Cardiotonic 'C' Ring Modified Isomazole Analogues

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Isomazole analogues which have achiral electron withdrawing substituents at the 4'-position and analogues with heterocyclic 'C' rings have been synthesized and evaluated as inotropic agents. It was found that pyridyl could replace phenyl in the 'C' ring without loss of activity. The 4'-methylsulphonyl, -cyano, -carboxamido, and acetyl analogues had similar inotropic potencies to Isomazole whilst displaying superior cardiovascular profiles in *in vivo* studies.

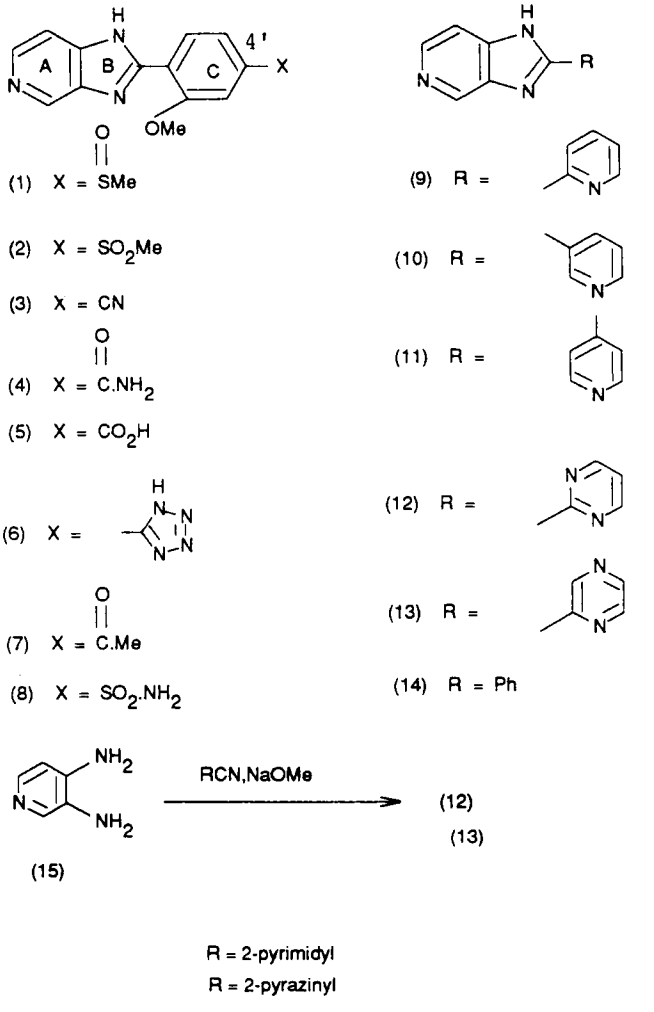
Kardiotonische 'C'-Ring modifizierte Isomazolanaloge

Isomazolanaloge mit achiralem, elektronenziehendem Substituenten an der 4'-Position und heterocyclischem 'C'-Ring wurden synthetisiert und als ionotrope Agentien evaluiert. Den Untersuchungsergebnissen zufolge kann der Pyridylrest ohne Aktivitätsverlust die Phenylgruppe im 'C'-Ring ersetzen. Die 4'-Methylsulfonyl, -Cyan, -Carboxamid und -Acetyl-Analogen wiesen vergleichbare ionotrope Wirksamkeiten wie Isomazol auf und zeigten im Rahmen von *in vivo* Studien das beste kardiovaskuläre Profil.

Isomazole **1**[1,2] is an inotropic vasodilator which may have utility in the management of congestive heart failure. As part of an exercise to develop some understanding of the structure-activity relationships for Isomazole we investigated the effects of replacing the 'C' aryl ring by heterocyclic groups. In addition we wanted to know whether replacement of the 4'-methylsulphinyl substituent by achiral electron-withdrawing groups would lead to analogues with a better pharmacological profile. The enantiomers of Isomazole have been prepared[3,4] and found[3] to have markedly different pharmacological properties. Studies of the inotropic properties of achiral Isomazole analogues would therefore be much simpler than those for Isomazole itself. Analogues **2** - **14** were therefore prepared in order to aid the above investigations.

Chemistry

Sulphone **2**[2,5], nitrile **3**[6], pyridines **9** - **11**[7,8], and imidazopyridine **14**[9] were prepared by described methods. Reaction of nitrile **3** with conc. H_2SO_4 at room temp. gave amide **4**. When this reaction was carried out in refluxing acetic acid the major product was the acid **5**. Tetrazole **6** was obtained by treating nitrile **3** with NaN_3 and ammonium chloride in DMF at 125°. Analogues **12** and **13** were obtained by base-catalysed condensation of 3,4-diaminopyridine **15** with the appropriate nitrile as shown in Scheme 1. Ketone **7** was prepared by a three stage process, beginning with the reaction of 3,4-diaminopyridine (**15**) with 4-acetyl-2-methoxybenzoic acid **19**, this acid being obtained as shown in Scheme 2. Acid **22**, prepared as shown in Scheme 3, underwent condensation with 3,4-diaminopyridine to give sulphonamide **8**.



SCHEME 1

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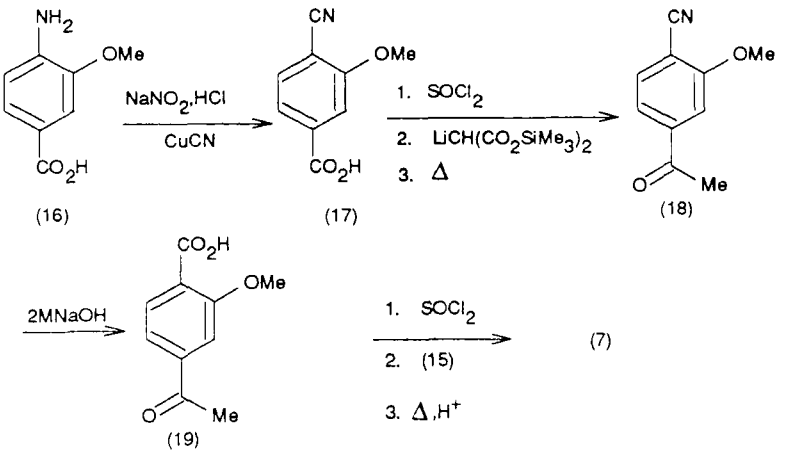

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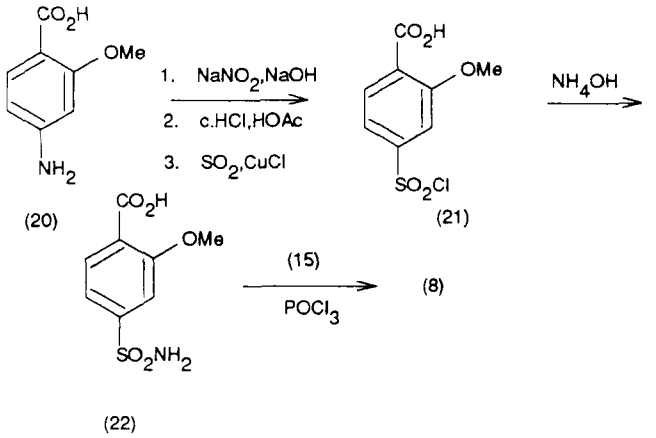

SCHEME 3

Structure-Activity Relationships

The *in vitro* inotropic activities of Isomazole analogues **2** - **14** are given in Table 1 along with some physicochemical parameters. For the heterocyclic analogues it can be seen that replacement of the 2-phenyl group of **14** by a 2-pyrimidinyl or 2-pyrazinyl moiety gives rise to less active analogues. A pyridyl group however appears to be a good bioisostere for phenyl at the 2-position as evidenced by the increased potency of analogues **9** and **11** relative to **14**. It will thus be of interest to see whether introduction of methoxy and/or electron withdrawing substituents into the 2-pyridyl group leads to a further increase in potency. The 2-pyridyl analogue **9** showed pronounced vasodilation and a hypotensive effect *in vivo*.

All the analogues possessing achiral 4'-electron withdrawing groups, with the exception of acid **5** and tetrazole **6**, displayed more potent inotropic activity than **14**. The drastic reduction in inotropic activity observed on replacing a 4'-cyano or 4'-carboxamido group by a 4'-carboxylate or 4'-

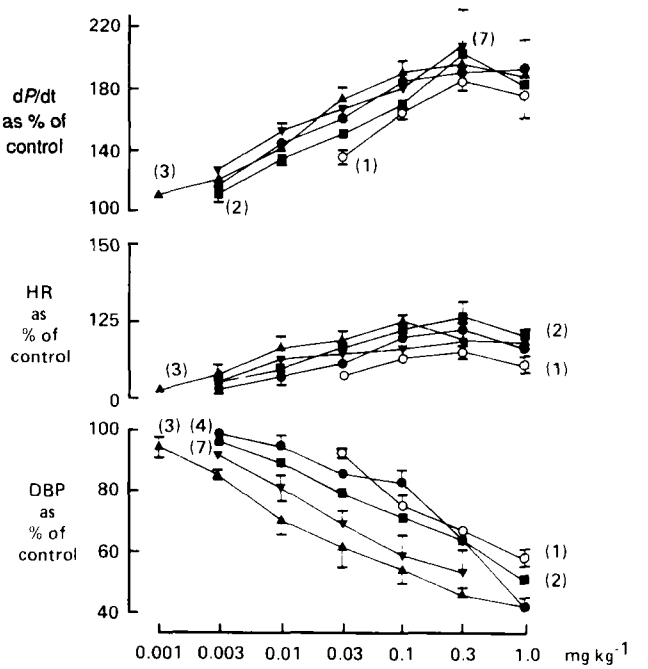

Fig. 1: Haemodynamic effects of Isomazole and analogues in the anaesthetised, open-chest dog. Dose-response curves are shown as mean ± s.e.m. O Isomazole **1**, n=9; ■ sulphone **2**, n=3; ▲ nitrile **3**, n=3; ● carboxamide **4**, n=3; ▼ ketone **7**, n=3.

tetrazol-5-yl anion may be due in part to a sharp fall in lipophilicity. It has been found that inotropic activities in both the 1*H*-imidazo[4,5-*b*]- and [4,5-*c*]pyridine series are sensitive to gross changes in log P[10,11]. No correlation was observed however between *in vitro* inotropism and lipophilicity as modelled by log P. Similarly for analogues **2** - **8** no correlation was observed between the electron-withdrawing capability of the 4'-substituent (as modelled by σp) and *in vitro* inotropic activity.

Analogues **2**,**3**,**4**, and **7** displayed the most potent *in vitro* inotropic activity and were thus chosen for further *in vivo*

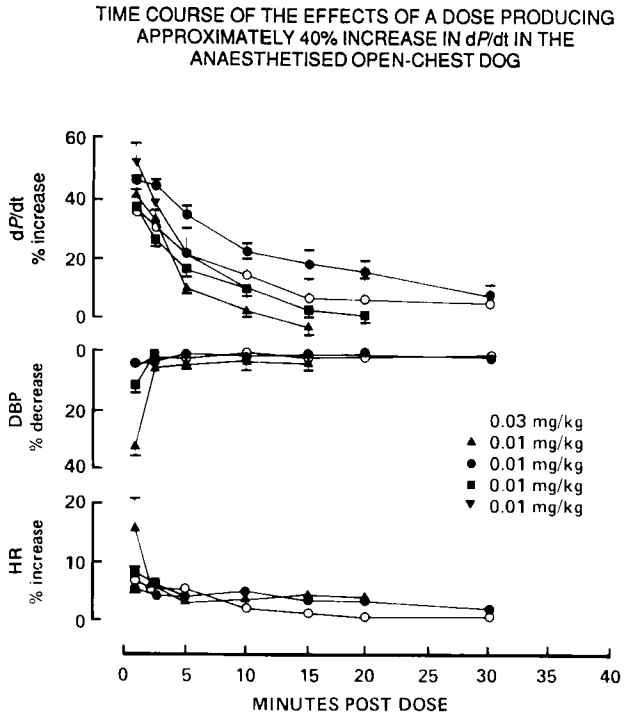

Fig. 2: Time course of the haemodynamic effects of Isomazole and analogues in the anaesthetised, open chest dog. ○ Isomazole **1**, 0.03 mg kg^{-1}; ■ sulphone **2**, 0.01 mg kg^{-1}; ▲ nitrile **3**, 0.01 mg kg^{-1}; ● carboxamide **4**, 0.01 mg kg^{-1}; ▼ ketone **7**, 0.01 mg kg^{-1}.

evaluation. Cardiovascular profiles after i.v. administration to anaesthetised dogs were determined and compared to Isomazole (Figures 1 and 2 and Table 2). The principal effects of analogues **2** - **4** and **7** were a dose-dependent inotropic effect (increase in dP/dt where P is left ventricular pressure), a rise in heart rate, and fall in diastolic blood pressure (DBP). The inotropic and vasodilator effects of analogues **2** - **4** and **7** give rise to similar shaped dose-response curves to Isomazole but are apparent at lower doses. The 4'-carboxamido analogue **4** shows a greater separation between these two actions (in favour of inotropism) than does Isomazole (low ED_{50} dP/dt: ED_{30} DBP ratio). A greater separation in favour of vasodilation is shown by the 4'-cyano analogue **3**. The duration of these effects is shown in Figure 2. The positive inotropic effects of the carboxamide **4** were still evident 90 min after dosing up to 0.3 mg kg^{-1} and persisted longer than those of Isomazole.

Comparison of the cardiovascular profiles of analogues **2** - **4**, and **7** suggested that carboxamide **4** was the most attractive candidate inotrope for further study. It is interesting to note that those analogues displaying potent *in vivo* inotropic properties all have 4'-substituents capable of being hydrogen bond acceptors, in agreement with previous studies[2].

Experimental Part

Details of the instruments employed and general methodology are given in the preceding paper.

Table 1: Inotropic Activity of Isomazole Analogues

Compound	$pA_{50\%}$[a]	log P[b]	p*K*a[c]	πp[d,e]	σp[e,f]
1	4.64 ± 0.15 (17)	1.23	6.2	-1.58	0.49
2	4.40 ± 0.26	1.35	6.1	-1.63	0.72
3	< 6 g	2.09		-0.57	0.66
4	4.43 ± 0.08	1.18		-1.49	0.36
5	i (2)			-4.36	0.00
6	2.62 ± 0.21				
7	4.65 ± 0.15			-0.55	0.50
8	4.26 ± 0.31			-1.82	0.57
9	3.30 ± 0.0	1.64	5.9		
10	3.10 ± 0.10				
11	3.20 ± 0.0	1.18	3.0,4.9		
12	i (2)	0.45			
13	2.73 ± 0.15	0.84			
14	3.03 ± 0.14	2.19	6.0	0.00	0.00

a $pA_{50\%}$ is the negative logarithm to base 10 of the drug concentration required to give a 50% increase in basal contractile force for (n) experiments where n=3 unless otherwise stated. Paced guinea pig papillary muscle preparations were employed; i=inactive; 50% increase not achieved.

b P=octanol-aqueous phosphate buffer partition coefficient at 25°C and pH 7.4. Values were determined experimentally by the shake-flask method and are accurate to within ± 0.05.

c Relates to equilibrium for proton addition(s) to heterocycle (pK_1, pK_2). Values determined spectrophotometrically in water are within ± 0.04.

d πp is the hydrophobicity constant for the 4'-substituent.

e Values taken from 'Substituent Constants for Correlation Analysis in Chemistry and Biology' ed. *C. Hansch* and *A. Leo*, Wiley-Interscience N.Y.

f σp is the *Hammett* constant for the 4'-substituent.

g Variable biphasic dose-response curve observed; accurate determination not possible.

2-(4-Carbamoyl-2-methoxyphenyl)-1H-imidazo[4,5-c]pyridine (**4**)

2-(4-Cyano-2-methoxyphenyl)-1H-imidazo[4,5-c]pyridine (**3**)[6] (1.0 g) was added to conc. H_2SO_4 (15 ml) and stirred for 1 h. The resulting solution was allowed to stand at room temp. for 48 h and then poured onto ice. The mixture was neutralised by 10 M NaOH with cooling. The resulting solid **4** was collected, washed with water, and dried. A solution of **4** in methanol was treated with methanolic HCl to give 0.73 g (52%) of **4**-dihydrochloride mp. 225-235°C.- $C_{14}H_{12}N_4O_2 \cdot 2HCl \cdot 0.4\ H_2O$ (348.4) Calcd. C 48.3 H 4.28 N 16.1 Cl 20.4 Found C 48.5 H 4.11 N 15.8 Cl 20.1.- ^{1}H-NMR (200 MHz, dmso-d_6): δ = 3.36 (2H, br. peak, NH_2, exchangeable), 4.16 (3H, s, OMe), 7.6 (1H, br. s, NH, exchangeable), 7.70 (1H, d, J = 8.2 Hz, H-5'), 7.79 (1H, br. s, H-3'), 8.20 (1H, d, J = 6.5 Hz, H-7), 8.26 (1H, br. s, NH, exchangeable), 8.43 (1H, d, J = 8.1 Hz, H-6'), 8.58 (1H, d, J = 6.5 Hz, H-6), 9.42 (1H, br. s, H-4).

2-(4-Carboxy-2-methoxyphenyl)-1H-imidazo[4,5-c]pyridine (**5**)

Nitrile **3** (1.0 g), water (2 ml), conc. H_2SO_4 (2 ml) and acetic acid (2 ml) were heated at reflux for 1 h. The cooled mixture was poured onto ice and neutralised with 10 M NaOH. The resulting solid was collected by filtration, washed with water and dried to give 0.66 g (61%) of acid **5**. Treatment with one equivalent of methanolic NaOH gave **5**-monosodium salt mp > 300°C.- $C_{14}H_{10}N_3O_3Na \cdot 1.25\ H_2O$ (313.8) Calcd. C 53.6 H 4.02 N 13.4 Na 7.33 Found C 53.9 H 3.96 N 13.0 Na 6.97.

2-[2-Methoxy-4-(5-tetrazolyl)phenyl]-1H-imidazo[4,5-c]pyridine (**6**)

Nitrile **3** (1.0 g, 4 mmol), NH_4Cl (0.29 g; 5 mmol), NaN_3(0.33 g; 5 mmol) and dry DMF (3 ml) were stirred and heated at 125° until **3** was

Table 2: Haemodynamic Responses to Isomazole Analogues[a]

Compound	ED_{50}/mg kg^{-1}[b]	ED_{30}/mg kg^{-1}[c]	ED_{10}/mg kg^{-1}[d]
1	0.051	0.16	0.056
(n=9)	+88% at 0.3 mg kg^{-1}	-43% at 1 mg kg^{-1}	+15% at 0.3 mg kg^{-1}
2	0.029	0.11	0.014
(n=3)	+105% at 0.3 mg kg^{-1}	-51% at 1 mg kg^{-1}	+26% at 0.3 mg kg^{-1}
3	0.014	0.01	0.004
(n=3)	+98% at 0.3 mg kg^{-1}	-61% at 1 mg kg^{-1}	+24% at 0.1 mg kg^{-1}
4	0.014	0.20	0.019
(n=3)	+95% at 1 mg kg^{-1}	-61% at 1 mg kg^{-1}	+21% at 0.3 mg kg^{-1}
7	0.009	0.025	0.007
(n=3)	+107% at 0.3 mg kg^{-1}	-60% at 1 mg kg^{-1}	+18% at 0.3 mg kg^{-1}

a Experiments using anaesthetised, open-chest dogs.
b ED_{50} is the dose required to produce a 50% increase in d*P*/dt. The maximum percentage increase observed in d*P*/dt is given below the ED_{50} value.
c ED_{30} is the dose required to produce a 30% decrease in diastolic blood pressure (DBP). The maximum decrease observed is given below the ED_{30} value.
d ED_{10} is the dose required to produce a 10% increase in heart rate (HR). The maximum increase observed is given below the ED_{10} value.

absent (as indicated by tlc, ca 6 h). The cooled mixture was poured onto ice - conc. HCl, and then heated on a steam bath for 1 h to convert the gelatinous precipitate of **6**-HCl to granular form. After cooling the resulting solid was collected by filtration, suspended in water and the mixture treated dropwise with excess 2 M NaOH. The insoluble material was removed by filtration and the filtrate neutralised with 2 M HCl. The resulting solid was collected, washed with water and dried to give 0.94 g (80%) of **6** mp. 228-231°C.- $C_{14}H_{11}N_7O \cdot 1.5\ H_2O$ (320.3) Calcd. C 52.5 H 4.40 N 30.6 Found C 52.8 H 4.44 N 30.8. Treatment with one equivalent of methanolic sodium methoxide gave the sodium salt of **6**, mp. 300-305°C (decomp.).- ^{1}H-NMR (200 MHz, dmso-d_6): δ = 4.10 (3H, s, OMe), 7.61 (1H, d, J = 5.5 Hz, H-7), 7.79 (1H, dd, J = 8.1, 1.2 Hz, H-5'), 7.86 (1H, d, J = 1.2 Hz, H-3'), 8.30 (1H, d, J = 5.5 Hz, H-6), 8.38 (1H, d, J = 8.1 Hz, H-6'), 8.94 (1H, s, H-4).

2-(2-Pyrimidinyl)-1H-imidazo[4,5-c]pyridine (**12**)

To a freshly prepared solution of sodium (230 mg, 0.01 mol) in dry methanol (30 ml) was added 2-cyanopyrimidine[12] (1.05 g, 0.01 mol). The resulting solution was stirred at room temp. for 5 h then the methanol was removed *in vacuo* to give the crude imidate as an oil. This oil, 2-methoxyethanol (30 ml) and 3,4-diaminopyridine HCl (1.45 g, 0.01 mol) were stirred and heated at reflux for 6 h. The cooled mixture was evaporated to dryness, water was added and the aqueous phase extracted with $CHCl_3$ (4x). Organic extracts were dried, the solvent was removed *in vacuo*, and the oily residue was triturated in ether. The solid was collected and treated with methanolic HCl to yield 220 mg (8%) of **12**-dihydrochloride, mp. 345-346°C.- $C_{10}H_7N_5 \cdot 2HCl$ (270.1) Calcd. C 44.4 H 3.36 N 25.9 Found C 44.6 H 3.37 N 25.9.- ^{1}H-NMR (200 MHz, dmso-d_6): δ = 7.78 (1H, "t", J = 4.9 Hz, pyrimidine-H), 8.12 (1H, d, J = 6.5 Hz, H-7), 8.63 (1H, d, J = 6.5 Hz, H-6), 9.14 (2H, d, J = 4.9 Hz, pyrimidine-H), 9.56 (1H, s, H-4).

2-(2-Pyrazinyl)-1H-imidazo[4,5-c]pyridine (**13**)

Reaction of 2-cyanopyrazine[13] with 3,4-diaminopyridine HCl under the conditions employed above gave 15% of **13**-hydrochloride mp. 255-257°C.- $C_{10}H_7N_5 \cdot HCl \cdot 1.5\ H_2O$ (260.7) Calcd. C 46.1 H 4.25 N 26.9 Found C 46.1 H 3.89 N 26.7.- ^{1}H-NMR (200 MHz, dmso-d_6): δ = 8.14 (1H, d, J = 6.5 Hz, H-7), 8.62 (1H, d, J = 6.5 Hz, H-6), 8.93 (2H, s, pyrazine-H), 9.54 (1H, s, H-4), 9.58 (1H, s, CHN).

4-Cyano-3-methoxybenzoic Acid (**17**)

A solution of CuCN was prepared as follows. 2 N HCl was added to a stirred solution of $CuSO_4 \cdot 5H_2O$ (34.2 g, 0.14 mol) in water (115 ml) until acid to Congo Red. To the resulting solution at 60°C was added a solution of sodium metabisulphite (8.7 g) in water (38 ml) over 5 min followed by a solution of KCN (9.6 g) in water (38 ml). The resulting suspension was stirred at 60°C for 0.5 h, filtered and the white solid washed with water and sucked dry at the pump. This solid (CuCN) was dissolved in a solution of KCN (17.9 g) in water (60 ml) and used as described below.

A solution of $NaNO_2$ (8.30 g; 0.12 mol) in water (18 ml) was added dropwise to a stirred mixture of 4-amino-3-methoxybenzoic acid (**16**)[14] (19.0 g; 0.11 mol), conc. HCl (12 ml) and water (245 ml) at 0-5°C. The mixture was then stirred at 0-5°C for 1 h and the resulting diazonium salt solution added in portions to the freshly prepared CuCN solution at 50°C. After addition was complete the mixture was stirred and heated at reflux for 2 h, cooled and then filtered to remove insoluble by-products. The filtrate was acidified with conc. HCl and the resulting solid collected. This crude acid was stirred with an excess of 5% $NaHCO_3$ solution for 1 h, the suspension filtered, and the filtrate acidified with conc. HCl. After standing for 1 h the yellow solid was collected, washed with water and dried to give 11.0 g (55%) of **17**, mp. 237-240°C.- $C_9H_7NO_3$ (177.2) Calcd. C 61.0 H 3.98 N 7.91 Found C 60.8 H 4.17 N 7.79.- ^{1}H-NMR (200 MHz, $CDCl_3$): δ = 4.01 (3H, s, OMe), 7.64-7.72 (3H, m, aromat. H).

4'-Cyano-3'-methoxyacetophenone (**18**)

Acid **17** (14.0 g, 0.08 mol), dry toluene (800 ml) and $SOCl_2$ (16 ml) were stirred and heated at reflux for 3 h. Any remaining insoluble material was removed by filtration, and the filtrate was evaporated to dryness to give crude 3-methoxy-4-cyanobenzoyl chloride. This acid chloride was dissolved in dry THF (80 ml) and the resulting solution was then added over 10 min to a stirred solution of lithium bis (trimethylsilyl) malonate in dry ether (250 ml) at 0°C [(prepared from bis (trimethylsilyl) malonate (40.4 g, 0.163 mol) and n-butyllithium (98.5 ml of 1.6 M solution, 0.158 mol)]. Stirring was continued at 0-5°C for 1 h. The resulting mixture was acidified with conc. HCl and extracted with EtOAc. Org. extracts were washed with water, dried and evaporated *in vacuo*. The residual brown solid was dissolved in dioxan (100 ml) and the solution heated at reflux for 2 h. Volatile material was removed *in vacuo*, the residue dissolved in CH_2Cl_2 (200 ml), and the solution washed with 5% $NaHCO_3$ solution and then water. The

CH_2Cl_2 extract was dried and evaporated to dryness to give 9.3 g (67%) of **18**, mp. 110-111°C.- $C_{10}H_9NO_2$ (175.2) Calcd. C 68.6 H 5.18 N 8.00 Found C 68.4 H 5.07 N 8.14.- MS: m/z 175 (M^+).- ^{1}H-NMR (200 MHz, $CDCl_3$): δ = 2.64 (3H, s, H_3C-CO), 4.06 (3H, s, OMe), 7.57 (2H, m, aromat. H), 7.68 (1H, d, J = 8.2 Hz, aromat. H).

4-Acetyl-2-methoxybenzoic acid (**19**)

Nitrile **18** (6.0 g, 0.03 mol) and 2 M aqueous NaOH solution (60 ml, 0.12 mol) were heated at reflux for 40 min. The cooled mixture was filtered to remove traces of insoluble material. The filtrate was acidified with acetic acid and extracted with $CHCl_3$. The org. phase was dried, treated with decolourising charcoal, and evaporated *in vacuo* to give 4.6 g (69%) of **19** as a cream solid mp. 127-128°C.- $C_{10}H_{10}O_4$ (194.2) Calcd. C 61.9 H 5.19 Found C 62.1 H 5.30.- ^{1}H-NMR (200 MHz, $CDCl_3$): δ = 2.66 (3H, s, H_3C-CO), 4.14 (3H, s, OMe), 7.65 (2H, m, aromat. H), 8.26 (1H, d, J = 8.4 Hz, aromat. H).

2-(4-Acetyl-2-methoxyphenyl)-1H-imidazo[4,5-c]pyridine (**7**)

Acid **19** (2.56 g, 0.013 mol), $SOCl_2$ (2.45 ml, 0.03 mol) and dry toluene (122 ml) were heated at reflux for 4 h. After cooling the volatiles were removed *in vacuo*. The residual solid was dissolved in dry toluene (15 ml) and the resulting solution added dropwise to a stirred mixture of 3,4-diaminopyridine (1.44 g, 0.013 mol), triethylamine (13 ml) and dry pyridine (40 ml). Stirring was continued at room temp. for 18 h and then the precipitate was collected by filtration, washed with water and dried. This crude monoamide (2.70 g), ethanediol (17 ml) and conc. HCl (3 drops) were stirred and heated together at 150°C for 1 h. The cooled mixture was diluted with ice-water (150 ml), triturated, and the solid collected by filtration. This solid, crude ethylene ketal derivative of **7**, and 2 M HCl solution (120 ml) were stirred at 50° for 3 h. The mixture was cooled, brought to pH 9 by addition of 2 M NaOH and the resulting precipitate filtered off, washed with water and dried. Yield 1.7 g (48%), mp. 181-183°C (methanol-ether). Treatment with methanolic HCl gave **7**-dihydrochloride, mp. 194-196°C.- $C_{15}H_{13}N_3O_2 \cdot 2HCl \cdot 0.25\ H_2O$ (344.7) Calcd. C 52.3 H 4.49 N 12.2 Cl 20.6 Found C 52.2 H 4.30 N 11.9 Cl 20.6.- ^{1}H-NMR (200 MHz, dmso-d_6): δ = 2.69 (3H, s, H_3C-CO), 4.21 (3H, s, OMe), 7.74-7.83 (2H, m, H-3', H-5'), 8.24 (1H, d, J = 7 Hz, H-7), 8.45-8.62 (2H, m, H-6', H-6), 9.45 (1H, s, H-4).

4-Chlorosulphonyl-2-methoxybenzoic acid (**21**)

4-Amino-2-methoxybenzoic acid **20**[15,16] (23.7 g, 0.14 mol) was dissolved in NaOH solution (5.68 g, 0.14 mol, 48 ml H_2O). 10% NaOH solution (2 ml) and $NaNO_2$ (10.5 g, 0.15 mol) in water (25 ml) were added and the mixture poured slowly into conc. HCl (90 ml) and glacial acetic acid (45 ml) at -10°C with vigorous stirring. The mixture was stirred for 5 min and then poured slowly into glacial acetic acid (75 ml) containing CuCl (1.2 g) and then saturated with SO_2 at 0-5°C. Stirring was continued for 18 h at room temp. and then the precipitate filtered off and washed with water. The crude product was taken up in ether, the org. extract dried and evaporated to give 20.0 g (56%) of **21** mp. 147-149°C (toluene), lit.[17]: mp. 149.5°C.

2-Methoxy-4-sulphamoylbenzoic acid (**22**)

Reaction of acid **21** (18 g) with cold aqueous NH_3 (100 ml, d = 0.88) gave, after acidification with conc. HCl, 14.0 g (84%) of **22**, mp. 197-200°C, lit.[17]: 201°C.

2-(2-Methoxy-4-sulphamoylphenyl)-1H-imidazo[4,5-c]pyridine (**8**)

3,4-Diaminopyridine (4.36 g, 0.04 mol) and acid **22** (9.24 g, 0.04 mol) were pulverised to a fine powder and added in portions to $POCl_3$ (190 ml) with stirring. The resulting mixture was stirred and heated at 135°C for 4 h. After cooling volatile material was removed *in vacuo* and the residue treated with water and then NH_3 solution (d = 0.88). The suspension obtained was evaporated to dryness and the residue extracted with boiling alcohol (2x). Removal of the solvent *in vacuo* gave 3.6 g of a syrup which was purified by extensive SiO_2 chromatography (CH_2Cl_2/MeOH 95:5 to 4:1) yielding **8** (350 mg, 3%). Treatment with methanolic HCl gave **8** hydrochloride, mp. 228-230°C (decomp.).- $C_{13}H_{12}N_4O_3S \cdot 1.6\ HCl \cdot 1.25\ H_2O$ (385.2) Calcd. C 40.5 H 4.21 N 14.6 Cl 14.8 S 8.32 Found C 40.4 H 3.90 N 14.3 Cl 14.5 S 8.31.- ^{1}H-NMR (200 MHz, dmso-d_6): δ = 4.15 (3H, s, OMe), 7.64 (1H, dd, J = 8.2, 1.5 Hz, H-5'), 7.74 (1H, d, J = 1.5 Hz, H-3'), 7.6 (2H, br. s, NH_2, exchangeable), 8.22 (1H, d, J = 6.5 Hz, H-7), 8.53 (1H, d, J = 8.2 Hz, H-6'), 8.59 (1H, d, J = 6.5 Hz, H-6), 9.45 (1H, s, H-4).

In vitro inotropism

Details of the *in vitro* inotropic assay are given in the preceding paper. The inotropic potency is expressed as the negative logarithm of the concentration required to increase basal contractility by 50% ($pA_{50\%}$).

Experiments using Anaesthetised Dogs

Beagle dogs of either sex, weighing between 11 and 15 kg were used. Anaesthesia was induced by intravenous thiopentone and maintained with intravenous chloralose/pentobarbitone as required. Arterial blood pressure was measured by a catheter in the left carotid artery. The trachea was cannulated and all animals artificially ventilated with room air. Body temp. was maintained by a heated under-blanket. The chest was opened (sternal approach) and the pericardium removed to expose the heart. The ascending aortic arch was cleared of fat and an electromagnetic flow-probe attached near the heart. Cardiac output and stroke volume were calculated by the integration of aortic flow. A catheter was introduced into the left ventricle via the apex of the heart to measure left ventricular pressure (abbreviated to L.V.P. and symbolised by *P*). The rate of change of left ventricular pressure (dP/dt) was obtained by differentiation. Periodically the L.V.P. recording was electrically amplified by a factor of 10 to facilitate the measurement of left ventricular end diastolic pressure (LVEDP). Heart rate was obtained using a tachograph. Lead II ECG (using subcutaneous needle electrodes) was periodically recorded. All recordings were made using Grass Polygraphs. All drugs were administered as a bolus intravenous injection using a femoral venous catheter. A dose volume of 0.1 ml kg^{-1} was standard. Further details relating to these experiments have been described[18].

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