Inotropic "A" Ring Substituted Sulmazole and Isomazole Analogues†

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A series of "A" ring substituted sulmazole and isomazole analogues have been prepared and evaluated as inotropic agents. pK_A's, protonation sites, and log P values were measured for selected compounds and their electronic properties were calculated. No simple correlation between inotropic activity and pK_A, protonation site, or log P value was observed. However, in vitro inotropism did correlate with the calculated charge density of the "B" ring imidazo nitrogen atom. The 6-position of sulmazole appeared to be the most tolerant toward substituents, the 6-amino derivative **7** being a more potent inotrope than sulmazole itself. 4-Methoxyisomazole **13** had comparable in vivo inotropic properties to those of isomazole.

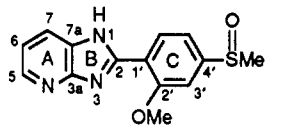
The inotropic agent sulmazole (**1**,[1,2] Chart I) has been tested clinically as an orally active replacement for digoxin in the treatment of heart failure. Further development of sulmazole, for chronic oral use, has been suspended,[3,4] however, because of undesirable toxicological effects and substantial metabolism. In an attempt to obtain an inotropic agent with a better profile of pharmacological activity than sulmazole, we[5] and others[6,7] have prepared 1*H*-imidazo[4,5-*c*]pyridine isomer **2** (Chart I), isomazole,[37] and found[8] it to be more potent than sulmazole as an inotrope. Because of this encouraging result we wished to develop some understanding of the structure–activity relationships (SAR's) for sulmazole analogues. In particular we required to know what effects substituents in the "A" ring would have on the inotropic activities of sulmazole and isomazole and whether any effects were related to the physicochemical properties of these heterocyclic bases.

Our previous study[5] of sulmazole analogues having differing heterocyclic ring systems suggested a correlation (correlation coefficient 0.81) between in vitro inotropic activity and the charge density of the "B" ring imidazo nitrogen atom. Guided by these results we initially chose methoxyl as an "A" ring substituent for the present work for two main reasons. Initially, we speculated that electron-releasing substituents might increase the charge density of the imidazo nitrogen atom, and hence in vitro inotropism, if a correlation exists between these two parameters for these analogues. We also hypothesized that a small, noncharged, nonlipophilic "A" ring substituent such as methoxyl (MR = 7.87, π = –0.02)[9] would be compatible with good activity as is the case for "C" ring methoxyl groups. Having defined the "A" ring positions which were the most tolerant of the introduction of a methoxyl group, further analogues having a selected range of substituents at these positions were then prepared to help test our hypotheses. This investigation thus resulted in the synthesis of the sulmazole analogues **3–21** (Tables I and II).

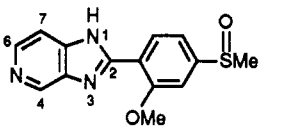
Results and Discussion

Chemistry. 1*H*-Imidazo[4,5-*b*]pyridines **3–12** and **29–36** and the 1*H*-imidazo[4,5-*c*]pyridines **13–21**, **42–45**, and **63** synthesized in this study are shown in Tables I and II,

Chart I



1, sulmazole



2, isomazole

respectively. Analogues **3-10** and **13-18** were prepared (Scheme I) via condensation of the requisite diaminopyridines **22–28** and **37–41** with either 2,4-dimethoxybenzoic acid, 2-methoxy-4-methylthiobenzoic acid[5,6] (methods A or D), or their acid chlorides (methods B or C). Sulfide intermediates **29–31**, **33**, **35**, **36**, and **42–44** were then oxidized routinely with H_2O_2/HOAc (method E) or with MCPBA (method F) to give the corresponding sulfoxides in good yields. The 6-amino derivatives **6** and **7** were obtained by hydrogenation of the corresponding 6-nitrosulmazole analogues **32** and **34**, respectively (method G). Similarly hydrogenation of nitro compound **45** gave amine **16** (method H). Diamines **22**,[10] **25**,[11] **26**,[12] **27**,[13] **37**,[14] **40**,[15] and **41**[15] were prepared by previously reported methods. 2,3-Diamino-4-methoxypyridine (**24**) was obtained from 2-amino-4-chloro-3-nitropyridine (**46**)[16] as

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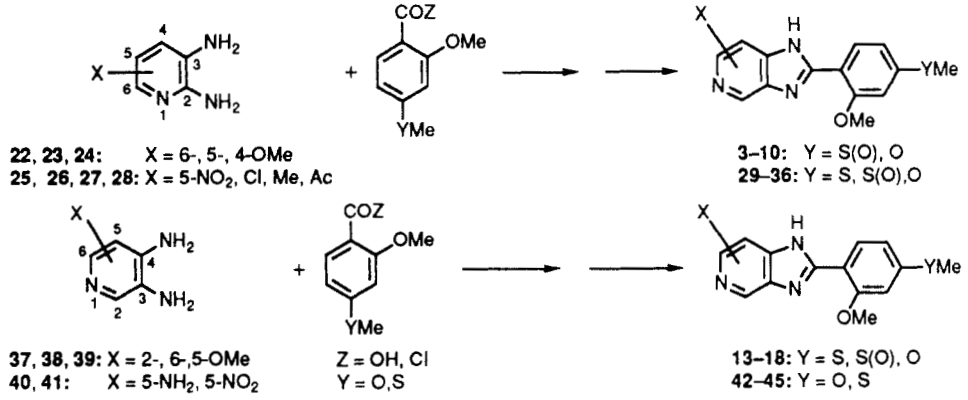
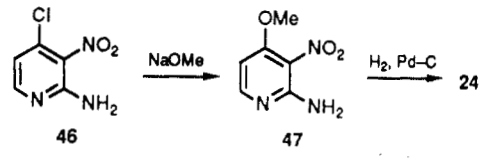
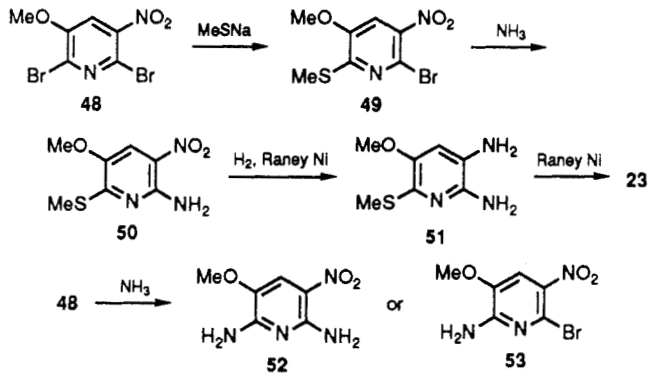
Scheme I



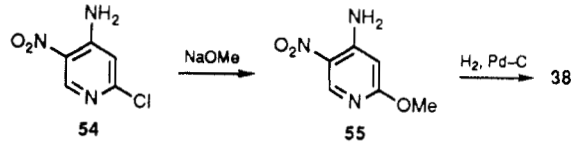
Scheme II



Scheme III

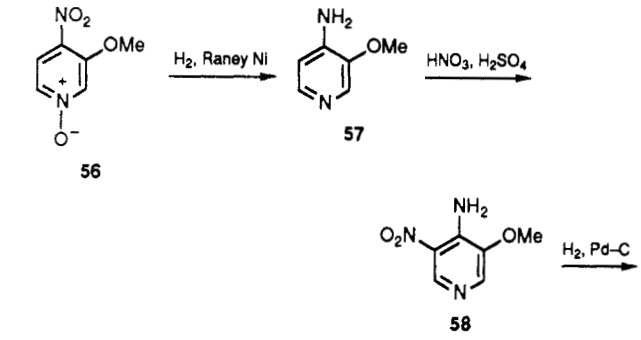


Scheme IV

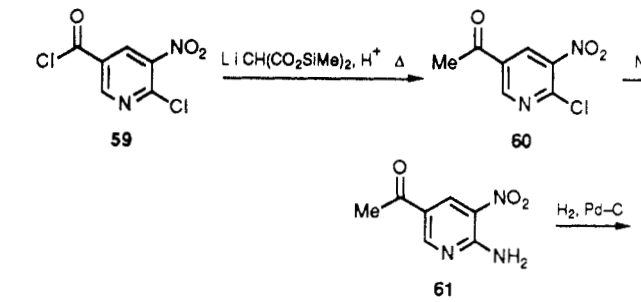


shown in Scheme II. 2,3-Diamino-5-methoxypyridine (**23**) proved to be the most difficult isomer to obtain. After many unsuccessful attempts to prepare **23**, which involved putative nitrations of numerous aminomethoxy- and halomethoxypyridines, the four-step process outlined in Scheme III proved to be a workable route. Direct reaction of dibromide **48**[17] with ammonia gave under vigorous conditions diamine **52**, while under milder conditions (room temperature) the undesired monoamine **53** was obtained. The assignment of structure **53** to the amine obtained was based on the IR absorption band at 1310 cm^{-1} indicative of a "para" nitroamine.[18] The use of a blocking group such as methylthio was hence required to give the required substitution pattern as in nitroamine **50**, whose IR spectrum showed a band at 1230 cm^{-1} characteristic of a hydrogen-bonded "ortho" nitroamine.[18] 3,4-Diamino-6-methoxypyridine (**38**) was prepared from 4-amino-2-chloro-5-nitropyridine (**54**)[19,20] as shown in Scheme IV.

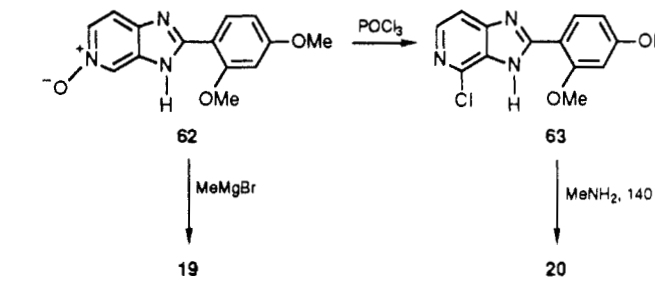
Scheme V



Scheme VI



Scheme VII



The route to 3,4-diamino-5-methoxypyridine (**39**) is outlined in Scheme V. The key step is the nitration of 4-amino-3-methoxypyridine (**57**)[21] to give **58**.

5-Acetyl-2,3-diaminopyridine (**28**) was prepared from 6-chloro-5-nitronicotinoyl chloride (**59**)[22] by the three-stage

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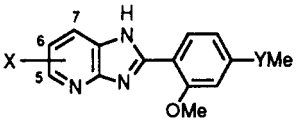
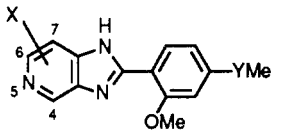
Table I. Structure and Properties of 1*H*-Imidazo[4,5-*b*]pyridines



no.	X	Y	method,[d] intermediates	% yield	formula	mp, °C	anal.
3	5-OMe	S(O)	F, 29	53	$C_{15}H_{15}N_3O_3S$	135–137	C, H, N
4	6-OMe	S(O)	E, 30	62	$C_{15}H_{15}N_3O_3S{\cdot}0.33H_2O$	212–215	C, H, N, S
5	7-OMe	S(O)	E, 31	77	$C_{15}H_{15}N_3O_3S$	ca. 110[a]	C, H, N
6	6-NH_2	O	G, 32	88	$C_{14}H_{14}N_4O_2{\cdot}2HCl$	232–234 dec	C, H, N, Cl
7	6-NH_2	S(O)	G, 34	43	$C_{14}H_{14}N_4O_2S{\cdot}0.4H_2O$	>220 dec	C, H, N
8	6-Cl	S(O)	F, 35	32	$C_{14}H_{12}ClN_3O_2S{\cdot}0.5HCl$	232–234	C, H, N, Cl
9	6-Me	S(O)	F, 36	66	$C_{15}H_{15}N_3O_2S$	216–219	C, H, N
10	6-Ac	O	C, 28	19	$C_{16}H_{15}N_3O_3{\cdot}HCl$	216–218	C, H, N, Cl
11	6-OH	S(O)		5	$C_{14}H_{13}N_3O_3S$	227–230	
12[c]	H	O					
29	5-OMe	S	A, 22	52	$C_{15}H_{15}N_3O_2S$	158–160	C, H, N
30	6-OMe	S	A, 23	30	$C_{15}H_{15}N_3O_2S$	177–180	C, H, N
31	7-OMe	S	A, 24	61	$C_{15}H_{15}N_3O_2S$	133–136	C, H, N
32	6-NO_2	O	A, 25	58	$C_{14}H_{12}N_4O_4$	272–274 dec	C, H, N
33	6-NO_2	S	A,[b] 25	70	$C_{14}H_{12}N_4O_3S$		
34	6-NO_2	S(O)	F, 33	70[b]	$C_{14}H_{12}N_4O_4S$		
35	6-Cl	S	A, 26	50	$C_{14}H_{12}ClN_3OS$	155–157	C, H, N
36	6-Me	S	A, 27	38	$C_{15}H_{15}N_3OS$	65–70	C, H, N

[a] Hygroscopic; value determined in vacuo. [b] This intermediate proved difficult to purify and the crude material was used; estimated yield given. [c] References 5, 6, 30. [d] Method A, X(diNH_2)C_5H_2N, ArCO_2H, $POCl_3$; method B, X(diNH_2)C_5H_2N, ArCOCl, NEt_3, C_5H_5N; $POCl_3$; method C, X(diNH_2)C_5H_2N, ArCOCl, NEt_3, C_5H_5N; trace of concentrated HCl, ethanediol, 180 °C; method D, X(diNH_2)C_5H_2N, ArCO_2H, PPA; method E, sulfide oxidation with H_2O_2, HOAc; method F, sulfide oxidation with MCPBA, CH_2Cl_2, −15 °C; method G, H_2, Pd–C, EtOH; method H, H_2, PtO_2, EtOH.

Table II. Structure and Properties of 1*H*-Imidazo[4,5-*c*]pyridines



no.	X	Y	method,[c] intermediates	% yield	formula	mp, °C	anal.
13	4-OMe	S(O)	F, 42	76	$C_{15}H_{15}N_3O_3S$	189–190	C, H, N
14	6-OMe	S(O)	E, 43	65	$C_{15}H_{15}N_3O_3S$	205–206	C, H, N, S
15	7-OMe	S(O)	F, 44	72	$C_{15}H_{15}N_3O_3S$	168–171	C, H, N
16	7-NH_2	O	H, 45	93	$C_{14}H_{14}N_4O_2{\cdot}HCl{\cdot}0.25H_2O$	269–270	C, H, N
17	7-NH_2	S	D, 40	12	$C_{14}H_{14}N_4OS{\cdot}2HCl{\cdot}H_2O$	278–280	C, H, N
18	7-NH_2	S(O)	E, 17	42	$C_{14}H_{14}N_4O_2S{\cdot}0.75H_2O$	198–200	C, H, N
19	4-Me	O	62	31	$C_{15}H_{15}N_3O_2{\cdot}1.33HCl{\cdot}H_2O$	234–235	C, H, N, Cl
20	4-NHMe	O	63	38	$C_{15}H_{16}N_4O_2{\cdot}HCl$	204–207 dec	C, H, N, Cl
21[b]	H	O					
42	4-OMe	S	B, 37	18	$C_{15}H_{15}N_3O_2S$	189–190	C, H, N
43	6-OMe	S	A, 38	16	$C_{15}H_{15}N_3O_2S$	250–252	C, H, N
44	7-OMe	S	A, 39	25	$C_{15}H_{15}N_3O_2S$	140–142[a]	C, H, N
45	7-NO_2	O	A, 41	68	$C_{14}H_{12}N_4O_4{\cdot}0.75H_2O$	260–262	C, H, N
63	4-Cl	O	62	67	$C_{14}H_{12}ClN_3O_2$	203–204	C, H, N, Cl

[a] Melts at 107–110 °C, resolidifies, and then remelts at this temperature. [b] References 5, 6, and 30. [c] Methods A–F: see Table I, footnote d.

process shown in Scheme VI. 6-Hydroxysulmazole **11**[23] was obtained in low yield by Fenton's oxidation of sulmazole. Analogues **19** and **20** were prepared from *N*-oxide **62**[24] and the 4-chloro heterocycle **63**, respectively, as shown in Scheme VII.

Protonation Equilibria. pK_A's were measured by a rapid spectrophotometric method as described previously.[5] ^{13}C NMR methods[5] were employed to determine the major protonation sites of the "A" ring methoxy analogues, and these studies will be reported in detail elsewhere.[25] The ^{13}C technique is illustrated by the determination of the protonation site of amine **6**. The chemical shifts for the base and salt of analogue **6** are given in Table VI of the supplementary material and indicate protonation of the 6-amino group.

Calculation of Electronic Properties. Initial calculations were performed on a VAX 11/750 computer. The geometries and conformations of the heterocycles were deduced by molecular mechanics calculations. The SYBYL[26] software package was utilized to perform geometry opti-

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(26) SYBYL molecular modelling system. Tripos Associates, St. Louis, MO.

Table III. In Vitro Inotropic Activities and Charge Densities of Sulmazole Analogues

compd	inotropism; pA_{50}[a]	n[b]	charge[c]
1	4.70 ± 0.10	9	−0.266
3	3.00 ± 0.12	3	−0.225
4	3.63 ± 0.02	3	−0.246
5	4.10 ± 0.02	3	−0.281
2	4.64 ± 0.15	17	−0.256
13	3.91 ± 0.12	3	−0.279
14	3.76 ± 0.43	3	−0.258
15	3.00 ± 0.08	3	−0.226
6	4.80 ± 0.03	3	−0.276
7	4.01 ± 0.07	3	−0.263
8	3.61 ± 0.08	3	−0.258
9	3.73 ± 0.02	3	−0.243
10	i[d]	2	−0.248
11	3.75 ± 0.09	3	−0.249
12	5.08 ± 0.09	4	−0.282
16	i[e]	3	
17	i[f]		
18	3.72 ± 0.12		−0.238
19	i[g]		−0.281
20	i[f]		−0.283
21	4.01 ± 0.09	9	−0.259

[a] pA_{50} = −log c where c = drug concentration required to give a 50% increase in basal contractile force; i = inactive; 50% increase not achieved. Further details are given in ref 30. [b] n = no. of determinations employing paced guinea pig papillary muscle preparations. [c] Charge of imidazo nitrogen calculated by CNDO/2. [d] Tested at concentrations up to 1 mM; weak negative inotropic effects observed. [e] Tested at concentrations up to 5 mM. [f] Tested at concentrations up to 1 mM. [g] Tested at concentrations up to 0.2 mM.

mizations. The resulting structures were further geometry optimized with MOPAC[27] (AMI, PM3) on a CRAY X-MP28 supercomputer. Atom-centered charge densities were calculated by using the semiempirical molecular orbital method CNDO/2.[28] Electrostatic potentials were calculated from the ab initio STO-3G wave function with Gaussian 80-UCSF (CRAY)[29] in a plane through the imidazopyridine and also in a series of planes parallel to the plane of this ring system. Isopotential maps were then constructed in the usual way by drawing isopotential contours. A complicating factor in this computational study was the tautomerism of the imidazopyridines. The above calculations were performed on the 3*H* tautomers. The choice of tautomer was based on ab initio (3-21G basis set) calculations which predicted (in agreement with experiment) 3*H*-imidazo[4,5-*b*]pyridine to be of lower energy than the 1*H* tautomer. Since the imidazo[4,5-*b*] series generally shows[30] the most potent in vitro inotropism, the 3*H* tautomer was chosen for calculation. The energy difference between the imidazopyridine tautomers was calculated to be small, e.g. 0.5 kcal mol^{-1} (depending on the basis set used) for 1*H*- and 3*H*-imidazo[4,5-*c*]pyridine.

Pharmacology. Inotropic activity was determined in vitro by using a isolated paced guinea pig papillary muscle preparation set up under isometric conditions. Cumulative concentration effect curves were constructed for each test compound. The response parameter chosen was pA_{50}, the negative logarithm of the drug concentration required to give a 50% increase in basal contractile force.

Those compounds which displayed significant inotropic effects in vitro, were routinely evaluated in vivo by determining the effects of giving single bolus (iv) injections to anesthetized open-chest dogs. Inotropic responses were indexed by a ED_{50} value, i.e. the dose (mg kg^{-1}) of drug required to produce a maximum increase of 50% in dP/dt. [dP/dt is the first time derivative of left ventricular pressure (P)].

Structure–Activity Relationships. The in vitro inotropic activities of the compounds are collected together in Table III. Comparison of the inotropic activities of the sulmazole analogues **3–5** with that of sulmazole shows that introduction of a methoxy substituent in the 6- or 7-position is better tolerated than in the 5-position. Using these results as a guide we prepared and evaluated other 6- and 7-substituted sulmazole analogues. Analogue **5** and other 7-(alkyloxy)- or 7-(alkylamino)sulmazole analogues[31] (data not given) consistently displayed poor in vivo inotropic activity despite showing good in vitro inotropism. The reasons for this difference are not known, although the reduced in vivo inotropism is probably in part a reflection of increased lipophilicity for the higher homologues. Previous studies[1,32] and our own work[30,33] on "C" ring modified analogues have suggested that both in vivo and in vitro inotropic activities of the 1*H*-imidazopyridines are sensitive to gross changes in log P. In addition there may also be an upper limit[5,34] to log P (ca. 2.5) for good in vivo inotropic activity. These observations in addition to synthetic difficulties precluded a more detailed study of 7-substituted analogues.

The 6-position of sulmazole appeared tolerant of amino, chloro, methyl, and hydroxyl substituents and the resulting analogues **6–9** and **11** all displayed in vitro inotropism. A 6-acetyl substituent was not tolerated, however, and the reasons for the inactivity of analogue **10** are at present not clear.

The activities of the isomazole analogues **13–15** indicate that a methoxy substituent is better tolerated in the 4- or 6-position than in the 7-position. In addition, for isomazole, introduction of methyl or methylamino substituents at the 4-position led to inactive analogues (i.e. **19** and **20**). Of the 7-amino analogues investigated, sulfoxide **18** did display inotropic activity, unlike 4′-methoxy (**16**) and 4′-methylthio (**17**) analogues, which were inactive. These results differ from those obtained with sulmazole, where introduction of an amino group β to the "A" ring nitrogen atom leads to active analogues for both sulfoxide **7** and ether **6**.

Substituent effects were quite complex for isomazole and we have no simple explanation for them. Lipophilicity changes may have some influence on inotropic activity, but such changes alone cannot account for the inactivities observed in the present set of compounds. Thus, although the observed and calculated π values for several "A" ring substituents were found to be different (Table V, sup-

(27) MOPAC. QCPE 455, Quantum Chemical Program Exchange, Department of Chemistry, University of Indiana, Bloomington, Indiana (PM3 parameterization of MOPAC, J. J. P. Stewart, private communication).
(28) CNDO/2. QCPE 91, Quantum Chemical Program Exchange, Department of Chemistry, University of Indiana, Bloomington, Indiana.
(29) Gaussian-80 UCSF. QCPE 446, Quantum Chemical Program Exchange, Department of Chemistry, University of Indiana, Bloomington, Indiana.
(30) Barraclough, P.; Black, J. W.; Cambridge, D.; Gerskowitch, V. P.; Hull, R. A. D.; Iyer, R.; King, W. R.; Kneen, C. O.; Nobbs, M. S.; Shah, G.; Smith, S.; Vine, S. J.; Whiting, M. V. *Arch. Pharm. (Weinheim)*, in press.
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(33) Barraclough, P.; Black, J. W.; Cambridge, D.; Demaine, D. A.; Gerskowitch, V. P.; Giles, H.; Hill, A. P.; Hull, R. A. D.; Iyer, R.; King, W. R.; Livingstone, D. J.; Nobbs, M. S.; Randall, P.; Shah, G. P.; Whiting, M. V. *Arch. Pharm. (Weinheim)*, in press.
(34) Kutter, E.; Austel, V. *Arzneim-Forsch.* **1981**, *31*, 135.

Table IV. In Vivo Cardiovascular Effects[a] of Isomazole and Analogues

compd	ED_{50} dP/dt	% max increase (dose, mg kg^{-1})	ED_{30} DBP	% max decrease (dose, mg kg^{-1})	ratio ($ED_{50}dP/dt$)/(ED_{30}DBP)	ED_{10} HR	% max increase (dose, mg kg^{-1})
sulmazole (n = 4)	0.80	73 (3.0)	1.0	36 (3.0)	0.80	0.50	20 (3)
7 (n = 2)	0.50	87 (3.0)	>3.0	25 (3.0)	<0.17	1.41	15 (3)
isomazole (n = 9)	0.05	88 (0.3)	0.16	43 (1)	0.32	0.056	15 (0.3)
13 (n = 3)	0.13	125 (1)	0.20	70 (3)	0.65	0.28	10 (0.3)

[a] Maximum percentage change in dP/dt, diastolic blood pressure and heart rate, produced by selected analogues and the effective dose (mg kg^{-1} iv) of these compounds to produce a maximum increase of 50% in dP/dt (ED_{50} dP/dt), 30% decrease in diastolic blood pressure (ED_{30}DBP), and 10% increase in heart rate (ED_{10} HR) as compared to those of isomazole and sulmazole in anesthetized, open-chest dogs. The ED's were derived from the mean values shown in Figure 3 (supplementary material).

plementary material), the log P values of inactive analogues were still within the range where in vitro inotropic activity is observed for other imidazopyridines.[30]

Overall no simple relationship between in vitro inotropic activity and pK_A, protonation site, or log P is apparent. At physiological pH (7.4) none of the analogues in the present set are significantly protonated or deprotonated. This suggests that the active species are the un-ionized molecules, although the possibility that protonation occurs within the active-site cleft of their target receptor (e.g. phosphodiesterase III) cannot be excluded.

Molecular mechanics calculations predict that the most stable conformers of the molecules are essentially planar with little (<5°) or no deviation of the aryl ring from the plane of the heterocyclic ring system. The calculated geometries are consistent with experimental values. X-ray crystallographic studies show isomazole[6] to be planar in the crystal. In the solid state a hydrogen bond exists between the imidazo nitrogen and the oxygen of the *o*-methoxy substituent for isomazole and this further stabilizes the planar conformer. Ab initio calculations show that the 3*H* tautomer of imidazo[4,5-*b*]pyridine is of lower energy than the 1*H* tautomer. For imidazo[4,5-*c*]pyridine, however, the 1*H* tautomer was found to be of lower energy than the 3*H* tautomer. ^{15}N and ^{13}C NMR studies[5] are in broad agreement with the ab initio calculations and show that the 3*H* tautomer of imidazo[4,5-*b*]pyridine is the predominant form in solution whereas for imidazo[4,5-*c*]pyridine the 1*H* tautomer is the major species. The 1*H* tautomers of sulmazole and isomazole are however the predominant species in solution as determined by NMR studies.[5] These observations reflect the importance of solvent effects in determining the preponderance of the major tautomeric species.

Calculations of the electrostatic potential field in the plane of the imidazopyridine ring and in a series of planes parallel to the system revealed the trend that active molecules had an electrostatic potential minimum adjacent to the formally sp^2 imidazo nitrogen. Since this trend was observed quite clearly, and without exception, when calculations were performed in the plane of the imidazopyridine ring, isopotential maps were constructed from this reference plane rather than from some other arbitrarily chosen plane above the molecule. Examination of the atom-centered charge density calculated for the imidazo nitrogen (Table III) shows that the most potent in vitro inotropism is most often associated with a relatively high charge density on this imidazo nitrogen atom. This is more clearly illustrated by a plot between these two parameters (Figure 1) and indicates a correlation between them (correlation coefficient is 0.81). Because the differences in charge densities are small (max 0.06) this correlation is best described as reasonable, rather than excellent, for this subset of active analogues. This correlation does however explain the good in vitro activities of analogues **6** and **13** and is consistent with our earlier findings.[5] Further studies undertaken with a larger set of isomazole analogues, having a wider range of charge densities, do give an excellent correlation between the two parameters.[31]

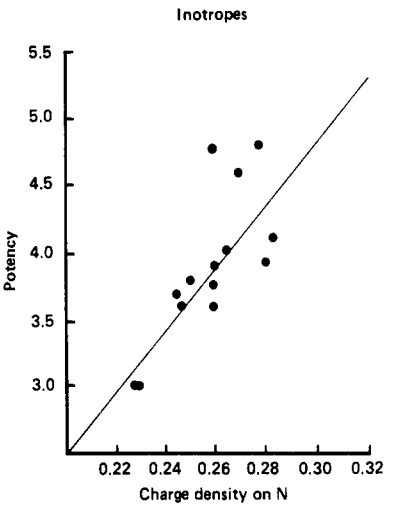

Figure 1. Plot of in vitro inotropic potency vs imidazo N charge density for the active analogues in Table III.

The isopotential maps of the 4′-methoxy analogues of isomazole and **18** (i.e. **21** and **16** respectively, Table II) were obtained by employing the procedures described above (Figure 2, supplementary material). Calculations of the electronic properties of these achiral ethers are much simpler and involve fewer approximations than those for the sulfoxides (i.e. isomazole and **18**). It was found that the electrostatic potential miminum is much farther away from the imidazo nitrogen in **16** than in **21**. This property of **16** has been found to be quite common among inactive analogues and may reflect an inability to adopt a favorable orientation at the inotropic receptor site(s). Details of isopotential maps for a range of isomazole and sulmazole analogues will be reported elsewhere.[31]

Additional Pharmacological Studies. Cardiovascular profiles after iv administration to anesthetized dogs were determined for selected analogues and compared to those of sulmazole and isomazole (see Figure 3, supplementary material, and Table IV). Analogue **5** displayed only very weak inotropic effects (ED_{50} 8.4 mg kg^{-1}) and was not studied further. The major effects of all other compounds were a dose-dependent inotropic effect (increase in dP/dt), a rise in heart rate, and a fall in diastolic blood pressure. Analogue **13** produced the largest maximum percentage increase in dP/dt. However, at lower doses (up to 0.3 mg kg^{-1}) isomazole produced a larger percentage increase in dP/dt relative to the other compounds. The dose–response curves (Figure 3, supplementary material) for the inotropic and vasodilator effects of analogues **7** and **13** were similar but not identical with those of sulmazole and isomazole, respectively. The 6-amino analogue **7** shows a greater separation between these two effects (in favor of inotropism) than does sulmazole itself (cf. lower (ED_{50} dP/dt)/(ED_{50} DBP) ratio, Table IV).

In some additional animals the time courses of the responses to single large bolus doses of drugs were observed. The maximal responses to these agents were achieved after 5-10 min of administration. Despite an initial decline in these responses the inotropic and vasodilator effects of isomazole, but not of sulmazole, were still evident 180 min after administration.[8] These studies[31] indicate that the inotropic effects of **7** persist longer than those of sulmazole. This observation may reflect a slower or different mode of metabolism induced by the 6-amino substituent. It is well-known[2] that a major metabolic pathway for sulmazole in several species involves hydroxylation at the 6-position and subsequent oxidative cleavage of the pyridine ring. No marked differences between the duration of the inotropic effects of analogue **13** and isomazole were found.

Conclusions

The present study has shown that the inotropic activities of the 1*H*-imidazopyridines are sensitive to both the nature and the position of "A" ring substituents. Overall, electron-releasing substituents, such as methoxy and amino, were better tolerated than lipophilic or electron-withdrawing groups. The 6-position of sulmazole appeared to be the most tolerant toward substituents, and a methoxy substituent was best tolerated at the 4-position of isomazole.

None of the analogues investigated were appreciably protonated or deprotonated at physiological pH, neither was there any obvious relationship between inotropic activity and the lipophilicities (log *P*) or the basicity properties (pK_A's, protonation sites) of these heterocycles. A reasonable correlation, between in vitro inotropism and the calculated charge density of the "B" ring imidazo nitrogen, was observed, however, for a subset of active analogues. The 7-amino derivatives of isomazole, **16** and **17**, each had an electrostatic potential minimum relatively remote from the "B" ring nitrogen atom and, in common with other analogues possessing this property, were inactive. 6-Aminosulmazole **7** and 4-methoxyisomazole **13** displayed the most potent inotropic effects in vivo. While the cardiovascular profile of **13** in experiments using dogs was similar to that of isomazole, **7** displayed weaker vasodilator effects but more prolonged potent inotropic effects than sulmazole. The ability of "A" ring substituents, such as methoxy, to confer better in vivo inotropic properties on the resulting isomazole analogues has also been observed for some imidazo[1,2-*a*]pyrazines. These studies will be reported elsewhere.

Experimental Section

Melting points were determined on a Koffler hot-stage apparatus and are uncorrected. The identity of all compounds was confirmed by ^1H NMR, mass spectra, and combustion analysis unless stated otherwise. All reactions were followed by TLC carried out on Merck F254 silica gel plates. Microanalytical data were provided by the Physical Chemistry Department of the Wellcome Research Laboratories; only symbols of elements analyzed are given and their percentages were within 0.4 of calculated values. Details of the ^1H and ^{13}C NMR spectra of compounds **1–5** and **13–15** are given in ref 25.

Synthesis of Diaminopyridines. 2,3-Diamino-4-methoxypyridine (24). 2-Amino-4-chloro-3-nitropyridine (**46**,[16] 1.75 g, 10.1 mmol) was added to a solution freshly prepared by dissolving sodium (0.59 g, 25.7 mmol) in dry methanol (45 mL) and the mixture so obtained was stirred and heated at reflux for 2 h. The reaction mixture was allowed to stand at room temperature overnight, treated with concentrated hydrochloric acid to adjust the pH to 6, chilled in ice, and then filtered. The solid obtained was boiled with benzene and filtered, and the filtrate was diluted with hexane. On standing, yellow crystals deposited which were collected by filtration, yielding 0.95 g (56%) of **2-amino-4-methoxy-3-nitropyridine (47)**, mp 183–185 °C. Anal. ($C_6H_7N_3O_3$) C, H, N.

A solution of **47** (0.70 g) in methanol (120 mL) containing 10% palladium–charcoal catalyst (0.17 g) was shaken under 1 atm of hydrogen until uptake ceased (ca. 1 h). The catalyst was removed by filtration through Celite and the filtrate was concentrated in vacuo, yielding 0.50 g (87%) of **24** as a tan oil. This intermediate was utilized for the condensation stage (method A) without further purification because of its instability.

2,6-Diamino-3-methoxy-5-nitropyridine (52). 2,6-Dibromo-3-methoxy-5-nitropyridine (**48**;[17] 0.5 g) and aqueous ammonia (15 mL, d = 0.88) were heated at 80 °C in an autoclave for 16 h. After cooling, yellow crystals were removed by filtration and washed with water, yielding 0.25 g (85%) of **52**, mp 265–268 °C. Anal. ($C_6H_8N_4O_3$) C, H, N.

2-Amino-6-bromo-3-methoxy-5-nitropyridine (53). Dibromide **48** (0.50 g, 1.7 mmol), aqueous ammonia (0.18 mL, d = 0.88, 3.4 mmol), and methanol (20 mL) were heated at 60 °C in a sealed glass tube for 60 h. On cooling to room temperature and standing, fine yellow crystals deposited which were removed by filtration to yield 50 mg (14%) of **53**: mp 227–229 °C; IR (KBr) ν_{max} 3400, 3300, 1640, 1580, 1520, 1310 cm^{-1}; ^1H NMR (90 MHz, Me_2SO-d_6) δ 3.89 (3 H, s, OMe), 7.5–8.0 (2 H, v br peak, NH_2, exchangeable), 7.66 (1 H, s, H4). Anal. ($C_6H_6N_3O_3Br$) C, H, N, Br.

2-Bromo-5-methoxy-6-(methylthio)-3-nitropyridine (49). A slow stream of methanethiol was passed for ca. 2 h through a solution freshly prepared by dissolving sodium (0.96 g, 0.042 mol) in methanol (135 mL). The resulting solution of MeSNa was then transferred under a positive pressure of nitrogen into a refluxing solution of dibromide **48** (12.0 g, 0.038 mol) in methanol (250 mL). The reaction mixture was heated at reflux for 5 min further and cooled to room temperature, and the solvent was removed in vacuo. The residual solid was shaken with dichloromethane and water, the organic layer was separated and dried over $MgSO_4$, and the solvent was removed in vacuo, yielding 10.7 g of crude product. This was purified by column chromatography on silica gel eluting with dichloromethane–trichloroethylene (1:1) to give 9.1 g (85%) of pure **49** as yellow crystals: mp 140–142 °C (hexane); ^1H NMR (200 MHz, $CDCl_3$) δ 2.59 (3 H, s, SMe), 3.97 (3 H, s, OMe), 7.52 (1 H, s, H4). Anal. ($C_7H_7BrN_2O_3S$) C, H, N. 2,6-Bis(methylthio)-3-methoxy-5-nitropyridine (0.6 g, 6%) was also isolated from this experiment: mp 165–168 °C; MS m/z 246 (M^+); ^1H NMR (200 MHz $CDCl_3$) δ 2.61 (3 H, s, SMe), 2.64 (3 H, s, SMe), 3.96 (3 H, s, OMe), 7.77 (1 H, s, H4). Anal. ($C_8H_{10}N_2O_3S_2$) C, H, N.

2-Amino-5-methoxy-6-(methylthio)-3-nitropyridine (50). A solution of bromopyridine **49** (9.0 g, 0.03 mol) in propan-2-ol (160 mL) and alcoholic ammonia (50 mL of 6% w/v, 0.18 mol) was heated in a PTFE-lined autoclave at 100 °C for 72 h. The contents of the autoclave were cooled to room temperature, volatile material was removed in vacuo, and the residue was extracted with dichloromethane. The combined extracts were evaporated to dryness and the crude product was purified by flash chromatography on silica gel eluting with dichloromethane to yield some recovered starting material (R_f 0.7) and 3.8 g (55%) of **50** (R_f 0.4) as a yellow solid: mp 163–165 °C; ^1H NMR (90 MHz, Me_2SO-d_6) δ 2.50 (3 H, s, SMe), 3.88 (3 H, s, OMe), 7.63 (1 H, s, H4), 7.90 (2 H, br s, NH_2); IR ν_{max} (KBr) 1230 cm^{-1}. Anal. ($C_7H_9N_3O_3S$) C, H, N.

2,3-Diamino-5-methoxy-6-(methylthio)pyridine (51) Hydrochloride. A solution of **50** (2.90 g) in methanol (375 mL) and fresh Raney nickel (ca. 6 g of slurry) was shaken under hydrogen at 1 atm of pressure and room temperature until 2 equiv of hydrogen had been consumed (ca. 2 h). The reaction mixture was filtered through Celite into a flask containing concentrated hydrochloric acid (2.25 mL) and the solvent was removed in vacuo. The residue was stirred with propan-2-ol, the solid was collected by filtration and recrystallized from 2 N hydrochloric acid, yielding 2.3 g (77%) of the hydrochloride of **51**: mp 208–210 °C; ^1H NMR (90 MHz, Me_2SO-d_6) δ 2.42 (3 H, s, SMe), 3.78 (3 H, s, OMe), 5.8–6.8 (5 H, v br peak, 5 × NH, exchangeable), 7.10 (1 H, s, H4). Anal. ($C_7H_{11}N_3OS$·HCl) C, H, N, Cl.

2,3-Diamino-5-methoxypyridine (23) Dihydrochloride. A solution of **51**·HCl (2.5 g) in water (250 mL) and freshly prepared Raney nickel (ca. 35 g of slurry) was stirred and heated at 100 °C until TLC indicated that the reaction was complete (2–4 h).

The reaction mixture was filtered through Celite into a flask containing concentrated hydrochloric acid (2 mL) and the filtrate was evaporated to dryness in vacuo. The residue was dissolved in a little water, a chloroform and 10 N sodium hydroxide solution was added, and the chloroform layer was then extracted with 2 N hydrochloric acid. The acidic extracts were evaporated in vacuo, and the residual solid recrystallized from propan-2-ol, yielding 1.25 g (52%) of the title dihydrochloride **23** as fawn crystals: mp 166–168 °C; ^{1}H NMR (90 MHz, Me_2SO-d_6) δ 3.65 (3 H, s, OMe), 6.78 (1 H, d, J = 2 Hz, H6 or H4), 6.94 (1 H, d, J = 2 Hz, H4 or H6), 7.2–8.0 (6 H, v br peak, 6 × NH, exchangeable). Anal. ($C_6H_9N_3O$·2HCl) C, H, N, Cl.

4-Amino-2-methoxy-5-nitropyridine (55). Reaction of 4-amino-2-chloro-5-nitropyridine (**54**)[19,20] with sodium methoxide utilizing a procedure analogous to that used for preparing **47** afforded a 55% yield of **55**: mp 138–141 °C; ^{1}H NMR (60 MHz, $CDCl_3$) δ 3.96 (3 H, s, OMe), 6.0–6.5 (2 H, v br peak, exchangeable NH_2), 5.96 (1 H, s, H3), 8.96 (1 H, s, H6). Anal. ($C_6H_7N_3O_3$) C, H, N.

3,4-Diamino-6-methoxypyridine (38). Catalytic hydrogenation of **55** was achieved by employing the same procedure as that used for preparing diamine **24**. By this means **38** (85%) was obtained as an unstable red oil which was used immediately for the condensation step.

4-Amino-3-methoxypyridine (57). 3-Methoxy-4-nitropyridine 1-oxide (**56**,[35] 10.0 g), methanol (100 mL), and Raney nickel (ca. 2 g) were stirred at 40 °C under 40 atm of hydrogen for 2.5 h. After cooling to room temperature, the catalyst was removed by filtration through Celite, and the filtrate was evaporated. Recrystallization of the residue from benzene–hexane yielded 4.0 g (55%) of **57**: mp 89–90 °C (lit.[21] mp 94.5–95.5 °C).

4-Amino-3-methoxy-5-nitropyridine (58). Amine **57** (4.0 g, 0.03 mol) was added in small portions to concentrated sulfuric acid (34 mL) at 0 °C so that the temperature remained below 10 °C. The resulting solution was stirred at 0 °C and fuming nitric acid (1.65 mL, 0.04 mol) was added dropwise with external cooling to maintain the temperature at 0 °C. The reaction mixture was stirred for 1 h at 0 °C, then 1 h at room temperature, and finally at 60 °C for 1 h. After cooling, the mixture was poured onto ice and neutralized with 10 N NaOH solution, and the solid was collected by filtration. Charcoal decolorization of a chloroform solution of this solid and subsequent crystallization gave 2.0 g (37%) of **58**: mp 180–182 °C; ^{1}H NMR (60 MHz, Me_2SO-d_6) δ 3.92 (3 H, s, OMe), 7.6 (2 H, br s, NH_2, exchangeable), 7.96 (1 H, s, H2), 8.64 (1 H, s, H6). Anal. ($C_6H_7N_3O_3$) C, H, N.

3,4-Diamino-5-methoxypyridine (39). Catalytic hydrogenation of nitroamine **58** (2.0 g) in methanol (200 mL) with 10% Pd–C catalyst gave 1.4 g (85%) of **39** as an unstable tan syrup: ^{1}H NMR (60 MHz, Me_2SO-d_6) δ 2.8–3.8 (4 H, v br peak, 2 × NH_2, exchangeable), 3.88 (3 H, s, OMe), 7.68 (2 H, s, H2, H6).

5-Acetyl-2-chloro-3-nitropyridine (60). 6-Hydroxy-5-nitronicotinic acid (5.0 g, 27 mmol) was chlorinated with phosphorus pentachloride and phosphorus oxychloride as described[22] for the preparation of methyl 6-chloro-5-nitronicotinate. The methanolysis step was omitted, however, and acid chloride intermediate **59** was converted to the methyl ketone by the following method. Bis(trimethylsilyl) malonate (13.4 g, 54 mmol) in dry ether (100 mL) was stirred under N_2 and cooled to −70 °C as n-BuLi in hexane (33.8 mL of 1.6 M solution, 54 mmol) was added during 20 min. The temperature of the resulting suspension was raised to 0 °C and acid chloride **59**, dissolved in dry THF (20 mL), was added. The mixture was stirred for 1 h at 0 °C and then poured into aqueous $NaHCO_3$ (300 mL). The aqueous phase was acidified with dilute H_2SO_4 and extracted three times with EtOAc and once with $CHCl_3$. These extracts were combined and evaporated under reduced pressure. The residue was dissolved in dioxane (150 mL) and refluxed for 0.5 h. The solvent was evaporated and the crude product was dissolved in EtOAc, washed with $NaHCO_3$ and brine, decolorized with charcoal, dried over Na_2SO_4, and evaporated to give (2.55 g, 47%) **60** as a slowly crystallizing oil: ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.68 (3 H, s, Me), 8.52 (1 H, d, J = 2 Hz, H4 or H6) 8.96 (1 H, d, J = 2 Hz, H6 or H4); MS m/z 202, 200 (M^+). This material decomposed on standing and was therefore used while fresh for the next step.

5-Acetyl-2-amino-3-nitropyridine (61). Chloropyridine **60** (2.50 g, 12.5 mmol), an aqueous ammonia solution (25 mL, d = 0.88), and ethanol (25 mL) were stirred at room temperature for 3 h. The solvent was evaporated and the residue was stirred with water for 0.5 h. The solid was filtered off, washed with water, and dried to give (1.87 g, 83%) **61**: mp 238–242 °C (EtOH); ^{1}H NMR (60 MHz, Me_2SO-d_6) δ 2.50 (3 H, s, Me), 8.30 (2 H, br s, NH_2, exchangeable), 8.60 (1 H, d, J = 2.5 Hz, H4 or H6), 8.76 (d, 1 H, J = 2.5 Hz, H6 or H4); MS m/z 181 (M^+). Anal. ($C_7H_7N_3O_3$) C, H, N.

5-Acetyl-2,3-diaminopyridine (28). Catalytic hydrogenation of **61** (3.0 g) in methanol (300 mL) with 10% Pd–C catalyst gave, after purification of the crude product by flash chromatography on silica ($CHCl_3$–MeOH 4:1), 0.97 g (39%) of **28** as an oil: ^{1}H NMR (90 MHz, Me_2SO-d_6) δ 2.41 (3 H, s, Me), 4.90 (2 H, s, NH_2, exchangeable), 6.35 (2 H, br s, NH_2, exchangeable), 7.14 (1 H, d, J = 2 Hz, H4), 8.03 (1 H, d, J = 2 Hz, H6); MS m/z 151 (M^+).

Synthesis of Imidazopyridines. Method A. 6-Methoxy-2-[2-methoxy-4-(methylthio)phenyl]-1*H*-imidazo[4,5-*b*]pyridine (30). The dihydrochloride of amine **23** (1.15 g, 5.4 mmol) and 2-methoxy-4-(methylthio)benzoic acid[5,6] (1.08 g, 5.5 mmol) were ground together in a mortar and added in portions to phosphorus oxychloride (23 mL) with stirring. The reaction mixture was stirred at room temperature for 0.5 h, heated at reflux for 6 h, and then cooled to room temperature. Excess phosphorus oxychloride was removed in vacuo; the residue was treated with ice–water and then 10 N NaOH solution until the mixture was at pH 14. After warming to 50 °C, the mixture was filtered, the filtrate was brought to pH 7 with concentrated HCl, and after chilling, the solid was collected by filtration. This crude product (1.2 g) was purified by flash chromatography eluting with $CHCl_3$–MeOH (14:1), collecting fractions of R_f 0.6 on TLC in $CHCl_3$–MeOH 9:1, and recrystallized from propan-2-ol to give 490 mg (30%) of **30**: mp 177–180 °C; ^{1}H NMR (360 MHz, $CDCl_3$, 60 °C) δ 2.52 (3 H, s, SMe), 3.88 (3 H, s, OMe), 4.00 (3 H, s, OMe), 6.86 (1 H, br s, H3′), 6.95 (1 H, dd, J = 2, 8 Hz, H5′), 7.52 (1 H, br d, J = 2.4 Hz, H7), 8.11 (1 H, br d, J = 2.4 Hz, H5), 8.42 (1 H, d, J = 8 Hz, H6′). Anal. ($C_{15}H_{15}N_3O_2S$) C, H, N.

Method B. 4-Methoxy-2-[2-methoxy-4-(methylthio)-phenyl]-1*H*-imidazo[4,5-*c*]pyridine (42). 2-Methoxy-4-(methylthio)benzoic acid (4.35 g, 21.9 mmol), thionyl chloride (30 mL), and benzene (60 mL) were stirred at room temperature overnight and then heated at reflux for 1 h. The volatile material was then removed in vacuo and the residual crystalline crude acid chloride was dissolved in dry pyridine (25 mL). To this solution was added 2-methoxy-3,4-diaminopyridine (**37**; 3.18 g, 22.9 mmol) in dry pyridine (50 mL); the reaction mixture was stirred at room temerature for 3 days, and then concentrated in vacuo. The residue was triturated in water (100 mL), and the tan solid was collected by filtration and, after drying, chromatographed on silica. Elution with $CHCl_3$–MeOH (99:1) gave 2.3 g (33%) of **3-amino-4-[[2-methoxy-4-(methylthio)benzoyl]amino]-2-methoxypyridine**: mp 174–175 °C (MeOH–H_2O). Anal. ($C_{15}H_{17}N_3O_3S$) C, H, N. Phosphorus oxychloride (2.0 mL) was added dropwise to a stirred solution of the above amide intermediate (1.65 g) in dry pyridine (40 mL) cooled to 0 °C. The reaction mixture was stirred at 0 °C for 1.5 h, poured onto ice–water (200 mL), and stirred at room temperature for 1.5 h further. On standing, a solid deposited which was removed by filtration and recrystallized from aqueous methanol, yielding 0.85 g (55%) of **42** as pale yellow crystals: mp 189–190 °C; ^{1}H NMR (90 MHz, Me_2SO-d_6) δ 2.58 (3 H, s, SMe), 4.02 (3 H, s, OMe), 4.04 (3 H, s, OMe), 6.97–7.08 (2 H, m, H3′, H5′), 7.22 (1 H, d, J = 6 Hz, H7), 7.83 (1 H, d, J = 6 Hz, H6), 8.21 (1 H, d, J = 8 Hz, H6′). Anal. ($C_{15}H_{15}N_3O_2S$) C, H, N.

Method C. 6-Acetyl-2-(2,4-dimethoxyphenyl)-1*H*-imidazo[4,5-*b*]pyridine Hydrochloride (10). To 5-acetyl-2,3-diaminopyridine (**28**, 1.33 g, 8.8 mmol) in dry acetone (250 mL) was added dry pyridine (4 mL) and triethylamine (1.2 mL, 8.8 mmol), and the mixture was stirred under N_2 as 2,4-dimethoxybenzoyl chloride (1.77 g, 8.8 mmol) in dry acetone was added dropwise. The mixture was stirred for 3 h and filtered, and the solvent was removed in vacuo. The residue was dissolved, with warming, in $CHCl_3$, washed with water, and dried over Na_2SO_4 and the solvent was evaporated. Chromatography on silica

(35) Talik, T.; Talik, Z. *Rocz. Chem.* **1964**, *38*, 777.

($CHCl_3$–MeOH, 40:1) gave 1.99 g (64%) of **5-acetyl-2-amino-3-[(2,4-dimethoxybenzoyl)amino]pyridine hydrochloride** as a pale yellow solid, mp 210 °C dec. Anal. ($C_{16}H_{17}N_3O_4$·HCl) C, H, N. The above monoamide (1.7 g, 4.8 mmol) in ethylene glycol (25 mL) was treated with a catalytic amount (3 drops) of concentrated hydrochloric acid. The mixture was heated at 180 °C for 7 h under N_2. The cooled reaction mixture was poured into 2 M HCl (200 mL) and allowed to stand for 1 h. It was then basified with $NaHCO_3$ and extracted three times with $CHCl_3$. The extracts were washed with water and dried over Na_2SO_4, and the solvent was evaporated. The product was purified by chromatography on silica, eluting with $CHCl_3$ to give 0.47 g (29%) of **10**. It was then converted to the hydrochloride salt by dissolving in EtOH and adding one equiv of ethereal HCl to give **10**·HCl: mp 216–218 °C; ^{1}H NMR (90 MHz, Me_2SO-d_6) δ 2.72 (3 H, s, CMe), 3.92 (3 H, s, OMe), 4.10 (3 H, s, OMe), 6.0–7.0 (2 H, v br peak, 2 × NH, exchangeable), 6.77–6.87 (2 H, m, H3′, H5′), 8.34 (1 H, d, J = 9.5 Hz, H6′), 8.62 (1 H, d, J = 1.8 Hz, H7), 9.10 (1 H, d, J = 1.8 Hz, H5). Anal. ($C_{16}H_{15}N_3O_3$·HCl) C, H, N, Cl.

Method D. 7-Amino-2-[2-methoxy-4-(methylthio)phenyl]-1*H*-imidazo[4,5-*c*]pyridine (17). 3,4,5-Triaminopyridine trihydrochloride (**40**,[15] 6.69 g, 28.6 mmol) and 2-methoxy-4-(methylthio)benzoic acid (5.67 g, 28.6 mmol) were pulverized to a fine powder and added to polyphosphoric acid (137 g), and the mixture was stirred at 150 °C for 3 h. The dark reaction mixture was cooled slightly and poured onto ice, basified with ammonia solution (d = 0.88), and extracted with chloroform (3×). The combined extracts were dried over Na_2SO_4, the solvent was removed in vacuo, and the residue was triturated with ethanol–ether to give 960 mg (12%) of product. This was converted to the dihydrochloride in the usual way to give **17**·2HCl·H_2O, mp 278–280 °C. Anal. ($C_{14}H_{14}N_4OS$·2HCl·H_2O) C, H, N.

Method E. 6-Methoxy-2-[2-methoxy-4-(methylsulfinyl)phenyl]-1*H*-imidazo[4,5-*c*]pyridine (14). Hydrogen peroxide (0.26 mL of 30% w/v, 2.3 mmol) was added dropwise to a stirred solution of sulfide **43** (330 mg, 1.1 mmol) in acetic acid (10 mL) and the mixture was stirred at room temperature for 6 h. The volatile material was removed in vacuo and the residue was treated with water (15 mL) and then aqueous ammonia (d = 0.88) to bring the pH to 9. The resulting solution was saturated with salt and extracted thoroughly with chloroform, and the combined organic extracts were dried over $MgSO_4$. Removal of the solvent in vacuo and chromatography of the residue on silica eluting with $CHCl_3$–MeOH (98:2) gave 226 mg (65%) of **14**, mp 205–206 °C (from ether–hexane). Anal. ($C_{15}H_{15}N_3O_3S$) C, H, N, S.

Method F. 5-Methoxy-2-[2-methoxy-4-(methylsulfinyl)phenyl]-1*H*-imidazo[4,5-*b*]pyridine (3). A solution of *m*-chloroperbenzoic acid (0.72 g of 80%, 3.34 mmol) in chloroform (25 mL) was added over 10 min to a stirred solution of sulfide **29** (1.0 g, 3.32 mmol) in chloroform (35 mL) cooled to −15 °C. The reaction mixture was stirred at this temperature for 1.5 h, allowed to warm to room temperature, and then diluted with chloroform. The organic phase was washed twice with saturated $NaHCO_3$ solution and dried over $MgSO_4$, and the solvent was removed in vacuo. The residue was chromatographed on silica, eluting with $CHCl_3$–MeOH (96:4) to give [after removal of the sulfone by-product (R_f 0.4 on TLC in $CHCl_3$–MeOH, 9:1)] a foam (R_f 0.2, $CHCl_3$–MeOH 9:1) which was crystallized from ether, yielding 0.56 g (53%) of **3**, mp 135–137 °C. Anal. ($C_{15}H_{15}N_3O_3S$) C, H, N.

Method G. 6-Amino-2-[2-methoxy-4-(methylsulfinyl)phenyl]-1*H*-imidazo[4,5-*b*]pyridine (7). A solution of the nitro compound **34** (0.70 g) in ethanol (50 mL) in which was suspended 10% Pd–C catalyst (0.2 g) was shaken under hydrogen at atmospheric pressure until uptake ceased. The catalyst was removed by filtration and the filtrate was evaporated to dryness. The residue was carefully chromatographed on silica (EtOAc–$CHCl_3$–MeOH 2:2:1) to give 0.28 g (43%) of **7** as a yellow solid: mp >220 °C dec; ^{1}H NMR (90 MHz, Me_2SO-d_6) δ 2.83 (3 H, s, SMe), 4.09 (3 H, s, OMe), 5.24 (2 H, br s, NH_2, exchangeable), 7.11 (1 H, d, J = 2.5 Hz, H7), 7.30–7.50 (2 H, m, H3′, H5′), 7.88 (1 H, d, J = 2.5 Hz, H5), 8.43 (1 H, d, J = 8.2 Hz, H6′), 11.9 (1 H, br s, NH, exchangeable). Anal. ($C_{14}H_{14}N_4O_2S$·0.4H_2O) C, H, N.

Method H. 7-Amino-2-(2,4-dimethoxyphenyl)-1*H*-imidazo[4,5-*c*]pyridine (16) Hydrochloride. Nitro compound **45** (1.0 g, 3 mmol), ethanol (200 mL), and platinum oxide catalyst (0.10 g) were stirred under hydrogen at 70 atm and 60 °C for 7 h. After cooling to room temperature, the mixture was filtered to remove the catalyst and the solvent was removed in vacuo. The residue was triturated in ether, filtered to remove insoluble material, and the filtrate was evaporated to dryness to give a tan solid. This material was dissolved in 2 N hydrochloric acid (6.3 mL) and ethanol was then added to induce crystallization, yielding 0.97 g (93%) of **16**·HCl·0.25H_2O, mp 269–270 °C. Anal. ($C_{14}H_{14}N_4O_2$·HCl·0.25H_2O) C, H, N.

4-Methyl-2-(2,4-dimethoxyphenyl)-1*H*-imidazo[4,5-*c*]pyridine (19). To anhydrous 2-(2,4-dimethoxyphenyl)-1*H*-imidazo[4,5-*c*]pyridine 5-oxide (**62**;[24] 540 mg, 2.0 mmol, dried at 120 °C under vacuum) in dry THF (50 mL) under nitrogen was added a 3 M solution of methylmagnesium bromide (7 mL, 21 mmol). After stirring for 16 h, the reaction mixture was quenched by addition of ethyl acetate and then 5% $NaHCO_3$ solution. The organic extract was separated, the aqueous layer was extracted twice with chloroform, and the combined organic extracts were dried over $MgSO_4$. Evaporation of the solvent gave a gum which was chromatographed on silica (eluant $CHCl_3$–MeOH 9:1) and treated with ethereal HCl to give 0.21 g (31%) of **19**·HCl: mp 234–235 °C; ^{1}H NMR (90 MHz, CD_3OD) δ 3.09 (3 H, s, CMe), 3.94 (3 H, s, OMe), 4.13 (3 H, s, OMe), 6.75–6.83 (2 H, m, H3′, H5′), 8.02 (1 H, d, J = 6.7 Hz, H7), 8.29–8.43 (2 H, m, H6, H6′). Anal. ($C_{15}H_{15}N_3O_2$·1.33HCl·H_2O) C, H, N, Cl.

4-Chloro-2-(2,4-dimethoxyphenyl)-1*H*-imidazo[4,5-*c*]pyridine (63). *N*-Oxide **62**[24] (5.0 g) and phosphorus oxychloride (60 mL) were stirred at 80 °C for 3 h and the reaction mixture was evaporated to dryness. The residue was partitioned between $CHCl_3$ and 5% $NaHCO_3$ solution; the $CHCl_3$ layer was washed with water and dried over $MgSO_4$. Removal of the solvent in vacuo and recrystallization of the residual solid from ethanol–water gave 3.6 g (67%) of **63**: mp 203–204 °C; ^{1}H NMR (90 MHz, Me_2SO-d_6) δ 3.92 (3 H, s, OMe), 4.08 (3 H, s, OMe), 6.64–7.00 (2 H, m, H3′, H5′), 7.64 (1 H, d, J = 6 Hz, H7), 8.12 (1 H, d, J = 6 Hz, H6), 8.34 (1 H, d, J = 9 Hz, H6′). Anal. ($C_{14}H_{12}ClN_3O_2$) C, H, N, Cl.

4-(Methylamino)-2-(2,4-dimethoxyphenyl)-1*H*-imidazo[4,5-*c*]pyridine (20). Chloride **63** (1.0 g) and a solution of methylamine in ethanol (20 mL of 33%) were stirred at 140 °C in an autoclave for 40 h. After cooling, volatile material was removed in vacuo, the residue was partitioned between $CHCl_3$ and 5% $NaHCO_3$ solution, and the $CHCl_3$ layer was washed with water and dried over $MgSO_4$. Evaporation of the solvent gave the crude product which was chromatographed on silica (eluant $CHCl_3$–MeOH, 4:1) and treated with ethereal HCl to give 0.42 g (38%) of **20**·HCl, mp 204–207 °C. Anal. ($C_{15}H_{16}N_4O_2$·HCl) C, H, N, Cl.

Pharmacological Methods. Paced Guinea Pig Papillary Muscles. Male guinea pigs (Halls 275–325 g) allowed free access to food and water were killed by a blow to the head. The heart was rapidly excised and washed in Krebs–Henseleit solution containing 2.5 mM Ca^{2+}, gassed with 95% O_2–5% CO_2 at 30 °C. The heart was transferred to a Petri dish containing the same buffer kept at approximately 34 °C throughout the dissection. Fresh buffer was used for each dissection and washings were discarded after use. A single right ventricular papillary muscle was employed from each heart, the tendinous end was ligated to a stainless steel hook, and the lower end was ligated and cut away from the ventricle wall and attached to a Perspex clamp such that the tissue was in contact with a platinum punctate electrode. The stainless steel hook was suspended from a Grass FT.03 transducer which recorded isometric tension. The preparation was placed in a 20 mL Pyrex organ bath containing buffer gassed with 95% O_2–5% CO_2 and maintained at 34 °C. A 500 mg loading tension was applied to the preparation. Stimulation was effected by rectangular pulses of 1-ms duration at 1.5 Hz at 20% above the threshold voltage (1–5 V) by a SRI stimulator. The transducer inputs were coupled to a potentiometric recording device by a six-channel Grass transducer coupler. A group of organ baths enabled up to six preparations to be utilized during one experimental run. After 60 min, preparations unable to sustain uniform contractions beyond this period were rejected. The total volume of compound containing solutions added generally amounted to less than 400 μL. Compound additions were made to the baths in a cumulative fashion in the following multiples of the initial dose (1, 3, 10, 30–100, etc), the individual responses being allowed

to attain a plateau before the next addition was made. The total volume added during a standard cumulative agonist dose response curve never exceeded 2% of the total bath volume. The inotropic potency is expressed as the negative log of the molar concentration required to increase basal contractility by 50% (pA_{50}).[30] Other workers have also recently used this parameter to express inotropic potency.[36]

Experiments Using Anesthetized Dogs. Beagle dogs (of either sex) weighing between 8.5 and 13 kg were initially anesthetized by an iv injection of thiopentone sodium (30 mg kg^{-1}) into a cephalic vein. Anesthesia was subsequently maintained by intravenous injection of α-chloralose (15 mg kg^{-1}) and pentobarbitone sodium (6 mg kg^{-1}) via a cannula placed in the right femoral vein. The trachea was then cannulated and the animal artificially ventilated with room air and a Palmer pump (stroke volume 200–250 mL and respiration rate 20 min^{-1}). Arterial blood samples were removed before beginning the experiment and analyzed (Radiometer blood gas analyzer) to ensure that the pump ventilation maintained blood gases within acceptable limits. The chest was opened along the length of the sternum and the pericardium opened to expose the heart. The root of the ascending aorta was located and cleared of fat and an electromagnetic flow probe (10–12 mm internal diameter) was placed around the ascending aorta and connected to a Statham flowmeter to measure aortic blood flow. Extracorporeal pressure transducers were used to measure blood and left ventricular pressure. Left ventricular pressure (P) and its first derivative, dP/dt, were measured by the insertion of a short cannula (containing heparinized saline and connected to a Statham pressure transducer) into the left ventricular chamber via the apex of the heart. This cannula was secured in place by a purse-string suture.

Arterial blood pressure was measured by means of a catheter (containing heparinized saline and connected to a Statham pressure transducer) inserted into the right femoral artery and a lead II electrocardiogram was obtained by use of subdermal needle electrodes. Heart rate was derived by use of a tachograph triggered either by the arterial pulse or the ECG–QRS complex. Body temperature was maintained at 37–38 °C by a heated under blanket. All recordings were made by use of a Grass Model 7D polygraph or a Gould 2800S recorder. In some animals the stability of this preparation was assessed by administering no drugs and monitoring the measured cardiovascular parameters for up to 300 min. In all animals the response to a bolus injection of vehicle only was observed.

A dose volume of 0.1 mL kg^{-1} was standard. In these experiments the dose of each drug to produce a 50% increase in dP/dt (ED_{50} dP/dt), 30% reduction in diastolic blood pressure (ED_{30} DBP), and a 10% increase in heart rate (ED_{10} HR) was calculated from the mean data shown in Figure 3 of the Supplementary Material. The values differ from those reported in ref 8.

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Registry No. 1, 73384-60-8; **2**, 86315-52-8; **3**, 127356-01-8; **4**, 127356-02-9; **5**, 127356-03-0; **6**, 127356-04-1; **6**·2HCl, 127356-43-8; **7**, 127356-05-2; **8**, 127382-94-9; **8**·$^1/_2$HCl, 107238-23-3; **9**, 107238-22-2; **10**, 127356-06-3; **10**·HCl, 127356-44-9; **11**, 77439-55-5; **12**, 77303-19-6; **13**, 127356-07-4; **14**, 127356-08-5; **15**, 127356-09-6; **16**, 127356-10-9; **16**·HCl, 127356-45-0; **17**, 127356-11-0; **17**·2HCl, 127356-46-1; **18**, 127356-12-1; **19**, 127356-13-2; **19**·xHCl, 127356-47-2; **20**, 127356-14-3; **20**·HCl, 127356-48-3; **21**, 87359-11-3; **22**, 28020-38-4; **23**·2HCl, 127356-15-4; **24**, 127356-16-5; **25**, 3537-14-2; **26**, 25710-20-7; **27**, 24638-29-7; **28**, 127356-17-6; **29**, 127356-18-7; **30**, 127356-19-8; **31**, 127356-20-1; **32**, 127356-21-2; **33**, 127356-22-3; **34**, 127356-23-4; **35**, 127356-24-5; **36**, 127356-25-6; **37**, 33631-04-8; **38**, 127356-26-7; **39**, 127356-27-8; **40**·3HCl, 127356-28-9; **41**, 4318-68-7; **42**, 127356-29-0; **43**, 127356-30-3; **44**, 127356-31-4; **45**, 127356-32-5; **46**, 6980-08-1; **47**, 84487-08-1; **48**, 79491-46-6; **49**, 127356-33-6; **50**, 127356-34-7; **51**·HCl, 127356-35-8; **52**, 127356-36-9; **53**, 127356-37-0; **54**, 2604-39-9; **55**, 127356-38-1; **56**, 19355-04-5; **57**, 52334-90-4; **58**, 127356-39-2; **59**, 23945-84-8; **60**, 127356-40-5; **61**, 127356-41-6; **62**, 87359-55-5; **63**, 87359-56-6; 2,4-$(MeO)_2C_6H_3CO_2H$, 91-52-1; 6-hydroxy-5-nitronicotinic acid, 6635-31-0; bis(trimethylsilyl) malonate, 18457-04-0; 2-methoxy-4-(methylthio)benzoic acid, 72856-73-6; 2,4-dimethoxybenzoyl chloride, 39828-35-8; 5-acetyl-2-amino-3-[(2,4-dimethoxybenzoyl)amino]pyridine hydrochloride, 127382-95-0; 3-amino-4-[[2-methoxy-4-(methylthio)benzoyl]amino]-2-methoxypyridine, 127356-42-7.

Supplementary Material Available: Tables V and VI listing physicochemical parameters of sulmazole and its analogues and proton and carbon chemical shift differences between the base and salt of 6-amino-2-(2,4-dimethoxyphenyl)-1*H*-imidazo[4,5-*b*]pyridine (**6**) and Figures 2 and 3 showing electrostatic potential diagrams for compounds **16** and **21** and dose–response curves for the hemodynamic effects of isomazole, sulmazole, and analogues **7** and **13** (5 pages). Ordering information is given on any current masthead page.

(36) Takeya, K.; Itoigawa, M.; Furukawa, H. *Eur. J. Pharmacol.* **1989**, *169*, 137.

(37) This compound was discovered independently by workers at Wellcome, E. Lilly, and E. Merck and is also known as B-W746C or LY-175326.