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SYNTHESIS AND PHARMACOLOGICAL PROPERTIES OF BW315C AND OTHER INOTROPIC 2-ARYLIMIDAZO[1,2-*a*]PYRAZINES

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Abstract: *A series of 2-aryl imidazo[1,2-a]pyrazines has been prepared and evaluated for inotropic activity. Sulphoxide* ***12****, BW315C, displayed potent inotropic effects having comparable in vitro and vivo inotropic potencies to those of isomazole. Structure-activity relationships are discussed.*

The inotropic agent digoxin and the vasodilator enalapril are important drugs[1] for the treatment of congestive heart failure(CHF). Although enalapril or digoxin may prolong the life of patients with heart failure, the use of digoxin is limited by its toxicity[1]. Attempts to enhance the impaired cardiac contractility of the hearts of such patients has led to clinical studies with new inotropic agents [2] such as milrinone **1**, vesnarinone (OPC-8212) **2**, sulmazole **3**, and isomazole **4**. However, when patients with severe chronic heart failure were treated with oral milrinone for long periods, an increase in mortality was observed [1,3]. Similar results [3] have been obtained with other inotropic phosphodiesterase (PDE) III inhibitors and consequently it has been postulated [3] that inotropic agents which elevate cAMP levels will accelerate the progression of heart disease by inducing severe ventricular arrhythmias. Sulmazole [4] and isomazole [5-7], also display undesirable toxicological effects, but some of these actions appear unrelated to PDE III inhibition. Furthermore, vesnarinone [8], a potassium channel blocker [9] and PDE III inhibitor [8], has been reported [10,11] to reduce the mortality of patients with moderate to severe heart failure. Thus there is much current interest in testing the above inotropic hypothesis and also investigating whether new inotropic agents having other biochemical [3,9,12-14] modes of action are more efficacious and less toxic especially in moderate CHF.

We hoped therefore that a non-toxic inotrope would emerge from our studies on the structure-activity relationships (SAR's) of isomazole analogues. Our previous investigations have shown that heterocycles **3-8** are potent inotropic agents [15] *in vitro*, and that the 4'-S(O)Me groups of isomazole [15] and **8** [16] can be replaced by achiral 4'-substitutents, (such as $C(O)NH_2$, OSO_2Me) without loss of inotropic activity. In addition the *1H*-imidazo[4,5-*c*]pyridines **4** and **7** were found [17] to elicit similar *in vivo* inotropic effects and be more potent and longer-acting in this respect than heterocycles **3** or **8** [16]. These SAR's led us to speculate that the imidazo[1,2-*a*]pyrazines **9-14** might display potent inotropic effects both *in vitro* and *in vivo*. Imidazo[1,2-*a*]pyrazine is not ionised [18] at physiological pH and, according to CNDO/2 calculations, possesses an electron-rich imidazo nitrogen atom. Both these molecular properties have been found [19] to be commonly associated with potent inotropism for a series of heterocyclic sulmazole analogues.

The imidazo[1,2-*a*]pyrazine analogues **9-14** were prepared *via* condensation of the requisite 2-bromoacetophenone derivative**15** [16],**16** [16] or **17** [16] with either 2-aminopyrazine (**18**) or 2-amino-3-chloropyrazine (**22**) [20] Heterocycles **19-21** and **23-25** (Schemes 1 and 2) were obtained in low yields (6-30%). Substantial amounts of uncharacterized polar by-products and/or intractable polymeric materials were also produced.

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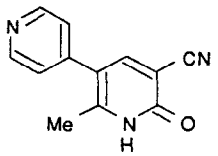
The formation of these undesired products is believed to arise *via* alkylation of **18** or **22** at N-4 [21]. Analogues **9-14** [22] were obtained from **19-21** and **23-25** by modifying the 8-chloro and 4'-substituents of these precursors. The interconversions all utilize standard reactions.



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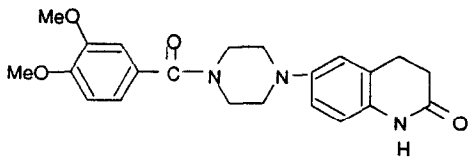

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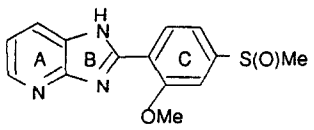
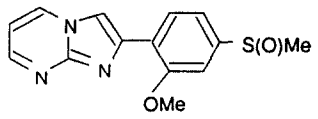


3 Sulmazole



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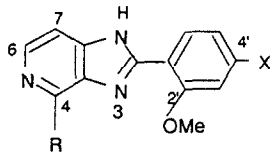
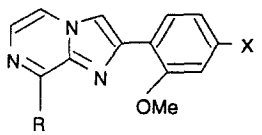
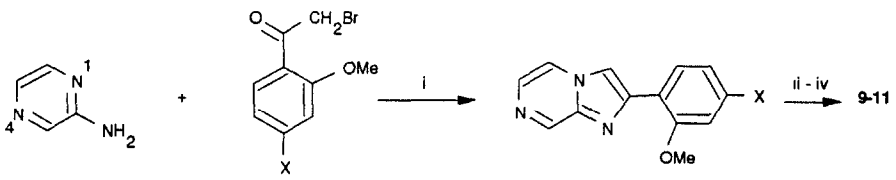


4	X = S(O)Me,	R=H	Isomazole
5	X = $C(O)NH_2$,	R=H	
6	X = OSO_2Me,	R=H	
7	X = S(O)Me,	R=OMe	

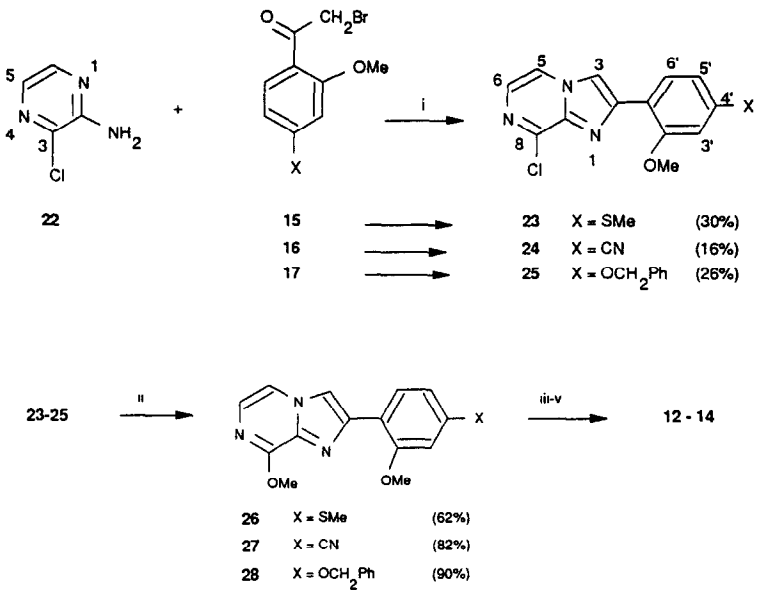


9	X = S(O) Me,	R=H
10	X = $C(O)NH_2$,	R=H
11	X = OSO_2Me,	R=H
12	X = S(O)Me,	R=OMe (BW315C)
13	X = $C(O)NH_2$,	R=OMe
14	X = OSO_2Me,	R=OMe



18	**15**	X = SMe	**19**	X = SMe	(8%)
	16	X = CN	**20**	X = CN	(6%)
	17	X = OCH_2Ph	**21**	X = OCH_2Ph	(8%)

SCHEME 1 (i)75°C,N_2 Solvent EtOH [**19**], DMF[**20**] or Me_2CHOH[**21**]
(ii)MCPBA, $CHCl_3$ [For **19**→**9**, 80%] (iii) conc. H_2SO_4 [for **20**→**10**, 70%]
(iv)CF_3CO_2H, PhSMe; $MeSO_2Cl$, C_5H_5N, [For **21**→**11**, 30%]



SCHEME 2 (i) 80C,°N_2, DMF (ii) NaOMe, MeOH, reflux
(iii)MCPBA, $CHCl_3$ [**26**→**12**, 67%](iv)Conc. H_2SO_4 [**27**→**13**, 75%]
(v)1 atmos. H_2, 5% Pd-C, NEt_3, DMF; $MeSO_2Cl$, C_5H_5N[**28**→**14**, 58%]

Table 1. **Inotropic Activities, phosphodiesterase inhibitory properties, and charge densities of sulmazole analogues.**

Compound	*In vitro* pA_{50}[a]	*In vivo* $ED_{50}I$[b]	pIC_{50} PDIII[c]	Charge CNDO/2[d]
Sulmazole	4.70±0.10	0.80	4.3	-0.266
Isomazole	4.64±0.15	0 06	4.5	-0.256
5	4.43±0.08	0.01		
6	<5[e]	0.002		
7	3.91±0.12	0.13		-0.279
8	4.63±0.09	2.4		-0.309
9	5.39±0.13	0.75	5.0	-0.296
10	4.68±0.23	0.02		-0 295
11	inactive[f]			
12	4 82±0.44[e]	0.09	4.4	-0.282
13	insol.[g]	0.06		-0.231
14	insol.[g]	0 07		-0.240

(a) Inotropic potency *in vitro*, pA_{50} = -logc where c = drug concentration required to give a 50% increase in basal contractile force of paced guinea pig papillary muscle preparations (n≥3); inactive; 50% increase not achieved. (b) Inotropic potency *in vivo*. $ED_{50}I$ is the dose (mgkg-1iv) of drug required to produce a maximum increase of 50% in d*P*/dt where *P* =left ventricular pressure.(c) Potency for phosphodiesterase III inhibition. PIC50 PDIII = -logc where c = concentration of drug required for 50% inhibition of type III cAMP phosphodiesterase from dog ventricle s.e.m is±

25%(n=3). Milrinone has pIC_{50} of 6.2. The four heterocycles investigated were weaker inhibitors of the type I enzyme (pIC_{50}'s 3.0-3.6) (d) Charge of imidazo nitrogen calculated by CNDO/2 (e). Biphasic dose-response curve observed; approximate value given. (f).Tested at concentrations up to 0.1mM; v. weak positive inotropic effects observed. (g.)The poor aqueous solubility of the compound precluded a quantitative pharmacological evaluation.

Table 2: ***In vivo* Cardiovascular Effects[a] of Sulmazole Analogues.**

Compound	$ED_{50}I$	$ED_{30}V$	$ED_{10}C$	I/V[b]	I-DUR[c]	V-DUR[d]
Sulmazole	0.80	1 25	0.50	0.64	30	30
Isomazole	0 06	0.19	0.06	0.32	20-30	15-30
9	0.75	0 03	0.28	25	5	>90
10	0.02	0 05	0.02	0.4	>30	<5
12(BW315C)	0 09	1.17	0.11	0.08	60	2
13	0 06	0.15	0.06	0.4	30-45	30-45
14	0 07	0 06	0.05	1.2	45-60	2-15
Milrinone	0 02	0 06	0 02	0 3	45-60	10-30

(a)The effective dose (mg kg^{-1}iv) of these compounds to produce a maximum increase of 50% in d*P*/dt ($ED_{50}I$), 30% decrease in diastolic blood pressure ($ED_{30}V$, V= vasodilation), and 10% increase in heart rate ($ED_{10}C$, C = chronotropism), as compared to those of sulmazole and isomazole in anaesthetized, open-chest dogs (b) I/V = $ED_{50}I/ED_{30}V$ (c) I-DUR = Duration (min) of inotropic response to ED_{50} I dose (d) V-DUR = Duration (min) of vasodilator response at $ED_{50}I$ dose The ED's were derived from the mean values for n≥3 experiments; s.e.m is ±≤ 40% e.g. sulmazole $ED_{50}I$ 0.80±0.34(n=4), isomazole $ED_{50}I$ 0 06±0.01(n=9), BW315C $ED_{50}I$ 0.09 ± 0.03 (n=3)

The inotropic activities of the isomazole analogues were determined *in vitro* and *vivo* by previously described methods[15,23] The ability of some of these heterocycles to inhibit the phosphodiesterase III isoenzyme was also evaluated [24,25] Electronic properties of compounds **3-14** were calculated by CNDO/2 [26,27] methods. The results are collected together in Table 1

Of the six imidazo[1,2-*a*]pyrazines investigated, analogues **10** and **12** were found to be approximately equipotent with sulmazole as inotropic agents *in vitro*. Mesylate **11** proved to be inactive and heterocycles **13** and **14** could not be evaluated satisfactorily *in vitro* because of their low aqueous solubilities[28] Sulphoxide **9** was found to be a more potent inotrope *in vitro* than any of the other imidazo[1,2-*a*]pyrazines, sulmazole, or other sulmazole analogues possessing a 4'-S(O)Me group. Interestingly, compound **9** is also a more potent inhibitor of cardiac phosphodiesterase III isoenzyme than isomazole.

A comparison of the *in vitro* inotropic activities of *1H*-imidazo[4,5-*c*]pyridines **4-7** and imidazo[1,2-*a*]pyrazines **9-12** indicates that the effects of the 4'-substitutuents on activity are different for the two series. 'A' ring methoxy substitution appears to induce similar changes *in vitro* in both series although a comparison can only be made (**4** and **7** vs. **9** and **12**) in one case. The different effects of 'C' ring substituents on inotropic properties may reflect changes in water solubility, electronic properties and biochemical mechanisms Most of the imidazo[1,2-*a*]pyrazine analogues were found to be far less water soluble than their *1H*-imidazo[4,5-*c*]pyridine isomers despite having similar log P values [29] No correlation was observed between the *in vitro* inotropic potencies of the imidazopyrazines and their imidazo nitrogen charge densities. This finding contrasts with the correlation observed between these parameters for a subset of the more water-soluble imidazopyridines [17,19] Nevertheless the three potent inotropic imidazopyrazines **9**, **10** and **12** did have higher charge densities at the imidazo nitrogen atom than sulmazole. Sulphoxide **9**, which has a higher water solubility than**10** or **12**, is the most potent of the three compounds

Changes in biochemical mechanisms may also contribute to the different SAR's for the two series. The mechanisms responsible for eliciting the inotropic effects of analogues **3 - 14** are at present poorly understood. Nonetheless P D E.III inhibition [24.] and possibly calcium sensitization [30] of cardiac myofibrils and adenosine antagonism [31] are believed to

account for a substantial part of the inotropism of **3** and **4** [19]. Analogue **12**, unlike the other sulmazole analogues, displays a biphasic dose-response curve *in vitro* and may have a different mode of action.

Heterocycles **10** and **12-14** were found to be potent inotropes *in vivo* [32] having potencies comparable to those of isomazole (table 1). Sulphoxide **9** proved to be a much less potent inotrope *in vivo* than isomazole although sulphoxides **7** and **12** have comparable *in vivo* potencies. The above results show that the imidazo[1,2-*a*]pyrazine ring is often a good bio-isostere for the *1H*-imidazo[4,5-*c*]pyridine moiety of isomazole analogues. A second important conclusion is that 'A' and 'C' ring substituent effects on the inotropic activities of the imidazo pyrazines are sometimes different from those in the imidazopyridine series. This latter finding contrasts with the results of Spitzer *et al* [33] who observed similar SAR's between four compounds of each series. The divergence in the results is probably due to the different sets of compounds studied.

The cardiovascular effects of the imidazopyrazines were studied *in vivo* in more detail and are given in table 2. Besides their inotropic actions the major effects of the compounds are a dose-related lowering of blood pressure and an increase in heart rate. The most potent vasodilator is sulphoxide **9** its blood pressure lowering effects being evident at lower doses than its inotropic actions. BW315C (**12**) is perhaps the most interesting inotrope since it shows the greatest separation between its inotropic and vasodilatory effects (in favour of inotropism) in terms of potency and duration of action.

Further evaluation of heterocycles **9,12, 13** and their metabolites should reveal whether they are suitable probes for testing hypotheses associated with the use of inotropic agents. Such investigations may provide information about the relative merits [34] of a 'pure' inotrope vs inotrope-vasodilator in heart failure and may also indicate the extent to which efficacy/toxicity is related to PDE III inhibition, adenosine antagonism [31], calcium sensitization of myofilaments [12], sodium channel activation [13] or neurohormonal stimulation [14]

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22. Physical properties of selected compounds· **9**, m p. 195-198°C. Anal.($C_{14}H_{13}N_3O_2S$) C,H.N;. **10**. HCl, m.p. 262-264°C. ^{1}H-NMR (200MHz, Me_2SO-d_6) δ4.03(3H,s,OMe), 4.70(2H,br.s,exchang.,NH_2), 7.56-7.68(2H,m,H3',H5'), 8.02(1H,brd, J=4Hz,H6), 8.27 (1H,d, J=8Hz,H6') 8.75 (1H,d,J=4Hz,H5), 8.86 (1H,s,H3)9.30 (1H, brs,H8) Anal. ($C_{14}H_{12}N_4O_2$.HCl.0.25H_2O) C, H, Cl,N. **12**, m p. 184-185°C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ2.78 (3H, s, SMe), 4.10(3H, s, OMe), 4 20 (3H, s, OMe) 7.18(1H,dd, J=8.3, 1.2 Hz,H5'), 7.38 (1H,d, J=4.6 Hz, H6) 7.46(1H, d, J=1.2 Hz, H3'), 7.73 (1H,d,J=4.6 Hz,H5), 8 26(1H,s,H3), 8.54(1H,d,J=8.3 Hz,H6') Anal. ($C_{15}H_{15}N_3O_3S$) C,H,N.; **13** HCl, m.p. 195-197°C. Anal. ($C_{15}H_{14}N_4O_3$ HCl.1.75H_2O, C.H,Cl,N,. **14**, m.p.220-222°C.^{1}H-NMR(200 MHz, Me_2SO-d_6)δ3.46(3H,s,SMe), 4.02(3H,s,OMe), 4.07(3H,s,OMe), 7.05-7 15(2H,m,H3',H5'), 7.42 (1H,d,J=4.6 Hz, H6),8.21 - 8.39 (2H,m,H5, H6'), 8.50 (1H,s,H3). m/z 349 (M+). Anal. ($C_{15}H_{15}N_3O_5S$), C,H,N; **20**, m.p. 199-201°C. Anal,.($C_{14}H_{10}N_4O$)C,H,N..**23**, m.p. 182-183°C. Anal ($C_{14}H_{12}ClN_3OS$) C,H,N,Cl; **24**, m.p. 272-275°C. ^{1}H-NMR (200 MHz, Me_2SO-d_6 δ-4.08 (3H, s, OMe), 7.57 (1H,dd, J=1,8 Hz, H5'), 7.69 (1H, d, J=1Hz, H3'), 7.76 (1H, d,J=4 Hz, H6), 8.47 (1H,d, J=8 Hz,H6'), 8.66 (1H,d, J=4Hz, H5), 8.81(1H, s,H3). Anal.($C_{14}H_9ClN_4O$:0.25H_2O)C.H.Cl,N, **25** m.p. 192-194°C, Anal. ($C_{20}H_{16}ClN_3O_2$) C,H,Cl,N; **26**, m.p. 144-146°C. Anal. ($C_{15}H_{15}N_3O_2S$) C,H,N;.**27**, m p. 237-240°C (Anal $C_{15}H_{12}N_4O_2$) C.H,N. **28**, m.p. 187-189°C. Anal. ($C_{21}H_{19}N_3O_3$) C,H,N.
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