Cardiotonic Agents. Synthesis and Cardiovascular Properties of Novel 2-Arylbenzimidazoles and Azabenzimidazoles

Timur Güngör,*,† André Fouquet,† Jean-Marie Teulon,† Daniel Provost,‡ Michèle Cazes,‡ and Alix Cloarec‡

CARPIBEM, 128 rue Danton, 92500 Rueil Malmaison, France, and UPSA, 128 rue Danton, 92500 Rueil Malmaison, France

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Novel 2-arylbenzimidazoles and azabenzimidazoles were synthesized, and their inotropic action was evaluated. Changes in left ventricular pressure, d*P*/d*t* max, were measured as an index of cardiac contractility. The structural features that impart optimal inotropic activity are presented. The most potent compounds were evaluated orally in conscious dogs with implanted Konigsberg pressure transducers. To investigate the mechanism of action, the most potent compounds were tested for their calcium-sensitizing properties and their potential for the inhibition of phosphodiesterase. Two compounds, 1 and 41, showed interesting in vitro and oral activity without side effects. They have a more potent calcium-sensitizing effect than MCI-154 and are under futher investigation.

For many years cardiac glycosides have been the only available cardiotonic agents for heart failure therapy. However, for many reasons their use is limited, especially because of their arrhythmogenic liability. In the last decade, a series of nonglycosidic, nonsympathomimetic, cardiotonic agents have been developed. Amrinone[1,2] and sulmazole,[3,4] which were prototypical compounds, were followed by a second generation of more active compounds including enoximone,[5,6] piroximone,[7] imazodan,[8,9] MCI-154,[10,11] indolidan,[12] and pimobendan[13] (Figure 1).

While many of the new cardiotonics appear to act by inhibition of a phosphodiesterase isozyme, resulting in an increase of intracellular cyclic AMP, sulmazole,[14] pimobendan,[15] and MCI-154[16] are known to act, at least in

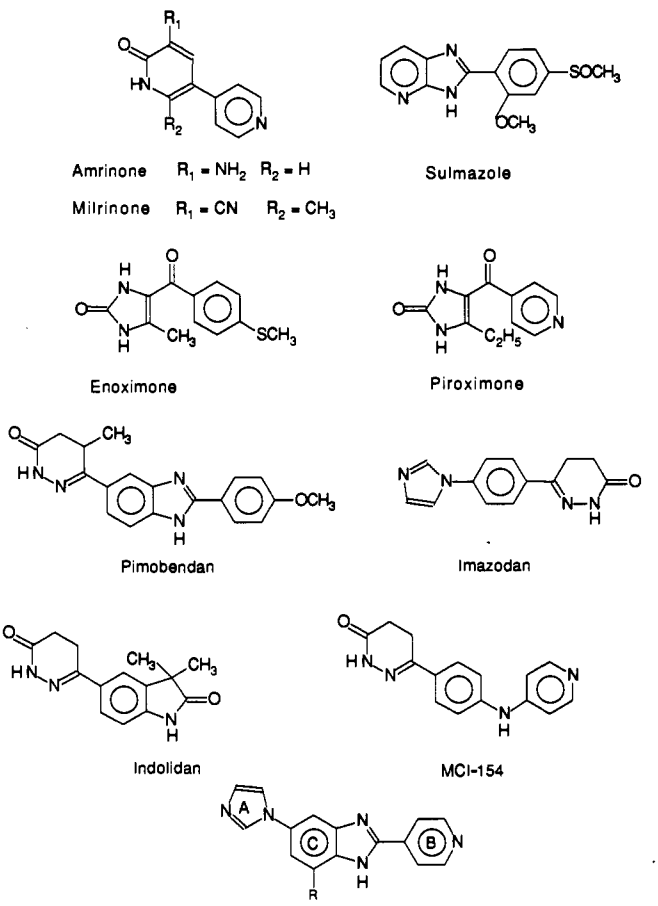

Figure 1.

* Author to whom correspondence should be addressed.
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‡ UPSA.

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part, by increasing the myofibrillar Ca^{2+} sensitivity. This is one of the most interesting new concepts underlying positive inotropism. It consists of increasing troponin C sensitivity to calcium. Such a mechanism appears to be very attractive since myocardial contractility is enhanced without further burdening the cardiac muscle through calcium overload.

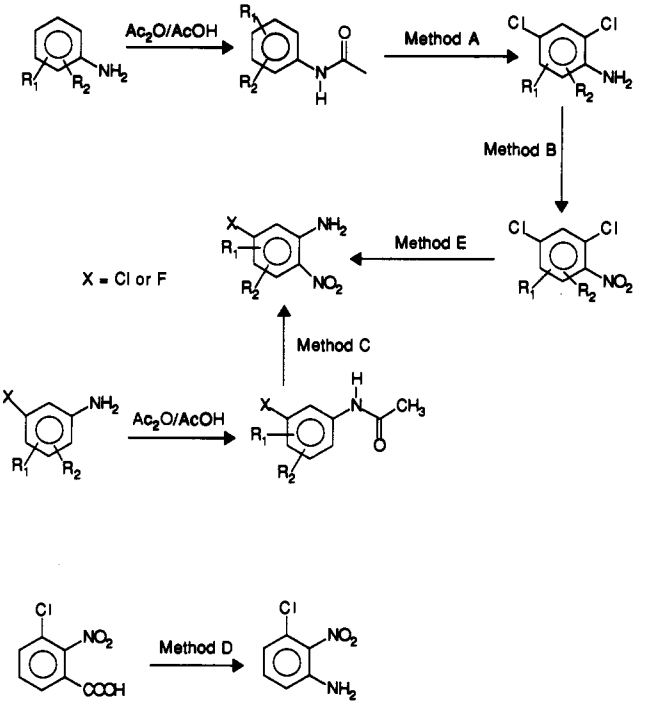
These new theoretical approaches for treatment of heart failure have encouraged us to develop new benzimidazoles derivatives possessing both phosphodiesterase inhibition and calcium sensitizing properties.

Molecular Modeling Studies among cardiotonics of diverse structural classes, such as amrinone, milrinone, enoximone, and imazodan, suggest spatial and electronic similarities between these compounds. This observation has resulted in a hypothetical five-point model for positive inotropic activity.[17] Recently, two similar models have been proposed[18,19] which differ from the first one known as the "generally flat topographic" model. We thought that one of the essential features required by those models, namely the presence of a strong dipole (carbonyl) at one end of the molecule, is responsible for the phosphodiesterase inhibition. As we wished to avoid selective inhibition of cAMP PDE III, we postulated to use an aromatic without any strong dipole (e.g. carbonyl or amide) since pyridazinone cardiotonics (e.g. imazodan) elicit a positive inotropic response by selectively inhibiting cAMP PDE III.[20]

It was also demonstrated[21] that the 1*H*-imidazol-1-ylphenyl moiety had a significant contribution to superior inotropic activity in comparison with other more conventional aromatic substituents, such as halogen, alkyl, alkyloxy, nitro, amine, etc.

With all this in mind, in order to prepare new cardiotonic agents acting by increasing the myofibrillar Ca^{2+} sensitivity, we decided to synthesize derivatives of 5-(1*H*-imidazol-1-yl)-2-aryl-substituted benzimidazoles. This resulted in a series of potent inotropes,[22,23] the synthesis and biological activity of which are described in this paper.

Scheme I [a]



[a] Method A: (1) Cl_2/AcOH, (2) C_2H_5OH/concentrated HCl. Method B: (1) HCl/$NaNO_2$/$NaBF_4$, (2) $NaNO_2/H_2O$/Cu. Method C: (1) Ac_2O/HNO_3/AcOH, (2) CH_3OH/Na. Method D: (1) $SOCl_2$/toluene; (2) aqueous NH_3; (3) Br_2/KOH/H_2O. Method E: liquid $NH_3/CH_3OCH_2CH_2OH$.

Chemistry

The key intermediates halonitroanilines, were prepared by the different ways outlined in Scheme I, depending on the nature and the position of the substituents.

Starting from acetanilides,[24,25] chlorination according to method A gave the dichloroanilines. The amino function was transformed into a nitro group according to method B. The molecule thus activated reacted according to method E to yield the 5-chloro-2-nitroanilines. The same compounds can be obtained by nitration of the already halogenated acetanilide compounds (method C).

In the case of 3-chloro-2-nitroaniline, the compound was obtained, starting from 3-chloro-2-nitrobenzoic acid, by Hofmann degradation[26] according to method D.

Activated by the *o*- or *p*-nitro group, the halogen atom was then displaced by the appropriate nucleophile according to one of the methods G–J (Scheme II).

Hydrogenation of the nitro group according to method K or L followed by condensation with aldehydes, acids, or acyl chlorides by methods M, N, or O gave the expected benzimidazoles (Scheme III).

Condensation of an acyl chloride on the amino nitro intermediate (Scheme III, method P) followed by reduction and cyclization in acidic medium gave the benzimidazoles with a similar yield.

The synthesis of the compound 14 is represented in Scheme IV. The imidazo[2,1-*b*]thiazole ring was built in

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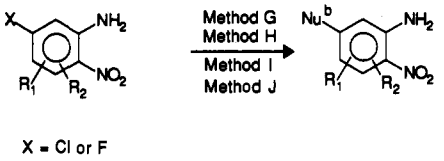
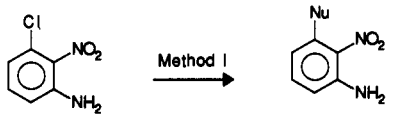
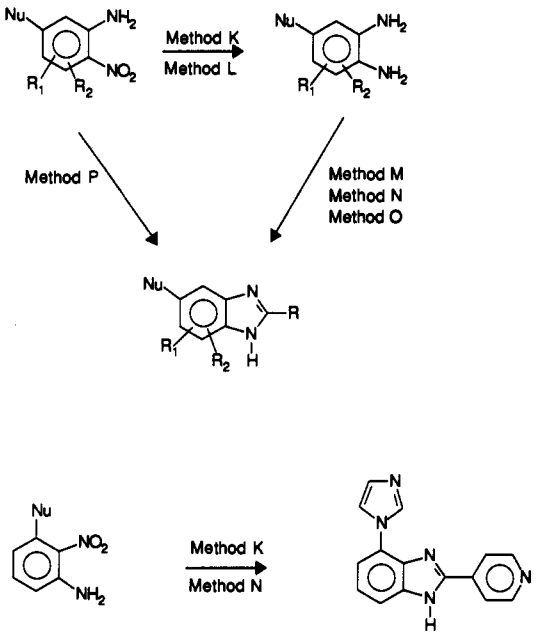
Scheme II [a]





[a] Method G: NuH/Na_2CO_3/DMF. Method H: NuH/NaH/DMF. Method I: NuH. Method J: NuNa/DMF.[b] NuH represents a nucleophilic substrate. Typical NuH is imidazole.

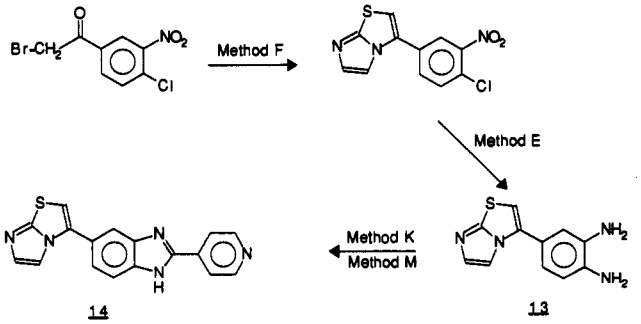
Scheme III [a]



26

[a] Method K: H_2/Raney nickel. Method L: Fe powder/HCl. Method M: RCHO/CH_3OH. Method N: (1) RCHO/CH_3OH, (2) nitrobenzene/heat. Method O: (1) RCOOH/$POCl_3$, (2) HCl. Method P: (1) RCOCl/pyridine, (2) H_2/Raney nickel; (3) CH_3COOH, reflux.[b] See footnote *b* of Scheme II.

Scheme IV [a]



14 13

[a] Method E: liquid NH_3/$CH_3OCH_2CH_2OH$. Method F: (1) 2-mercaptoimidazole/CH_3OH, (2) polyphosphoric acid (PPA). Method K: H_2/Raney nickel. Method M: 4-formylpyridine/MeOH.

two steps first by condensation of the mercapto function of the 2-mercaptoimidazole on the bromine of the α-bromoacetophenone and then cyclization performed in polyphosphoric acid (PPA) (method F).

Azabenzimidazoles such as compounds **34** and **36** (Table II) were synthesized according to Scheme V starting respectively from **13** and **15**, which were prepared by known methods reported in the literature.[27,28]

All physical data and preparation methods for the final products are summarized in Tables I and II.

Structure–Activity Relationships

Pharmacological data of the final products **1–41** are given in Table III. In order to better understand the structure–activity relationships, we decided to distinguish three regions (A, B, C) shown in formula **1** (Figure 1 UP 100-23).

Region A. Where the in vitro inotropic activity of compounds **4**, **9**, **16**, **17**, and **18** is concerned, the in vitro potency was relatively similar, except for **17**, which was inactive under our experimental conditions. A conformational study with the Sybyl system[29] (see Experimental Section) of the unsubstituted molecule **4** and the trimethyl-substituted compound **16** showed that in the unsubstituted molecule the imidazole ring was almost coplanar with the benzimidazole ring, while in the methylated compound the coplanar conformation had the highest energy. However this difference did not significantly affect the in vitro activity of compounds. This suggests that receptor interaction tolerates flexibility in this part of the molecule.

However, a most important differentiation appeared on in vivo (iv) testing, with a complete lack of activity for compounds **16** and **18**, which would suggest a limited distribution or an extensive metabolism for these compounds.

While the triazole and the unsubstituted imidazole derivatives (**10** and **4**, respectively) exhibited almost similar activity, the imidazothiazole compound **14** had no activity in vitro and the benzimidazole compound **20** was devoid of iv potency. For compounds **4**, **9**, and **10** the level of inotropic activity after oral administration (Table IV) was similar to that upon iv administration.

Region B. When the results of all the tests carried out on compounds **2–5**, **7**, **8**, **11**, **12**, **22**, **35**, and **40** were studied, we noted that the pyridinyl series gave the best results. Compounds **2**, **3**, and **5**, which may be regarded as analogs of sulmazole, were insufficient or inactive. Of the pyridinyl compounds, the 4-pyridyl appears to have the highest activity in dogs (compound **4**).

Consequently we suggested that the nitrogen atom can play an important role in this area. Therefore we tested different nitrogen-containing groups such as acetanilide (compound **22**) or imidazolylphenyl (compound **30**). Unfortunately, a dramatic decrease in vitro and iv activity was observed.

Region C. The in vitro results indicate that there is no difference between compounds **4**, **1**, **6**, **21**, **33**, **37–39**, and **41**. Nevertheless, on iv and oral administration, the substitents' effect on the benzimidazole ring plays an important role on the inotropic activity, depending on the

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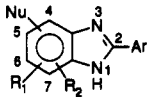
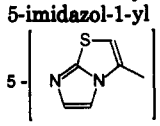
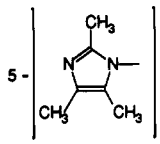
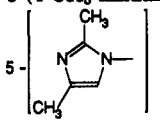
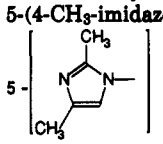
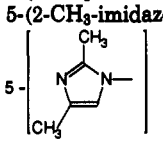
Table I. Structure and Properties of 2-Arylbenzimidazoles



compd no.	Nu	R_1	R_2	Ar	prep methods	% yield[a]	mp, °C[b]	recryst solvent[c]	formula[d]
1	5-imidazol-1-yl	7-CH_3	H	4-pyridyl	A,B,E,I,K,N	67	289	EtOH	$C_{16}H_{13}N_5$
2	5-imidazol-1-yl	H	H	2,4-$(OCH_3)_2C_6H_3$	E,G,K,M	20	188	CH_3CN	$C_{16}H_{16}N_4O_2$
3	5-imidazol-1-yl	H	H	2-(OCH_3)-4-(SCH_3)-C_6H_3	E,G,K,O	12	182	CH_3CN	$C_{16}H_{16}N_4OS$
4	5-imidazol-1-yl	H	H	4-pyridyl	E,G,K,N	42	265	MeOH	$C_{16}H_{11}N_5 \cdot 0.1H_2O$
5	5-imidazol-1-yl	H	H	2-(OCH_3)-4-$(SOCH_3)C_6H_3$	E,G,K,O	41	208	CH_3CN	$C_{16}H_{16}N_4O_2S \cdot 0.22H_2O$
6	5-imidazol-1-yl	6-CH_3	H	4-pyridyl	C,I,K,N	56	292	EtOH	$C_{16}H_{13}N_5$
7	5-imidazol-1-yl	H	H	2-pyridyl	E,I,K,N	32	219	CH_3CN	$C_{16}H_{11}N_5$
8	5-imidazol-1-yl	H	H	3-pyridyl	E,I,K,N	35	250	EtOH	$C_{15}H_{11}N_5$
9	5-(2-CH_3-imidazol-1-yl)	H	H	4-pyridyl	E,I,K,N	52	295	EtOH[j]	$C_{16}H_{13}N_5$
10	5-1,2,4 triazol-1-yl	H	H	4-pyridyl	E,J,K,N	31	160[e]	EtOH[j]	$C_{14}H_{10}N_6$
11	5-imidazol-1-yl	H	H	3-(2-OCH_3-pyridyl)	E,I,K,N	12	242	EtOH[j]	$C_{16}H_{13}N_5O$
12	5-imidazol-1-yl	H	H	3-(6-Cl-pyridyl)	E,I,P	51	>330	*f*	$C_{15}H_{10}ClN_5$
14		H	H	4-pyridyl	F,E,K,N	62	332	DMF	$C_{17}H_{11}N_5S$
16		H	H	4-pyridyl	E,I,K,N	33	278	EtOH[k]	$C_{18}H_{17}N_5$[d]
17	5-(4-CH_3-imidazol-1-yl)	H	H	4-pyridyl	E,I,K,N	51	264	EtOH	$C_{16}H_{13}N_5$
18		H	H	4-pyridyl	E,G,K,N	57	291	EtOH[j]	$C_{17}H_{15}N_5$
20	5-benzimidazol-1-yl	H	H	4-pyridyl	E,H,K,N	57	280-282	EtOH[j]	$C_{19}H_{13}N_5$
21	5-imidazol-1-yl	6-Cl	H	4-pyridyl	I,K,N	44	326	EtOH	$C_{15}H_{10}ClN_5$
22	5-imidazol-1-yl	H	H	4-acetanilide	E,I,K,N	40	362	DMSO	$C_{16}H_{15}N_5O$
24	5-(4-CH_3-imidazol-1-yl)	7-CH_3	H	4-pyridyl	A,B,E,I,K,N	76	285	EtOH[j]	$C_{17}H_{15}N_5$
25		7-CH_3	H	4-pyridyl	A,B,E,I,K,N	48	286	*g*	$C_{16}H_{17}N_5 \cdot 0.1H_2O$
26	4-imidazol-1-yl	H	H	4-pyridyl	D,I,K,N	63	313	DMF	$C_{15}H_{11}N_5$
27	5-(4-CH_3-imidazol-1-yl)	6-CH_3	H	4-pyridyl	C,I,K,N	44	332	EtOH[j]	$C_{17}H_{15}N_5$
28	5-(2-CH_3-imidazol-1-yl)	6-CH_3	H	4-pyridyl	C,I,K,N	51	308	EtOH	$C_{17}H_{15}N_5$
29		6-CH_3	H	4-pyridyl	C,I,K,N	41	295	EtOH	$C_{16}H_{17}N_5 \cdot 0.25H_2O$
30	5-imidazol-1-yl	6-CH_3	H	4-imidazol-1-ylphenyl	C,I,K,N	72	345[h]	MeOH	$C_{20}H_{16}N_6 \cdot 3HCl \cdot 0.4H_2O$
31	5-(2-CH_3-imidazol-1-yl)	6-Cl	H	4-pyridyl	I,K,N	54	324	EtOH	$C_{16}H_{12}ClN_5$
32	5-(4-CH_3-imidazol-1-yl)	6-Cl	H	4-pyridyl	I,K,N	56	285	EtOH	$C_{16}H_{12}ClN_5$
33	5-imidazol-1-yl	4-CH_3	H	4-pyridyl	C,I,K,N	49	283	EtOH	$C_{16}H_{13}N_5$
35	5-imidazol-1-yl	H	H	3-(2-SCH_3-pyridyl)	E,I,K,N	24	217–218	EtOH	$C_{16}H_{13}N_5S$
37	5-imidazol-1-yl	6-CF_3	H	4-pyridyl	E,I,K,N	48	315–320[i]	MeOH	$C_{16}H_{10}F_3N_5 \cdot 2HCl$
38	5-imidazol-1-yl	6-Cl	7-CH_3	4-pyridyl	A,B,E,I,K,N	53	316	EtOH	$C_{16}H_{12}ClN_5$
39	5-imidazol-1-yl	6-CH_3	7-CH_3	4-pyridyl	A,B,E,I,K,N	61	315–318	EtOH	$C_{17}H_{15}N_5$
40	5-imidazol-1-yl	H	H	3-[2-(N-phenylamino)-pyridyl]	E,I,K,N	36	309	DMF	$C_{21}H_{16}N_6 \cdot 0.2DMF$
41	5-imidazol-1-yl	7-C_2H_5	H	4-pyridyl	A,B,E,I,K,N	8	280	*g*	$C_{17}H_{15}N_5$

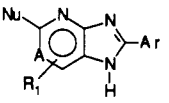
[a] Yields are not optimized and correspond to the recrystallized product in the final step (Method M, N, O, or P) except for 1, in which case the optimal yield is mentioned. [b] Melting points are uncorrected. [c] All compounds were purified first by column chromatography and then recrystallized except for **25** and **41**. [d] Analyses for C, H, and N were ±0.4% of the expected values for formula shown except for **16**; C: calcd, 71.27; found, 70.70. [e] The product dried at 100 °C under vacuum melted at 248 °C. [f] $CH_3OCH_2CH_2OH$. [g] Purification by column chromatographies. [h] As 3HCl salt. Melting point of the free base, 300 °C. [i] As 2HCl salt, melts with decomposition. Melting point of the free base, 337–340 °C. [j] 95% EtOH. [k] 50% EtOH.

nature and the position of the substituent. For example, on the 6 position the order of activity was $CH_3 > Cl \gg CF_3$ (see compounds **6**, **21**, and **37**).

In the case of the methyl group (compounds **1**, **6**, and **33**), the potency on iv testing for substitution on positions 4 and 6 was similar. However, the oral activity decreased dramatically for compound **33**. The best results were obtained with the methyl group on the 7 position (compound **1**). Another alkyl group at this position—i.e. ethyl (compound **41**)—showed similar activity.

When position 7 is substituted by a methyl group, additional substituents on position 6 (compounds **38** and

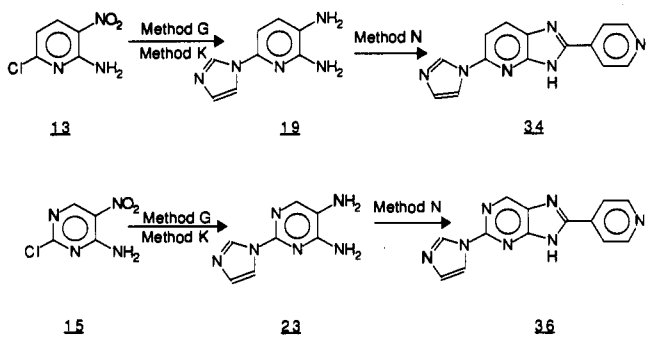
Table II. 2-Arylazabenzimidazoles



compd. no.	Nu	A	R_1	Ar	prep methods	% yield[a]	mp, °C[b]	recryst solvent[d]	formula
34	1-imidazolyl	CH	H	4-pyridyl	E,G,K,N	20	358–359	DMF	$C_{14}H_{10}N_6$
36	1-imidazolyl	N	H	4-pyridyl	E,G,K,N	60	>340	DMF	$C_{13}H_8N_7$

[a] Yields are not optimized and correspond to the recrystallized product in the final step. [b] Melting points are uncorrected. [c] All compounds were purified first by column chromatography and then recrystallization. [d] Analyses for C, H, and N were ±0.4% of the expected values for formula shown.

Scheme V [a]



[a] Method G: imidazole/Na_2CO_3/DMF. Method K: H_2/Raney nickel. Method N: 4-formylpyridine/CH_3OH/nitrobenzene/heat.

39) did not increase activity and the oral activity was slightly less potent.

Simultaneous changes on regions A and C, and, in particular, methylated or chlorinated compounds on the 6 or 7 position (**24**, **25**, **27–29**, **31**, **32**), had less potent activities compared to the unsubstituted compounds on the imidazole ring.

On the other hand, introduction of heteroatoms on the benzimidazole ring (compounds **34** and **36**) has not yet shown promise. The most potent compounds were under evaluation for their Ca^{2+}-sensitizing properties and for their cardiac phosphodiesterase inhibitor character. The complete results of these studies will be developed in a future paper. Nevertheless, the effects of compounds **1** and **41** on the calcium sensitivity of cardiac myofilaments were investigated on skinned muscle fibers from rats. The skinned-fiber technique allows the study of muscle contractility at the level of myofilaments without any membrane intervention (sarcoplasmic reticulum, mitochondria, or plasma membrane). The skinned fibers were activated by buffer solutions containing varying concentrations of Ca^{2+} (10^{-7}–10^{-4} M). The pCa–tension relationship characterizes the sensitivity to calcium of myofibrils. Compounds **1** and **41** shifted the sigmoidal tension versus pCa relation curve to the left as shown on figure 2. However, compound **1** was less potent than that of compound **41**. The Ca^{2+} concentration required for half-maximal activation (pCa_{50}) was decreased in the presence of tested compounds and these results suggest that the inotropic effect of compounds **1** and **41** may be due, in part, to an increased sensitivity of the myofilaments to Ca^{2+}. In addition these two compounds also showed cardiac phosphodiesterase inhibition activity, however, without pronounced selectivity.

In conclusion, we have prepared several new 2-arylbenzimidazoles and azabenzimidazoles and demonstrated that this series of compounds possesses interesting inotropic activity. This activity seems to be due at least in part to the Ca^{2+}-sensitizing effect of this family. Further investigations will be undertaken to assess this mechanism's contribution to the inotropic activity compared with that of the phosphodiesterase inhibition which was observed in this series. Compounds **1** and **41** showed promising activity on all inotropic tests and have been selected as lead compounds for further studies.

Experimental Section

The molecular models were built with the program Sybyl[29] and a Sybyl standard fragment Library. Conformations were analyzed by using the molecular orbital (MO) method (PM3).[31] The calculations of molecular mechanics and PM3[31] were made on Vax. The conformations were examined on an Evans and Sutherland PS 390 computer terminal by using the program Sybyl.

Melting points were determined on an Electrothermal capillary melting point apparatus and are not corrected. The identity of all compounds was confirmed by ^1H NMR (200 MHz, Bruker, solvent Me_2SO-d_6, TMS = 0 ppm) infrared (Perkin-Elmer 298) and microanalytical data (Carlo Erba, elemental analyzer, Model 1106). All reactions were followed by TLC on Merck silica gel plates (60F-254). Merck silica gel (0.063–0.200 mm) was used for column chromatography. Unless literature references are given, the starting materials were commercially available or were prepared according to the literature.[22] The following methods A–O are described for specific products. However, identical procedures may be applied to analogous compounds.

Method A. 2,4-Dichloro-6-methylaniline. Chlorine was passed during 2 h through a solution of 74.5 g (0.50 mol) of *N*-acetyl-2-methylaniline in 400 mL of glacial acetic acid and 400 mL of chloroform until 111 g (1.56 mol) was taken up. Excess chlorine was trapped with a concentrated aqueous sodium thiosulfate solution. Reaction was exothermic during the second chlorination step and the mixture was cooled with an ice bath. Distillation of chloroform and acetic acid under vacuum gave a crystalline product which was washed abundantly with water then with 2-propanol and pentane to give upon drying 63 g (0.31 mol) of 2,4-dichloro-6-methylacetanilide as a white solid: mp 186 °C. A second crop of 6.2 g was isolated from the mother liquor: mp 182 °C. The crude product (67 g, 0.31 mol) prepared as above was refluxed and stirred for 18 h with 740 mL of 95% ethanol and 820 mL of concentrated HCl. Upon cooling the solution was evaporated to dryness, 500 mL of water added and the mixture was then alkalinized with 200 mL of concentrated NaOH and extracted with ethyl acetate. The organic liquor was dried and the solvent was removed to give 55.8 g of a crude brown orange oil which was purified by distillation to give 44.7 g (80%) of 2,4-dichloro-6-methylaniline as a colorless oil: bp 135–140 °C (13 mmHg). This crystallized on standing as a white product: mp 42–45 °C; ^1H NMR δ 2.13 (s, 3 H, CH_3), 5.2 (s, 2 H, NH_2), 7 (d, 1 H, J = 2.1 Hz), 7.16 (d, 1 H, J = 2.1 Hz).

Method B: 2,4-Dichloro-6-methylnitrobenzene. 2,4-Dichloro-6-methylaniline (21.6 g, 0.12 mol), 32 mL of concentrated HCl, and 32 mL of H_2O were stirred at 50–60 °C for 30 min. Upon cooling with an ice bath to 5 °C, a solution of 9 g (0.13 mol) of $NaNO_2$ in 20 mL of water was introduced. The temperature was kept below 7 °C and the solution allowed to stay for 1 h at 5 °C. A mixture of 20 g (0.18 mol) of $NaBF_4$ in 40 mL of water was then

(31) Stewart, J. J. P. MOPAC: A semiempirical molecular orbital program. *J. Comput.-Aided Mol. Des.* **1990**, *4*, 1–105.

Table III. In Vitro and in Vivo Inotropic Activities of Novel 2-Arylbenzimidazoles and Azabenzimidazoles[a,c]

	isolated left atria, inotropic activity		isolated right atria, chronotropic activity		open-chest dog, iv			
					contractility		hypotension	
compd	EC_{50}, μM	tension max, mg	EC_{50}, μM	rate max, beat/min	ED_{50}, mg/kg	% dP/dt max	ED_{25}, mg/kg	% DAP
1	16	432 ± 48	50	35 ± 6	0.08	+50	2	−25
2	[50]	250 ± 38		NT	[3]	+22	[3]	−16
3		no effect [50 μM]		NT	[3]	+27	up to 3	no effect
4	88	558 ± 68	310	49 ± 11	0.75	+50	5	−25
5		no effect [1000 μM]		NT	3	+50	[10]	−20
6	24	670 ± 98	60	43 ± 12	1.4	+50	[3]	−22
7	[50]	170 ± 8		no effect [50 μM]	up to 3	no effect	up to 3	no effect
8	82	625 ± 69	[1000]	24 ± 15	3.5	+50	up to 10	no effect
9	43	708 ± 89		no effect [1000 μM]	2.4	+50	up to 10	no effect
10	150	455 ± 102	[500]	21 ± 1	1.8	+50	[10]	−18
11	140	496 ± 82		no effect [1000 μM]	[3]	+18	up to 3	no effect
12	33	398 ± 65		no effect [100 μM]	5	+50	up to 10	no effect
14		no effect [100 μM]		NT	[3]	+39	up to 3	no effect
16	15	518 ± 180	97	44 ± 7	up to 10	no effect	up to 10	no effect
17		no effect [500 μM]		NT	up to 3	no effect	up to 3	no effect
18	26	337 ± 77	230[b]	−30 ± 18[b]	up to 10	no effect	10	−25
20	14	378 ± 68		no effect [100 μM]	up to 3	no effect	up to 3	no effect
21	35	455 ± 41	23	48 ± 10	2	+50	3.4	−25
22		no effect [50 μM]		NT	up to 3	no effect	[3]	−44
24	[200]	178 ± 26		NT	4.2	+50	[10]	−10
25	[500]	222 ± 44		no effect [100 μM]	4.8	+50	10	−25
26	nl	350 ± 80		NT	up to 10	no effect	[10]	−48
27	56	490 ± 87		no effect [500 μM]	10	+50	[10]	−10
28	58	585 ± 81		no effect [1000 μM]	[10]	+25	[10]	−22
29	37	453 ± 52		NT	[10]	+33	[10]	−22
30		no effect [50 μM]		NT	up to 3	no effect	up to 3	no effect
31	39	620 ± 73		no effect [500 μM]	10	+50	[10]	−12
32	[20]	156 ± 19		NT	3	+50	up to 3	no effect
33	[50]	231 ± 23		NT	0.9	+50	[10] nl	−61
34	[50]	190 ± 42		NT	up to 10	no effect	1	−25
35	16	378 ± 78		no effect [200 μM]	10	+50	3	−25
36	NT			NT	NT		NT	
37	34	623 ± 65	101	36 ± 9	[10]	+28	10	−25
38	10	408 ± 27	24	50 ± 23	0.32	+50	0.75	−25
39	24	599 ± 102	[500]	29 ± 6	0.06	+50	2.3	−25
40		no effect [20 μM]		NT	NT		NT	
41	15	645 ± 100		no effect [50 μM]	0.1	+50	0.7	−25
MCI 154	34	410 ± 131	20	39 ± 12	0.009	+50	0.1	−25
sulmazole	31	600 ± 126	100	88 ± 18	1	+50	5	−25
milrinone	4.7	580 ± 99	22	70 ± 6	0.15	+50	1	−25

[a] First and second columns = isolated atria: The drug concentration producing 50% of the maximal inotropic or chronotropic response was expressed as the EC_{50}. When EC_{50} could not be calculated, values in brackets [x] indicate the maximal concentration used. No effect was recorded up to and including the concentration used in the test. NT = not tested. Values are means ± SEM. [b] EC_{50} for compound 18 was the drug concentration which produced 50% of the maximal decrease in heart rate. [c] Open-chest dog ED_{50} for contractility was expressed as the dose of compound which produced a 50% increase in dP/dt max from the control value. ED_{25} for hypotension was expressed as the dose of drug producing a 25% decrease in diastolic arterial pressure (DAP) from control value. The other doses (values in brackets [x]) correspond to the maximal percent effect. If there was no effect, the maximum intravenous dose used is given. NT = not tested, nl = non linear. For details, see pharmacological section.

Table IV. Cardiotonic Activity of Some 2-Arylbenzimidazoles and Azabenzimidazoles Administered Orally in Conscious Dog[a]

compound	contractility ED_{50}, mg/kg	compound	contractility ED_{50}, mg/kg
1	1	36	no effect
4	10		(up to 1 mg/kg)
6	10	38	3
9	dP/dt max at 10 mg/kg: +20%	39	3
10	≥10	41	1
21	10	MCI 154	0.1
33	no effect (up to 10 mg/kg)	Sulmazole	10
		Milrinone	2

[a] For explanation, see footnote c of Table III.

added dropwise (temperature = 5 °C) causing precipitation. The white solid was filtered, washed with a cold solution of 2.5 g of $NaBF_4$ in 25 mL of water and with cold water and 2-propanol and then dried to give 28.5 g (84%) of the corresponding diazonium fluoroborate: mp 200 °C dec.

Diazonium fluoroborate (28.5 g) prepared as above was added in small portions to a mechanically stirred solution of 100 g (1.45 mol) of $NaNO_2$, 300 mL of H_2O, 50 mL of ethyl ether, and 20 g (0.31 mol) of freshly prepared Cu powder.[32] The temperature of the mixture was maintained at 20 °C with an ice bath. Stirring was continued for 15 min; Cu powder was filtered and washed with ethyl ether and then water. The organic layer was separated and the aqueous layer extracted twice with ether. Organic phases were washed with water, dried on magnesium sulfate, and vacuum concentrated to give 19.1 g of a brown oil. Purification by distillation gave 5.6 g of a pale yellow oil [bp 94–106 °C (27 mmHg)] which was identified as 3,5-dichlorotoluene and 14.2 g (54%) of 2,4-dichloro-6-methylnitrobenzene as an oil [bp 142–150 °C (27 mmHg)] which crystallized: mp 68 °C; ^1H NMR δ 2.32 (s, 3 H, CH_3), 7.68 (d, 1 H, 1.7 Hz), 7.87 (d, 1 H, J = 1.7 Hz).

Method C. 5-Chloro-4-methyl-2-nitroaniline. 3-Chloro-4-methylacetanilide (358.5 g, 1.95 mol) acetic anhydride (800 mL) and acetic acid (400 mL) were cooled at −5 °C. To this solution was added dropwise (2 h) a mixture of concentrated nitric acid (128 mL, d = 1.51) and acetic acid (175 mL) so that the temperature never exceeded 0 °C. After additional stirring at 0 °C for 2 h the reaction mixture was poured into 800 mL of

(32) Vogel, A. I. *A text-book of Practical Organic Chemistry*, 3rd ed.; Longmans, Green and Co. Ltd.: London, 1956; p 192.

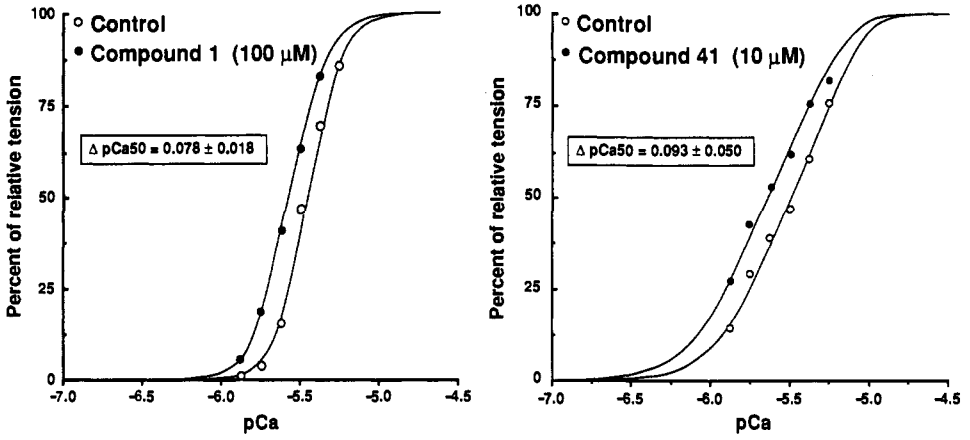


Figure 2. Relation between pCa and tension development of cardiac skinned muscle fibers from rats. The force–calcium relationship is determinated in the presence of the compound or the solvent (control curve). Data are fitted using the Hill equation; $n = 3$. pCa_{50} is the negative logarithm of the calcium concentration required for half-maximal activation. For MCI 154 (200 μM), the ΔpCa_{50} calculated was 0.052 ± 0.013.

water. The solid was filtered, washed with water and then with 2-propanol and pentane, and dried to give 288.2 g of 5-chloro-4-methyl-2-nitroacetanilide: mp 112 °C. Recrystallization in ethanol gave 244.9 g (55%) of the pure product: mp 116 °C; ^{1}H NMR δ 2.08 (s, 3 H, CH_3), 2.39 (s, 3 H, CH_3), 7.78 (s, 1 H), 8.01 (s, 1 H), 10.26 (s, 1 H, amide proton). 5-Chloro-4-methyl-2-nitroacetanilide (139 g, 0.61 mol) prepared as above was added to a methanol/sodium methanolate solution (prepared from 530 mL of methanol and 14.3 g (0.62 mol) of sodium). The mixture was stirred for 3 h and then poured into 2 L of water. The orange solid was filtered and washed with water, 2-propanol, and pentane to give 108.1 g (95%) of 5-chloro-4-methyl-2-nitroaniline: mp 166–168 °C; ^{1}H NMR δ 2.21 (s, 3 H, CH_3), 7.12 (s, 1 H, H_6), 7.37 (s, 2 H, NH_2), 7.94 (s, 1 H, H_3).

Method D. 3-Chloro-2-nitroaniline. 3-Chloro-2-nitrobenzoic acid (50 g, 0.25 mol), toluene (250 mL), and thionyl chloride (100 mL) were heated to reflux for 3 h. Distillation of the thionyl chloride under vacuum gave upon cooling a brown solid which can be purified by pentane washing. The acid chloride (51.1 g, 0.23 mol) (mp 54 °C) thus obtained was added to 500 mL of concentrated NH_3 (28%) and stirring continued for 20 min. The solid was filtered, washed abundantly with water and then with 2-propanol and pentane, and dried to give 44.8 g (96%) of 3-chloro-2-nitrobenzamide: mp 198 °C.

To an ice-bath-cooled solution of 33.6 g (0.6 mol) of potassium hydroxide in 350 mL of water was added dropwise 11.5 mL (0.22 mol) of bromine. Then 40.1 g (0.20 mol) of the benzamide prepared as above was introduced at once. Stirring was continued for an additional 30 min. This solution was then poured into a solution of 44.8 g (0.8 mol) of potassium hydroxide in 100 mL of water. The temperature was maintained at 70–75 °C for about 45 min. The solid obtained on cooling was filtered, washed with water, dried to give 21.7 g of the crude product which was purified by chromatography (SiO_2 toluene). 3-Chloro-2-nitroaniline (10.3 g, 30%) was obtained as an orange solid: mp 110 °C (lit.[33] mp 108–109 °C); ^{1}H NMR δ 6.34 (s, 2 H, NH_2), 6.73 (d, 1 H, $J = 7.8$ Hz), 6.86 (d, 1 H, $J = 8.36$ Hz), 7.22 (t, 1 H, $J_1 = 8.1$ Hz, $J_2 = 8.2$ Hz).

Method E. 5-Chloro-3-methyl-2-nitroaniline. A mixture of 2,4-dichloro-6-methylnitrobenzene (34.4 g, 0.17 mol) and 2-methoxyethanol (125 mL) was cooled to −40 °C. Liquid ammonia (45 mL) was then added and the whole was heated at 150 °C for 72 h under pressure (ca. 33 bar). The resulted solution was concentrated to dryness and purified by chromatography (SiO_2, toluene). With 8.4 g of the starting dichloro compound, 20.1 g (63%) of 5-chloro-3-methyl-2-nitroaniline was obtained as an orange solid: mp 82 °C; ^{1}H NMR δ 2.31 (s, 3 H, CH_3), 6.57 (m, 3 H, aromatic proton and NH_2), 6.8 (d, 1 H, $J = 2.1$ Hz).

Method F. 3-(4-Chloro-3-nitrophenyl)imidazo[2,1-*b*]thiazole. A mixture of 5.6 g (0.02 mol) of 5-(α-bromoacetyl)-2-chloro nitrobenzene and 2 g (0.02 mol) of 2-mercaptoimidazole in 120 mL of methanol was stirred at room temperature for 1 h. Evaporation of the solvent gave 7 g of the hydrobromide of the intermediate 5-(imidazol-2-ylthioacetyl)-2-chloronitrobenzene: mp 240 °C. The free base (4.8 g, 0.016 mol) was obtained as a white solid by neutralizing with a solution of Na_2CO_3: mp 165 °C. This was introduced into 200 g of polyphosphoric acid (PPA) at 110 °C. The whole was maintained at 110–120 °C for 7 h and then poured into 800 mL of water and neutralized with aqueous ammonia. The solid was filtered, washed with water and acetone, and dried to give 4.5 g (100% yield) of 3-(4-chloro-3-nitrophenyl)imidazo[2,1-*b*]thiazole: mp 206 °C; ^{1}H NMR δ 7.39 (s, 1 H), 7.77 (s, 1 H), 7.94 (d, 1 H, $J = 8.4$ Hz), 8.11 (m, 2 H), 8.45 (d, 1 H, $J = 1.95$ Hz).

Method G. 2-Amino-6-imidazol-1-yl-3-nitropyridine. 2-Amino-6-chloro-3-nitropyridine (20 g, 0.12 mol), 12.2 g (0.12 mol) of Na_2CO_3, and 13.6 g (0.20 mol) of imidazole in 200 mL of DMF were heated for 3 h at 110 °C. The solvent was evaporated in vacuo, water was added, and the solid filtered, washed with water and then 2-propanol, and dried to give 20.2 g (86%) of 2-amino-6-imidazol-1-yl-3-nitropyridine: mp 224 °C; ^{1}H NMR δ 7.16 (d, 2 H, pyridine proton and imidazole proton), 7.95 (s, 1 H, imidazole proton), 8.21 (s, 2 H, NH_2), 8.56 (d, 2 H, pyridine proton and imidazole proton).

Method H. 5-Benzimidazol-1-yl-2-nitroaniline. To 23.6 g (0.20 mol) of benzimidazole in 240 mL of DMF was added 8 g (0.20 mol) of 60% NaH and then stirring was continued at 50–60 °C for 1 h, the mixture was cooled, and 31.2 g (0.20 mol) of 5-fluoro-2-nitroaniline in 150 mL of DMF was added. After refluxing for 4 h, the DMF was evaporated in vacuo, water added, and the solid filtered. Purification of the crude product by two successive column chromatography runs (SiO_2, 5% methanol/chloroform) gave 19.3 g (38%) of 5-benzimidazol-1-yl-2-nitroaniline as an orange solid: mp 204–206 °C; ^{1}H NMR (Me_2SO-d_6/$CDCl_3$) δ 6.96 (dd, 1 H, $J_1 = 2.3$ Hz, $J_2 = 9.2$ Hz) 7.39 (m, 3 H), 7.68 (s, 2 H, NH_2), 7.78 (m, 2 H), 8.2 (d, 1 H, $J = 9.2$ Hz), 8.64 (s, 1 H).

Method I. 5-Imidazol-1-yl-3-methyl-2-nitroaniline. 5-Chloro-3-methyl-2-nitroaniline (19.3 g, 0.10 mol) and 42 g (0.63 mol) of imidazole were heated for 24 h at 180 °C. Water was added and the solid filtered, washed with water and ether, and dried to give 19.9 g (89%) of the crude 5-imidazol-1-yl-3-methyl-2-nitroaniline: mp 180–182 °C; ^{1}H NMR (Me_2SO-d_6/$CDCl_3$) δ 2.51 (s, 3 H, CH_3), 6.57 (s, 2 H, NH_2), 6.66 (d, 1 H, $J = 2.2$ Hz), 7 (d, 1 H, $J = 2.2$ Hz), 7.14 (s, 1 H), 7.42 (s, 1 H), 8 (s, 1 H).

Method J. 2-Nitro-5-(1,2,4-triazol-1-yl)aniline. 5-Fluoro-2-nitroaniline (20.9 g, 0.13 mol) and 14.6 g (0.16 mol) of 1,2,4-triazole sodium salt in 100 mL of DMF were refluxed for 4 h. Water was added and the precipitate filtered, washed with water and then with 2-propanol and dried to yield 24.2 g (88%) of 2-nitro-5-(1,2,4-triazol-1-yl)aniline: mp 264 °C; ^{1}H NMR δ 7.19 (dd, 1 H, $J_1 = 2.2$ Hz, $J_2 = 9.3$ Hz, H_4), 7.55 (d, 1 H, $J = 2.2$ Hz,

(33) White, W. N.; Klink, J. R. Products of Rearrangement of m-Chloro-N-nitro-N-methylaniline. *J. Org. Chem.* **1972**, *42*, 1, 166–169.

H_6), 7.72 (s, 2 H, NH_2), 8.16 (d, 1 H, J = 9.3 Hz, H_3), 8.32 (s, 1 H, triazole proton), 9.39 (s, 1 H, triazole proton).

Method K. 2-Amino-5-imidazol-1-yl-3-methylaniline. 5-Imidazol-1-yl-3-methyl-2-nitroaniline (19.9 g, 0.09 mol) in 600 mL of methanol was hydrogenated in the presence of Raney nickel at room temperature and atmospheric pressure for 3 h. The solution was filtered on Celite 545 and washed with methanol and the solvent removed in vacuo. After being triturated with isopropyl acetate, the precipitate was filtered and dried to yield 15.6 g (91%) of 2-amino-5-imidazol-1-yl-3-methylaniline: mp 148 °C; ^{1}H NMR δ 2.1 (s, 3 H, CH_3), 4.39 (s, 2 H, NH_2), 4.79 (s, 2 H, NH_2), 7 (s, 1 H, imidazole proton), 7.39 (s, 1 H, imidazole proton), 7.87 (s, 1 H, imidazole proton).

Method L. 2-Amino-4-imidazol-1-ylaniline. To 26.3 g (0.13 mol) of 5-imidazol-1-yl-2-nitroaniline[22] (mp 185–187 °C) in 150 mL of ethanol were added 35 mL of water and 120 g (2.15 mol) of iron powder. With stirring, 2 mL of concentrated HCl was introduced and the mixture refluxed for 4 h. The hot solution was filtered on Celite 545 and extracted with dichloromethane. The organic layers were dried on magnesium sulfate and then concentrated to give 17 g (75%) of 2-amino-4-imidazol-1-ylaniline: mp 168–170 °C; ^{1}H NMR δ 4.64 (s, 2 H, NH_2), 4.77 (s, 2 H, NH_2), 6.55 (m, 2 H, aromatic proton), 6.65 (s, 1 H, aromatic proton), 7 (s, 1 H, imidazole proton), 7.39 (s, 1 H, imidazole proton), 7.87 (s, 1 H, imidazole proton).

Method M. 5-Imidazol-1-yl-2-(2,4-dimethoxyphenyl)benzimidazole. 2-Amino-5-imidazol-1-ylaniline (9.1 g, 0.05 mol) and 8.6 g (0.05 mol) of 2,4-dimethoxybenzaldehyde in 150 mL of methanol were refluxed for 13 h. The solvent was evaporated and the residue triturated with ethyl acetate to give a solid which was chromatographed (SiO_2 10% methanol/chloroform) to yield 3.2 g (20%) of 5-imidazol-1-yl-2-(2,4-dimethoxyphenyl)benzimidazole. Crystallization from acetonitrile afforded pure product: mp 188 °C; ^{1}H NMR δ 3.87 (s, 3 H, OCH_3), 4.04 (s, 3 H, OCH_3), 6.75 (m, 2 H), 7.11 (s, 1 H), 7.4 (d, 1 H, J = 10.2 Hz), 7.75 (m, 3 H), 8.25 (m, 2 H), 12.13 (s, 1 H, NH).

Method N. 5-Imidazol-1-yl-7-methyl-2-(4-pyridyl)benzimidazole. 2-Amino-5-imidazol-1-yl-3-methylaniline (15.6 g, 0.08 mol) and 8.9 g (0.08 mol) of 4-formylpyridine in 210 mL of methanol were refluxed for 6 h. Nitrobenzene (50 mL) was then added and the methanol distilled. The mixture was heated for an additional 4 h at 180 °C, nitrobenzene distilled, acetonitrile added, and the mixture triturated to obtain 17.9 g of precipitate. Elution with 10% methanol/chloroform followed by recrystallization from ethanol yielded 5.8 g (25%) of the analytically pure 5-imidazol-1-yl-7-methyl-2-(4-pyridyl)benzimidazole: mp 289 °C; ^{1}H NMR δ 2.66 (s, 3 H, CH_3), 7.12 (s, 1 H), 7.39 (s, 1 H), 7.69 (s, 1 H), 7.77 (s, 1 H), 8.16 (d, 2 H, pyridine protons, J = 5.7 Hz), 8.26 (s, 1 H), 8.77 (d, 2 H, pyridine protons, J = 5.7 Hz), 13.4 (s, 1 H, NH).

Method O. 5-Imidazol-1-yl-2-[2-methoxy-4-(methylthio)phenyl]benzimidazole. A mixture of 8.7 g (0.05 mol) of 2-amino-5-imidazol-1-ylaniline and 9.9 g (0.05 mol) of 2-methoxy-4-mercaptobenzoic acid was added to 200 mL of $POCl_3$. After refluxing for 4 h the precipitate was filtered, acidified with 200 mL of 1 N HCl and then neutralized with 30% NaOH and extracted with chloroform. The extract was dried, concentrated, and, after partial purification by column chromatography (SiO_2, 20% methanol/chloroform), recrystallized from acetonitrile to yield 2.1 g (12%) of 5-imidazol-1-yl-2-[2-methoxy-4-(methylthio)phenyl]benzimidazole: mp 182 °C; ^{1}H NMR (Me_2SO-d_6/D_2O) δ 2.58 (s, 3 H, SCH_3), 4.06 (s, 3 H, OCH_3), 7.04 (m, 2 H), 7.15 (s, 1 H), 7.47 (d, 1 H, J = 8.6 Hz), 7.79 (m, 3 H), 8.24 (m, 2 H).

Method P. 5-Imidazol-1-yl-2-(6-chloro-3-pyridyl)benzimidazole. 6-Chloronicotinoyl chloride (8.8 g, 0.05 mol) in 50 mL of toluene was added dropwise to a solution of 10.2 g (0.05 mol) of 5-imidazol-1-yl-2-nitroaniline in 100 mL of pyridine. The mixture was stirred for 3 h at room temperature and then for 3 h at 50 °C, concentrated in vacuo. The oil obtained was triturated with water; the precipitate was filtered and washed with water and then with 2-propanol to give 13.7 g (0.04 mol) of 6-chloro-*N*-(5-imidazol-1-yl-2-nitrophenyl)nicotinamide: mp 180 °C.

Catalytic hydrogenation of this compound according to method K was followed by treatment in 100 mL of acetic acid under reflux for 3 h. The compound obtained was partially purified by chromatography (SiO_2, 10% methanol/chloroform) and recrystallized from 2-methoxyethanol to yield 4.8 g (51%) of 5-imidazol-1-yl-2-(6-chloro-3-pyridyl)benzimidazole: mp >330 °C; ^{1}H NMR δ 7.15 (s, 1 H), 7.52 (dd, 1 H, J_1 = 8.6 Hz, J_2 = 2 Hz), 7.76 (m, 3 H), 7.88 (d, 1 H, J = 1.7 Hz), 8.29 (s, 1 H), 8.56 (dd, 1 H, J_1 = 8.4 Hz, J_2 = 2.4 Hz), 9.19 (d, 1 H, J = 2.3 Hz).

Pharmacology. Isolated Tissue from Guinea Pig Hearts. Male guinea pigs (500–700 g) were killed by cervical dislocation. The heart was immediately removed, and the left or right atria was dissected free from surrounding tissue and suspended in individual 20-mL muscle baths. Each bath contained a pH 7.35–7.40 Krebs buffer of the following millimolar composition (mM): NaCl, 118; KCl, 4.7; $MgSO_4$, 1.6; KH_2PO_4, 2.2; $CaCl_2$, 2.5; $NaHCO_3$, 24.9; D-glucose, 12. Bath solution was bubbled with 95% O_2/5% CO_2 at 32 °C for right atria or 37 °C for left atria. A hook secured the muscle to a bipolar electrode in the bottom of the bath and a silk thread connected the tissues to an isometric force transducer. A baseline tension of 1.0 g was applied to each tissue. Left atria were stimulated electrically with square-wave pulses (2.7 Hz in frequency, 1 ms in duration and 20–30% above the threshold voltage) delivered through the hook electrode and the second electrode positioned near the top of the muscle. Right atria were beating spontaneously. Contraction wave forms were analyzed by a pulsatile contraction analyzer (Buxco Electronics).

The inotropic effect was accessed in paced electrically left atria and the chronotropic effect in spontaneously contracting right atria.

Organ baths were washed out at regular intervals during the 60-min equilibrating period prior to drug treatment. Aliquots (5-250 μL) of concentrated stock solutions of each drug were added cumulatively (10^{-6}–10^{-3} M) to the tissue baths every 5 min. The drug concentration producing 50% of the maximal inotropic or chronotropic response was calculated from three to six experiments and designated as the EC_{50}. The maximal increase in developed tension is referred to as tension max, and the maximal increase in rate is designated as rate max. EC_{50} was not calculated when tension max and rate max were below 250 mg and 30 beat/min, respectively.

Experiments in Anesthetized Dogs. Mongrel dogs of either sex (15–18 kg) were anesthetized with sodium pentobarbital (30 mg/kg IV + 3 mg/kg per h) and ventilated artificially with air and 40% O_2 using a volumetric respirator. Blood gases were analyzed at regular intervals and a heating pad maintained body temperature at 37–38 °C. The saphenous vein was cannulated for intravenous drug administration. Carotid arterial blood pressure was measured through a polyethylene tube filled with heparin solution (50 UI/mL) and connected to a Statham pressure transducer. Heart rate was recorded using a cardiotachometer triggered by the electrocardiogram waves (lead II). After thoracotomy at the left fifth intercostal space, the heart was suspended in a pericardial cradle and aortic blood flow was measured by an electromagnetic flowmeter (Statham SP 2202) with a probe placed at the root of the thoracic aorta. The total peripheral resistance, stroke volume, and left ventricular work were also calculated. A microtip catheter pressure transducer (Millar PC 350) was introduced into the left cavity to provide a measurement of left ventricular pressure and its first derivative, dP/dt max, as the main index of contractile state. All parameters were recorded on a polygraph (Gould ES 1000) and data were collected on computer from Data Logger and Buxco system analyzer. The parameters were allowed to stabilize for at least 30 min following surgical preparation. To assure that the preparation was functioning properly, a 0.125–0.25 μg/kg challenge of isoproterenol was given prior to experimental compound. Only one compound was administered to any one animal. Drugs were given intravenously as a bolus, in incremental doses (0.003–10 mg/kg) at 30-min intervals. Measurements of cardiac contractility, heart rate, and blood pressure were made both before drug administration and 3 min afterward. All compounds were dissolved in normal saline or 1 N HCl, pH 2–4.

Equieffective doses were obtained by extrapolation from dose–response curves. ED_{50} for contractility was expressed as the dose of drug producing a 50% increase in dP/dt max from control value. ED_{25} for hypotension was expressed as the dose of drug

producing a 25% decrease in diastolic arterial pressure (DAP) from the control value.

Experiments in Conscious Dogs. Male beagle dogs (12–15 kg) were chronically instrumented to monitor left ventricular pressure and arterial pressure. Under isoflurane/nitrous oxide anesthesia, a precalibrated Kõnigsberg P5-S pressure transducer was aseptically positioned in the left ventricle through a stab wound in the apex. The carotid artery was exteriorized over a length of 5–6 cm. A small piece of Dacron material (USCI, sauvage) around the artery permitted repeated intra-arterial insertion of cannula, without bleeding complications. After recovery from surgery, a minimum of 3 weeks was allowed to train dogs to lie quietly in a sling for 6-h periods. Dogs were fasted for 18 h before an experiment. The Königsberg pressure transducer was connected to a Beckman R 611 recorder to monitor left ventricular pressure and its first derivative (dP/dt max). Under local anesthesia, a Teflon cannula was percutaneously inserted into the carotid artery and coupled to a pressure transducer for systolic and diastolic pressure recording. Heart rate was recorded using a cardiotachometer triggered by the pulsatile waves. Drug or placebo was administered orally using a gastric tube, in a 1% gum arabic suspension. Effects of drugs were followed for 5 h. Equiactive doses were obtained by extrapolation from dose–response curves. ED_{50} for contractility was expressed as above.

Ca^{2+} Sensitivity of Cardiac Skinned Muscle Fibers. The effect on Ca^{2+} sensitivity of the myofilaments was tested on cardiac, chemically skinned, muscle fibers from rats, as previously described by Ventura-Clapier et al.[30] Briefly, subendocardial muscle fiber bundles were dissected from the left ventricle of rats. Muscles were skinned by exposure to the relaxing buffer solution (pCa = 9) containing 1% Triton X-100. After the skinning procedure, muscles were transferred to an organ bath containing the relaxing buffer solution, attached horizontally between two hooks, and connected to a AE801 strain gauge transducer for measurements of isometric tension. Muscles were activated by increasing Ca^{2+} until no further increase in calcium would produce an additional increase in tension, i.e. maximal Ca^{2+}-activated tension. The total salt concentrations necessary for obtaining the desired pCa ($-\log [Ca^{2+}]$), pMg, pMgATP, and pH of relaxing or activating buffer solutions were calculated with a computer program as described previously by Ventura-Clapier et al.[30] The pCa–tension relationships were expressed in percent relative force, the tension versus free Ca^{2+} concentration data were fitted to a modified Hill equation, and values of pCa_{50} (the $-\log [Ca^{2+}]$ producing 50% of maximal activation) were calculated. Muscles were subjected to repeat activation–relaxation cycles in the absence or presence of tested compound.

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