# Synthesis and Biological Activities of a New Set of Irreversibly Acting 2-(4'-Isothiocyanatobenzyl)imidazoline Analogs in Rat Thoracic Aorta

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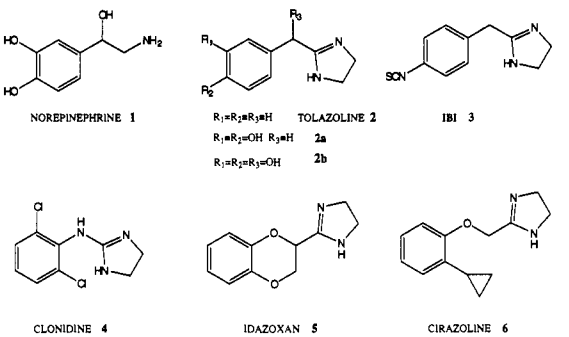
IBI [2-(4'-isothiocyanatobenzyl)imidazoline, **3**] has been shown to cause slow-onset, long-lasting contractions of rat thoracic aorta through a non-$\alpha$-adrenergic receptor (non-$\alpha$-AR) mediated mechanism. A series of IBI-related anlogs **7–14** and **16** was prepared to determine the structural requirements for the interaction with non-$\alpha$-AR in rat aortic strips. All IBI analogs produced concentration-dependent contractile responses on rat thoracic aorta. Whereas the actions of analogs **7**, **14**, and **16** were partly mediated by $\alpha$-ARs, the stimulatory activities of the remaining IBI analogs were unaffected by phenoxybenzamine pretreatment, suggesting that a non-$\alpha$-adrenergic mechanism is involved. We have shown that the contractile actions of IBI and analogs **10–13** were not blocked with the imidazoline/guanidinium receptive site (IGRS) ligands idazoxan, cirazoline, or clonidine. However, the calcium channel blockers nifedipine or verapamil shifted the concentration–response curve of IBI and its analogs **10–13** to the right and reduced the maximal contractile responses. The action of IBI on rat thoracic aorta was reduced by the omission of extracellular calcium in the medium. These results suggest that the stimulatory activities of IBI and analogs **10–13** are not related to the activation of $\alpha$-AR or IGRS receptors and are likely coupled to the voltage-dependent $Ca^{2+}$ channels.

## Introduction

It has been proposed that the two main classes of direct-acting $\alpha$-sympathomimetic drugs, phenethylamines (**1**) and imidazolines (**2**) (Chart 1), do not interact with $\alpha$-adrenergic receptors ($\alpha$-AR) in an identical manner. One of the first indications for a different mode of interaction at the $\alpha$-adrenergic receptors was a lack of cross desensitization between $\alpha$-agonists of the imidazoline and phenethylamine classes.[1] The major difference between these two classes of compounds is the lack of conformity with the Easson–Stedman hypothesis.[2] While the optically active phenethylamines follow the Easson–Stedman hypothesis and appear to interact with the $\alpha$-AR via three-point attachment (relative order of potency *R*-(−)-isomer > *S*-(+)-isomer = desoxy analog), the optically active imidazolines do not follow the Easson–Stedman hypothesis and interact with the receptor by only a two-point attachment (the order of potency desoxy analog > *R*-(−)-isomer > *S*-(+)-isomer).[3,4] These findings suggest the presence of different interacting sites on the $\alpha$-AR for compounds of the imidazoline and phenethylamine classes.

Recently, site-directed-mutagenesis experiments with $\alpha_2$-AR have identified an $Asp^{113}$ in transmembrane domain III and $Cys^{201}$ and $Ser^{204}$ in transmembrane domain V as potential sites of interaction with the charged nitrogen moiety and the catechol hydroxyl groups, respectively, of the phenethylamine class of compounds.[5] Furthermore, it has been proposed that similar to the phenethylamines the positively charged imidazoline ring of 2-substituted imidazolines interacts with $Asp^{113}$ in a similar manner. However, the remaining functional groups of imidazolines are thought to interact with $\alpha$-AR in a different manner.[5]

**Chart 1**




Affinity and photoaffinity probes have become one of the key approaches for studying the receptor binding sites. Designing affinity label probes of the imidazoline type for the $\alpha$-AR could provide useful information about the interaction of imidazolines with $\alpha$-AR. The preparation and effects of several affinity labels for the $\alpha$-AR have already been described in the literature and include (*N*-$\beta$-chloroethyl-*N*-methylaminomethyl)clonidine [(chloroethyl)clonidine, CEC], *p*-isothiocyanato and *p*-methylisothiocyanato clonidine.[6–8] To extend these studies a selective $\alpha$-adrenergic site-directed affinity probe 2-(4'-isothiocyanatobenzyl)imidazoline (IBI, **3**), the structure of which is based on tolazoline (**2**) rather than clonidine (**4**), was synthesized. The objective of this study is to investigate if 2-benzyl-substituted imidazolines behave in a manner similar to 2-aminoimidazolines like clonidine (**4**) (Chart 1). Interestingly, IBI produced a slow-onset and sustained contraction of rat thoracic aorta, with an $ED_{50}$ value of $1.63 \times 10^{-5}$ M.[9] However, these IBI-dependent effects were not blocked by the $\alpha_1$-AR blockers (phentolamine, prazosin), an $\alpha_2$-AR blocker (yohimbine), or irreversibly-acting $\alpha$-AR blockers [phenoxybenzamine (PBZ), CEC].[9,10] These results suggest that IBI does not interact with $\alpha$-AR in rat aorta and mediates long-lasting

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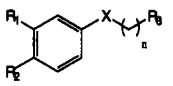
**Table 1.** Comparison of Maximal Contractile Tension Changes and Potencies of IBI and Analogs with and without Phenoxybenzamine (PBZ, 30 nM for 20 min) Pretreatment on Rat Thoracic Aorta Strips[a]



| compd | $R_1$ | $R_2$ | X | *n* | $R_3$ | $\%E_{max}$[b] control | $\%E_{max}$[b] +PBZ | $EC_{50}$ ($\mu$M)[c] control | $EC_{50}$ ($\mu$M)[c] +PBZ |
|---|---|---|---|---|---|---|---|---|---|
| **3** | H | NCS | $CH_2$ | 0 |  | 118 ± 12 | 100 ± 7 | 48 ± 7 | 52 ± 11 |
| **7** | H | $CH_3$ | $CH_2$ | 0 |  | 14 ± 3 | 5 ± 6 | 57 ± 5 | *e* |
| **8** | H | $NHCOCH_2Cl$ | $CH_2$ | 0 |  | 41 ± 14 | 32 ± 1 | 116 ± 9 | 129 ± 39 |
| **9** | H | $NHCOCH_2I$ | $CH_2$ | 0 |  | 25 ± 6 | 13 ± 7 | 47 ± 3 | *e* |
| **10** | H | NCS | $CH_2$ | 1 |  | 107 ± 2 | 102 ± 12 | 29 ± 7 | 29 ± 1 |
| **11** | H | NCS | O | 1 |  | 108 ± 7 | 117 ± 13 | 69 ± 41 | 47 ± 8 |
| **12** | H | NCS | $CH_2$ | 0 |  | 70 ± 6 | 68 ± 3 | 31 ± 8 | 36 ± 12 |
| **13** | H | NCS | $CH_2$ | 1 | $N(CH_3)_2$ | 98 ± 2 | 113 ± 10 | 41 ± 4 | 33 ± 6 |
| **14** | I | NCS | $CH_2$ | 0 |  | 31 ± 6 | 28 ± 6 | 10 ± 6 | 58 ± 5[d] |
| **16** | H | $NH_2$ | $CH_2$ | 0 |  | 69 ± 4 | 9 ± 3 | 3 ± 1 | 31 ± 20 |

[a] Tissues were pretreated with PBZ ($3 \times 10^{-8}$ M) as described in the methods. Analog-induced responses were monitored between 20 and 30 min after addition. [b] $\%E_{max}$ = percent of maximal contractile response to IBI analog, expressed relative to the maximal contraction produced by 30 $\mu$M phenylephrine. [c] Data represent the mean ± SEM of $n$ = 4–7. $EC_{50}$ value = drug concentration that produces 50% of the maximal response ($\mu$M). [d] Means are statistically different from the corresponding control value (IBI analog alone) at $p < 0.01$. [e] Not calculated. Maximal response was about 5–13% of the control preparation, and the $EC_{50}$ in PBZ-pretreated preparation cannot be determined accordingly.

contractions of aorta through a non-$\alpha$-AR mechanism. In this regard, preliminary experiments conducted by Wilson and Kendall[11] indicated that IBI competes with [$^3$H]-idazoxan for $\alpha_2$-AR and imidazoline-preferring non-$\alpha$-adrenergic sites of guinea pig cortex membrane.

It has been documented that imidazoline-containing compounds clonidine (**4**), idazoxan (**5**), and cirazoline (**6**) (Chart 1) interact not only with $\alpha$-AR but also with imidazoline, preferring sites in central and peripheral tissues.[12-16] Parini et al.[17] isolated a membrane receptor protein from rabbit renal cortex, which they call the imidazoline/guanidinium receptive site (IGRS), and separated it from $\alpha$-AR by heparin-agarose affinity chromatography. Wang et al.[18] isolated and partially purified imidazoline receptor protein from bovine adrenal chromaffin cells by affinity chromatography using imidazoline agents (e.g. idazoxan or *p*-aminoclonidine) as ligands. In addition to localization in plasma membranes, IGRS is found in high density in the outer mitochondrial membrane.[19] Limon el al.[20] have reported the purification of mitochondrial IGRS from rabbit kidney.

In the light of recent studies, subclassification of imidazoline receptors has been postulated.[21] Two distinct imidazoline binding sites have been identified using [$^3$H]-clonidine or [$^3$H]-*p*-aminoclonidine and [$^3$H]idazoxan.[22] There is speculation that the imidazoline-preferring receptor site may be involved in regulation of blood pressure; however, the physiological function of imidazoline binding sites has yet to be determined.[23]

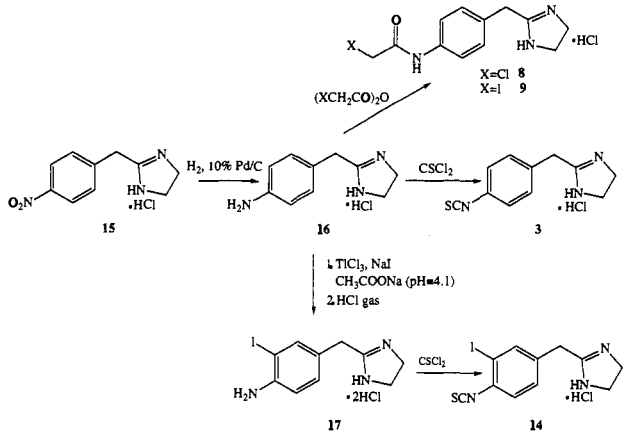
On the basis of the results of Wilson and Kendall[11] and the structural similarity between IBI and idazoxan, clonidine, and cirazoline, we proposed that the non-$\alpha$-adrenergic activity of IBI in rat aorta was related to the interaction of IBI with the imidazoline/guanidinium receptor site.

In the present study, a limited number of IBI-related analogs (Table 1) were prepared to determine the structural requirements for the interaction with non-$\alpha$-adrenergic receptors in rat thoracic aorta. Three types of chemical modifications were proposed: (1) modification of the imidazoline ring, (2) modifications of the electrophilic group, and (3) modifications of the distance between the aromatic and imidazoline rings. It was though that these studies would help us find highly potent and selective affinity labels for characterizing non-$\alpha$-AR. Subsequently, this work led to our discovery that IBI and related compounds do not mediate contraction of smooth muscle through the IGRS receptor but rather through a $Ca^{2+}$-dependent mechanism involving voltage-dependent $Ca^{2+}$ channels.

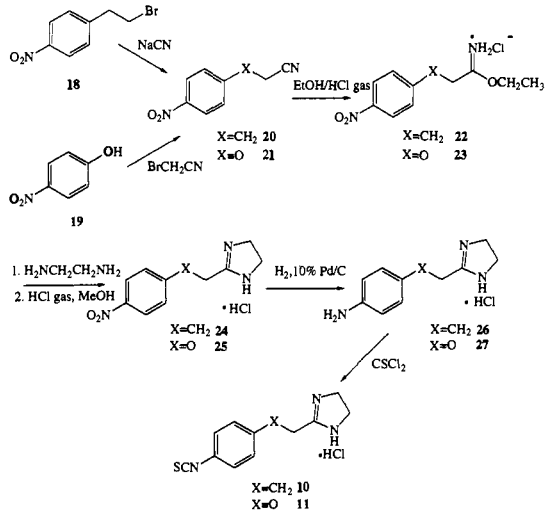
## Chemistry

The synthesis of IBI **3** is outlined in Scheme 1. The starting material 2-(4′-nitrobenzyl)imidazoline (**15**) was synthesized according to the procedure described previ-

**Scheme 1**



**Scheme 2**



ously by Cavallini et al.[24] Catalytic hydrogenation (10% palladium on carbon in methanol) of **15** afforded 2-(4′-aminobenzyl)imidazoline (**16**) which was treated with $CSCl_2$ to give IBI (**3**).[25] Compound **16** also served as a precursor for compounds **8**, **9**, and **14** (Scheme 1).

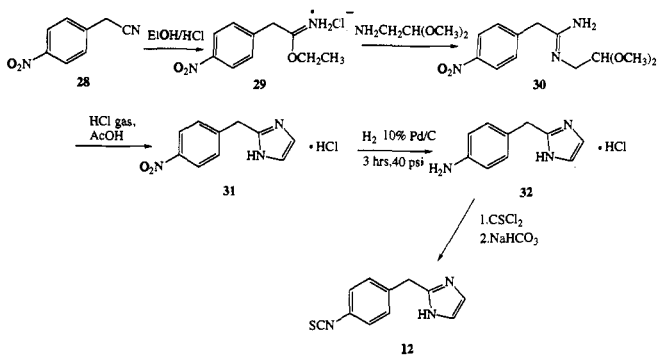
Stirring **16** in acetonitrile with chloroacetic or iodoacetic anhydride resulted in formation of 2-(4′-chloroacetamidobenzyl)imidazoline (**8**) or 2-(4′-iodoacetamidobenzyl)-imidazoline (**9**), respectively.

Iodination of **16** was performed according to the procedure for iodination of β-(4-aminophenyl)ethylamine described by Ruoho.[26] Heating a mixture of **16**, thallium trichloride, and sodium iodide in sodium acetate buffer (pH = 4.1) gave the desired 2-(4′-amino-3′-iodobenzyl)-imidazoline (**17**). Treatment of the imidazoline **17** with $CSCl_2$ gave **14**.

Compound **7**, 2-(4′-methylbenzyl)imidazoline, was synthesized according to the procedure previously described by Sonn.[27]

The synthesis of compounds **10** and **11** is outlined in Scheme 2. Treatment of 4′-nitrophenethyl bromide (**18**) with NaCN in EtOH, gave crude nitrile **20**.[28] Nitrile **21** was obtained by refluxing 4-nitrophenol (**19**), $K_2CO_3$, and bromoacetonitrile in acetone for 20 h.[29,30] Using the Pinner method,[31] nitriles **20** and **21**, in the presence of an anhydrous EtOH and HCl gas, were converted to the corresponding imidates **22** and **23**, respectively. Refluxing the imidates **22** and **23** with ethylendiamine in EtOH overnight gave imidazolines **24** and **25**, respectively, as

**Scheme 3**



free bases, which were then converted into monohydrochloride salts by treatment with HCl gas. Reduction of the nitro group of **24** and **25** by catalytic hydrogenation gave 2-(4′-aminophenethyl)imidazoline (**26**) and 2-[(4′-aminophenoxy)methyl]imidazoline (**27**), respectively. Treatment of **26** and **27** with $CSCl_2$ gave **10** and **11**, respectively. Compound **25** has been reported in literature as an insecticide and acaricide.[32]

Imidazole **12** was synthesized from 4′-nitrophenylacetonitrile (**28**) as outlined in Scheme 3. Reaction of **28** with anhydrous EtOH and HCl gas provided imidate **29**,[31] which was then treated with 2-aminoacetaldehyde dimethyl acetal to afford amidine **30**.[33,34] Addition of acetic acid and anhydrous HCl gas to **30** resulted in the formation of imidazole **31**, which precipitated from the reaction mixture. In this particular case the amount of HCl gas added does not make a significant difference, because of the electron-withdrawing nature of the nitro group in the para position. However, it was found that compounds with an electron-donating group para to the point of cyclization give aminobenzazepines if enough acid is present.[34] Catalytic hydrogenation (10% Pd–C in MeOH) of **31** afforded **32**, which was treated with $CSCl_2$ to give imidazole **12**.

The tertiary amine **13** was synthesized by treatment of *N*,*N*-dimethyl-2-(4-aminophenyl)ethylamine with $CSCl_2$ in acetone.[25] *N*,*N*-Dimethyl-2-(4-aminophenyl)ethylamine was synthesized as previously described from 4-nitro-β-haloethylbenzene and 40% aqueous $Me_2NH$ in EtOH, to give *N*,*N*-dimethyl-2-(4-nitrophenyl)ethylamine, which upon hydrogenation gave corresponding amine.[35] An alternative route to *N*,*N*-dimethyl-2-(4-aminophenyl)-ethylamine is Eschweiler–Clarke methylation of 4-nitrophenethylamine and subsequent hydrogenation to afford *N*,*N*-dimethyl-2-(4-aminophenyl)ethylamine.[36]

## Results and Discussion

**1. Effects of PBZ Pretreatment on the Contractile Activities of IBI Analogs on Rat Aorta.** All IBI analogs produced concentration-dependent contractile responses on rat thoracic aorta in a manner similar to that of IBI characterized by a slow onset and long duration of action. Table 1 summarizes the vascular effects of PBZ pretreatment on action of the IBI analogs. Pretreatment of rat thoracic aorta with 30 μM PBZ alkylates α-AR and the preparation was insensitive to α-AR ligands.[37] With the exception of analogs **7**, **14**, and **16**, the stimulatory activities of the remaining IBI analogs were unaffected by PBZ pretreatment (Table 1). These results suggest that a non-α-AR-mediated mechanism is involved for initiation of contractile responses by all analogs other than **7**, **14**, and

16. With the latter analogs, the replacement of 4'-NCS group in IBI with 4'-amino group 16 or 4'-methyl group 7 altered the stimulatory activity of IBI from a non-$\alpha$-AR-mediated to an $\alpha$-AR-mediated mechanism (Table 1). Compound 7 has previously been studied for its affect on blood pressure.[38] Introduction of an iodo atom in the 3' position on the aromatic ring of IBI led to analog 14; however, this compound shows mixed activity by interacting with both $\alpha$- and non-$\alpha$-AR sites in rat aorta. Thus, it appears that 14 will not be useful as a potential radioaffinity label for studying the non-$\alpha$-AR receptor sites in rat aorta. Lanier et al.[39] have recently prepared the photoaffinity label 2-[[3-azido-4-[$^{125}$I]iodophenoxy]-methyl]imidzoline (a cirazoline analog) and used it to covalently label the binding subunits of IGRS in various tissues such as kidney, brain, and liver. This photoaffinity label was found to be incorporated into two major peptides with apparent molecular weights of 55 and 61 kDa.[39]

Changing the electrophilic group from an isothiocyanato to a haloacetamido gave compounds 8 and 9. They produced very weak contractile responses (8, $EC_{50}$ = 116 $\mu$M with the maximal response only 40% relative to that caused with 30 $\mu$M phenylephrine); however, the affinity of 8 and 9 was not significantly affected by PBZ pretreatment. A possible explanation for the low activity of compounds 8 and 9 is that the highly reactive electrophile is needed for activity or the distance between the aromatic ring and the electrophile is important.

To examine the significance of the imidazoline ring on the action of IBI in rat aorta, the imidazoline was replaced with an imidazole ring in analog 12 and with tertiary amino group in compound 13. Both compounds produced a concentration-dependent response similar to that of IBI in rat aorta and their responses were not affected by the PBZ pretreatment. This finding suggests that the non-$\alpha$-AR-mediated contractile responses in smooth muscle are not soely due to the presence of an imidazoline ring, but rather the positive charge or potential of H-bonding is important for the production of contraction by IBI analogs in aortic smooth muscle. Furthermore, the data indicate that the maximal response but not potency of analog 12 is less than that of IBI (Table 1). This may be due to a conformational and/or an electronic factor. First, the imidazole ring is flat relative to the imidazoline ring due to the presence of an additional double bond, and secondly, the $pK_a$ of the imidazole group is lower than the $pK_a$ of the imidazoline, resulting in a smaller proportion of the protonated form of 12 at physiological pH.

In a search for the optimum distance between the imidazoline ring and the aromatic ring, we prepared 10 and 11. These compounds incorporate structural features of IBI, idazoxan, and cirazoline and they produced IBI-like responses which were not significantly blocked by PBZ pretreatment of rat aortic tissues. It appears that modification of the bridge does not greatly improve the non-$\alpha$-AR activity of IBI.

**2. Effect of Imidazoline Ligand (Idazoxan, Cirazoline) Pretreatment on Stimulatory Activities of IBI and Analogs 10–13 on Rat Thoracic Aorta.** Since Wilson and Kendall[11] have shown that IBI competes with [$^3$H]idazoxan for imidazoline-preferring non-$\alpha$-AR sites of guinea pig cortex membrane, we presumed that the non-$\alpha$-AR activity of IBI in rat aorta might be related to the interaction of IBI with the IGRS. To test this hypothesis, idazoxan or cirazoline were added to PBZ-pretreated rat aortic tissues, prior to the addition of IBI. In neither case did idazoxan (at 0.1 or 1 $\mu$M) or cirazoline (3 $\mu$M) affect the contractile activity of IBI on rat aorta in PBZ-pretreated tissues (Table 2). Furthermore, Table 2 summarizes the effect of cirazoline and idazoxan on the action of selected analogs 10–13 in rat aorta. These four compounds were chosen since they showed an affinity and intrinsic activity similar to IBI in PBZ-treated aortic strips. For these compounds no significant change in the concentration–response curve was observed in PBZ-pretreated tissues in the presence of cirazoline or idazoxan. These results show that non-$\alpha$-AR stimulatory activities of IBI and analogs 10–13 in rat aorta are not related to the activation of IGRS.

Table 2. Effect of Imidazole Ligands (Cirazoline and Idazoxan) on Contractile Responses of IBI and Its Analogs 10–13 in Rat Thoracic Aorta

| treatment | $n$ | $EC_{50}$ ($\mu$M)[c] | $\%E_{max}$[d] |
|---|---|---|---|
| IBI | 4 | 45.0 ± 3.0 | 100 ± 4 |
| IBI + PBZ + CIR[a] | 4 | 43.0 ± 6.0 | 94 ± 3 |
| IBI + PBZ + IDA (0.1 $\mu$M)[a] | 3 | 54.0 ± 3.0 | 104 ± 6 |
| IBI + PBZ + IDA (1 $\mu$M)[a] | 3 | 54.0 ± 6.0 | 105 ± 9 |
| 10 | 4 | 18.3 ± 0.7 | 100 ± 11 |
| PBZ + CIR + 10[b] | 3 | 14.9 ± 1.3 | 100 ± 13 |
| PBZ + IDA + 10[b] | 3 | 18.1 ± 0.6 | 114 ± 13 |
| 11 | 3 | 17.2 ± 0.9 | 100 ± 12 |
| PBZ + CIR + 11[b] | 3 | 16.4 ± 1.3 | 117 ± 21 |
| PBZ + IDA + 11[b] | 3 | 16.1 ± 1.1 | 110 ± 29 |
| 12 | 3 | 28.6 ± 11.7 | 100 ± 23 |
| PBZ + CIR + 12[b] | 3 | 25.5 ± 8.6 | 102 ± 32 |
| PBZ + IDA + 12[b] | 3 | 40.9 ± 9.5 | 79 ± 13 |
| 13 | 4 | 16.8 ± 0.2 | 100 ± 9 |
| PBZ + CIR + 13[b] | 3 | 16.9 ± 0.0 | 91 ± 6 |
| PBZ + IDA + 13[b] | 3 | 16.3 ± 0.6 | 94 ± 9 |

[a] Tissues were pretreated as follows: 30 nM PBZ for 20 min with washout followed by 3 $\mu$M cirazoline (CIR) for 1 h; 30 nM PBZ for 20 min washout followed by 0.1 or 1 $\mu$M idazoxan (IDA) for 1 h, as compared to 30 $\mu$M phenylephrine (100% maximal response) which was obtained prior to these treatments. IBI-induced responses were monitored between 20 and 30 min after addition. [b] Tissues were pretreated with PBZ ($10^{-8}$ M, 20 min) with washout followed by CIR ($10^{-6}$ M, 60 min) or IDA ($10^{-6}$ M, 60 min) as described in the methods. Analog-induced responses were monitored between 35 and 45 min after each addition. [c] Data represent the mean ± SEM of $n$ = 3–4. $EC_{50}$ value = drug concentration that products 50% of the maximal response ($\mu$M). [d] $\%_{Emax}$ = percent of maximal contractile response to IBI, expressed relative to the maximal contraction produced by the compound alone.

Binding studies carried out using [$^3$H]-$p$-aminoclonidine, [$^3$H]idazoxan, and [$^3$H]clonidine have shown the presence of putative IGRS in different tissues and species with the highest abundance in the brain, liver, and kidney.[13,19,40] Tesson et al.[41] have studied the distribution of the IGRS in different rabbit and human tissues. They could not detect any [$^3$H]idazoxan binding in heart homogenate, in neither rabbit nor human heart.[41] Only a very low density of IGRS was detected in mitochondrial fraction from rabbit and human heart.[41] We conclude that rat heart is relatively deficient of IGRS. If rat thoracic aorta also lacks IGRS, then this is the reason why our compounds cannot exert their contractile action through activation of IGRS. However, we plan to test our compounds in tissues rich with IGRS (brain, liver, and kidney).

We were curious as to the mechanism of action of IBI and its analogs in the heart. First, we tried to block the activity of IBI and its analogs in rat aorta with a variety of compounds including cinanserin (5-$HT_2$ blocker), cimetidine ($H_2$-histamine blocker), pyrilamine ($H_1$-histamine blocker), and 4-aminopyridine ($K^+$ channel block-

**Table 3.** Effect of Various Treatments on the Concentration-Dependent Responses to IBI and Selected IBI Analogs (10–13) in Rat Thoracic Aorta[a]

| treatment | $n$ | $EC_{50}$ ($\mu$M)[b] | $\%E_{max}$[c] |
|---|---|---|---|
| IBI | 4 | 5.8 ± 1.9 | 100 ± 9 |
| verapamil + IBI | 4 | 21.4 ± 9.7[d] | 48 ± 10[d] |
| nifedipine + IBI | 3 | 19.8 ± 8.7[d] | 42 ± 12[d] |
| $Ca^{2+}$ deficient buffer + IBI | 4 | 12.7 ± 0.3[d] | 34 ± 9[d] |
| 10 | 3 | 18.3 ± 0.7 | 100 ± 11 |
| nifedipine + 10 | 3 | 67.0 ± 16.5[d] | 21 ± 6[d] |
| 11 | 3 | 17.2 ± 0.9 | 100 ± 12 |
| nifedipine + 11 | 3 | 52.1 ± 0.0[d] | 11 ± 6[d] |
| 12 | 3 | 28.6 ± 11.7 | 100 ± 23 |
| nifedipine + 12 | 3 | 38.9 ± 13.2 | 35 ± 5[d] |
| 13 | 4 | 16.8 ± 0.2 | 100 ± 9 |
| nifedipine + 13 | 3 | 48.5 ± 6.1[d] | 29 ± 9[d] |

[a] Tissues were pretreated with verapamil ($10^{-6}$ M, 60 min), nifedipine ($10^{-6}$ M, 60 min), or calcium-deficient PSS as described in the methods. Analog-induced responses were monitored between 35 and 45 min after each addition. [b] Data represents the mean ± SEM of $n$ = 3–4, $EC_{50}$ value = drug concentration that produces 50% of the maximal response ($\mu$M). [c] $\%E_{max}$ = percent of maximal contractile response to IBI analog, expressed relative to the maximal contraction produced by the compound alone. [d] Means are statistically different from the corresponding control value (IBI analog alone) at $p < 0.05$ (paired observations).

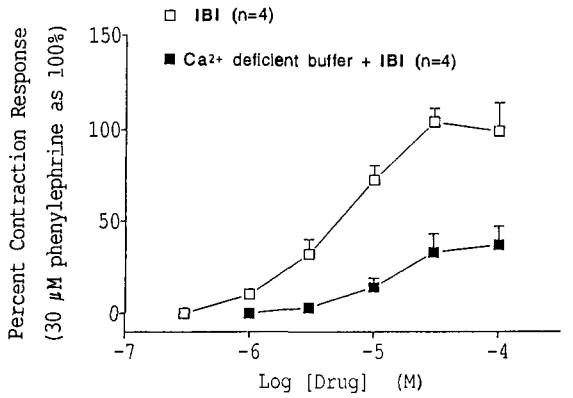



**Figure 1.** The action of IBI on rat thoracic aorta in PSS (physiological salt solution) as a control (□) and in $Ca^{2+}$-deficient buffer (■).

er) (data not shown). However, the agonist effect of IBI and related analogs is not mediated by these different receptor systems.

**3. Effect of $Ca^{2+}$ Channel Blocker (Nifedipine, Verapamil) Pretreatment on the Stimulatory Activities of IBI and Analogs 10–13 on Rat Thoracic Aorta.** Pretreatment of rat aortic tissues with nifedipine ($10^{-7}$ M) or verapamil ($10^{-6}$ M) shifted the concentration response curve of IBI as well as its analogs **10–13** to the right and reduced the maximal contractile response (Table 3).[9] It appears that the effect of IBI and analogs **10–13** depends on a calcium-sensitive mechanism. Furthermore, when IBI was incubated with rat aortic strips in $Ca^{2+}$-deficient media, the concentration–response curve was shifted to the right with a marked reduction of the maximal response (35% of control, Table 3, Figure 1). This is an indication that the contractile action of IBI in rat aorta is mainly dependent on extracellular calcium and the translocation of extracellular calcium through a voltage-dependent $Ca^{2+}$ channel. Does IBI activate $Ca^{2+}$ channels directly or does it act via receptors coupled to membrane $Ca^{2+}$ channels? In this regard, $\alpha_1$- and $\alpha_2$-AR are examples of receptor systems coupled to membrane $Ca^{2+}$ channels, and activation of these receptors involves the translocation of extracellular $Ca^{2+}$ through channels.[42] Since pretreatment of the rat aortic tissues with these receptor blockers still failed to block the effect of IBI and its analogs **10–13**, we suggest that these analogs do not act via receptors copuled to membrane $Ca^{2+}$ channels. At the moment we are exploring a possibility that IBI and its analogs exert their action, in rat aortic tissue, by activating $Ca^{2+}$ channels directly. We compared the structure of IBI and its analog **10** with the $Ca^{2+}$ channel blocker nifedipine[43] and with the $Ca^{2+}$ channel activator (agonist) Bay K 8644[43] using molecular modeling (Sybyl) (not shown) in order to find out if there is any possibility that IBI and **10** can accommodate the 1,4-dihydropyridine active site of the $Ca^{2+}$ channel. The reason for the selection of the 1,4-dihydropyridine system was that Bay K 8644 and closely related molecules were the only activators of $Ca^{2+}$ channels and IBI and its analogs also act as activators of $Ca^{2+}$ channel. Very recently a new set of competitive $Ca^{2+}$ channel agonists have been reported.[44]

The structures of 1,4-dihydropyridines (nifedipine and Bay K 8644) and IBI are chemically quite different. However, certain structural similarities are noted: all compounds are comprised of an aromatic ring and a N–H group as a part of either the 1,4-dihydropyridine ring or the imidazoline ring. The results of superposition (not shown) indicate that the aromatic rings of Bay K 8644 or nifedipine are in the same plane with aromatic ring of IBI or that of **10**. Another striking feature is the N atom overlay of the hydrogen-bond-donor group $N^1$–H in dihydropyridines with one of the N–H groups in the 2-substituted imidazolines. The only feature that is missing in IBI and analog **10** that is present in both nifedipine and Bay K 8644 is a hydrogen-bond-acceptor group (ester or nitro group). In contrast, IBI and analog **10** have an additional hydrogen bond donor group (that is another N–H group).

These results depict that IBI and its analog **10** are able to mimic, to a reasonable extent, the spacial orientation of calcium channel ligands (Bay K 8644 as well as nifedipine), and therefore there is a distinct possibility that IBI and its analogs exert their action in rat aorta by increasing the influx of extracellular $Ca^{2+}$ through the activation of $Ca^{2+}$ channels. At the moment we are conducting biological studies that will test this hypothesis.

In summary, the data presented demonstrates that IBI and related 4-substituted isothiocyanato analogs **10–13** produce concentration-dependent contractions of rat thoracic aorta via a non-$\alpha$-adrenergic-mediated mechanism. Furthermore, we have shown that the functional effects observed in rat aorta are not related to the activation of the IGRS receptors, but likely coupled to the voltage dependent $Ca^{2+}$ channels.

## Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared data were collected on a Analect RFX-40 FTIR spectrophotometer. The NMR spectra were recorded on an IBM AF-250 FTNMR spectrometer (250 Hz) and are reported in pats per million. Mass spectra were obtained at the College of Pharmacy by use of a Kratos MS25 RFA mass spectrophotometer or at the Ohio State University Campus Chemical Instrumentation Center by use of a VG 70-250S or a Kratos MS-30 mass spectrometer. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN, and were within ±0.4% of the theoretical values for the elements indicated. All solvents were dried and/or purified prior to use.

**2-(4'-Aminobenzyl)imidazoline Hydrochloride (16).** A mixture of 2-(4'-nitrobenzyl)imidazoline hydrochloride (**15**) (5.00 g, 21 mmol) and 10% Pd/C (0.50 g) in MeOH (50 ml) was hydrogenated using a Parr apparatus at room temperature, 40 psi, for 4 h. The resulting mixture was filtered over Celite and evaporated to give white residue, which was recrystallized from MeOH–$Et_2O$ to afford 4.09 g (93%) of **16** as colorless crystals: mp 217–218 °C; $^1H$ NMR ($CD_3OD$) $\delta$ 7.06–7.02 (d, $J_{AB}$ = 8.5 Hz, 2H, ArH), 6.72–6.69 (d, 2H, ArH), 3.89 (s, 4H, 2 × $CH_2$), 3.71 (s, 2H, $CH_2$); IR (KBr, $cm^{-1}$) 3400, 3320, 1605. Anal. ($C_{10}H_{14}ClN_3 \cdot 0.5H_2O$) C, H, N.

**2-(4'-Isothiocyanatobenzyl)imidazoline Hydrochloride (3).** To a solution of $CSCl_2$ (5.43 g, 47 mmol) in acetone (30 mL) was added 2-(4'-aminobenzyl)imidazoline (**16**) (1.00 g, 5 mmol) in $H_2O$ (10 mL) dropwise over 10 min, while cooling in an ice–water bath. The stirring was continued at room temperature for additional 1.5 h. Removing the solvent *in vacuo* at room temperature gave a brown solid residue which was taken into $CH_2Cl_2$ (50 mL), washed with $H_2O$, and dried over $Na_2SO_4$. The resulting $CH_2Cl_2$ solution was concentrated under reduced pressure to afford a viscous oil which was crystallized with acetone. Recrystallization from acetone–$Et_2O$ gave white crystals (0.51 g, 43%): mp 153–155 °C; $^1H$ NMR ($CDCl_3$), TMS) $\delta$ 7.67–7.64 (d, $J_{AB}$ = 8.2 Hz, 2H, ArH), 7.11–7.08 (d, 2H, ArH), 4.10 (s, 2H, $CH_2$), 3.87 (s, 4H, 2 × $CH_2$); IR (KBr, $cm^{-1}$) 3060, 2175, 2104, 1618. Anal. ($C_{11}H_{12}ClN_3S$) C, H, N.

**2-[4'-(Chloroacetamido)benzyl]imidazoline Hydrochloride (8).** To a suspension of 2-(4'-aminobenzyl)imidazoline hydrochloride (**16**) (0.20 g, 0.9 mmol) in $CH_3CN$ (15 mL) was added a solution of chloroacetic anhydride (0.19 g, 1.1 mmol) in acetonitrile (5 mL). The mixture was stirred overnight at room temperature and then concentrated under reduced pressure, yielding white solid. Recrystallization from MeOH–$Et_2O$ afforded **8** (0.19 g, 73%) as white needles: mp 214.6–216 °C; $^1H$ NMR ($CD_3OD$) $\delta$ 7.64–7.61 (d, $J_{AB}$ = 8.6 Hz, 2H, ArH), 7.33–7.30 (d, 2H, ArH), 4.18 (s, 2H, $CH_2$), 3.91 (s, 4H, 2 × $CH_2$), 3.86 (s, 2H, $CH_2$); IR (KBr, $cm^{-1}$) 3113, 1683; MS *m/z* 251/253 ($M^+$ – HCl), 250 (base). Anal. ($C_{12}H_{15}Cl_2N_3O$) C, H, N.

**2-[4'-(Iodoacetamido)benzyl]imidazoline Hydroiodide (9).** To a suspension of 2-(4'-aminobenzyl)imidazoline hydrochloride (**16**) (0.53 g, 2 mmol) in $CH_3CN$ (20 mL) was added a solution of iodoacetic anhydride (1.00 g, 2 mmol) in $CH_3CN$ (5 mL). The mixture was stirred overnight at room temperature and then concentrated under reduced pressure, yielding a yellow solid. Recrystallization from MeOH–$Et_2O$ gave **9** (0.19 g, 20%) as yellow crystals: mp > 180 °C dec; $^1H$ NMR ($CD_3OD$) $\delta$ 7.61–7.57 (d, $J_{AB}$ = 8.6 Hz, 2H, ArH), 7.32–7.28 (d, 2H, ArH), 3.92 (s, 4H, 2 × $CH_2$), 3.86 (s, 4H, 2 × $CH_2$); IR (KBr, $cm^{-1}$) 3129, 1652; MS-FAB 344 ($MH^+$ – HI). Anal. ($C_{12}H_{15}I_2N_3O$) C, H, N.

**2-(4'-Amino-3'-iodobenzyl)imidazoline Dihydrochloride (17).** To a solution of NaI (2.16 g, 14 mmol) and **16** in sodium acetate buffer (0.1 M, pH = 4.1) was added a solution of thallium trichloride ($TlCl_3$) (5.78 g, 17 mmol) in $H_2O$ (50 mL), over a 30-min period. The brown mixture was heated on an oil bath for 2 h under argon. The reaction mixture was stopped by the addition of $Na_2SO_3$ (1.79 g, 14 mmol) in $H_2O$ (20 mL). After the mixture had cooled to room temperature, the solution was alkalinized (pH = 9) by adding $Na_2CO_3$ and then extracted with $CHCl_3$ (4 × 20 mL). The organic extracts were combined, washed with brine, dried over $Na_2SO_4$ and concentrated under reduced pressure to give **17**, as a free base (3.56 g, 83%), as yellow solid. Imidazoline **17**, free base, (3.4 g, 11 mmol) was dissolved in MeOH (50 mL) and saturated with HCl gas. The mixture was then concentrated under reduced pressure to give yellow solid which was recrystallized from MeOH–$Et_2O$ to afford **17** (3.31 g, 78%): mp 209–210 °C; $^1H$ NMR (DMSO-$d_6$) $\delta$ 10.27 (s, 2H, 2NH), 7.69–7.68 (d, $J$ = 2 Hz, 1H, ArH), 7.22–7.18 (dd, $J$ = 2 and 8 Hz, 1H, ArH), 6.92–6.89 (d, $J$ = 8 Hz, 1H, ArH), 5.66 (br s, 3H, $ArNH_3^+$) 3.77 (s, 4H, 2 × $CH_2$), 3.7 (s, 2H, $CH_2$); IR (KBr, $cm^{-1}$) 3135, 1612, 1488; MS *m/z* 301 ($M^+$, base), 174 ($M^+$ – I). Anal. ($C_{10}H_{14}Cl_2IN_3$) C, H, N.

**2-(3'-Iodo-4'-isothiocyanatobenzyl)imidazoline Hydrochloride (14).** To a cold solution of $CSCl_2$ (1.16 g, 10 mmol) in acetone (20 mL) was added a solution of $NaHCO_3$ (0.10 g, 1 mmol) and imidazoline **17** (0.40 g, 1 mmol) in $H_2O$ (7 mL). The mixture was stirred at room temperature for 2 h and the solvent removed *in vacuo* to give a residue which was taken up in $CH_2Cl_2$ to afford a pink solid. Recrystallization from MeOH–$Et_2O$ gave **14** as white crystals (0.22 g, 54%): mp 218–220 °C; $^1H$ NMR (DMSO-$d_6$) $\delta$ 10.25 (s, 2H, 2NH), 7.99–7.98 (d, $J$ = 1.6 Hz, 1H, ArH), 7.54–7.45 (m, 2H, ArH), 3.88 (s, 2H, $CH_2$), 3.79 (s, 4H, 2 × $CH_2$); IR (KBr, $cm^{-1}$) 3272, 2189, 2094, 1639; MS *m/z* 343 ($M^+$), 342 (base), 216 ($M^+$ – I), 284 ($M^+$ – NCS). Anal. ($C_{11}H_{11}ClIN_3S$) C, H, N.

**2-(4'-Nitrophenethyl)imidazoline Hydrochloride (24).** To a solution of 4'-nitrobenzylacetonitrile (**20**) (3.06 g, 17 mmol) in 20 mL of benzene–$CH_2Cl_2$ (1:1) was added EtOH (0.8 g, 17 mmol), and an excess of HCl gas was passed into the solution while it was cooled in an ice bath. The resulting solution was stirred at room temperature of 1 h and then kept in a refrigerator overnight. The mixture was then poured into $Et_2O$ (100 mL) while cooling in an ice–water bath. The resulting white precipitate was filtered, washed with $Et_2O$, and dried to afford imido ester hydrochloride **22**, (4.12 g, 92%), mp 117–118 °C. To a solution of imidate **22** (3.80 g, 15 mmol) in EtOH (50 mL) was added a solution of ethylendiamine (0.93 g, 16 mmol) in EtOH (10 mL). The mixture was refluxed overnight, cooled, acidified with concentrated, HCl, and concentrated under reduced pressure. The brown residue was then dissolved in $H_2O$ (100 mL) and washed with $CH_2Cl_2$ (50 mL). The water layer was made alkaline with 10% NaOH and extracted with $CH_2Cl_2$ (3 × 50 mL). The organic extracts were combined, washed with brine, dried over $Na_2SO_4$, and evaporated in vacuo to give a crude solide, which was dissolved in MeOH (50 mL) and saturated with HCl gas. Evaporation of the solvent in vacuo gave a crude solid which was recrystallized from MeOH–$Et_2O$, yielding 2.6 g (74%) of **24** as yellow crystals: mp 182–184 °C; $^1H$ NMR (DMSO-$d_6$) $\delta$ 8.21–8.17 (d, $J_{AB}$ = 8.8 Hz, 2H, ArH), 7.56–7.53 (d, 2H, ArH), 3.77 (s, 4H, 2 × $CH_2$), 3.16–3.10 (t, $J$ = 7.8 Hz, 2H, $CH_2$), 2.89–2.83 (t, 2H, $CH_2$); MS *m/z* 219 ($M^+$ – HCl), 218 (base), 172 ($M^+$ – HCl, $NO_2$). Anal. ($C_{11}H_{14}ClN_3O_2$) C, H. N.

**2-(4'-Aminophenethyl)imidazoline Dihydrochloride (26).** According to the same procedure as described for compound **16**: hydrogenation of **24** (1.50 g, 6 mmol) gave 1.27 g (95%) of **26** as a monohydrochloride salt, which was bubbled with HCl gas to obtain **26** as a dihydrochloride salt (1.07 g, 73%) in the form of white crystals: mp >250 °C dec; $^1H$ NMR ($D_2O$) $\delta$ 10.26 (s, 2H, 2NH) 7.35–7.26 (m, 4H, ArH), 3.77 (s, 4H, 2 × $CH_2$), 2.98–2.95 (t, $J$ = 8 Hz, 2H, $CH_2$), 2.81–2.79 (t, 2H, $CH_2$); IR (KBr, $cm^{-1}$) 2969, 1588; MS *m/z* 189 $M^+$ – 2HCl, base). Anal. ($C_{11}H_{17}Cl_2N_3$) C, H, N.

**2-(4'-Isothiocyanatophenethyl)imidazoline Hydrochloride (10).** The procedure was same as that for **3**; from **26** (0.50 g, 2 mmol) and $CSCl_2$ (0.30 g, 3 mmol) was obtained 0.29 g (49%) of **10**: mp 170–171 °C; $^1H$ NMR (DMSO-$d_6$) $\delta$ 10.27 (s, 2H, 2NH), 7.41–7.31 (q, $J$ = 8.5 Hz, 4H, ArH), 3.76 (s, 4H, 2 × $CH_2$), 3.02–2.96 (t, $J$ = 7.7 Hz, 2H, $CH_2$), 2.82–2.76 (t, 2H, $CH_2$); IR (KBr, $cm^{-1}$) 3098, 2179, 2120, 1600; MS *m/z* 231 ($M^+$ – HCl, base) 230 ($M^+$ – HCl, H). Anal. ($C_{12}H_{14}ClN_3S \cdot {}^1/_2H_2O$) C, H, N.

**2-[(4'-Nitrophenoxy)methyl]imidazoline Hydrochloride (25).** The same procedure was used as described for compound **24**; from **21** (9.00 g, 51 mmol), EtOH (2.33 g, 51 mmol), and HCl gas was obtained imidate **23** (12.3 g 93%). Imidate **23** (12 g, 46 mmol) was reacted with ethylendiamine (2.79 g, 46 mmol) to give 8.9 g (87%) of **25**: mp 219–220 °C; $^1H$ NMR (DMSO-$d_6$) $\delta$ 10.56 (s, 2H, 2NH), 8.29–8.25 (d, $J$ = 9.2 Hz, 2H, ArH), 7.26–7.22 (d, 2H, ArH), 5.28 (s, 2H, $OCH_2$), 3.89 (s, 4H, 2 × $CH_2$); IR (KBr, $cm^{-1}$) 3422, 1587, 1504, 1337, 1251. Anal. ($C_{10}H_{12}N_3O_3Cl \cdot {}^1/_2H_2O$) C, H, N.

**2-[(4'-Aminophenoxy)methyl]imidazoline Dihydrochloride (27).** The same procedure was used as described for compound **16**; starting with compound **25** (5.00 g, 19 mmol), imidazoline **27** was obtained in the form of white crystals (4.11 g, 93%): mp >250 °C; $^1H$ NMR ($D_2O$ + DSS) $\delta$ 7.45–7.42 (d, $J$ = 9 Hz, 2H, ArH), 7.19–7.16 (d, 2H, ArH), 5.17 (s, 2H, $OCH_2$), 4.03 (s, 4H, 2 × $CH_2$); IR (KBr, $cm^{-1}$) 3138, 2597, 1603, 1261; MS *m/z* 191 ($M^+$ – HCl). Anal. ($C_{10}H_{15}Cl_2N_3O$) C, H, N.

**2-[(4'-Isothiocyanatophenoxy)methyl]imidazoline Hydrochloride (11).** To a solution of $CSCl_2$ (0.76 g, 7 mmol) in acetone was added a solution of imidazoline **27** (1.00 g, 4 mmol) in 10 mL of $H_2O$-acetone (1:1). The mixture was stirred for 1.5 h at room temperature, and acetone was removed under reduced pressure. The $H_2O$ layer was alkalyzed with $Na_2CO_3$ and

extracted with $CH_2Cl_2$ (3 × 70 mL). Organic extracts were combined washed with brine (2 × 50 mL), dried over $Na_2SO_4$, and poured into $Et_2O$ saturated previously with HCl gas. Precipitate was collected and recrystallized from MeOH–$Et_2O$ to give white crystals of **11** as the hydrochloride salt (0.4 g, 37%): mp 138–140 °C; $^1$H NMR (DMSO-$d_6$) δ 10.4 (s, 2H, 2NH), 7.48–7.44 (d, $J_{AB}$ = 8.9 Hz, 2H, ArH), 7.09–7.06 (d, 2H, ArH), 5.13 (s, 2H, $OCH_2$), 3.88 (s, 4H, 2 × $CH_2$); IR (KBr, cm$^{-1}$) 3353, 3110, 2084, 1610; MS *m/z* 233 ($M^+$ – HCl, base). Anal. ($C_{11}H_{12}ClN_3OSH_2O$) C, H, N.

**2-(4′-Nitrobenzyl)imidazole Hydrochloride (31).** To a suspension of imidate **29** (11.20 g, 46 mmol) in glyme (25 mL) was added dropwise aminoacetaldehyde dimethyl acetal (4.84 g, 46 mmol), while cooling in an ice–water bath. The resulting mixture was stirred at room temperature overnight, acidified by adding AcOH (25 mL), and bubbled with HCl gas (≈1.7 g). The solution was then heated at 50 °C on an oil bath for 3 days, cooled down, poured into ether (100 mL), and filtered to give brown solid. Recrystallization from MeOH–$Et_2O$ gave 5.21 g (47%) of brown crystals of **31**: mp 209–211 °C; $^1$H NMR ($CD_3OD$) δ 8.28–8.25 (d, *J* = 8.8 Hz, 2H, ArH), 7.57–7.53 (d, 2H, ArH), 7.50 (s, 2H, 2 × CH), 4.51 (s, 2H, $CH_2$); IR (KBr, cm$^{-1}$) 3127, 1526, 1344; MS *m/z* 203 ($M^+$ – HCl) 156 ($M^+$ – HCl, $NO_2$), 55 (base). Anal. ($C_{10}H_{10}ClN_3O_2$) C, H, N.

**2-(4′-Aminobenzyl)imidazole Hydrochloride (32).** The procedure was the same as that for **16**; from 2.3 g (10 mmol) imidazole **31** was obtained **32** (1.75 g, 87%) as the monohydrochloride salt: mp 225–227 °C; $^1$H NMR ($CD_3OD$) δ 7.39 (s, 2H, 2 × CH), 7.03–7.00 (d, *J* = 8.6 Hz, 2H, ArH), 6.74–6.70 (d, 2H, ArH), 4.17 (s, 2H, $CH_2$); IR (KBr, cm$^{-}$) 3346, 3153, 1624; MS *m/z* 173 ($M^+$ – HCl), 55 (base). Anal. ($C_{10}H_{12}ClN_3$) C, H, N.

**2-(4′-Isothiocyanatobenzyl)imidazole (12).** The same procedure was used for **11**; from 1.00 g (5 mmol) of **32** and 5.48 g (50 mmol) of $CSCl_2$ was obtained **12** (0.71 g, 69%): mp 201.5–203 °C; $^1$H NMR ($CD_3OD$) δ 7.27–7.18 (m, 4H, ArH), 6.94 (s, 2H, 2 × CH), 4.05 (s, 2H, $CH_2$); IR (KBr, cm$^{-1}$) 2189, 2140, 1453; MS *m/z* 215 ($M^+$, base). Anal. ($C_{11}H_9N_3S^{1/4}H_2O$) C, H, N.

***N,N*-Dimethyl-2-(4-isothiocyanatophenyl)ethylamine Hydrochloride (13).** The procedure was the same as that for **11**; from *N,N*-dimethyl-2-(4′-aminophenyl)ethylamine (0.50 g, 3 mmol) and $CSCl_2$ (2.90 g, 30 mmol) was obtained 0.24 g (52%) of **13** as the hydrochloride salt in the form of white fluffy crystals: mp 174–175 °C; $^1$H NMR (DMSO-$d_6$) δ 7.42–7.33 (m, 4H, ArH), 3.27–3.21 (m, 2H, $CH_2$), 3.07–3.00 (m, 2H, $CH_2$), 2.76 (s, 6H, 2 × $CH_3$); IR (KBr, cm$^{-1}$) 2042; MS *m/z* 206 ($M^+$), 58 (base). Anal. ($C_{11}H_{15}ClN_2S$) C, H, N.

**Pharmacological Studies in Rat Thoracic Aorta.** Tissues from male Sprague-Dawley albino rats ranging from 300 to 450 g were used. Rats were killed by $CO_2$ asphyxiation. Helically-cut thoracic aorta strips (2 mm width × 20 mm length, two to three strips per animal) were placed into a 10-mL water-jacketed tissue bath containing a physiological salt solution (PSS) [composition (mM): NaCl, 118.07; KCl, 4.7; $CaCl_2·2H_2O$, 2.5; $MgSO_4·7H_2O$, 0.57; $NaH_2PO_4$, 1.01; $NaHCO_3$, 25; and dextrose, 11.01] maintained at 37 ± 0.5 °C with aeration (95% $O_2$ and 5% $CO_2$). Tissues were mounted with a resting tension of 1 g and tension charges were monitored with a force displacement transducer (FT-03) connected to a Grass polygraph (Model 7).

After a 1-h equilibrium, each strip was primed with $10^{-6}$ M phenylephrine and washed, followed with the construction of a concentration–response curve to phenylephrine ($10^{-9}$ to 3 × $10^{-5}$ M). After a washout and equilibrium for 1 h, tissues were incubated with PSS alone or in the presence of phenoxybenzamine (PBZ, 3 × $10^{-6}$ M) for 20 min and washed. In other experiments, PBZ-pretreated tissues were incubated with cirazoline ($10^{-6}$ M) or idazoxan ($10^{-7}$ or $10^{-6}$ M) for 60 min, followed by the construction of a concentration–response curve for each IBI analog (3 × $10^{-6}$–$10^{-4}$ M). Some tissues contracted slightly (5–15% of maximal phenylephrine contractile responses) after addition of cirazoline or idazoxan. Contractile responses to IBI were measured in the presence of verapamil ($10^{-5}$ M, 60 min) or nifedipine ($10^{-6}$ M, 60 min) or in calcium-deficient PSS. The activity of selected IBI analogs was also tested in the presence of nifedipine ($10^{-6}$ M, 60 min).

Cumulative concentration–response curves for each drug were compiled according to the procedure described by van Rossum.[45] In order to examine the slow-onset, long-lasting contractions produced by these synthesized compounds, the concentration-dependent drug responses were monitored between 20 and 30 min (for data of IBI analogs in Tables 1 and 2) or between 35 and 45 min (for data of IBI analogs in Tables 2 and 3) after each addition. Maximal contractile responses to IBI analogs were normalized relative to the maximal response to phenylephrine (30 μM). A Student's *t* test was used for data analysis. Data were expressed as the mean ± SEM of $n$ = 3–4.

Nifedipine and phenoxybenzamine were dissolved in EtOH as 0.01 M stock solutions and diluted with 0.01 M sodium phosphate buffer, pH 7.4, before use. With the exception of analog **12** (0.01 M MeOH stock solution) IBI analogs, phenylephrine, verapamil, cirazoline, and idazoxan were dissolved in double-distilled $H_2O$ and diluted in the phosphate buffer.

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