5. Isolated guinea pig ileum was placed in a bath containing 15 mL of oxygenated Tyrode solution at 36.5 ± 0.5 °C; acetylcholine and histamine were used as standard spasmogens.

6. The guinea pig intradermal wheal method[26] was used for testing the local anesthetic activity with procaine and propranolol as standard drugs. The response to each of the six pinpricks made at intervals of 5 s was recorded as one plus for one negative response. Table IV shows an average of six such observations.

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Synthesis and Adrenergic Activity of Benzimidazole Bioisosteres of Norepinephrine and Isoproterenol[1]

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The concept of bioisosterism between benzimidazole and catechol was applied to the design and synthesis of benzimidazole analogues of norepinephrine, (*R,S*)-1-[5(6)-benzimidazolyl]-2-aminoethanol (**2**), and of isoproterenol, (*R,S*)-1-[5(6)-benzimidazolyl]-2-isopropylaminoethanol (**4**). Compound **2** was shown to be a partial bioisostere of norepinephrine, with direct agonist activity at the α-adrenergic receptor. The ED_{50} for **2** in contracting the guinea pig isolated aortic strip was determined to be 8.0×10^{-6} M. Compound **4** was shown to be a partial bioisostere of isoproterenol, with direct activity as a β-adrenergic agonist. The ED_{50} values for positive chronotropic and inotropic effects of **4** on the isolated guinea pig atrial preparation were determined to be 6.2×10^{-6} and 3.8×10^{-6} M, respectively. The ED_{50} for **4** on the isolated guinea pig tracheal preparation was determined to be 1.6×10^{-6} M. These results indicate that **4** shows greater selectivity for the β-2 adrenergic receptor than does isoproterenol. The chemical stability of benzimidazole, compared with that of catechol, suggests that benzimidazole bioisosteres of catecholamines may be of value as adrenergic drugs.

In prior studies of structure–activity relationships of compounds which have direct agonist activity at adrenergic receptors, emphasis has been placed on alterations in the ethanolamine side-chain portion of the basic catecholamine agonist. Replacement of the catechol portion of the molecule has met with limited success. We have been interested in the relationship between catechol and a possible nitrogen bioisostere, benzimidazole, especially as it relates to catecholamines.

Several 5(6)-substituted benzimidazoles have been synthesized and tested for adrenergic activity prior to our work in this area. Vaughan and Blodinger[3] reported the syntheses of two benzimidazole analogues of epinephrine, 1-[2-methyl-5(6)-benzimidazolyl]-2-methylaminoethanol and 1-[2-oxo-5(6)-benzimidazolyl]-2-methylaminoethanol. Both compounds were tested for bronchodilator activity, but neither was found to be active. Chodnekar et al.[4] synthesized 1-[2-oxo-5(6)-benzimidazolyl]-2-isopropylaminoethanol as a possible β-adrenergic blocking agent. They reported that an infusion of 100 μg/kg per minute of this compound produced a 35% inhibition of isoproterenol-induced tachycardia in the cat, indicating weak

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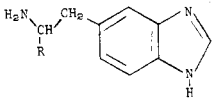
β-adrenergic blockade. It should be noted that they were studying the compound as an analogue of the β-antagonist pronethalol, not as an analogue of the β-agonist isoproterenol. A possible explanation for the lack of significant agonist activity of the compounds reported by these workers is the steric or electronic effects of the substituent in the 2 position of the benzimidazole ring in these derivatives. The synthesis and hypertensive property of 2-[5(6)-benzimidazolyl]ethylamine (**1a**) was first described by Maron[5] in 1914. No analogy was drawn at that time



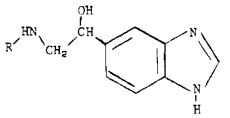
1a, R = H
b, R = COOH

between **1a** and the structurally similar catecholamine, dopamine, possibly because dopamine itself was a newly discovered compound.[6]

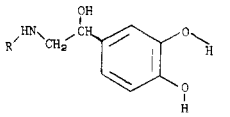
Previous studies from this laboratory have shown some 5(6)-substituted benzimidazoles to have activity in adrenergic systems. A possible metabolic precursor to **1a**, 3-[5(6)-benzimidazolyl]alanine (**1b**), was first synthesized by Milkowski[7] and tested as a possible inhibitor of tyrosine hydroxylase and phenylalanine hydroxylase. The activity of this compound as a competitive inhibitor of tyrosine hydroxylase ($K_i = 2 \times 10^{-5}$ M)[7,8] and in the depletion of brain and heart catecholamines[8] first established the analogy between benzimidazoles and catechols. Further investigations of the activity of **1b** led to the synthesis of **1a** by Wright[9] and subsequent confirmation of its hypertensive properties by King.[10] An initial test of bioisosterism between benzimidazole and catechol was designed using the enzyme catechol *O*-methyltransferase (COMT). This test[11] demonstrated that benzimidazole can serve as a substrate for COMT, an enzyme known previously to be catechol-specific.[12]

The importance for direct adrenergic activity of an acidic proton in the aromatic position meta to the side chain has been suggested by Larsen et al.[13] Increased acidity of the meta proton correlated with increased adrenergic activity in a series of catecholamine analogues in which the *m*-hydroxyl was replaced with the N–H of a substituted sulfonamido group. Recently Yoshizaki et al.[14] have demonstrated potent sympathomimetic activity in a series of 8-hydroxycarbostyril analogues, compounds which have an acidic proton attached to nitrogen in the approximate position of the *m*-hydroxyl group of the corresponding catecholamines. While a benzimidazole analogue would have only one acidic proton, this proton would tautomerize between the positions equivalent to meta and para relative to the side chain in the benzenoid portion of the aromatic nucleus, thus allowing at least the minimum necessary interaction with the receptor.

In light of the above discussion of potential bioisosterism between catechols and benzimidazoles, and of the adrenergic activities of some of the known benzimidazole derivatives, it was deemed worthwhile to synthesize (*R*,*S*)-1-[5(6)-benzimidazolyl]-2-aminoethanol (**2**) as a benzimidazole analogue of norepinephrine (**3**) and (*R*,*S*)-1-[5(6)-benzimidazolyl]-2-isopropylaminoethanol (**4**) as a benzimidazole analogue of isoproterenol (**5**) and to evaluate their activities in well-characterized adrenergic systems.

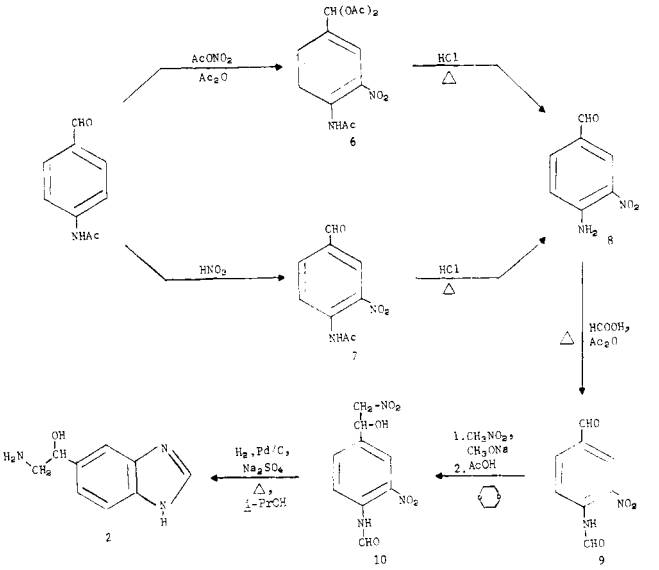


2, R = H
4, R = $CHMe_2$



3, R = H
5, R = $CHMe_2$

Scheme I



Chemistry. The synthesis of **2** was accomplished according to the synthetic pathway outlined in Scheme I. The synthesis of 3-nitro-4-aminobenzaldehyde (**8**) from 4-acetamidobenzaldehyde was achieved via either simple nitration to produce 3-nitro-4-acetamidobenzaldehyde (**7**) or by a single-step acetylation of the aldehyde and nitration of the benzylidene diacetate derivative to produce 3-nitro-4-acetamidobenzylidene diacetate (**6**).[15] Subsequent acid hydrolysis of either of these intermediates produced **8**.

The formylation of **8** to produce 3-nitro-4-formamidobenzaldehyde (**9**) was difficult. An attempt to use *N*-formylimidazole[16] for this reaction produced quantitative recovery of **8**. A Dean-Stark type condensation using formic acid in refluxing benzene gave no reaction. The use of a mixture of acetic anhydride and formic acid (or acetic–formic anhydride)[17] resulted in a number of side reactions involving primarily acylation of either the amine or the aldehyde group or both until optimal reaction conditions (time, temperature, and molar ratios) were established.

The Knoevenagel condensation of **9** with nitromethane was carried out according to the general procedure of Boileau[18] to produce (*R*,*S*)-1-(3-nitro-4-formamidophenyl)-2-nitroethanol (**10**). The reduction and cyclization of **10** to form (*R*,*S*)-1-[5(6)-benzimidazolyl]-2-aminoethanol (**2**) was attempted by several different procedures. Using formic acid as the solvent in this reduction gave little cyclization at room temperature. Increasing the temperature by jacketing the reduction bottle with steam gave a product, the ^{1}H NMR spectrum of which showed two distinct singlets at about 8.2 ppm. Both signals shifted downfield to about 9.7 ppm on addition of DCl, suggesting formation of at least two benzimidazoles with magnetically different C-2 protons.

Performing this reduction and cyclization in methanol or ethanol gave complex mixtures containing little, if any, benzimidazole; the water formed during the reduction in alcoholic solvents was apparently sufficient to hydrolyze the formamide. This hydrolysis was prevented by using anhydrous 2-propanol in the presence of anhydrous sodium sulfate, the reaction being complete in this solvent after 16 h at steam temperature.

The isoproterenol analogue, (*R*,*S*)-1-[5(6)-benzimidazolyl]-2-isopropylaminoethanol (**4**), was synthesized by reductive alkylation[19] of **2** with acetone.

Table I. Pressor Effect of (*R,S*)-1-[5(6)-Benzimidazolyl]-2-aminoethanol (2) in Control and Reserpine-Pretreated Rats

	Pretreatment (no. of animals)	Dose of 2, μmol/kg	Preinjection mean arterial pressure ± SEM, mmHg	Mean pressor response ± SEM,[a,b] mmHg
A	Control (3)	1	82 ± 11	8 ± 3
B	Reserpine[c] (6)	1	73 ± 3	9 ± 2
C	Control (5)	10	98 ± 8	39 ± 4
D	Reserpine[c] (5)	10	70 ± 5	40 ± 7
E	Control + phentolamine[d] (4)	10	46 ± 4	−4 ± 1
F	Reserpine[c] + phentolamine[d] (3)	10	48 ± 2	−5 ± 2

[a] Corrected for saline injection artifact. [b] Significance A vs. B, C vs. D: $p > 0.05$. [c] 2.5 mg/kg of reserpine, ip, 48 and 24 h prior to the study. [d] 10 mg/kg of phentolamine, iv, 1 min prior to 2.

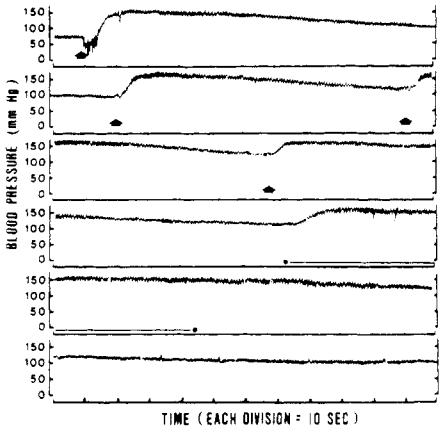


Figure 1. Effects of repeated injections of 20 μmol/kg (arrows) and an infusion of 128 μmol/kg (solid line) of (*R,S*)-1-[5(6)-benzimidazolyl]-2-aminoethanol (2) on blood pressure in a reserpine-pretreated rat (continuous tracing).

Results and Discussion

(*R,S*)-1-[5(6)-Benzimidazolyl]-2-aminoethanol (2) as an α-Adrenergic Agonist. The effect of 2 on rat blood pressure is shown in Table I. The compound produced a pressor response at a dose of 1.0 μmol/kg. A dose of 10 μmol/kg resulted in a consistent 40% rise in blood pressure. Pretreatment with reserpine failed to reduce this hypertensive effect at either dose level. Blockade of the α-adrenergic receptors by pretreatment of the animals with 10 mg/kg of phentolamine completely prevented the hypertensive effect of 10 μmol/kg of 2 in both the control and the reserpine-pretreated rats.

In the experiment depicted in Figure 1, repeated injections of 20 μmol/kg doses of 2 in a reserpine-pretreated rat elevated the blood pressure to a consistent maximum of approximately 166/150 mmHg. When an infusion (during 90 s) of 128 μmol/kg of 2 was injected (indicated by a solid line on the bottom of the tracing), the blood pressure was again elevated to the 166/150 mmHg maximum, remained at that level during the period of infusion, and then slowly began to decrease. The return to preinjection blood pressure required 15 min.

The in vivo study of the effect of 2 on blood pressure in the rat demonstrates that the compound is a direct-acting agonist at the α-adrenergic receptor. The fact that reserpine pretreatment does not affect the pressor response to 2 at two dose levels suggests that a major (if not total) component of its action is direct. Total blockade of the pressor response to 10 μmol/kg of 2 by preinjection of the α-blocker phentolamine demonstrates that this response is mediated by the α-adrenergic receptor. The direct activity of 2 is substantiated by the results of the experiment depicted in Figure 1. Repeated injections of a high dose of 2 (as well as a continuous infusion of 2) in a reserpine-pretreated rat failed to show any tachyphylaxis associated with its hypertensive effect.

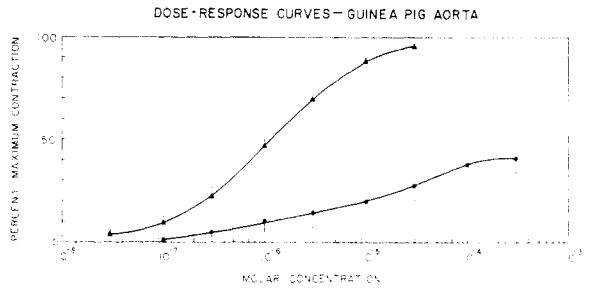


Figure 2. Cumulative dose–response curves for (*R*)-norepinephrine (▲) and (*R,S*)-1-[5(6)-benzimidazolyl]-2-aminoethanol (●) in contraction of the guinea pig aortic strip. Each point on the (*R*)-norepinephrine plot represents the mean of five determinations in four guinea pigs. Each point on the (*R,S*)-1-[5-(6)-benzimidazolyl]-2-aminoethanol plot represents the mean of four determinations in four guinea pigs. Vertical bars, SEM.

Figure 2 compares the cumulative dose–response curve obtained for 2 with that obtained for (*R*)-norepinephrine on contraction of the guinea pig aortic strip. The ED_{50} values for norepinephrine and 2 obtained from these data are 1.1×10^{-6} and 8.0×10^{-6} M, respectively. The maximum response obtained with 2 was 41% of the maximum response obtained with norepinephrine. The addition of 10^{-7} M phentolamine to the bath completely prevented the contraction caused by 2 at doses up to 3×10^{-3} M (data not shown).

In vitro evaluation of 2 on the guinea pig aortic strip allows quantitative comparison of this compound's potency and intrinsic activity with that of the reference drug, norepinephrine. If maximum effect may be used as a measure of intrinsic activity, 2 has an intrinsic activity equal to 41% of that of norepinephrine. If the assumption is made that the effect seen with racemic 2 is due only to the *R* isomer (as is the case for all known direct adrenergic agonists with a β-hydroxyl[20]), then comparison of the ED_{50} of (*R*)-2 [corrected for one-half the total concentration of 2 being the inactive (*S*)-2] with that of (*R*)-norepinephrine gives a potency for (*R*)-2 of one-fourth that of (*R*)-norepinephrine. The activity of 2 in contracting the guinea pig aorta is also blocked by phentolamine. These results indicate that 2 is a potent, direct-acting, partial α-adrenergic agonist.

(*R,S*)-1-[5(6)-Benzimidazolyl]-2-isopropylaminoethanol (4) as a β-Adrenergic Agonist. The in vivo evaluation of the effects of 4 on heart rate and blood pressure in the intact rat demonstrated that the compound is a potent, long-lasting β-adrenergic agonist. Because this was a preliminary evaluation, a comparison with the effects of isoproterenol was not performed. Table II lists the results of injecting various doses of 4 in the intact rat. At 100 nmol/kg the compound effected a 20% increase in heart rate and a 24% decrease in blood pressure. The depressor effect at this dose was transient, lasting only about 1 min. The positive chronotropic effect, however, was of at least 15-min duration. Both effects of 4 at a dose of 1.0 μmol/kg were blocked by prior injection of 1 mg/kg of the β-adrenergic blocker propranolol. Injection of propranolol after 1.0 μmol/kg of 4 reversed both the depressor and positive chronotropic effects of 4.

Table II. Effects of (*R,S*)-1-[5(6)-Benzimidazolyl]-2-isopropylaminoethanol (4) on Heart Rate and Blood Pressure in the Rat

Dose of 4, nmol/kg (no. of animals)	Increase in heart rate, beats/min ± SEM	Change in mean arterial pressure, mmHg ± SEM
Saline (4)	1 ± 0.1	+7 ± 2
1 (4)	1 ± 3	+4 ± 2
10 (4)	12 ± 6	−6 ± 7
100 (4)	65 ± 17[a]	−12 ± 5[b]
1000 (4)	59 ± 15[a]	−30 ± 3[a]
Propranolol[c] + 1000 (3)	13 ± 4	+3 ± 4

[a] Significantly different from saline control, $p < 0.01$. [b] Significantly different from saline control, $p < 0.05$. [c] 1.0 mg/kg of propranolol injected 5 min prior to 1.0 μmol/kg of 4.

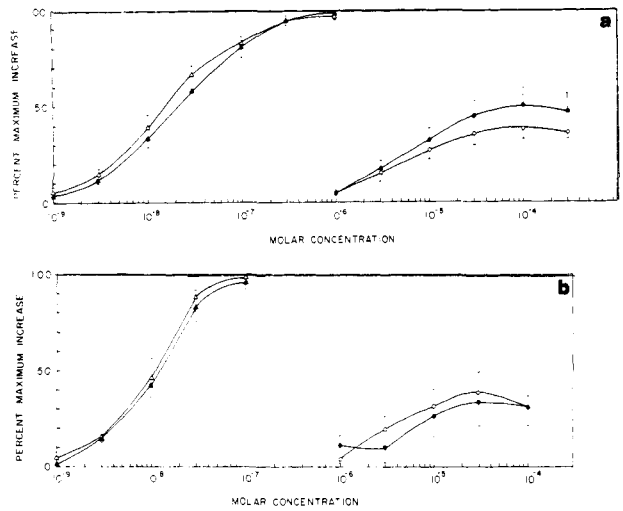


Figure 3. Comparison of cumulative dose–response curves for (*R,S*)-isoproterenol in control (▲) and reserpine-pretreated (△) guinea pig atrial rate (a) and force (b) of contraction with those obtained for (*R,S*)-1-[5(6)-benzimidazolyl]-2-isopropylaminoethanol (4) in control (●) and reserpine-pretreated (○) preparations. Each point represents the mean of at least four determinations in four guinea pig atria. Vertical bars, SEM.

Figure 3 shows the cumulative dose–response curves obtained for 4 and (*R,S*)-isoproterenol on control and reserpine-pretreated guinea pig atrial rate (Figure 3a) and force (Figure 3b) of contraction. ED_{50} values obtained from these data for isoproterenol and 4 on rate are 2.2×10^{-8} and 6.2×10^{-6} M, respectively, and are not significantly changed by reserpine pretreatment. ED_{50} values for isoproterenol and 4 on force of contraction are 1.3×10^{-8} and 3.8×10^{-6} M, respectively, and are not altered by reserpine pretreatment. Because increased frequency of contraction at high agonist concentrations may reduce the inotropic effect observed in this type of atrial preparation,[21] these values may be artificially low. The maximum response obtained with 4 on atrial rate was 50% of that obtained with isoproterenol. The maximum effect of 4 on force of contraction was 33% of that obtained with isoproterenol. Neither maximum was significantly changed by reserpine pretreatment. The addition of 10^{-6} M propranolol to the bath blocked both inotropic and positive chronotropic effects of 4 at concentrations up to 3×10^{-4} M (not shown).

The cumulative dose–response curves obtained for 4 and (*R,S*)-isoproterenol in control and reserpine-pretreated guinea pig tracheal chain preparations are depicted in Figure 4. The ED_{50} values obtained from these data for isoproterenol and 4 on tracheal relaxation are 7.8×10^{-8} and 1.6×10^{-6} M, respectively. The ED_{50} for 4 was not significantly changed with reserpine pretreatment, but that for isoproterenol was significantly ($p < 0.01$) lowered to 2.1×10^{-8} M. Tye et al.[22] reported no significant difference in ED_{50} values obtained for (*R*)-isoproterenol between normal and reserpine-pretreated guinea pig tracheal chain preparations. This inconsistency may be due to our method of pretreatment differing sufficiently so that we observed supersensitivity with this preparation and they did not. The maximum effect of 4 on tracheal relaxation was 58% of the maximum obtained with isoproterenol, and this was not significantly affected by reserpine pretreatment. Addition of 10^{-6} M propranolol to the bath shifted the dose–response curve for 4 at least two log concentration units higher (not shown).

The in vitro studies of the effects of 4 on guinea pig atrial and tracheal preparations allow accurate quantitative comparison with known agonists. The use of reserpine-pretreated preparations demonstrates that the activity of 4 is mainly (if not totally) direct. Table III presents a comparison of the activities of 4 with those of the known β-adrenergic agonist isoproterenol in the same system. It can be seen from this table that 4 is a potent β-adrenergic agonist which shows a greater selectivity for the β-2 receptor than does isoproterenol. Its potency as a β-1 agonist

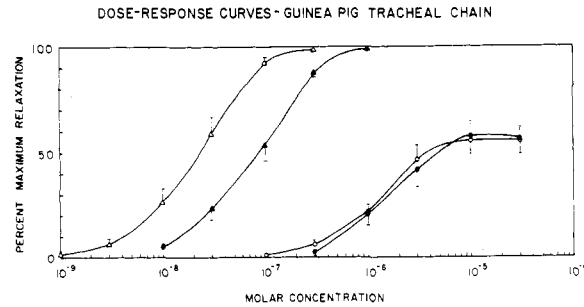


Figure 4. Comparison of cumulative dose–response curves for (*R,S*)-isoproterenol in control (▲) and reserpine-pretreated (△) tracheal chain preparations with those obtained for (*R,S*)-1-[5(6)-benzimidazolyl]-2-isopropylaminoethanol (4) in control (●) and reserpine-pretreated (○) preparations. Each point represents the mean of at least four determinations in four guinea pig tracheae. Vertical bars, SEM.

Table III. Comparison of the Guinea Pig β-Receptor Agonistic Activity of (*R,S*)-1-[5(6)-Benzimidazolyl]-2-isopropylaminoethanol (4) with the Activity of the Known Agonist (*R,S*)-Isoproterenol[e]

	(*R,S*)-Isoproterenol	4
Atrial rate (β-1)		
pD_2[a]	7.66 ± 0.13 (6)	5.21 ± 0.08 (5)
Rel act.[b]	100	0.355
% max response[c]	100	50 ± 9
Atrial force (β-1)		
pD_2	7.87 ± 0.10 (6)	5.42 ± 0.20 (3)
Rel act.	100	0.355
% max response	100	33 ± 12
Tracheal relaxation (β-2)		
pD_2	7.10 ± 0.09 (5)	5.78 ± 0.08 (4)
Rel act.	100	4.79
% max response	100	58 ± 7
Ratio, β-2/β-1[d]	1.0	13.5

[a] pD_2 = negative logarithm of ED_{50} (molar concentration required to produce half-maximal effect). [b] Relative activity = $[ED_{50}$ [(*R,S*)-isoproterenol]/ED_{50} (compound)] × 100. [c] Percent maximum response = [maximum response (compound)/maximum response [(*R,S*)-isoproterenol]] × 100. [d] Ratio, β-2/β-1 = relative activity (tracheal relaxation)/relative activity (atrial rate). [e] Values are means ± SEM, with the number of experiments in parentheses.

is only $^1/_{300}$th that of isoproterenol.

Bioisosterism between Benzimidazole Amines and Catecholamines. In discussing bioisosterism, Burger makes the following point: "By closing or opening cyclic structures one may arrive at compounds that have comparable shapes, especially if such features as rigid bonds or intramolecular hydrogen bonds provide an impetus for pseudocyclic conformation."[23] In 1936 Pauling used infrared spectral evidence to show that the preferred conformation of the catechol molecule is such that intramolecular hydrogen bonding forms a five-membered planar ring of the substituent hydroxyl groups.[24] By replacing the hydroxyl groups of catechol with amino groups ($-NH_2$ to replace $-OH$ by Grimm's "hydride displacement law") and cyclizing the resulting unstable phenylenediamine to form the more chemically stable benzimidazole, one would be following Burger's suggestion by making a cyclic structure (benzimidazole) as a bioisostere of a pseudocyclic conformation (catechol). The octanol–water partition coefficients for benzimidazole (log P = 1.34[25]) and catechol (log P = 0.88[25]) support the suggestion of bioisosterism between the two structures. An additional benefit of substituting benzimidazole for catechol, as far as drug design is concerned, would be a considerable increase in chemical stability. Compounds **2** and **4** are chemically much more stable than the corresponding catecholamines. Dilute aqueous solutions of either **2** or **4** exhibited high chemical stability when stored at 4 °C for more than 1 week, as demonstrated by bioassay (mentioned in the Experimental Section). No change in color of any solution of either **2** or **4** was noted during these investigations.

The possibility of central nervous system activity by these compounds deserves mention. Johnson has shown that intraperitoneal injection of 100 mg/kg of 2-[5(6)-benzimidazolyl]ethylamine (**1a**) significantly lowers brain levels of norepinephrine in the rat.[8] Since dopamine is known not to cross the blood–brain barrier in significant amounts, the central activity of this analogue of dopamine indicates that replacement of the catechol moiety of catecholamines with a benzimidazole ring system may enhance blood–brain transport. The acute in vivo studies presented here cannot be used to argue for or against significant blood–brain transport, but this possibility should not be ruled out.

The present study has introduced benzimidazole analogues of norepinephrine and isoproterenol and demonstrated direct agonist activity for these compounds at the α- and β-adrenergic receptors, respectively. Because of their relatively lower intrinsic activities, they may be classed as partial bioisosteres[26] of the corresponding catecholamines. The unique chemical stability and potent direct agonist activity of these compounds demonstrate the potential usefulness of benzimidazole bioisosteres of catecholamines in the evaluation of structure–activity relationships in adrenergic systems and as adrenergic drugs.

Experimental Section

All melting points were determined on a Thomas-Hoover melting point apparatus and are corrected. Unless specified, all melting ranges were less than 1 °C. IR spectra were recorded as a 0.5% KBr disk on a Perkin-Elmer Model 257 grating infrared spectrophotometer. ^{1}H NMR spectra were recorded as ca. 10% solutions in the indicated solvents with tetramethylsilane (for Me_2SO-d_6 spectra) or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (for D_2O spectra) as internal standard on a Jeolco C-60HL high-resolution NMR spectrometer. Electron-impact mass spectra were determined on a Du Pont 21-490 mass spectrometer interfaced with a Du Pont 21-094 data system for normalization of the spectra and operating at 70-eV ionizing voltage. Mass spectra are not corrected for background. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.

3-Nitro-4-acetamidobenzylidene Diacetate (6).[15] Acetyl nitrate was prepared by adding 25.2 mL (540 mmol) of red, fuming HNO_3 dropwise to 80.0 mL (846 mmol) of Ac_2O maintained at −20 °C (acetone–dry ice bath). This reagent was added dropwise to a stirred suspension of 80.0 g (490 mmol) of 4-acetamidobenzaldehyde (Aldrich) in 200 mL of Ac_2O at −20 °C. The resulting suspension was allowed to warm slowly (vigorous exothermic reaction) to 45 °C (H_2O bath) and maintained at this temperature for 1 h. The solution was then poured into 1 L of cold H_2O, and the resulting precipitate crystallized from EtOH to yield 62.0 g (41%) of **6** as orange-yellow leaflets: mp 114 °C (lit.[15] mp 114 °C).

3-Nitro-4-acetamidobenzaldehyde (7).[27] To 100 mL (2.14 mol) of red, fuming HNO_3 maintained at 0 °C was added, portionwise over a 1-h period, 40.0 g (245 mmol) of 4-acetamidobenzaldehyde. The solution was stirred at 0 °C for 1 h more and then poured into 2 L of cold (4 °C) H_2O. The resulting solid was filtered off, triturated with 1 L of cold H_2O, and refiltered. The product was crystallized from 2-propanol to yield 29.0 g (57%) of **7**: yellow needles; mp 155 °C (lit.[15,27] mp 155 °C).

3-Nitro-4-aminobenzaldehyde (8).[15] To 200 mL of concentrated HCl was added 60.0 g (194 mmol) of **6** or an equivalent amount of **7** and the suspension was stirred at 65 °C for 15 min. The resulting solid was triturated with 1 L of cold H_2O for 15 min. This solid was filtered and triturated for 30 min in 1 L of cold H_2O to which 40 g of $NaHCO_3$ had been added. The resulting solid was crystallized from 2-propanol to yield 29.5 g (92%) of **8** as orange-brown needles: mp 191–192 °C (lit.[15] mp 191 °C).

3-Nitro-4-formamidobenzaldehyde (9). To 56.6 mL (1.50 mol) of HCOOH was added 24.9 g (150 mmol) of **8**. The solution was heated to reflux, 28.4 mL (300 mmol) of Ac_2O was cautiously added dropwise, and the solution was refluxed for 30 min. The resulting suspension was cooled and poured into 400 mL of cold H_2O with rapid stirring. The product was filtered, air-dried, and crystallized from 2-propanol to yield 23.2 g (80%) of **9** as a bright yellow powder: mp 183–191 °C; IR 3292 (m, secondary amide ν N–H), 2925 and 2870 (w, aldehyde ν C–H), 1710 (s, aromatic aldehyde ν C=O), 1690 (s, secondary amide ν C=O), 1570 and 1343 (s, aromatic nitro ν N–O), and 1514 cm^{-1} (s, secondary amide δ N–H); ^{1}H NMR (Me_2SO-d_6) δ 8.24 (d of d, 1, J_{AB} = 9 Hz, J_{AC} = 1.8 Hz, ArH at C-6), 8.53 (d, 1, J = 9 Hz, ArH at C-5), 8.67 (d, 1, J = 1.8 Hz, ArH at C-2), 8.69 (s, 1, NHC*H*O), 10.07 (s, 1, ArCHO), and 10.87 (br s, 1, exchanges with D_2O, NH); mass spectrum (70 eV) m/e (rel intensity) 194 (51, M$^+$), 166 (100), 165 (92), 120 (39), 119 (50), 91 (36), 90 (28), 65 (68), 64 (28), and 63 (40). An analytical sample of **9** was prepared by recrystallizing this product four times from 2-propanol: mp 187–192 °C. Anal. ($C_8H_6N_2O_4$) C, H, N.

(*R*,*S*)-1-(3-Nitro-4-formamidophenyl)-2-nitroethanol (10). To 23.8 mL of 25% $NaOCH_3$ in MeOH (Aldrich, 110 mmol of $NaOCH_3$) and 250 mL of 1,4-dioxane was added 27.0 mL (500 mmol) of CH_3NO_2 (Aldrich, 96%). The suspension was stirred at 0 °C during the addition of 19.4 g (100 mmol) of **9**. After 1 h 6.3 mL (110 mmol) of AcOH in 50 mL of 1,4-dioxane was added; the suspension was stirred for 10 min and filtered. The solvent was evaporated under vacuum and the product crystallized from $CHCl_3$ to yield 7.9 g (31%) of **10** as bright orange crystals: mp 129–131 °C; IR 3445 (m, alcohol ν O–H), 3353 (m, secondary amide ν N–H), 1694 (s, secondary amide ν C=O), 1570–1540 and 1355–1335 (s, nitro ν N–O), 1514 (s, secondary amide δ N–H), and 1085 cm^{-1} (m, secondary alcohol ν C–O); ^{1}H NMR (Me_2SO-d_6) δ 4.81 (m, 2, CHC*H*$_2$), 5.46 (m, 1, CH$_2$C*H*), 6.43 (d, 1, J = 5 Hz, exchanges with D_2O, OH), 7.85 (d of d, 1, J_{AB} = 9 Hz, J_{AC} = 2 Hz, ArH at C-6), 8.20 (d, 1, J = 9 Hz, ArH at C-5), 8.25 (d, 1, J = 2 Hz, ArH at C-2), 8.55 (s, 1, CHO), and 10.62 (br s, 1, exchanges with D_2O, NH); mass spectrum (70 eV) m/e (rel intensity) 255 (4, M$^+$), 237 (4, M$^+$ − H_2O), 194 (53), 166 (100), 165 (86), 120 (26), 119 (37), 65 (53), 63 (29), and 61 (35). Anal. ($C_9H_9N_3O_6$) C, H, N.

(*R*,*S*)-1-[5(6)-Benzimidazolyl]-2-aminoethanol (2). To 2.0 g of anhydrous Na_2SO_4 in 200 mL of 2-propanol was added 2.0 g (7.8 mmol) of **10**, followed by 2.0 g of 5% Pd/C. The mixture was reduced at 2.8 kg/cm^2 of H_2 for 16 h in a steam-jacketed

bottle. The solution was cooled, filtered, and evaporated under vacuum, and the residue was converted to its dihydrochloride salt by the addition of 10 mL of 4 N HCl. After evaporating H_2O under vacuum, the solid was cystallized from 95% EtOH to yield 600 mg (30%) of **2** as its dihydrochloride salt: mp 248 °C dec; IR 3500–2500 (s, ν O–H and ν N–H), 1620, 1600, and 1495 cm^{-1} [m, bands characteristic of a 5(6)-substituted benzimidazole[28]], lack of IR bands in the 1850–1620-cm^{-1} region indicates absence of a carbonyl; ^1H NMR (D_2O) δ 3.51 (m, 2, AB portion of ABX system, $CHCH_2$), 5.50 [d of d, 1, J_{AX} (app) = 8 Hz, J_{BX} (app) = 4.5 Hz, CH_2CH], 7.90 (d of d, 1, J_{AB} = 9.5 Hz, J_{AC} = 1.8 Hz, ArH at C-6), 8.17 (d, 1, J = 9.5 Hz, ArH at C-7), 8.21 (d, 1, J = 1.8 Hz, ArH at C-4), and 9.43 (s, 1, shifts to 8.25 on addition of NaOD, ArH at C-2); mass spectrum (70 eV) m/e (rel intensity) 148 (100), 147 (66), 146 (18), 145 (19), 131 (15), 122 (15), 121 (14), 119 (61), 92 (24), and 65 (17). Anal. ($C_9H_{13}Cl_2N_3O$) C, H, N, Cl.

(*R,S*)-1-[5(6)-Benzimidazolyl]-2-isopropylaminoethanol (4). To 625 mg (2.5 mmol) of **2**·2HCl in 20 mL of H_2O was added 14 g of Amberlite IR-45 ion-exchange resin (hydroxide form). The suspension was filtered after 10 min, and the H_2O was evaporated under vacuum to yield 426 mg (96%) of **2** as the free base. To this light yellow oil in 50 mL of MeOH was added 500 mg of anhydrous Na_2SO_4 and 3.7 mL (50 mmol) of acetone, followed by 500 mg of 10% Pd/C. This mixture was reduced at 2.8 kg/cm^2 of H_2 for 2 h in a steam-jacketed bottle, cooled, and filtered to remove the catalyst. The solvent was evaporated under vacuum, and the product was converted to its dihydrochloride salt by the addition of 10 mL of 4 N HCl. After evaporating H_2O under vacuum, the product was crystallized from 2-propanol to yield 213 mg (29%) of **4** dihydrochloride: mp 217 °C dec; IR 3500–2700 (s, ν O–H and ν N–H), 1620, 1593, 1485 [m, bands characteristic of a 5(6)-substituted benzimidazole[28]], 1399 and 1382 cm^{-1} (m, *gem*-dimethyl δ C–H); ^1H NMR (D_2O) δ 1.34 [d, 6, J = 6.5 Hz, $CH(CH_3)_2$], 3.45 (d, 2, J = 6.5 Hz, $CHCH_2$), 3.63 [septet, 1, J = 6.5 Hz, $CH(CH_3)_2$], 5.36 (t, 1, J = 6.5 Hz, $CHCH_2$), 7.90 (d of d, 1, J_{AB} = 8.5 Hz, J_{AC} = 1.7 Hz, ArH at C-6), 8.17 (d, 1, J = 8.5 Hz, ArH at C-7), 8.21 (d, 1, J = 1.7 Hz, ArH at C-4), and 9.43 (s, 1, shifts to 8.25 on addition of NaOD, ArH at C-2); mass spectrum (70 eV) m/e (rel intensity) 149 (16), 148 (100), and 147 (26). Anal. ($C_{12}H_{19}Cl_2N_3O$) C, H, N, Cl.

In Vivo Rat Blood Pressure Studies. Male Wistar rats weighing 230–380 g were obtained from Microbiological Associates, Inc., Walkersville, Md. For testing the effects of catecholamine depletion on the responses, some of the rats received reserpine (Sigma Chemical Co.), 2.5 mg/kg ip, 48 and 24 h prior to the study. The rats were anesthetized with urethane (Sigma), 1.1–1.5 g/kg ip. The trachea was cannulated to maintain adequate ventilation. The right carotid artery was cannulated (PE-50 Intramedic polyethylene tubing, Clay-Adams, Inc., New York, N.Y.), and the cannula was connected, using PE-100 tubing, to a Statham P23AC transducer. A Gilson Model K-IC oxygraph with a modified adapter A-4010 was used to monitor arterial blood pressure. Heart rate and pulse pressure were also obtained from this recording. Either the left jugular vein or the right femoral vein was cannulated to be used for all drug injections. Solutions to be injected were prepared fresh daily by dissolving the compounds in saline at a concentration to allow a 0.5 mL/kg bolus injection volume. Each injection was followed by a 0.1-mL flush of heparinized saline. (Total dead volume of the injection cannula was determined to be less than 0.08 mL.) A volume of saline equal to the volume of drug injection was injected at the beginning of each run to allow a correction for the injection artifact.

In Vitro Guinea Pig Studies. Male guinea pigs weighing 400–900 g were obtained from Bar-F-Rabbitry, New Park, Pa. For testing the effects of catecholamine depletion on the responses, some of the guinea pigs were administered reserpine (Sigma Chemical Co.), 5.0 mg/kg ip, 24 h prior to the study. The animals were killed by decapitation with a guillotine (Harvard Instruments Co.). The heart was rapidly removed and placed in a modified Ringer–Locke solution of the following composition in g/L: NaCl, 9.0; KCl, 0.42; $NaHCO_3$, 0.5; $CaCl_2$, 0.12; glucose, 1.0; and ascorbic acid, 0.20. Isolated, spontaneously beating atria were prepared[29] and suspended in a 30-mL tissue bath containing the same Ringer–Locke solution. The bath was oxygenated with a mixture of 95% O_2 and 5% CO_2 and maintained at 37.0 ± 0.5 °C. Contractions were measured isometrically with a Grass FT 03 force-displacement transducer connected to a Grass Model 5D polygraph. The diastolic tension was adjusted to 1 g. The preparation was allowed to beat until a steady rate (counted manually) and force of contraction (measured as the pen displacement per beat) were obtained. This usually required 20 min. At least one dose–response curve each for isoproterenol and **4** was determined on each preparation. The sequence was alternated to ensure that the curves were independent of each other. The volumes of drug doses added to the bath ranged from 7 to 200 μL. The time between doses was 1 min for both compounds. The bath solution was changed between cumulative dose–response curves by draining and refilling with fresh buffer. At least four changes in a 30-min period were allowed for recovery. Each response (both chronotropic and inotropic) was measured as a percentage of the maximum effect produced by 10^{-6} M isoproterenol, added after the last dose of compound.

The tracheae were dissected free of extraneous tissue, and tracheal chains were prepared as described by Timmerman and Scheffer.[30] Each preparation, containing 10–12 cartilage rings, was suspended in a 30-mL tissue bath containing a modified Krebs–bicarbonate solution of the following composition in g/L: NaCl, 6.9; KCl, 0.35; $MgSO_4$, 0.14; KH_2PO_4, 0.16; $NaHCO_3$, 2.1; $CaCl_2$, 0.28; glucose, 2.0; and ascorbic acid, 0.40. The solution was oxygenated with a mixture of 95% O_2 and 5% CO_2 and maintained at 37.0 ± 0.5 °C. Responses were measured isometrically as changes in tension with a Grass FT 03 force-displacement transducer connected to a Grass Model 5D polygraph. After 30-min equilibration, the tension was adjusted to 1 g and the tissue brought into tonic contraction by the addition of 3.0 × 10^{-6} M methacholine to the bath. Ten minutes was allowed to achieve maximum contraction before beginning cumulative dose–response curves. The volumes of drug doses added to the bath ranged from 7 to 200 μL. The time interval between doses was 3 min for isoproterenol and 4 min for **4**. The bath solution was changed between cumulative dose–response curves by draining and refilling with fresh buffer. At least six changes in a 60-min period were allowed for recovery. At least one dose–response curve each for isoproterenol and **4** was determined on each preparation. The sequence was alternated to ensure that the curves were independent of each other. Each response was measured as a percentage of the maximum relaxation produced by 10^{-5} M isoproterenol, added after the last dose of compound.

Spirally cut thoracic aortic strips were prepared by the method of Furchgott and Bhadrakom.[31] Each strip, measuring 3 cm by 2 mm, was suspended in a 30-mL tissue bath containing a modified Krebs solution as described for the tracheal chain preparation. The bath was oxygenated with a mixture of 95% O_2 and 5% CO_2 and maintained at 37.0 ± 0.5 °C. Contractions were measured isometrically as changes in tension with a Grass FT 03 force-displacement transducer connected to a Grass Model 5D polygraph. The initial tension was adjusted to 0.5 g. To achieve maximum sensitivity, each strip was equilibrated in the bath for at least 2 h before beginning cumulative dose–response studies.[31] The volumes of drug doses added to the bath ranged from 7 to 200 μL. After each dose the tissue was allowed to contract until a leveling off of the response was observed. This required 3 min for norepinephrine and 10 min for **2**. The bath solution was changed between cumulative dose–response curves by draining and refilling with fresh buffer. At least four changes over a 1-h period allowed for full recovery of the tissue. One dose–response curve for norepinephrine and one for **2** were obtained on each tissue. The sequence of these curves was alternated to ensure that one compound did not influence the dose–response curve of the other by changes in sensitivity of the tissue or other aftereffects.[32] Each response was measured as a percentage of the maximum contraction produced by 10^{-4} M norepinephrine, added after the last dose of compound.

Solutions of isoproterenol were prepared fresh daily in saline containing 0.04% ascorbic acid and were stored at 4 °C. Solutions of **4** were prepared in saline and gave consistent responses after storage for 1 week at 4 °C. Solutions of norepinephrine were prepared in saline containing 0.04% ascorbic acid and were made fresh for each dose–response curve from a stock solution at pH 3, which was prepared fresh daily and stored at 4 °C. Solutions of **2** were prepared in saline and gave consistent responses after storage for 2 weeks at 4 °C. (*R,S*)-Isoproterenol hydrochloride

was obtained from Sigma Chemical Co. Propranolol hydrochloride (Inderal HCl) was supplied by Ayerst Laboratories, Inc. (*R*)-Norepinephrine was obtained from Sigma Chemical Co. Phentolamine hydrochloride (Regitine HCl) was supplied by CIBA Pharmaceuticals. Methacholine chloride (Mecholyl) was obtained from Merck and Co.

References and Notes

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Antidepressant Agents. 9.[1] 3,3-Diphenylcyclobutylamines, a New Class of Central Stimulants

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3,3-Diphenylcyclobutylamine (**4**), *N*-methyl-3,3-diphenylcyclobutylamine (**6**), and *N*,*N*-dimethyl-3,3-diphenylcyclobutylamine (**7**) have been prepared and tested as potential antidepressant agents. The secondary (**6**) and tertiary (**7**) amines strongly decrease the accumulation of NA and 5-HT in brain slices in vitro and in vivo. The cyclobutylamines also cause motor stimulation. The most potent compound in this respect is the tertiary amine **7**. The increase in locomotion is not blocked by pretreatment with phenoxybenzamine, methergoline, or α-methyltyrosine. Pretreatment with pimozide or reserpine reduces the hyperactivity induced by **7**. This hyperstimulation seems to be caused by a mechanism of action which differs from that of amphetamine. **7** may cause increase in locomotion by release of dopamine from granular stores.

In a research program aiming at new antidepressant agents, we have investigated compounds showing an inhibitory activity on the neuronal accumulation of noradrenaline (NA) and of 5-hydroxytryptamine (5-HT). The preparation and pharmacological effects of a series of diphenylcycloalkylamines, e.g., 3,3-diphenylcyclopentylamines, 3,3- and 4,4-diphenylcyclohexylamines,[2] and 4,4-diphenyl-1-methylcyclohexylamines,[3] were recently reported. We have now included the four-membered ring analogues in our investigation and wish to report the synthesis and some pharmacological properties of 3,3-diphenylcyclobutylamines of general structure **1**.

The new compounds have been examined for reduction of the accumulation of NA, dopamine (DA), and 5-HT in mouse brain slices. The behavioral and toxicological effects of these compounds, including the interaction with 5-hydroxytryptophan (5-HTP), have also been studied in mice. Furthermore, an experimental comparison of the

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