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Original article

Inotropic activity of heterocyclic analogues of isomazole

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Summary — Aryl-substituted benzimidazole, imidazopyridine, imidazopyrazine, imidazopyridazine, oxazolopyridine, purine, pyrollopyridine and thiazolopyridine derivatives have been prepared and evaluated as inotropic agents. Purine **8** and the 1*H*-imidazo[4,5-*c*]- and [4,5-*d*]pyridazines **9** and **10** proved to be of most interest, having similar *in vivo* inotropic potencies to sulmazole. The p*K*a's, protonation sites and lipophilicities for most heterocycles were determined experimentally and some of their electronic properties calculated. For a subset of active heterocycles a correlation was observed between *in vitro* inotropism and the charge density of the imidazo nitrogen adjacent to the electrostatic potential minimum. Structure–activity relationships are discussed in some detail.

Résumé — Activité inotrope de quelques analogues hétérocycliques d'isomazole. *Des dérivés arylsubstitués de benzimidazole, imidazopyridine, imidazopyrazine, imidazopyridazine, oxazolopyridine, purine, pyrrolopyridine et thiazolopyridine ont été préparés et évalués comme agents inotropes. La purine **8** et les 1*H*-imidazo[4,5-c]- et [4,5-d]pyridazines **9** et **10** se sont montrés les plus intéressants dans la mesure où ils possèdent des pouvoirs inotropes similaires à ceux du sulmazole* in vivo. *Pour la plupart des hétérocycles, le p*K*a, les sites de fixation de protons et la lipophilie ont été déterminés expérimentalement et certaines de leurs propriétés électroniques ont été calculées. Une corrélation a été observée entre l'activité inotrope* in vitro *et la densité de charge de l'azote imidazolique à proximité du minimum de potentiel électrostatique, pour un sous-ensemble d'hétérocycles actifs. Les relations structure–activité sont discutées.*

cardiotonics / inotropes / benzimidazoles / imidazopyridines / imidazopyridazines / purines / isomazole / sulmazole

Introduction

Despite intense efforts in recent years, few orally effective positive inotropic agents have shown advantage over digoxin for the treatment of heart failure [1]. Undesirable side effects or problems associated with demonstrating efficacy have limited the development of candidate drugs [2]. Sulmazole **1** [3] is a cardiotonic agent whose development has been suspended because of undesirable toxicological effects. In a search for inotropic agents which are better tolerated and more efficacious we [4, 5] and others [6] discovered isomazole **2** [7] and found [8] it to be more potent *in vivo* than sulmazole as an inotrope.

We thus sought to gain some understanding of the structure–activity relationships (SARs) of sulmazole and isomazole with a view to obtaining inotropic agents with a better pharmacological profile. As part of this exercise we wished to investigate the effects of replacing the imidazopyridine ring of sulmazole by other (6,5)-fused heterocyclic systems. In addition, we wanted to determine whether the basicity or electronic properties of these heterocycles was related to any observed inotropic activity. We now report the results of these studies which involved the syntheses of heterocycles **3–17**, the determination of their pKa's and protonation sites, and calculation of their electronic properties.

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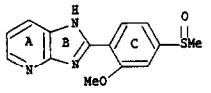

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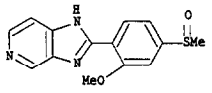

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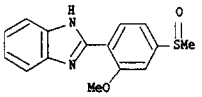

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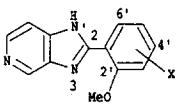

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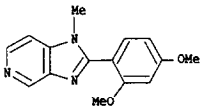
5 X = 6'-OMe



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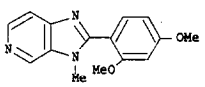

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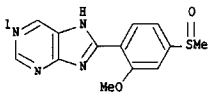

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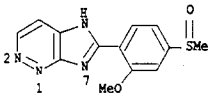

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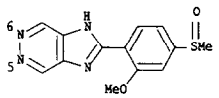

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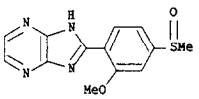

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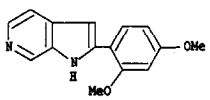

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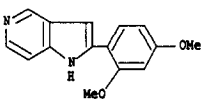

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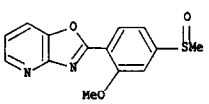

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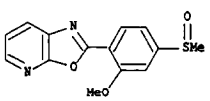

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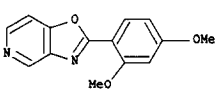

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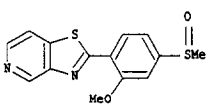

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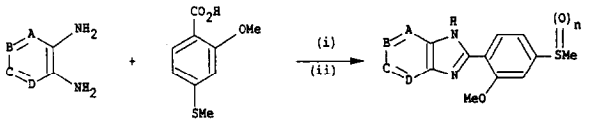

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Scheme 1. Reagents: (i) $POCl_3$, reflux; (ii) either Br_2, HOAc or MCPBA.

	Sulphide	Sulphoxide
	$n = 0$	$n = 1$
A, B, C, D are CH or N		3, 8–11
A, B, C, D is CH	19	
B, D is N; A, C is CH	20	
C, D is N; A, B is CH	21	
B, C is N; A, D is CH	22	
A, D is N; B, C is CH	23	

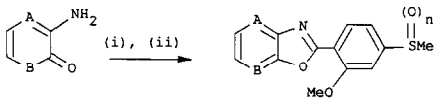
Results and Discussion

Chemistry

Sulphoxides **3**, **8** [9], **9** [10], **10** [11], **11** [11], **14**, **15** and **17** were prepared by oxidation [12] of the corresponding sulphides **19–25** and **27** respectively; details are given in table I. The sulphide intermediates **19–23** were obtained by condensation of the appropriate diamine with 2-methoxy-4-methylthiobenzoic acid **18** (scheme 1). Similarly, condensation of the requisite aminopyridone with 2-methoxy-4-methylthiobenzoyl chloride **30** gave sulphides **24**, **25** and **27** (schemes 2, 3). Acid **18** and acid chloride **30** were prepared on a large scale by the route shown in scheme 4.

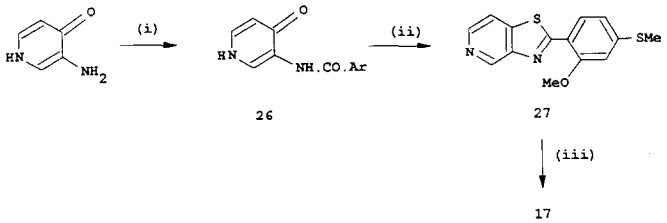
Table I. Preparation and properties of sulphoxides. Method A: MCPBA, CH_2Cl_2. Method B: Br_2, HOAc, NaOAc; see also [12]. [a] ^1H and ^{13}C NMR spectra given in [5, 17]. [b]Lit [9] mp 234–236°C. [c]Lit [10] mp 213–215°C. [d]Reported in [11]. [e]δ (DMSO–d_6) 2.80 (3H, s, SMe), 4.10 (3H, s, OMe). [f]δ (DMSO–d_6) 2.82 (3H, s, SMe), 4.15 (3H, s, OMe). [g]δ (DMSO–d_6) 2.82 (3H, s, SMe), 4.18 (3H, s, OMe), 9.55 (1H, s, CHN).

Compound	Method	% yield	Formula	m.p. °C	Anal.
3[a]	A	57	$C_{15}H_{14}N_2O_2S.H_2O$	no sharp m.p.	C,H,N
8[a,b]	B	80	$C_{13}H_{12}N_4O_2S$	245-246	C,H,N
9[a,c]	B	44	$C_{13}H_{12}N_4O_2S.0.2EtOH$	215-217	C,H,N,S
10[a,d]	B	70	$C_{13}H_{12}N_4O_2S$	236-238	C,H,N
11[a,d]	B	47	$C_{13}H_{12}N_4O_2S.0.1EtOH$	238-240	C,H,N
14[e]	A	87	$C_{14}H_{12}N_2O_3S$	152-153	C,H,N
15[f]	A	77	$C_{14}H_{12}N_2O_3S$	149-150	C,H,N
17[g]	A	81	$C_{14}H_{12}N_2O_2S_2$	246-247(dec)	C,H,N

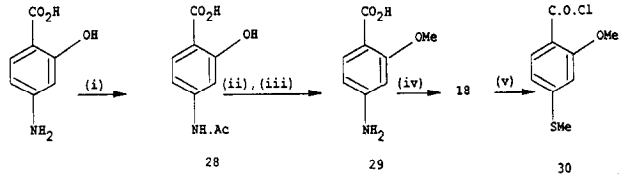


Scheme 2. Reagents: (i) cpd 30, $POCl_3$; (ii) MCPBA for $n = 1$.

		$n = 0$	$n = 1$
A is N; B is CH_2	A is N; B is CH	24	14
A is CH; B is NH	A is CH; B is N	25	15

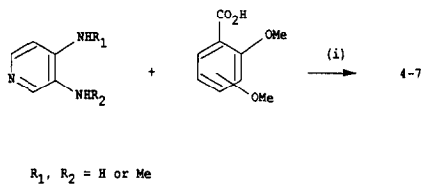


Scheme 3. Reagents: (i) cpd 30, $POCl_3$; (ii) P_2S_5, C_5H_5N, 130°C; (iii) MCPBA.



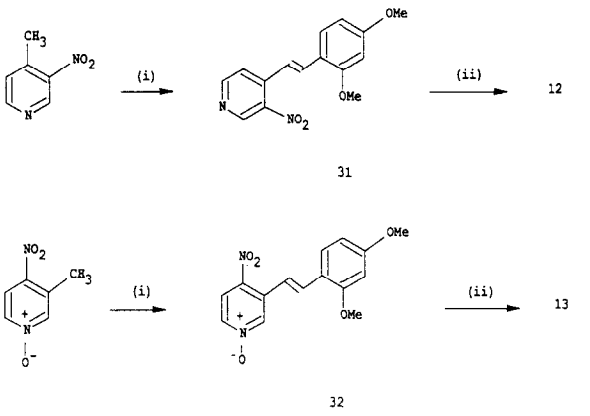
Scheme 4. Reagents: (i) Ac_2O; (ii) Me_2SO_4, K_2CO_3; (iii) 10% NaOH; (iv) $NaNO_2$, HCl, 0°C, Cu, MeSNa; (v) $SOCl_2$, C_6H_6, reflux.

The synthesis of heterocycles **4–7** (scheme 5) was accomplished by condensation of 3,4-diaminopyridine or its *N*-methyl derivatives with the requisite dimethoxybenzoic acid. In a similar manner, 3-amino-pyrid-4-one condensed with 2,4-dimethoxybenzoyl chloride to give heterocycle **16**. 1*H*-pyrrolopyridines **12** and **13** were obtained as shown in scheme 6. The appropriate picoline underwent base-catalysed con-



Scheme 5. Reagents (i) $POCl_3$, Δ.

densation with 2,4-dimethoxybenzaldehyde to give **31** and **32**, and these nitropyridines underwent deoxygenation with triethylphosphite to give **12** and **13** respectively.



Scheme 6. Reagents: (i) 2,4-(DiOMe) C_6H_3·CHO, Δ; (ii) $P(OEt)_3$.

Protonation equilibria

p*K*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 heterocycles **1–13**; these studies have been reported in detail elsewhere [5].

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 [13] software package was utilised to perform geometry optimisations. The resulting structures were further geometry optimised using MOPAC* [14] (AM1, PM3) on a Cray X-MP28 supercomputer. Atom-centred charge densities were calculated using the semi-empirical molecular orbital method CNDO/2 [15]. Electrostatic potentials were calculated from the *ab-initio* STO-3G wavefunction using Gaussian 80-UCSF (Cray) [16] in the plane of the 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 hetero-cycles **1**, **2**, **4**, **5**, **8** and **9**. In these cases the above calculations were performed on the tautomer which had the imidazo NH most adjacent to the pyridyl nitrogen *eg* the 3H

*PM3 parameterisation for MOPAC, JJP Stewart, private communication

tautomer of **4** and the 7H tautomer of **9**. The choice of tautomer was based on *ab initio* (3-21G basis set) calculations [16] which predicted (in agreement with experiment [5]) that 3*H*-imidazo[4,5-*b*]pyridine is of lower energy than the 1H-tautomer. Since the imidazo[4,5-*b*]pyridine series generally shows the most potent *in vitro* inotropism the 3H tautomer was chosen for calculation. In many cases *eg* 1*H*-imidazo[4,5-*c*]pyridine the energy difference between the tautomers was calculated to be small: 0.5 kcal/mol (depending on the basis set used).

Pharmacology

Inotropic activity was determined *in vitro* by using an 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 anaesthetised open-chest dogs. Inotropic responses were indexed by an ED_{50} value *ie* the dose (mg/kg) of drug required to produce a maximum increase of 50% above basal in LVd*P*/d*t* where LVP is the left ventricular pressure. LVd*P*/d*t* is the first time differential of LVP.

Structure–activity relationships

The inotropic activities, p*K*a's, major protonation sites, lipophilicities and imidazo nitrogen atom charge densities are collected together in table II. From a scrutiny of this data the following salient points emerge.

Table II. Inotropic activities and physicochemical properties of isomazole analogues. [a] $-\log_{10} c$ where c = drug concentration required to give a 50% increase in basal contractile force for (n) paced guinea pig papillary muscle preparations. i = inactive, 50% increase not achieved. [b]Effective dose to produce a maximum increase of 50% above basal LVd*P*/d*t* in anaesthetised, open-chest dogs. LVP is the left ventricular pressure. [c]Relates to equilibrium between non-ionised heterocycle and monocation or monoanion. BH^+ = proton addition; B^- = proton loss. p*K*a values (± 0.01–0.08) were determined in aqueous solution at 25°C. Further details are given in [5]. [d]Determined by ^{13}C NMR method. [e]P(D) = octanol–aqueous buffer partition (distribution) coefficient at 25°C and pH 7.4. Experimental values are within ± 0.02 (shake flask method). [f]Calculated by CNDO/2. [g]log P of neutral species is 3.50. [h]Estimated value. 2-(2,4-Dimethoxyphenyl)thiazolo[4,5-*c*]pyridine has p*K*a values of 4.64 (BH^+) and 11.2 (B^-) in H_2O–EtOH (9:1).

Compound	Inotropism	ED_{50} [b]	pKa [c]		Protonation [d]	log P (D) [e]	Charge [f]
	pA_{50} [a]	(mg/kg i.v.)	BH+	B-	Site		imidazo-N
Sulmazole	4.70±0.10 (9)	0.8	3.91	>11.5	pyridyl	1.22	-0.266
Isomazole	4.64±0.15 (17)	0.06	6.17	>11.5	pyridyl	1.23	-0.256
3	4.13±0.17 (3)	>10	4.74	>11.5	imidazo	2.15	-0.262
4	4.01±0.09 (9)	0.12	6.52	>11.0	pyridyl	2.56	-0.259
5	inactive (2)		6.38	10.84	pyridyl	1.66	
6	2.77±0.15 (3)		6.13			2.66	-0.228
7	3.07±0.13 (3)					2.68	
8	3.52±0.12 (3)	0.8	2.69	8.92	N1	0.53	-0.242
9	3.45±0.33 (3)	0.49	3.65	8.73	N2	0.23	-0.251
10	3.58±0.14 (3)	0.20	3.83	8.47	N6	0.39	-0.242
11	4.03±0.05 (3)	>10	<1	9.00	imidazo	0.66	-0.249
12	inactive (2)		8.27	>12.0	pyridyl		
13	inactive (2)		8.47	>12.0	pyridyl	(2.39) [g]	
14	3.23±0.19 (3)	2.0	1.8	>12.0		-0.40	-0.231
15	inactive (2)		<2.0				
16	inactive (2)		4.31			2.42	
17	inactive (2)		4.3 [h]			2.21	

The relative inotropic potencies of the imidazopyridines appear to be species dependent. In our present study employing guinea pig papillary muscle preparations, sulmazole and isomazole are approximately equipotent *in vitro*. Comparative studies of a large series of 2-aryl-1*H*-imidazo[4,5-*b*]- and [4,5-*c*]-pyridines, however, shows that in general the [4,5-*b*] series displays the more potent *in vitro* inotropic activity (Barraclough *et al*, unpublished results). 1*H*-2-(2,4-Dimethoxyphenyl)–imidazo[4,5-*b*]pyridine, for example, has a pA_{50} value of 5.08 ± 0.10 *cf* **4**, pA_{50} 4.01. These findings differ markedly from results [6] obtained with cat papillary muscle which show the [4,5-*c*] series to be more potent, *eg* isomazole is *ca* 5-fold more potent than sulmazole.

For the imidazo[4,5-*c*]pyridine series the greater *in vitro* inotropic potency of the 3-methyl analogue **7** relative to the 1-methyl isomer **6** is of interest. Although both compounds **6** and **7** are less active than analogue **4**, these potencies suggest that for isomazole the 3H-tautomer may be more active than the 1H-tautomer. Our previous study [5] of isomazole analogues showed that for isomazole and **4** the 1H-tautomers are the major species. These findings are consistent with the calculations in the present work which predict 1*H*-imidazo[4,5-*c*]pyridine to be of lower energy than its 3H-tautomer. Changes in the isomazole structure which give rise to a greater preponderance of the 3H-tautomer may therefore lead to analogues with greater inotropic potency.

Analogue **5** is inactive as an inotrope. This compound is non-planar, there being a 42° angle [5] between the planes of the phenyl and heterocyclic ring system. This result suggests that for this series of heterocycles substantial distortion from planarity leads to marked reduction of activity.

Benzimidazole **3**, purine **8**, 1*H*-imidazopyridazines **9** and **10**, and 1*H*-imidazo[4,5-*b*]pyrazine **11** all display moderately potent inotropic activity *in vitro*. The inactivity of **3** *in vivo*, however, suggests that the presence of a heterocyclic nitrogen atom in the "A" ring is desirable for good *in vivo* activity. Unfortunately, this suggestion cannot account for the observation that imidazopyrazine **11** is also inactive *in vivo*. Thus it may be that a requirement for *in vivo* activity is the presence of a certain type of "A" ring heterocyclic nitrogen atom, such as a basic ($pKa > 1$) or electron-rich atom. This hypothesis may then explain why the presence of a pyridyl, pyrimidyl or pyridazinyl "A" ring nitrogen atom is more compatible with *in vivo* inotropism than a pyrazinyl nitrogen. A basic nitrogen atom may retard oxidative metabolism by preventing hydroxylation in ring A and subsequent ring cleavage. These processes occur during the metabolism [3] of sulmazole in some animal species and man.

The pyrollopyridines **12**, **13**, oxazolopyridines **15**, **16** and thiazolo[4,5-*c*]-pyridine **17** are all devoid of inotropic activity. Thus, replacement of the imidazo nitrogen atom of sulmazole or isomazole by a methine group, oxygen or sulphur atom gives rise to analogues with poorer activity. These observations suggest the presence of a electron rich nitrogen atom in the B ring is essential for good *in vitro* inotropism as for the fused imidazoles **1–4** and **8–11**. The oxazolo[4,5-*b*]pyridine **14** did display moderate *in vitro* inotropic activity, however. For **14** the duration of *in vivo* inotropic effects is short-lived (less than 30 min) possibly due to rapid metabolism. Studies of SAR's for **14** and other oxazolo[4,5-*b*]pyridines (Barraclough *et al*, unpublished results) suggest that they have a different mode of action to the imidazole derivatives. Support for this hypothesis is provided by the inability of **14** to inhibit cAMP phosphodiesterase (rat heart, type III) in contrast to isomazole which displays inhibitory properties, IC_{50} 3.0×10^{-5} M [8].

The range of lipophilicities observed $0.2 < \log P < 2.7$, for this set of analogues, with the exception of **12**, **13** and **14**, is similar to that determined ($1 < \log P < 2.6$) for active members of a series of 2-aryl-1*H*-imidazo[4,5-*b*]pyridines [17]. This finding suggests that the inactivity of analogues **5**, and **15–17** is not primarily a reflection of a change of lipophilicity. Thus, sulmazole and analogues **3** and **4** display good *in vitro* inotropism and have similar log P values to those of analogues **5**, **16** and **17**. Further support for this hypothesis is provided by studies of a wide range of "A" and "C" ring substituted 1*H*-imidazo[4,5-*c*]-pyridines and imidazo[1,2-*a*]pyrazines. Many of these isomazole analogues have identical log P values to those of **15–17** and yet display potent inotropism (Barraclough *et al*, unpublished results). Lipophilicity may influence inotropism to a lesser extent for heterocycles **1–17** however, such as by effecting binding to plasma proteins (and increasing ED_{50}'s) or by changing distribution at its effector sites. No simple relationship between *in vitro* inotropic activity and p*K*a or protonation site is apparent.

Pyrrollopyridines **12** and **13**, which are devoid of inotropic activity are predominantly protonated at pH 7.4. Also at physiological pH all active heterocycles are not appreciably protonated or deprotonated. These findings suggest that the active species are the non-ionised molecules.

With the exception of analogue **5**, MOPAC (AM1, PM3) calculations and molecular mechanics calculations (SYBYL) 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*. X-ray

*Older versions of MOPAC having the earlier parameterisation (not PM3) tend to twist the ring system by *ca* 10°.

crystallographic studies [6] of isomazole indicate that the molecule is planar in the crystal and are hence consistent with the calculated geometries.

Calculations of the (STO-3G *ab initio*) electrostatic potential in the plane of the imidazo-pyridine ring system indicated that in all cases, active molecules had an electrostatic potential minimum adjacent to the formally sp^2 imidazo nitrogen. This electronegative region can be clearly seen in figure 1 which shows, for example, the isopotential map for analogue **4**. Active analogues may therefore be able to adopt an orientation similar to isomazole at the inotropic receptor site(s).

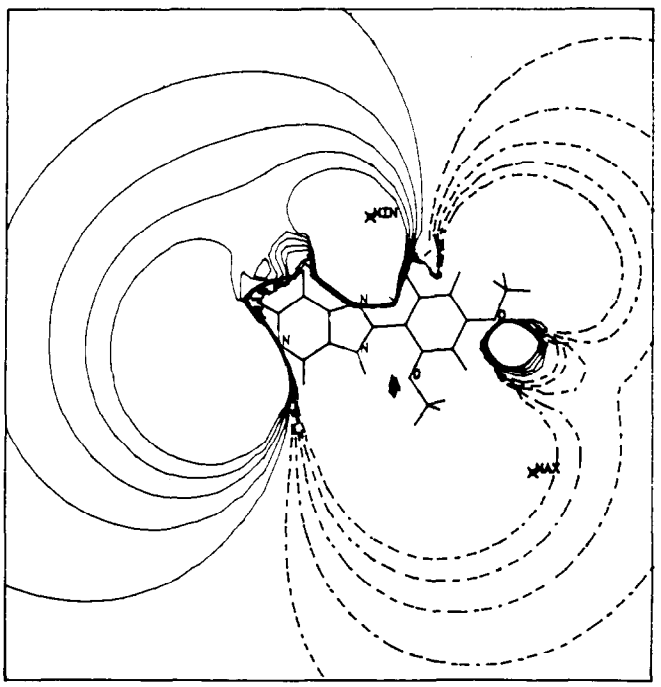


Fig 1. Isopotential map for analogue **4**.

Examination of the atom centred charge densities (CNDO/2) calculated for the imidazo nitrogen shows that the most potent *in vitro* inotropism is most often associated with a relatively high charge density on this nitrogen (table II). This is more clearly illustrated by a plot between these 2 parameters (fig 2) and indicates a correlation between them. Because the differences in charge density are small relative to the differences in lipophilicity the correlation for this set of heterocycles (correlation coefficient 0.81) is not as good as for other sulmazole analogues. Further studies with a much larger set of analogues (*eg*, "A" and "C" ring substituted imidazopyridines and imidazopyrimidines) having a wider range of charge densities but of more similar lipophilicity strongly support a good correlation however (Barraclough *et al*, unpublished results). An understanding in molecular terms of this correlation may be facilitated by a knowledge of the biochemical or pharmacological mechanisms by which sulmazole and its analogues exhibit their inotropic effects. At present these mechanisms are poorly understood. cAMP phosphodiesterase inhibition [8], adenosine antagonism [18] and effects on intracellular calcium [19] may contribute to the overall effect.

Conclusions

Analogues **8**, **9** and **10** proved to be of most interest and their cardiovascular profiles were determined *in vivo* and are shown in table III. The major effects of these compounds are a dose-dependent inotropic effect (increase in contractility), a rise in heart rate, and a fall in diastolic blood pressure. Analogues **8** and **9** have similar potencies to sulmazole in eliciting these pharmacological effects. The 1*H*-imidazo–[4,5-*d*]pyridazine **10** was approximately 4-fold more potent as an inotrope than sulmazole. However, analogue **10** did not display an increased separation between its inotropism and its effects on blood pressure and heart rate, relative to sulmazole. This lack of selectivity of inotropic action may not be detrimental therapeutically since a vasodilator component could reduce the loading on a "sick" heart.

The observations of the present SAR study give rise to the following hypotheses: (a) the primary requirement for activity is a structure composed of a "Z" shaped 4 atom system which is incorporated in a combination of aromatic rings. The extremities of the Z are suggested as being a hetero-atom and a conjugated atom where the first is generally nitrogen. This hypothesis is consistent with a previous computational study [20]; (b) heterocycles with the highest imidazo nitrogen charge density should display the most potent *in vitro* inotropism; (c) the p*K*a's of the heterocycle should be such that at pH 7.4 there is a high proportion of the uncharged species present. Further studies designed to test these hypotheses and provide more potent inotropic isomazole analogues will be reported elsewhere.

Experimental protocols

Chemistry

Melting points are uncorrected. TLC separations were effected on Merck silica gel 60F-254 and column chromatography on Merck silica gel (230–400 mesh). $MgSO_4$ was used for drying solutions in organic solvents. All products were shown homogeneous by TLC. The ^1H and ^{13}C NMR spectra of heterocycles **1–13** are given in references [5] and [21].

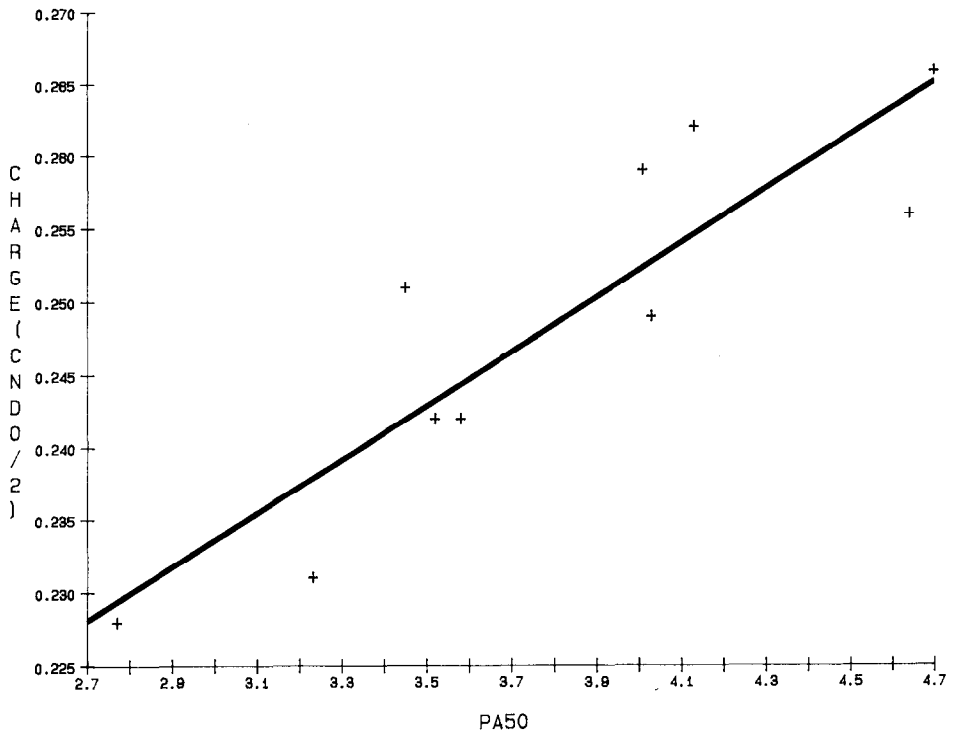


Fig 2. Inotropic activity *vs* charge of imidazo nitrogen of selected analogues.

Analyses indicated by the symbols of the elements or functions were within ± 0.4% of the theoretical values.

Condensation of heterocyclic diamines with substituted benzoic acids

*2-(2,4-Dimethoxyphenyl)-1*H*-imidazo[4,5-c]pyridine* **4**
3,4-diaminopyridine (1.50 g, 13.7 mmol) and 2,4-dimethoxybenzoic acid (2.50 g, 13.7 mmol) were pulverised together to a fine powder and then added in portions to phosphoryl chloride (50 ml) with stirring. The mixture was heated to reflux with stirring for 4 h and then the phosphoryl chloride distilled off *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 brought to pH 7 with concentrated HCl and after chilling the solid collected by filtration. Recrystallisation from EtOH–H_2O gave 2.50 g (71%) of **4**, mp 196–198°C; *m/z* 255 (M^+). Anal $C_{14}H_{13}N_3O_2$ (C, H, N). The following heterocycles were obtained in a similar manner.

*2-(2,6-Dimethoxyphenyl)-1*H*-imidazo[4,5-c]pyridine* **5** *dihydrochloride dihydrate*
mp 167–170°C. Anal $C_{14}H_{13}N_3O_2 \cdot 2\,HCl \cdot 2\,H_2O$(C, H, N, Cl) in 18% yield from 3,4-diaminopyridine and 2,6-dimethoxybenzoic acid.

2-(2,4-Dimethoxyphenyl)-1-methylimidazo[4,5-c]pyridine **6** *dihydrochloride*
mp 225–227°C. Anal $C_{15}H_{15}N_3O_2 \cdot 2\,HCl$ (C, H, N, Cl) in 15% yield from 3-amino-4-methylaminopyridine [22] and 2,4-dimethoxybenzoic acid (with modified work up and chromatography of crude product).

2-(2,4-Dimethoxyphenyl)-3-methylimidazo[4,5-c]pyridine **7** *hydrochloride*
mp 235–237°C. Anal $C_{15}H_{15}N_3O_2 \cdot HCl$ (C, H, N, Cl) in 30% yield from 4-amino-3-methylaminopyridine [22] and 2,4-dimethoxybenzoic acid.

2-(2-Methoxy-4-methylthiophenyl)benzimidazole **19**
mp 182–183°C. *m/z* 270 (M^+). Anal $C_{15}H_{14}N_2OS$ (C, H, N) in 73% yield from *o*-phenylenediamine and **18**.

8-(2-Methoxy-4-methylthiophenyl)purine **20**
mp 220–221°C. *m/z* 272 (M^+). Anal $C_{13}H_{12}N_4OS$ (C, H, N) in 35% yield from 4,5-diaminopyrimidine and **18**. lit [9] mp 212°C.

*6-(2-Methoxy-4-methylthiophenyl)-1*H*-imidazo[4,5-c]pyridazine* **21**
mp 215–217°C. *m/z* 272 (M^+). Anal $C_{13}H_{12}N_4OS$ (C, H, N) in 86% yield from 3,4-diaminopyridazine hydrochloride [23, 24] and **18**. lit [10] mp 190–192°C.

*2-(2-Methoxy-4-methylthiophenyl)-1*H*-imidazo[4,5-d]pyridazine* **22** [11]
mp 237–238°C. *m/z* 272 (M^+). Anal $C_{13}H_{12}N_4OS$ (C, H, N) in 35% yield from 4,5-diaminopyridazine hydrochloride [23, 24] and **18**.

*2-(2-Methoxy-4-methylthiophenyl)-1*H*-imidazo[4,5-b]pyrazine* **23** [11]
mp 202–204°C. *m/z* 272 (M^+). Anal $C_{13}H_{12}N_4OS$ (C, H, N) in 45% yield from 2,3-diaminopyrazine [25, 26] and **18**.

Table III. Cardiovascular effects of selected isomazole analogues. [a]Compounds administered to anaesthetised, open-chest dogs. All values quoted are derived from the mean data. [b]Effective dose (mg·kg^{-1} iv) of compound required to produce a maximum increase of 50% above basal in LV$dP/dt$, where LVP is left ventricular pressure. [c]Maximum percentage increase in LV$dP/dt$ observed (dose at which this change occurred). [d]Effective dose (mg·kg^{-1} iv) of compound required to produce a maximum decrease of 30% in diastolic blood pressure (DBP). [e]Maximum percentage decrease in DBP observed. [f]This ratio is a measure of the selectivity between inotropic and hypotensive effects. [g]Effective dose (mg·kg^{-1} iv) of compound required to produce a maximum increase of 10% in heart rate. [h]Maximum percentage increase in heart rate observed.

Compound [a]	ED_{50} [b] LV$dP/dt$	Max. Increase [c] (Dose)	ED_{30} [d] DBP	Max. Decrease [e] (Dose)	Ratio [f] ED_{50} LV$dP/dt$ / ED_{30} DBP	ED_{10} [g] HR	Max. Increase [h] (Dose)
Isomazole (n = 9)	0.05	88% (0.3 mgkg^{-1})	0.16	43% (1 mgkg^{-1})	0.32	0.05	15% (0.3 mgkg^{-1})
Sulmazole (n = 4)	0.80	73% (3 mgkg^{-1})	1.00	36% (3.0 mgkg^{-1})	0.80	0.50	20% (3 mgkg^{-1})
Analogue 8 (n = 2)	0.84	73% (3 mgkg^{-1})	0.76	47% (3 mgkg^{-1})	1.11	0.55	15% (1 mgkg^{-1})
Analogue 9 (n = 2)	0.49	88% (3 mgkg^{-1})	0.52	65% (3 mgkg^{-1})	0.94	0.19	16% (1 mgkg^{-1})
Analogue 10 (n = 2)	0.20	140% (1 mgkg^{-1})	-	24% (1 mgkg^{-1})	-	0.23	18% (1 mgkg^{-1})

2-Methoxy-4-methylthiobenzoic acid ***18***

Step 1: 4-Acetamidosalicyclic acid ***28***. 4-Aminosalicyclic acid (500 g) was added portionwise to acetic anhydride (2.5l) during 2 h at 20–25°C. After stirring at room temperature for a further 3 h the mixture was filtered. The solid was stirred with water (3 x 11), filtering each time and then dried at 50°C to constant weight to yield 629 g (98%) of crude product mp 234–235°C (decomp).

Step 2: 4-Amino-2-methoxybenzoic acid ***29***. Dimethyl sulphate (290 g, 2.3 mol) was added in a slow stream to a stirred mixture of **28** (195 g, 1.0 mol), anhydrous potassium carbonate (290 g, 2.1 mol) and acetone (2.5l, 4A molecular sieve dried). After stirring for 1 h at room temperature the mixture was carefully heated to reflux and held at reflux for 26 h. The cooled suspension was filtered, the residual inorganic solid washed with fresh acetone, and the filtrate concentrated *in vacuo* to give 230 g of crude methyl 4-acetamido-2-methoxybenzoate as a cream solid, mp 115–125°C. The crude ester was stirred with 10% NaOH solution (1.3l) at reflux for 3 h. The cooled hazy solution was clarified by filtration through celite, the filtrate treated with concentrated HCl (107 ml) to pH 8 with ice cooling followed by glacial acetic acid (175 ml) to pH 5 and the cream precipitate filtered off, washed with water and dried in a vacuum oven at 60°C to yield 145 g (87%) of product mp 140–142°C decomp. This product was carried through to the next stage without further purification. Crystallization from methanol gave the analytical sample. Anal $C_8H_9NO_3$ (C, H, N).

Step 3: Diazotisation. Finely ground **29** (80 g, 0.48 mol) was added to a stirred mixture of concentrated HCl (50 ml) and water (1025 ml). The suspension was cooled to 0–5°C and a solution of sodium nitrite (33.3 g, 0.48 mol) in water (74 ml) was added dropwise below the suspension surface for a period of 2 h. At the end of the addition the mixture was stirred at 0–5°C for a further 30 min, after which the pH was adjusted to 5 by the addition of sodium acetate trihydrate (21 g). During the above procedure, a solution of methanethiol (*ca* 36 g, 0.75 mol) in 15% NaOH solution (624 ml) at 0 ± 2°C was prepared. The diazonium salt suspension was blown over by means of nitrogen pressure dropwise into the thiol solution during 3 h, simultaneously adding portionwise copper powder (63 g), the reaction temperature being maintained at 0°C. After the addition was complete the dark solution was stirred, the temperature allowed to rise to 20°C for 3 h and then stood overnight at room temperature. The reaction mixture was filtered through celite to remove the copper residues, the filtrate cooled in ice, acidified to pH 1 with 6 N HCl and extracted

with chloroform. A solid collects at the interphase and the separation is made easier if the whole mixture is filtered. The chloroform solution was back extracted several times with saturated $NaHCO_3$ solution. The alkaline extract was stirred in ice, acidified with 6 N HCl to pH 5 and then with 2 N hydrochloric acid to pH 1. The brown oily precipitate was dissolved in chloroform, dried, treated with decolourising charcoal and concentrated *in vacuo* to give an oily brown solid (75 g). This material was triturated with ether and crystallised from isopropanol to give 39 g (41%) of **18**; mp 112–114°C (lit [6] mp 94–96°C).

*2-Methoxy-4-methylthiobenzoyl chloride **30***
Acid **18** (3.07 g, 15.5 mmol), thionyl chloride (10 ml, 0.14 mol) and dry benzene (30 ml) were stirred at room temperature for 0.5 h and then slowly heated to 80°C over 1 h. After a further 0.5 h at this temperature the volatiles were removed *in vacuo* to yield 3.20 g (95%) of crude **30** as an off-white solid. This material was used without further purification.

Condensation of heterocyclic amines with substituted benzoyl chlorides

*2-(2-Methoxy-4-methylthiophenyl)oxazolo[4,5-b]pyridine **24***
To a solution of **30** (3.35 g, 15.5 mmol) in phosphoryl chloride (30 ml) containing pyrophosphoryl tetrachloride (1 ml) was added 2-amino-pyrid-3-one (2.05 g, 18.6 mmol). The resulting mixture was heated at reflux for 16 h under N_2. After cooling to 0°C for 1 h, ether (60 ml) was then added and the solid collected by filtration. This solid was then partitioned between chloroform and 5% $NaHCO_3$ solution. The organic layer was then washed with 5% $NaHCO_3$ and then water. After drying the solvent was removed *in vacuo* and the residual gum chromatographed on silica (250 g). Elution with chloroform and then recrystallisation from benzene–hexane gave 1.93 g (46%) of **24**. mp 111–112°C. *m/z* 272 (M^+). Anal $C_{14}H_{12}N_2O_2S$ (C, H, N).

*2-(2-Methoxy-4-methylthiophenyl)oxazolo[5,4-b]pyridine **25***
mp 134–135°C. *m/z* 272 (M^+). Anal $C_{14}H_{12}N_2O_2S$ (C, H, N) in 22% yield from 3-aminopyrid-2-one [27] and **30**.

*2-(2,4-Dimethoxyphenyl)oxazolo[4,5-c]pyridine **16** hydrochloride*
mp 226–228°C, δ (CD_3OD) 3.94 (3H, s, OMe), 4.03 (3H, s, OMe), 6.82 (2H, m, aromatic), 8.26 (2H, m, aromatic), 8.78 (1H, brd, *J*, 6 Hz, aromatic), 9.20 (1H, br s, C, H, N). Anal $C_{14}H_{12}N_2O_3$·HCl (C, H, N, Cl) in 25% yield from 3-aminopyrid-4-one [28] and 2,4-dimethoxybenzoyl chloride.

*3-(2-Methoxy-4-methylthiobenzoyl)aminopyrid-4-one **26***
To a solution of 3-aminopyrid-4-one [28] (3.30 g, 30 mmol) in dry pyridine (80 ml) was added **30** (6.49 g, 30 mmol). The reaction mixture was stirred at room temperature for 8 h and then the volatiles removed *in vacuo*. Trituration of the residue in water gave a solid which was filtered off, washed with water and dried. This solid was chromatographed on silica (400 g) eluting with $CHCl_3$–MeOH (9:1) to give after crystallization from $CHCl_3$ 3.9 g (45%) of **26**. mp 276–278°C. *m/z* 290 (M^+). Anal $C_{14}H_{14}N_2O_3S$ (C, H, N).

*2-(2-Methoxy-4-methylthiophenyl)thiazolo[4,5-c]pyridine **27***
26 (2.90 g, 10.0 mmol), phosphorus pentasulphide (10.0 g, 45.0 mmol), dry xylene (90 ml) and dry pyridine (25 ml) were heated at reflux for 16 h. The hot solution was decanted from resinous material and evaporated to dryness. Recrystallisation (3x) of the residue from EtOH–H_2O gave 0.67 g (23%) of **27**. mp 196–197°C. *m/z* 288 (M^+). Anal $C_{14}H_{12}N_2OS_2$ (C, H, N).

Oxidation of sulphides to sulfoxides

*Method A: preparation of 2-(2-methoxy-4-methylsulphinylphenyl)benzimidazole **3***
To sulphide **19** (3.00 g, 0.011 mol) in dichloromethane (80 ml), stirred and cooled at –78°C, was added a solution of *m*-chloroperbenzoic acid (2.39 g, 80%, 0.011 mol) in dichloromethane (50 ml) over 2 min. The reaction mixture was stirred at –78°C for 2 h, allowed to warm up to room temperature, and then washed with aqueous saturated $NaHCO_3$ solution. The organic layer was dried, the solvent removed *in vacuo* and the residual oil chromatographed on silica gel (150 g). Elution with 1% MeOH-$CHCl_3$ gave firstly 2-(2-methoxy-4-methylsulphonylphenyl)benzimidazole. mp 240–242°C. Anal $C_{15}H_{14}N_2O_3S$ (C, H, N). Further elution gave 1.92 g (57%) of sulphoxide **3** as a buff solid of no sharp mp. Anal $C_{15}H_{14}N_2O_2S,H_2O$ (C, H, N). In a similar manner were obtained: 2-(2-Methoxy-4-methylsulphinylphenyl)oxazolo[4,5-b]pyridine **14**, 2-(2-methoxy-4-methylsulphinylphenyl)oxa-zolo[5,4-b]pyridine **15** and 2-(2-methoxy-4-methylsulphinyl-phenyl)thiazolo[4,5-c]-pyridine **17**. Details are given in table I.

*Method B [12] preparation of 2-(2-Methoxy-4-methylsulphinylphenyl)-1H-imidazo[4,5-b]-pyrazine **11***
Sulphide **23** (3.00 g, 11.0 mmol) and anhydrous sodium acetate (0.90 g, 11.0 mmol) was added to glacial acetic acid (25 ml). After stirring for ≈ 15 min to obtain a solution, bromine (0.60 ml, 11.6 mmol) in glacial acetic acid (3.6 ml) was added dropwise during 35 min with vigorous stirring, maintaining the reaction temperature between 20–23°C with water cooling. After the addition was complete the suspension was stirred for a further 1 h, poured onto ice (30 g) and treated with 0.880 NH_3 to pH 9 with ice cooling. The resulting mixture was saturated with sodium chloride and then extracted with chloroform. The extract was dried, treated with decolourising charcoal, filtered and the filtrate concentrated *in vacuo*. The residual solid was chromatographed on silica gel eluting with $CHCl_3$–MeOH (93:7) to give 1.50 g (47%) of **11** [11] mp 238–240°C (EtOH). Anal $C_{13}H_{12}N_4O_2S$·0.1 EtOH (C, H, N). In a similar manner were obtained 8-(2-methoxy-4-methylsulphinylphenyl)purine **8** [9], 6-(2-methoxy-4-methyl-sulphinylphenyl), 1-H-imidazo[4,5-c]pyridazine **9** [10], and 2-(2-methoxy-4-methylsulphinyl-phenyl)-1H-imidazo-[4,5-d]pyridazine **10** [11]. Details are given in table I.

Preparation of pyrollopyridines

*3-Nitro-4-[2-(2,4-dimethoxyphenyl)vinyl]pyridine **31***
A solution of 2,4-dimethoxybenzaldehyde (5.89 g, 35.5 mmol), 4-methyl-3-nitropyridine [29] (4.70 g, 34.1 mmol) and piperidine (1.5 ml) in methanol (20 ml) was heated at reflux for 5.5 h and then cooled in ice. The resulting precipitate was removed by filtration and recrystallised from methanol yielding 4.2 g (43%) orange needles, mp 98–99°C, *m/z* 286 (M^+). Anal $C_{15}H_{14}N_2O_4$ (C, H, N).

*3-[2-(2,4-Dimethoxyphenyl)vinyl]-4-nitropyridine-1-oxide **32***
2,4-Dimethoxybenzaldehyde (10.3 g, 62.0 mmol), 3-methyl-4-nitropyridine-1-oxide [30] (9.50 g, 62.0 mmol), piperidine (3 ml) and methanol (40 ml) were heated at reflux for 16 h with efficient stirring, and then cooled in ice. The resulting precipitate was removed by filtration and recrystallised from

dimethylformamide–water yielding 10.9 g (58%) orange crystals, mp 191–193°C. Anal $C_{15}H_{14}N_2O_5$ (C, H, N).

2-(2,4-Dimethoxyphenyl)-1H-pyrrolo[2,3-c]pyridine **12**
Nitropyridine **31** (4.20 g, 14.7 mmol), triethylphosphite (16.3 g, 98.2 mmol) and dry benzene (200 ml) were heated at reflux under a slow stream of nitrogen for 7 days. The solvent was removed *in vacuo* and the residual oil stirred with water (160 ml) for 10 min to decompose the excess phosphite. The resulting mixture was acidified with acetic acid, stirred 5 min, and then washed with chloroform (2x) to remove unreacted **31**. The aqueous layer was basified with $NaHCO_3$ solution, evaporated *in vacuo* to dryness, and the residue stirred with chloroform. Inorganic salts were removed by filtration and the chloroform extract concentrated *in vacuo* to yield crude **12** as an oil (2 g). This was purified by column chromatography on silica using $CHCl_3$–MeOH (1/1) and then MeOH as eluant followed by further chromatography on a column of deactivated alumina (6%, H_2O), CH_2Cl_2 as eluant and finally recrystallisation from aqueous dimethoxyethane yielding 130 mg (4%) of **12** as colourless needles, mp 150–152°C. Anal $C_{15}H_{14}N_2O_2$ (C, H, N).

2-(2,4-Dimethoxyphenyl)-1H-pyrrolo[3,2-c]pyridine **13** *hydrochloride*
Nitropyridine-1-oxide **32** (4.55 g, 15.0 mmol) triethylphosphite (25 g, 0.15 mol), and dry benzene (225 ml) were heated at reflux under a slow stream of nitrogen for 10 days. The reaction mixture was worked up in a similar manner to that of **12** yielding 1.1 g of crude **13**. This was purified by column chromatography on silica with $CHCl_3$–MeOH (4:1) as eluant followed by further chromatography on neutral alumina (grade 4) eluting with CH_2Cl_2 yielding 410 mg (11%) of **13**. This was converted to the hydrochloride, mp 234–236°C. Anal $C_{15}H_{14}N_2O_2$·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. A single right ventricular papillary muscle was obtained by dissection from each heart, the tendinous end ligated to a stainless steel hook and the lower end 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 and 5% CO_2 maintained at 34°C; 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 volts) by an SRI stimulator. The transducer inputs were coupled to a potentiometric recording device by a 6-channel Grass transducer coupler. A group of organ baths enabled up to 6 preparations to be utilised 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.

Experiments in anaesthetized dogs
Beagle dogs (of either sex) weighing between 8.5–13 kg were initially anaesthetized by an iv injection of thiopentone sodium (30 $mg \cdot kg^{-1}$) into a cephalic vein. Anaesthesia was subsequently maintained by intravenous injection of α-chloralose (15 $mg \cdot kg^{-1}$) and pentobarbitone sodium (6 $mg \cdot kg^{-1}$) *via* a cannula placed in the right femoral vein. The trachea was then cannulated and the animal artificially ventilated with room air using a Palmer pump (stroke volume 200–250 ml and respiration rate 20 min^{-1}). Arterial blood samples were removed before beginning the experiment and analysed (Radiometer Blood Gas Analyser) 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 proble (10–12 mm internal diameter) placed around the ascending aorta and connected to a Statham flowmeter to measure aortic blood flow. Extra-corporeal pressure transducers were used to measure blood and left ventricular pressure.

Left ventricular pressure (LVP) and its first derivative, $LVdP/dt$, were measured by the insertion of a short cannula (containing heparinised-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 heparinised 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 administrering 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.

Other parameters (aortic blood flow, ECG) were also measured as part of a general cardiotoxicity evaluation. Some further details are given in reference [8].

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