O_2$) C, H, N.

2-(2-*tert*-Butoxyphenyl)-1*H*-imidazo[4,5-*b*]pyridine (1h). 2-(2-*tert*-Butoxyphenyl)benzaldehyde (**2a**) (R = $OC(CH_3)_3$) was converted into the title compound in 51% yield after recrystallization from EtOAc, mp 195–196 °C. Anal. ($C_{16}H_{17}N_3O$) C, H, N.

2-(2-(Benzyloxy)phenyl)-1*H*-imidazo[4,5-*b*]pyridine (1j). 2-(2-(Benzyloxy)phenyl)benzaldehyde (**2b**) (R = OCH_2Ph) was converted into the title compound in 27% yield after recrystallization from cyclohexane/toluene, mp 136–137 °C. Anal. ($C_{19}H_{15}N_3O$) C, H, N.

Preparation of Substituted Imidazo[4,5-*b*]pyridines: Method C. 2-(2-(Cyclopropylmethoxy)phenyl)-1*H*-imidazo[4,5-*b*]pyridine (14). Ethyl 2-(cyclopropylmethoxy)benzoate (5.5 g, 25 mmol), prepared from ethyl salicylate as in method A, was saponified by heating with NaOH (1.1 g, 27.5 mmol) in MeOH/water solution and subsequent acidification. The acid produced was dissolved in dry CH_2Cl_2 (75 mL) and treated sequentially with DMF (0.2 g, 2.5 mmol) and oxalyl chloride (3.5 g, 27.5 mmol). After 4 h the solution was added to a stirred mixture of 2,3-diaminopyridine (2.7 g, 25 mmol) and Et_3N (10.1 g, 0.1 mol) in dry CH_2Cl_2 (50 mL). After 24 h the reaction was partitioned between water and CH_2Cl_2, and the organic layer was separated, dried ($MgSO_4$), and evaporated. The residual oil was dissolved in $POCl_3$ (5 mL) and refluxed for 6 h before pouring into ice/water. The solution was basified to pH 9 with 2 N KOH and extracted with CH_2Cl_2. The organic extracts were dried ($MgSO_4$), evaporated onto silica gel, and purified by flash chromatography using 95:5 CH_2Cl_2/MeOH as the eluting solvent. Evaporation of the appropriate fractions gave a gum which was treated with activated charcoal and recrystallized from aqueous EtOH to give the title compound (1.5 g, 23%), mp 140 °C. Anal. ($C_{16}H_{15}N_3O$) C, H, N.

Similarly, **2-(2-(cyclopentyloxy)phenyl)-1*H*-imidazo[4,5-*b*]pyridine (13)** was prepared from 2-(cyclopentyloxy)benzoic acid in 23.5% yield after recrystallization from EtOH, mp 163–164 °C. Anal. ($C_{17}H_{17}N_3O$) C, H, N.

2-(2-(3-Chloropropoxy)phenyl)-1*H*-imidazo[4,5-*b*]pyridine (8). CCl_4 (0.31 g, 2 mmol) was added to a stirred mixture of triphenylphosphine (0.52 g, 2 mmol) and 2-(2-(3-hydroxypropoxy)phenyl)-1*H*-imidazo[4,5-*b*]pyridine (**5a**) (0.27 g, 1 mmol) in dry CH_2Cl_2 (5 mL). The reaction was allowed to proceed for 16 h and partitioned between pH 7 phosphate buffer and CH_2-

Cl_2. The organic layer was separated and extracted with 2 N HCl, and the aqueous extract was washed with CH_2Cl_2 and neutralized with 2 N NaOH. Extraction with CH_2Cl_2 and evaporation of the dried ($MgSO_4$) extract gave a solid which was recrystallized from EtOAc to give the title compound (0.15 g, 52%), mp 153 °C. Anal. ($C_{15}H_{14}ClN_3O$) C, H, N.

2-(2-(3-(Methylamino)propoxy)phenyl)-1*H*-imidazo[4,5-*b*]pyridine (9). A mixture of alcoholic methylamine (15 mL) and 2-(2-(3-chloropropoxy)phenyl)-1*H*-imidazo[4,5-*b*]pyridine (8) (1.0 g, 3.5 mmol), in EtOH (10 mL) was heated at 100 °C for 24 h. Evaporation of volatiles gave an oil which was purified by recrystallization from EtOH to give the title compound as the HCl salt (0.3 g, 27%), mp 169 °C. Anal. ($C_{16}H_{18}N_4O{\cdot}HCl$) C, H, N.

2-(2-(3-(Dimethylamino)propoxy)phenyl)-1*H*-imidazo-[4,5-*b*]pyridine (10). A mixture of alcoholic dimethylamine (15 mL) and 2-(2-(3-chloropropoxy)phenyl)-1*H*-imidazo[4,5-*b*]pyridine (8) (1.0 g, 3.5 mmol) in EtOH (10 mL) was heated at 100 °C for 18 h. Evaporation of volatiles gave an oil which was purified by recrystallization from EtOH to give the title compound as the HCl salt (0.6 g, 52%), mp 215 °C. Anal. ($C_{17}H_{20}N_4O{\cdot}HCl$) C, H, N.

2-(2-Aminophenyl)-1*H*-imidazo[4,5-*b*]pyridine (11). A solution of 2-(2-nitrophenyl)-1*H*-imidazo[4,5-*b*]pyridine (7) (3.0 g, 12.5 mmol) in 2 M aqueous AcOH (100 mL) and 10% Pd/C (2.0 g) was hydrogenated at 50 psi for 9 h. The catalyst was removed by filtration and the filtrate neutralized with 2 N NaOH. The precipitated product was isolated (2.3 g, 87%), mp 323 °C, and converted to the dihydrochloride salt which was recrystallized from $EtOH/Et_2O$, mp 267 °C. Anal. ($C_{12}H_{10}N_4{\cdot}2.1HCl{\cdot}0.5H_2O$) C, H, N.

2-(2-(Propylamino)phenyl)-1*H*-imidazo[4,5-*b*]pyridine (12). 2-(2-Aminophenyl)-1*H*-imidazo[4,5-*b*]pyridine (11) (1.0 g, 5 mmol) was added to propanal (0.3 g, 5 mmol) and 4-Å molecular sieves (1 g) in dry EtOH (50 mL). The mixture was stirred at room temperature for 6 days before $NaBH_4$ (0.5 g, 13 mmol) was added. After a further 48 h the filtered solution was poured into water and extracted with CH_2Cl_2. The organic extracts were dried ($MgSO_4$) and evaporated. The residue was recrystallized from $EtOH/Et_2O$ to give the title compound (0.6 g, 46%), mp 240 °C. Anal. ($C_{15}H_{16}N_4{\cdot}0.5H_2O$) C, H, N.

2-(2-Hydroxyphenyl)-1*H*-imidazo[4,5-*b*]pyridine (15). An intimate mixture of salicylic acid (12.0 g, 87 mmol) and 2,3-diaminopyridine (10.0 g, 87 mmol) in polyphosphoric acid (230 g) was heated at 180 °C for 4 h. The reaction was cooled and added to ice/water, and the resulting solution was washed with CH_2Cl_2. Neutralization with aqueous KOH solution gave a solid which was isolated and recrystallized from EtOH to give the title compound (9.7 g, 53%), mp >300 °C. Anal. ($C_{12}H_9N_3O{\cdot}0.1H_2O$) C, H, N.

2-(2-Acetoxyphenyl)-1*H*-imidazo[4,5-*b*]pyridine (16). 2-(2-Hydroxyphenyl)-1*H*-imidazo[4,5-*b*]pyridine (15) (7.7 g, 36 mmol) was added to a solution of NaOH (2.2 g, 55 mmol) in water (40 mL). Ice was added, and the resulting slurry was treated with Ac_2O (5.6 g, 55 mmol). $CHCl_3$ was added to dissolve the resulting solid, and the organic layer was separated, dried ($MgSO_4$), and evaporated. The residue was recrystallized from $CHCl_3/Et_2O$ to give the title compound (7.6 g, 83%) mp 202 °C. Anal. ($C_{14}H_{11}N_3O_2$) C, H, N.

2-(2-Propoxyphenyl)imidazo[1,2-*a*]pyrimidine (17). A stirred mixture of 2-hydroxyacetophenone (68 g, 0.5 mol), 1-bromopropane (67 g, 0.55 mol), KI (8 g, 0.05 mol), and K_2CO_3 (80 g, 0.58 mol) in acetone (500 mL) was refluxed for 120 h. The residue left after evaporation of the filtered mixture was dissolved in ether (300 mL), and the solution was washed with 1 N NaOH, water, and brine, dried ($MgSO_4$), and evaporated to give a solid (77 g, 86%). The solid (5.0 g, 28 mmol), dissolved in $CHCl_3$ (20 mL), was treated dropwise with Br_2 (4.5 g, 28 mmol), and after 90 min the reaction was poured into water and allowed to stir for a further 10 min. The organic layer was separated, washed with water and brine, dried ($MgSO_4$), and evaporated. The residual oil and 2-aminopyrimidine (1.8 g, 19 mmol) were dissolved in DMF (40 mL) and stirred for 40 h. The reaction was diluted with ethyl acetate, washed with aqueous $NaHCO_3$ and brine, and dried ($MgSO_4$) before evaporation. The residue was purified by chromatography on silica using EtOAc/petroleum ether (3:1) as eluant. Evaporation of the appropriate fractions gave a solid which was recrystallized from EtOH to give the title compound as cream-colored crystals (0.75 g, 10%), mp 133 °C. Anal. ($C_{15}H_{15}N_3O$) C, H, N.

Phosphodiesterase Inhibition Studies. The concentration of drug required to produce a 50% inhibition of enzymic activity (IC_{50}) was determined by the boronate column method[33] using 1 μM cGMP as a substrate for PDE V and 1 μM cAMP as a substrate for PDE III and PDE IV. PDE V was isolated from porcine pulmonary arteries by anion-exchange chromatography and resolved from calmodulin-activated PDEs by a calmodulin affinity column. PDE V specifically hydrolyzed cGMP (K_m = 1 μM) was insensitive to calmodulin and was potently inhibited by M&B 22948 (IC_{50} = 0.9 μM). PDE III and PDE IV were both prepared from guinea pig ventricle by anion-exchange chromatography. PDE III utilized both cAMP ($K_m > 1$ μM) and cGMP ($K_m > 1$ μM); the hydrolysis of cAMP was inhibited by cGMP (IC_{50} = 1 μM), siguazodan (IC_{50} = 3 μM) but not rolipram (IC_{50} > 100 μM). Conversely, PDE IV was inhibited by rolipram (IC_{50} = 0.6 μM) but not by cGMP or siguazodan (IC_{50} > 100 μM for both).

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Supplementary Material Available: Listings of physical data (^{1}H NMR) for compounds **1d**, **3**, **5b**, **14**, **8–10**, **12**, **16**, and **17** together with elemental analyses for compounds **1a–j**, **5a**,**b**, and **6–19** (7 pages). Ordering information is given on any current masthead page.

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