Novel Hypotensive Agents from *Verbesina caracasana*. 8. Synthesis and Pharmacology of (3,4-Dimethoxycinnamoyl)-N^1-agmatine and Synthetic Analogues[1]

Marco Carmignani,*,† Anna Rita Volpe,‡ Bruno Botta,*,§ Romulo Espinal,|| Stella C. De Bonnevaux,|| Carlo De Luca,⊥ Maurizio Botta,*,▽ Federico Corelli,▽ Andrea Tafi,▽ Rosario Sacco,○ and Giuliano Delle Monache*,‡

Dipartimento di Biologia di Base e Applicata, Sezione di Farmacologia, Università di L'Aquila, 67010 Coppito (AQ), Italy, Centro Chimica dei Recettori, Università Cattolica, Largo F. Vito 1, 00168 Rome, Italy, Dipartimento di Chimica e Tecnologia delle Molecole Biologicamente Attive, Università La Sapienza, Rome, Italy, Departamento de Farmacologia, Universidad de Carabobo, Carabobo, Venezuela, Centro Elettrochimica e Chimica Fisica delle Interfasi, Rome, Italy, Dipartimento Farmaco Chimico Tecnologico, Università di Siena, Siena, Italy, and Istituto di Chirurgia, Università di Catanzaro, Catanzaro, Italy

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The more polar metabolites from the Venezuelan plant *Verbesina caracasana*, i.e., N^3-prenylagmatine, (3,4-dimethoxycinnamoyl)-N^1-agmatine, agmatine, and galegine (prenylguanidine), previously reported (Delle Monache, G.; et al. *BioMed. Chem. Lett.* **1999**, *9*, 3249−3254), have been synthesized following a biosynthetic strategy. The pharmacologic profiles of various synthetic analogues of (3,4-dimethoxycinnamoyl)-N^1-agmatine (G5) were also analyzed, to shed some light on the structure−activity relationship of these compounds. Derivatives with the (*E*)-configuration and/or with a *p*-methoxybenzoyl moiety were found to be responsible for higher hypotensive effects, which were associated with a slight and, in some cases, not dose-related increase of cardiac inotropism, with variable and not significant chronotopic responses, and, only at higher doses, with effects of respiratory depression. Either an increase (to six) or a decrease (to two) of the number of methylene groups in the alkyl chain of (*E*)-G5 did not change blood pressure responses, while slightly increasing the positive inotropic ones. At pharmacological doses, all the studied compounds showed hypotensive and slight positive inotropic effects without relevant chronotropic and respiratory actions.

Introduction

Many synthetic guanidine derivatives have attracted pharmacologists in search of new antihypertensive drugs for their ability to block adrenergic nerve activity through central and/or peripheral mechanisms.[2−4] As a result, compounds such as guanethidine,[5] guanabenz,[6] guanfacine,[7] and pinacidil[8] have been introduced in antihypertensive drug therapy. In this respect, while guanethidine induces arterial hypotension only by peripheral mechanisms including depletion of noradrenaline in the postganglionic adrenergic endings,[9,10] the long-lasting hypotensive effects of guanabenz and guanfacine are due to a central mechanism, analogous to that of clonidine and involving α_2-adrenoreceptors in the central sympathetic pathways.[11,12] Conversely pinacidil, devoid of actions in the central nervous system, is able to lower blood pressure through arterial vasodilation not related to changes of the peripheral adrenergic nerve activity[13,14] but depending on its activity as a potassium channel opener.[15] A series of studies on structure−activity relationships revealed that small changes in the structures of guanidine derivatives may lead to wide variations of activity.[16−18]

A crude methanol extract of the Venezuelan plant *Verbesina caracasana* Fries (Compositae), intravenously administered to mice, was found to induce biological effects such as erection of hair and initial stimulation and subsequent blockage of breathing. Biologically controlled purification, culminating in silica gel chromatography, yielded a series of active compounds, the least polar of which was named caracasanamide (G1) and assigned the structure 1-[(3,4-dimethoxycinnamoyl)amino]-4-[(3-methyl-2-butenyl)guanidino]butane (**1**), as a mixture of (*Z*)- and (*E*)-forms.[19] The pharmacological profile of the water-soluble (*Z*)-form and the synthesis of the (*E*)-form of caracasanamide (**1**) have been reported in a previous paper.[20] A second metabolite, named caracasandiamide (G2), was isolated and shown to be the truxinic-type dimer of **1**.[21] Similarly to, and more strongly than, G1, G2 may be considered a hypotensive and an antihypertensive drug, devoid of the negative side effects, e.g., the reflex tachycardia and decreased cardiac inotropism, shown by the majority of the antihypertensive and vasodilator drugs.[22]

Four other hypotensive agents, namely, G3, G5, G6, and G7, have been isolated and assigned the structures N^3-prenylagmatine (**2**), (*E*)-(3,4-dimethoxycinnamoyl)-N^1-agmatine (**3**), agmatine (**4**), and galegine (prenylguanidine, **5**), respectively.[23] This paper deals with the synthesis of the above components of the methanol extract of *V. caracasana*. In an effort to shed some light

* To whom correspondence should be addressed. (G.D.M.) Phone: +39 06 3057612. Fax: +39 06 3053598. E-mail: g.dellemonache@uniserv.ccr.rm.cnr.it.
† Università di L'Aquila.
‡ Università Cattolica.
§ Università La Sapienza.
|| Universidad de Carabobo.
⊥ Centro Elettrochimica e Chimica Fisica delle Interfasi.
▽ Università di Siena.
○ Università di Catanzaro.

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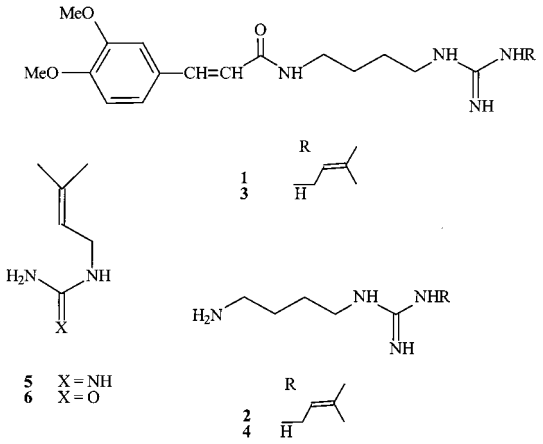
on structure−acivity relationships for these compounds, the pharmacological profiles of G5 and a number of synthetic analogues were studied.



Results and Discussion

Structure. Extended chromatography of the more polar fractions of the extract[20] afforded the metabolites G3, G5, G6, and G7, named according to the elution order and based on a common guanidino structure.[23] On the basis of the spectral data, the metabolite G3 was assigned the structure 1-amino-4-[(3-methyl-2-butenyl)-guanidino]butane (**2**). The isolation of *N*-(3-methyl-2-butenyl)urea (**6**) in the mixture from alkaline hydrolysis established the position of the isoprenyl chain in G3.[19,20] Since in the ^{1}H and ^{13}C NMR spectra of G5 (M^+ 320 in the electron impact mass spectrum), compared with those of G1 (M^+ 388), the signals of the 3-methyl-2-butenyl chain were absent, G5 was assigned the structure 1-(3,4-dimethoxycinnamoyl)amino-4-guanidinobutane (**3**). Accordingly, compound **6** was absent in the hydrolysis mixture. Just as G1 (**1**), the natural G5 was a mixture of (*Z*)- and (*E*)-forms (in a ratio of 2:1), and pharmacological tests were run with the (*Z*)-form, more soluble in water.[20]

The natural product G6 was identical with a commercial sample of agmatine (**4**), whereas the last isolated metabolite, G7, was coincident with galegine (*N*-(3-methyl-2-butenyl)guanidine, **5**), the toxic principle of *Verbesina enceloioides*[24] and *Galega officinalis* (goats rue).[25]

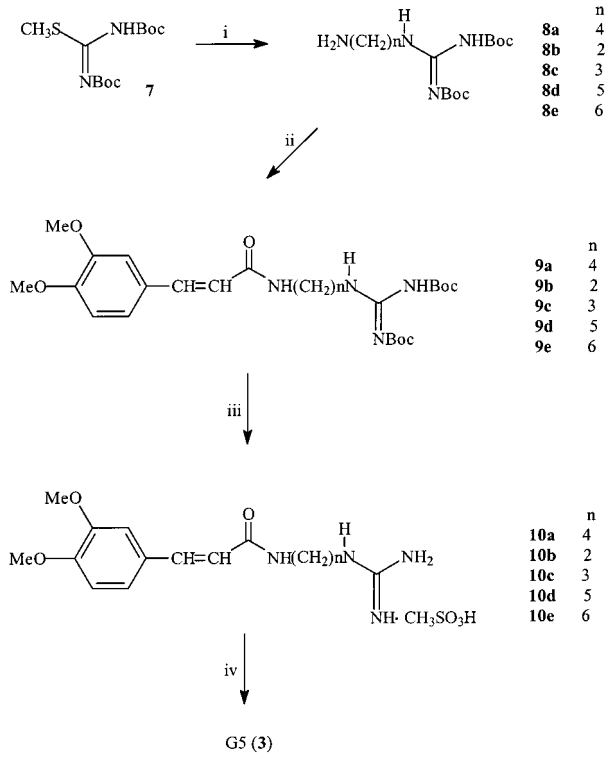
In summary, the metabolites of *V. caracasana* appear to derive from the combination of four elements, which are all present in G1: the guanidine group, the 1,4-diaminobutane base, the 3,4-dimethoxycinnamoyl residue, and the prenyl substituent. Biogenetically, G1 seems to be originated from guanidine by three operations: in detail, the addition of the base, the prenylation, and the acylation. The first two reactions may happen at each level, whereas the last one must obviously follow the addition of the base.

A similar strategy addressed our synthetic routes to G3, G5, and G7.

Synthesis. To confirm the assigned stuctures, compounds **2**, **3**, and **5** were synthesized according to the pathways summarized in Schemes 1 and 2.

The reaction of *N*,*N*′-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea (**7**)[20] with 1,4-diaminobutane in THF (Scheme 1) gave in 75% yield compound **8a**, which was

Scheme 1[a]



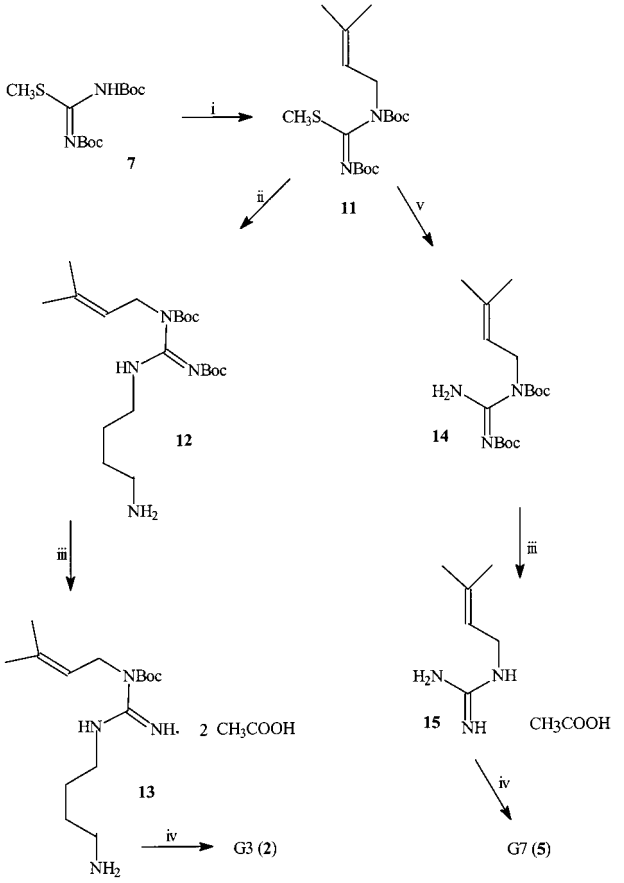
[a] Reaction conditions: (i) α,ω-diaminoalkane, THF/H_2O; (ii) 3,4-dimethoxycinnamic acid, $(EtO)_2P(O)Cl$, Et_3N; for **13b**−**e**, Et_3N, CH_2Cl_2; (iii) CH_3SO_3H, 1,4-dioxane, reflux; (iv) ion-exchange resin (OH^- form).

in turn acylated with the mixed anhydride obtained from (*E*)-3,4-dimethoxycinnamic acid and diethyl chlorophosphate to afford **9a**. Removal of the Boc groups with excess trifluoroacetic acid at room temperature gave a complex mixture of products, whereas treatment of **9a** with a catalytic amount of *p*-toluensulfonic acid (PTSA) in 1,4-dioxane at reflux, followed by filtration through anionic exchange resin, afforded (*E*)-G5 (**3**) as the free base in poor yield. However, when **9a** was heated in 1,4-dioxane in the presence of a stoichiometric quantity of methanesulfonic acid, the mesylate salt of (*E*)-G5 (**10a**) was obtained in good yield and easily purified by column chromatography on silica gel. Filtration through anionic exchange resin gave (*E*)-G5 (**3**), identical with the (*E*)- component of the natural product.

Phase-transfer-catalyzed (PTC) *N*-alkylation of **7**[20] with 4-bromo-2-methyl-2-butene at room temperature (Scheme 2) afforded **11** in 97% yield. The reaction of **11** (Scheme 2) with 1,4-diaminobutane in THF gave the intermediate **12**, which by heating in 1,4-dioxane with methanesulfonic acid yielded the diacetate salt **13**. Treatment of the last one with anionic exchange resin yielded G3 (**2**), identical with the natural sample. Conversely, the reaction of **11** with 25% NH_3 solution in ethanol provided **14**, which was deprotected under the same conditions as used for **13** to afford **12**. The acetate **12** was again neutralized by ion-exchange resin to give G7 (**5**), identical with the natural product.

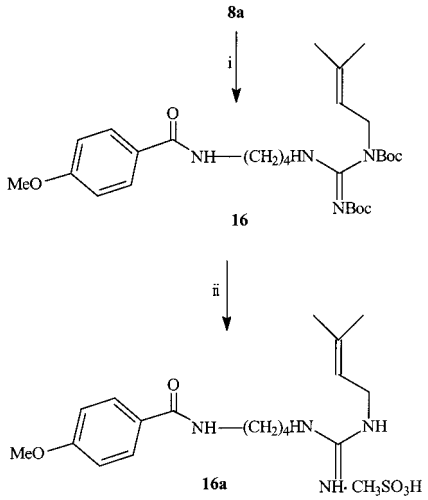
With the aim at both developing new hypotensive agents and gaining an insight into the structure−activity relationships for the series of these guanidino derivatives, we have synthesized homologues of **10a** (the mesylate salt of G5) bearing a diaminoalkane spacer,

Scheme 2[a]



[a] Reaction conditions: (i) 4-bromo-2-methyl-2-butene, KOH, Bu_4NBr, CH_2Cl_2/MeCN; (ii) 1,4-diaminobutane, THF/H_2O; (iii) CH_3SO_3H, 1,4-dioxane, reflux, ion-exchange resin ($MeCO_2^-$ form); (iv) ion-exchange resin (OH^- form); (v) NH_3, EtOH.

Scheme 3[a]



[a] Reaction conditions: (i) 4-methoxybenzoic acid, $(EtO)_2P(O)Cl$, Et_3N, CH_2Cl_2; (ii) CH_3SO_3H, 1,4-dioxane, reflux.

shorter (**10b**, **10c**) or longer (**10d**, **10e**) than that of the natural compound. Moreover, the analogue compound **16a**, modified in the acyl moiety, was prepared from **8a** as outlined in Scheme 3.

Pharmacology. All the natural compounds,[23] when administered by an intravenous (iv) route in anesthetized rats, were able to reduce blood pressure (BP) and to increase cardiac inotropism in a dose-related manner,

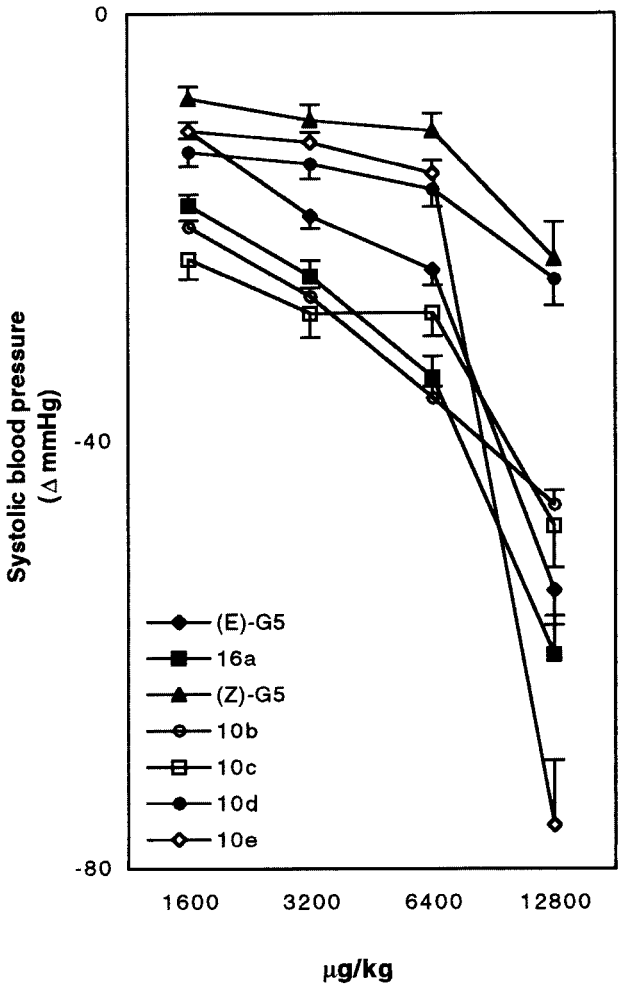


Figure 1. Changes in systolic blood pressure following iv administration of (*E*)-G5, its synthetic analogues **16a** and **10b**−**e**, and Natural (*Z*)-G5 in Anesthetized rats (means ± SE, $n = 8$ for each point).

G1[20] and G2[22] being the most potent among them (G2 > G1). In general, these compounds affected breathing moderately and biphasically (stimulation at low/middle doses, depression at high doses), without changing consistently the heart rate (HR), with the exception of G2, which induced dose-related bradycardia.

In the present study, all the synthetic compounds were tested as mesylate salts. Either (*E*)-G5 (**10a**) or its analogue **16a** was found, in urethane-anesthetized rats, to lower BP and to increase cardiac inotropism (as the maximum rate of rise of the left ventricular isovolumetric pressure, d*P*/d*t*) and, slightly, HR; an evident dose−response relationship was observed, for the hypotensive effect, in the dose range 1600−12800 μg/kg (Figures 1−4). The averaged basal values of the measured cardiovascular parameters were 106 ± 6 mmHg (systolic BP), 91 ± 5 mmHg (diastolic BP), 313 ± 24 beats/min (HR), and 4910 ± 463 mmHg/s (d*P*/d*t*) (means ± SE; $n = 56$ for each parameter). Whereas natural (*Z*)-G5 slightly increased the respiratory frequency (RF) and tidal volume (TV) at lower iv doses (200−1600 μg/kg) and depressed these two parameters not dose-dependently at higher doses (3200−12800 μg/kg),[23] synthetic (*E*)-G5 and **16a** reduced the RF and TV in a dose-related manner (1600−12800 μg/kg): such effects began a few seconds after iv administration of both drugs, lasted

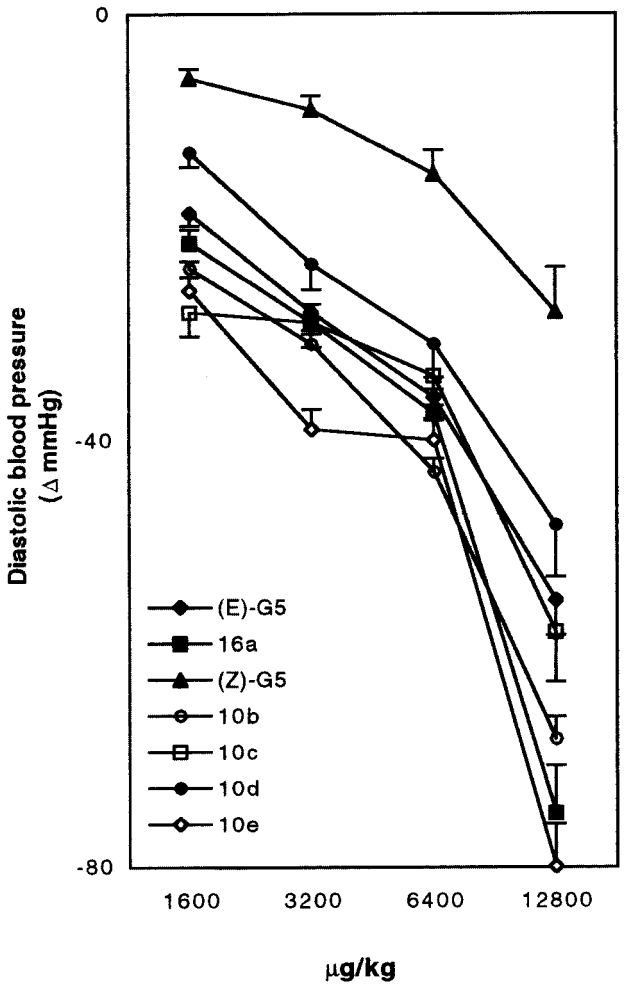


Figure 2. Changes in diastolic blood pressure following iv administration of (*E*)-G5, its synthetic analogues **16a** and **10b**−**e**, and natural (*Z*)-G5 in anesthetized rats (means ± SE, $n = 8$ for each point).

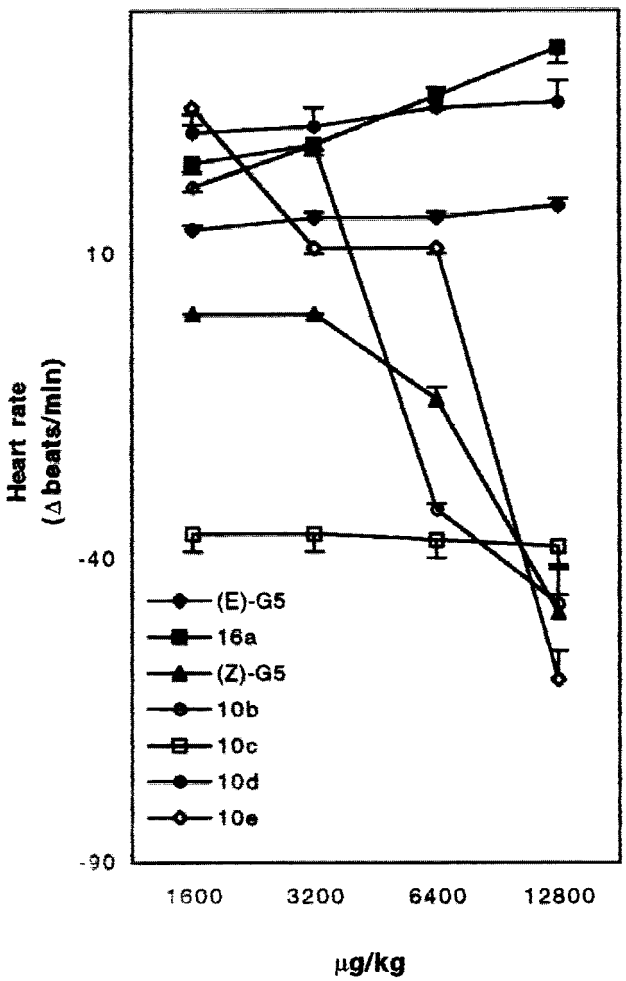


Figure 3. Changes in heart rate following iv administration of (*E*)-G5, its synthetic analogues **16a** and **10b**−**e**, and natural (*Z*)-G5 in anesthetized rats (means ± SE, $n = 8$ for each point).

from 16 to 110 s, and were concomitant to reduction of HR and d*P*/d*t* (which followed the early increase, lasting from 11 to 23 s, of these cardiac indices). On the whole, **16a** was slightly more active than **10a** in determining tachycardia and arterial hypotension (at higher doses) as well as in depressing breathing; moreover, these effects of **16a** were slightly longer than those of (*E*)-G5. As compared with natural (*Z*)-G5, which decreased HR (6400−12800 μg/kg), **16a** and (*E*)-G5 were more potent in lowering BP, induced slight tachycardia instead of bradycardia, and depressed breathing at all doses, while (*Z*)-G5 showed biphasic respiratory effects (see above); on the other hand, there were no substantial differences in the positive inotropic effects of the three compounds (Figures 1−4).

The analysis of the cardiovascular effects of **16a** and (*E*)-G5 showed that a slight increase of HR and d*P*/d*t*, induced by the lowest doses (50 and 100 μg/kg), was concomitant to the maximal hypotensive effect, whereas a slight bradycardia was observed when BP was returning to its basal levels. Such a bradycardia was not present at a dose of 200 μg/kg, which induced sporadic periods of ventricular extrasystolic firing lasting about 3 min. Tachycardia, followed by bradycardia and extrasystolia, reappeared in the dose range 400−1600 μg/kg. On the other hand, higher doses of **16a** and (*E*)-G5 continued to induce biphasic chronotropic responses (with prevalent bradycardia), and as a consequence of the primary effect of respiratory depression, also the increased chronotropic response was followed by a phase of reduced inotropism. For instance, the initial increase of HR and d*P*/d*t*, which was observed with either **16a** or (*E*)-G5 at a dose of 12800 μg/kg, was soon followed by an about 80% reduction of both HR and d*P*/d*t* (with respect to the basal values); HR, d*P*/d*t*, BP, RF, and TV all required about 7 min to return to the levels preceding administration of this dose of (*E*)-G5.

The synthetic homologues of (*E*)-G5, with two (**10b**), three (**10c**), five (**10d**), or six (**10e**) methylene groups in the alkyl chain, were all able to decrease systolic and diastolic BPs, the latter giving in all cases the highest response; the four compounds showed positive inotropic effects at all the tested doses, but only for **10c** and **10d** was it possible to establish a dose−response relationship (Figures 1−4). On the other hand, the effects of these drugs on HR were quite different: **10c** and **10d** were provided with slight bradycardic and tachycardic actions, respectively, without a dose−response relationship. **10b** did not show effects on HR until a dose of 400 μg/kg, increasing it until a dose of 3200 mg/kg and decreasing it at doses of 6400−12800 μg/kg (which induced lower positive inotropic responses). **10e** increased HR until a dose of 1600 μg/kg and then induced a lower tachycardic response which, at the highest dose

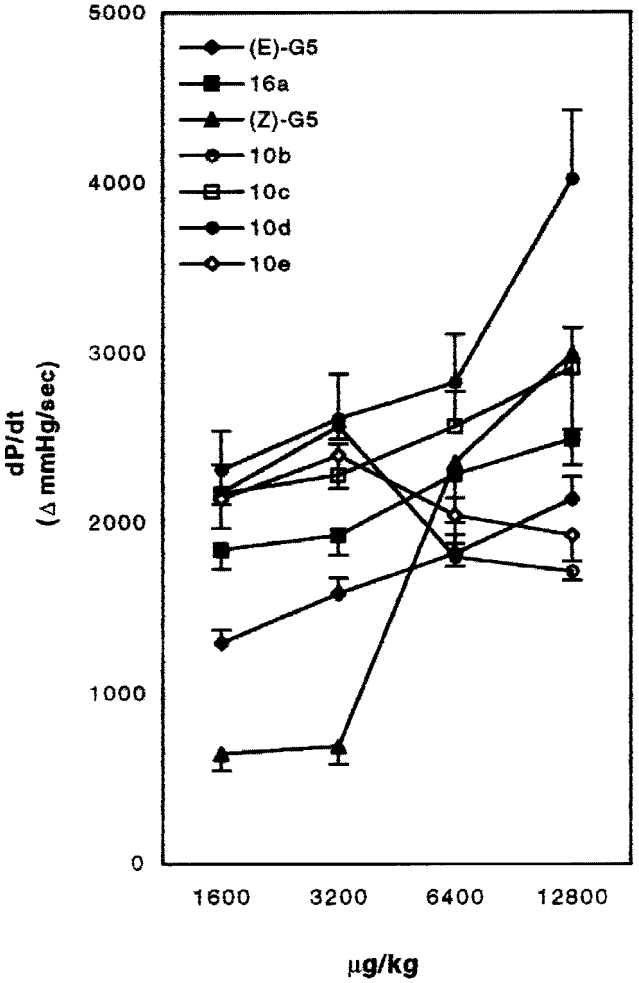


Figure 4. Changes in the maximum rate of rise of the left ventricular isovolumetric pressure (d*P*/d*t*) following iv administration of (*E*)-G5, its synthetic analogues **16a** and **10b**−**e**, and natural (*Z*)-G5 in anesthetized rats (means ± SE, $n = 8$ for each point).

(12800 μg/kg), was reversed with the appearance of a bradycardic effect; also in this case, doses of 6400−12800 μg/kg were characterized by lower positive inotropic responses (Figures 1−4). In conclusion **10a**, **16a,** and **10b**−**e** appeared to be, within a wide dose range, drugs lowering BP according to a small-slope dose−response curve. The hypotensive effect coexisted with variable and negligible chronotropic ones not exceeding, at the highest dose, a 10% or a 20% change (positive or negative, respectively) with regard to the basal values. The same compounds showed stimulatory actions on cardiac inotropism not related to the doses (**10b**, **10e**), not according to clear dose−response relationships (**10a**, **16a**, **10c**, **10d**), or accompanied by negative chronotropic responses associated with respiratory depression (**10a**, **16a**). In this regard, the depressive effects on breathing of all the studied compounds were slight and similar (in the dose range 50−1600 μg/kg). Only at the highest (subtoxic) doses, **10b** and **10e** induced lower increases of d*P*/d*t*, in the presence of reversal of the tachycardic response and potentiation of the hypotensive effect. Therefore, as compared with **10a** in the dose range 50−3200 μg/kg, its analogues **10b**−**e** were found to have similar effects on BP and to be slightly more active in increasing cardiac inotropism. As far as the chronotropic effects were concerned, **10d** and **10e** (50−6400 μg/kg) resembled either (*E*)-G5 or **16a** in determining slight tachycardia, while **10b** and, mostly, **10c** (at doses higher than 3200 μg/kg) resembled (*Z*)-G5 in inducing an appreciable bradycardia.

When considering structure−activity relationships, the (*E*)-configuration [**10a** vs natural (*Z*)-G5] and/or a *p*-methoxybenzoyl substituent [**16a** vs (*Z*)-G5] appeared to be responsible for a higher hypotensive action (mostly diastolic), a reversal of the chronotropic effect (tachycardic instead of bradycardic), and a dose-related depression of breathing. On the other hand, the decrease to two (**10b**) or three (**10c**) and the increase to five (**10d**) or six (**10e**) methylene groups in the alkyl chain did not allow, in the dose range 50−3200 μg/kg (without considering the highest doses depressing breathing), for effects on BP significantly different from those of (*E*)-G5 (**15a**, with four $=CH_2$ groups). Nevertheless, in this dose range, **10b**−**e** appeared to be slightly more active than **10a** in increasing cardiac inotropism. Moreover, when comparing chemical structures of the studied compounds with that of guanethidine, it appears quite likely that these compounds are provided with higher lipophility because of their higher hydrocarburic component (thus being able to cross the blood−brain barrier much more than guanethidine).[9,10]

Either natural[23] or synthetic guanidine compounds,[20−22] including those of the present study, have been characterized for their respiratory and, mostly, cardiovascular effects on the basis of a preliminary extensive investigation, carried out on mice (see above) and dogs, showing the crude methanol extract of *V. caracasana* to have the respiratory and cardiovascular systems as selective targets.[20,22] In particular, this extract (0.5−4.0 mg/kg of body mass by the iv route) was able to reduce the mean BP in a dose-related manner, in anesthetized dogs, with the maximum effect at a dose of 2.0 mg/kg (−58% ± 4% with respect to the basal BP values, mean ± SE; $n = 6$) and not differing under barbiturate or chloralose anesthesia.[19,20,22] Synthesis of the water-soluble (*Z*)-forms of a series of guanidine compounds (G1−G3, G5−G7), previously obtained as a mixture of the (*E*)- and (*Z*)-forms from *V. caracasana*, allowed in anesthetized rats (1) their prevalent cardiovascular effects to be confirmed, (2) such compounds to be characterized as hypotensive drugs through a vasodilating action (depending on central and/or peripheral mechanisms), (3) their pharmacological potencies to be determined (in the dose range 50−3200 μg/kg, by the iv route), (4) their pharmacological activities to be compared with those of standard (hypotensive and/or antihypertensive) drugs, ranging from clonidine to papaverine, and (5) chronotropic and/or inotropic components to be excluded (in the above dose range) in the hypotensive effect.[19−23] Therefore, these compounds have been proposed, in such a dose range, as hypotensive drugs of high (G2),[22,23] mild (G1, G7),[20,23] or low potency (G3, G5, G7)[23] not presenting significant chronotropic effects. In this regard, only G1, G2, and G7 (G2 > G1 > G7) showed appreciable positive inotropic effects together with stimulatory (G7 > G1) or inhibitory effects (G2) of variable degree on breathing.[20−23] Thus, G5 being one of the studied guanidine derivatives provided with hypotensive activity but devoid of consistent inotropic and respiratory actions,[23] the aim of this study

Table 1. Changes[a] in Systolic and Diastolic Blood Pressures Following iv Administration of Several Guanidine Derivatives and Vasodilating or Antyhypertensive Drugs in Anesthetized Rats

drug	blood pressure	
	systolic	diastolic
(*Z*)-G5 (4.12 μmol/kg)	−12 ± 2	−10 ± 3
(*E*)-G5 (10a) (4.12 μmol/kg)	−21 ± 2	−30 ± 3
16a (4.12 μmol/kg)	−28 ± 4	−31 ± 2
10b (4.12 μmol/kg)	−29 ± 3	−33 ± 4
10c (4.12 μmol/kg)	−24 ± 3	−28 ± 4
10d (4.12 μmol/kg)	−17 ± 2	−27 ± 2
10e (4.12 μmol/kg)	−14 ± 3	−36 ± 3
guanethidine (25 μmol/kg)	−32 ± 4	−23 ± 4
clonidine (0.108 μmol/kg)	−21 ± 2	−15 ± 2
hexamethonium (12 μmol/kg)	−46 ± 6	−30 ± 3
reserpine (8 μmol/kg)	−34 ± 5	−27 ± 4
papaverine (5 μmol/kg)	−21 ± 3	−16 ± 2
histamine (0.044 μmol/kg)	−28 ± 4	−23 ± 2

[a] Values are means ± SE (n = 4 in each group).

was also to assess the pharmacological profile of its (*E*)-homologue **10a** and analogues **16a** and **10b**−**e** to obtain compounds provided with a higher hypotensive activity and potentially more useful as antihypertensive agents. As previously shown,[20−23] some of the tested natural compounds were more potent, on a molar basis, than guanethidine, papaverine, reserpine, or hexamethonium in reducing BP, evidenced more prolonged hypotensive actions, and did not reveal the negative inotropic and chronotropic properties of such vasodilating or antihypertensive drugs (with the only exception of G2). On the whole, as discussed above in comparison with natural (*Z*)-G5, its (*E*)-homologue **10a** and analogue **16a** showed a higher hypotensive activity and confirmed properties of consistent respiratory depression only at high (subtoxic) doses. On the other hand, **10b**−**e** did not differ from **10a** and **16a** in the mean hypotensive effect, when considering the dose range 50−3200 μg/kg (excluding the highest doses depressing breathing), despite a slightly higher positive inotropic activity. In this regard, Table 1 compares, on a molar basis, the hypotensive effects of (*Z*)-G5, **10a**, **16a**, **10b**−**e**, and several vasodilating or antihypertensive drugs.

From a pharmacodynamic point of view, it was found that (*E*)-G5, **16a**, and **10b**−**e** decreased BP (lasting a few seconds after their iv administration) before affecting HR, d*P*/d*t*, RF, and TV, thus suggesting a vascular level of action in determining systemic arterial vasodilation, in a way similar to that shown by the natural products G2,[22] G3, G5, G6, and G7,[23] but not G1.[20] This last compound showed a peripheral (cardiac) β_1-adrenoreceptor-like action (causing an increase of HR and d*P*/d*t*) and reduced both the central sympathetic tone and baroreceptor reflex activity (causing a reduction of BP). On the other hand, G2 showed only peripheral mechanisms including reserpine- and guanethidine-like actions, β_1- and β_2-adrenoreceptor-like and M_{2-4}-cholinergic receptor-like components, and α_2-adrenoreceptor antagonistic properties. Moreover, the cardiovascular effects of G1[20] and G2[22] were not related, in the dose range 50−3200 μg/kg by the iv route, to the respiratory ones (consisting of an increase of RF and TV at lower doses and a decrease of RF and TV at higher doses). Similar effects were shown by G3 and G5−G7,[23] which resembled G1 and G2 in lowering BP, G1 in increasing RF and TV, and G2 in reducing HR. Differences in pharmacological potency were found for these natural compounds, G2 being the most active substance in lowering BP and increasing cardiac inotropism (only G2 depressed breathing also at nontoxic doses)[22] and G1[20] and G7[23] the most active substances in stimulating breathing. It seems quite likely that also (*E*)-G5, **16a**, and **10b**−**e** act by central and/or peripheral mechanisms already found for the natural guanidine derivatives obtained from *V. caracasana*. In this respect, pharmacodynamic differences among the synthesized drugs and between the synthetic mesylate salts with an (*E*)-configuration and the natural products with a (*Z*)-configuration are likely to depend on their ability to cross the blood−brain barrier, on the lower or higher lipophilicity, and on the pharmacokinetic properties.[20,22,23]

All the synthetic guanidine compounds presented in this study (as well as their natural homologue and analogues from *V. caracasana*) appear to be drugs provided with selective dose-related hypotensive effects within a wide range (50−3200 μg/kg by the iv route). These effects are generally accompanied, mostly in the cases of **10a** and **16a**, by a slight increase of cardiac inotropism but not by significant respiratory or chronotropic changes. If a hypotensive response (mostly when dependent on arterial vasodilation) should be expected to be followed by a reverse effect (associated with tachycardia), due to baroreceptor-dependent sympathetic activation, the absence of such an effect for the compounds of this study may be explained as the result of interactions among the above central and peripheral mechanisms previously described for the natural compounds.[19−23] Accordingly, while the hypotensive responses observed in this study are constantly accompanied by increased d*P*/d*t*, HR does not change consistently [(*E*)-G5] or appears to decrease [(*Z*)-G5, **10c**], to increase (**16a**, **10d**), or to change biphasically (**10b**, **10e**) as, in general, the respiratory responses. Therefore, the increased inotropism by the studied compounds does not seem to depend on a reflex increase of sympathetic activity by itself, their hypotensive effects being related to arterial vasodilation and not to reduction of HR. In this respect **10a**, **16a**, and **10b**−**e** seem to be, in a clinical perspective, more suitable hypotensive (antihypertensive) drugs because of the lack, as compared with some natural analogues, of consistent positive inotropic (as G1 and G2),[20,22] respiratory, and chronotropic side effects (as G2 and G7).[22,23] Although this study has not been directed to investigate the action mechanisms of the tested guanidine compounds, it is likely, considering the results obtained with their natural analogues,[20−23] that they too are provided with multiple action mechanisms (as also found for other guanidine derivatives such as guanethidine[5] and guanabenz[6]). Moreover, although a guanidine moiety is also shown by complex with nitric oxide (NO) synthase to have inhibitory activity [like L-NAME (nitroarginine methyl ester) and aminoguanidine], a similar mechanism is quite unlikely for our guanidine compounds, inducing, on the contrary, arterial vasodilation and slight positive inotropic effects (NO is known to induce vascular relaxation, to depress cardiac activity, and to be an antiarrhythmic agent).[26−28]

A further study will deal with structure−activity relationships among the compounds of this study, their natural analogues from *V. caracasana*, and other synthetic analogues to select some of them for a clinical evaluation in the treatment of arterial hypotension of various genesis.

Experimental Section

General Procedures. Melting points were determined with a Kofler apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were determined with a Varian Gemini instrument (300 and 75 MHz, respectively), using tetramethylsilane as an internal standard in the reported solvents. IR spectra were determined with a Perkin-Elmer 237 spectrophotometer. Electron impact (EI) and fast atom bombardment (FAB) mass spectra were determined on a VG 7070EQ spectrometer.

^{1}H NMR, IR, and FABMS spectra of the synthetic intermediates are reported in the Supporting Information.

Plant Material. Leaves of *V. caracasana* were collected in Valencia, Venezuela. A voucher specimen is deposited at the herbarium of the Departamento de Physiologia vegetal of the Universidad Central de Venezuela, Maracaibo, Venezuela.

Extraction and Fractionation. Extraction and the first fractionation on silica gel with $CHCl_3$/MeOH mixtures have been described in our previous paper.[20] Extended chromatography on silica gel with $CHCl_3$/MeOH mixtures of fractions containing products more polar than G1 G2 gave the new compounds G3−G7, G4 having not been identified. [23]

Synthesis. 1-Amino-4-[*N*^{2},*N*^{3}-bis(tert-butoxycarbonyl)-guanidino]butane (8a). A solution of *N*^{2},*N*^{3}-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea[20] (**7**; 2.9 g, 10 mmol) in THF (25 mL) was added dropwise to a stirred solution of 1,4-diaminobutane (2.29 g, 26 mmol) in THF/H_2O (40 mL; 20:1, v/v). After being stirred for 1 h at 50 °C, the reaction mixture was concentrated in vacuo, and the residue was partitioned between $CHCl_3$ and 10% aqueous $NaHCO_3$. The organic layer was dried (Na_2SO_4) and evaporated. The residue by chromatography on a Si gel column ($CHCl_3$/Et_3N, 19:1) gave **8a** (2.48 g, 75%) as an oil. Anal. ($C_{15}H_{30}N_4O_4$) C, H, N.

(*E*)-4-[*N*^{2},*N*^{3}-Bis(tert-butoxycarbonyl)guanidino]-1-[(3,4-dimethoxycinnamoyl)amino]butane (9a). To a solution of (*E*)-3,4-dimethoxycinnamic acid (825 mg, 4.2 mmol) and triethylamine (1.3 mL, 9.3 mmol) in dry THF (30 mL) was added slowly a solution of diethyl chlorophosphate (870 mg, 5 mmol) in THF (30 mL). After the resulting solution was stirred for 2 h at room temperature, the precipitate was filtered off and the filtrate was added dropwise to a solution of **8a** (2.0 g, 6 mmol) and triethylamine (1.3 mL, 9.3 mmol) in dry CH_2Cl_2 (30 mL). The reaction mixture was stirred for 2 h at room temperature, concentrated to a small volume, and diluted with CH_2Cl_2 and 10% aqueous Na_2CO_3. The organic layer was washed with water, dried (Na_2SO_4), and evaporated. The residue was purified on a silica gel column with AcOEt to afford **9a** (1.88 g, 60%) as a viscous oil. Trituration with Et_2O gave a colorless powder, mp 136−138 °C. Anal. ($C_{26}H_{40}N_4O_7$) C, H, N.

(*E*)-1-[(3,4-Dimethoxycinnamoyl)amino]-4-guanidinobutane Mesylate (10a). To a solution of **9a** (416 mg, 0.8 mmol) in dry 1,4-dioxane (20 mL) was added methanesulfonic acid (52 mL, 0.8 mmol) at 0 °C with stirring. The reaction mixture was held at reflux for 20 h and concentrated in vacuo. The residue was chromatographed on silica gel (CH_2Cl_2/MeOH, 7:3) to give **10a** (263 mg, 79%) as a pale yellow glass: IR (Nujol) ν_{max} 3310, 1620 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 8.90 (1H, br t, J = 6 Hz, NH), 8.24 (1H, t, J = 5 Hz, NH), 8.06 (1H, d, J = 15.5 Hz, H-α), 7.25 (1H, d, J = 2 Hz, H-2′), 7.22 (1H, dd, J = 8 and 2 Hz, H-6′), 7.18 (1H, d, J = 15.5 Hz, H-β), 6.87 (1H, d, J = 8 Hz, H-5′), 3.59 (2H, q, J = 6 Hz, 1-CH_2N), 3.82, 3.72 (3H each, s, 2 × OMe), 3.41 (2H, br q, J = 5.5 Hz, 4-CH_2N), 3.01 (3H, s, Me), 1.76 (4H, m, 2 × CH_2); ^{13}C NMR δ 167.18 (s, CO), 158.56 (s, C=NH), 150.57, 149.15 (s each, C-3′, C-4′), 140.05 (d, C-α), 129.04 (s, C-1′), 122.27, 120.87 (d each, C-6′, C-β), 112.21 (d, C-5′), 110.87 (d, C-3′), 55.90, 55.82 (q each, 2 × OMe), 41.76 (t, C-1), 40.03 (q, Me), 38.95 (t, C-4), 27.15, 26.22 (t each, 2 × CH_2); FABMS (TDEG/Gly) *m*/*z* 321 ($M^+ + 1$). Anal. ($C_{17}H_{28}N_4O_6S$) C, H, N.

(*E*)-(3,4-Dimethoxycinnamoyl)-*N*^{1}-agmatine (5). Treatment of **10a** (126 mg) with anion-exchange resin gave (*E*)-G5 (**5**; 92 mg), identical (^{1}H, ^{13}C NMR, FABMS) with the natural (*E*)-component.

***N*^{2},*N*^{3}-Bis(tert-butoxycarbonyl)-*N*-(γ,γ-dimethylallyl)-*S*-methylisothiourea (11).** A solution of **7**[20] (3.5 g, 12 mmol) in CH_2Cl_2/CH_3CN (60 mL; 19:1, v/v) was added dropwise to a stirred mixture of KOH (1.9 g, 34.3 mmol) and Bu_4NBr (720 mg, 2.27 mmol) in the same solvent (60 mL). After the resulting solution was stirred for 15 min, a solution of 4-bromo-2-methyl-2-butene (4.3 g, 28.8 mmol) in CH_2Cl_2/CH_3CN (100 mL, 19:1) was slowly added over 1 h. After being stirred for 2 h further, the reaction mixture was diluted with water, and the organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was filtered through a short pad of silica gel eluting with hexanes/EtOAc (9:1) to yield **11** (5.6 g, 97%) as a clear oil. Anal. ($C_{17}H_{30}N_2O_4S$) C, H, N.

1-Amino-4-[*N*^{2},*N*^{3}-bis(tert-butoxycarbonyl)-*N*^{3}-(γ,γ-dimethylallyl)guanidino]butane (12). A solution of **11** (3.58 g, 10 mmol) in THF (25 mL) was added dropwise to a stirred solution of 1,4-diaminobutane (2.29 g, 26 mmol) in THF/H_2O (40 mL; 20:1, v/v). After being stirred for 1 h at 50 °C, the reaction mixture was concentrated in vacuo, and the residue was partitioned between $CHCl_3$ and 10% aqueous $NaHCO_3$. The organic layer was dried (Na_2SO_4) and evaporated. The residue by chromatography on a silica gel column ($CHCl_3$/Et_3N, 19:1) gave **12** (2.79 g, 70%) as a clear oil. Anal. ($C_{20}H_{38}N_4O_4$) C, H, N.

***N*-(4-Aminobutyl)-*N*′-(γ,γ-dimethylallyl)guanidine Diacetate (13).** A solution of **12** (518 mg, 1.3 mmol) and methanesulfonic acid (0.17 mL, 2.6 mmol) in dry 1,4-dioxane (15 mL) was held at reflux overnight, then and concentrated in vacuo. The residue was applied to a column packed with Dowex 1x2-200 ion-exchange resin (CH_3COO^- form). Elution with MeOH afforded crude **13**, which was further purified by a column of Sephadex LH-20 preswelled in MeOH, to give pure **13** (125 mg, 30%) as an oil. Anal. ($C_{14}H_{30}N_4O_4$) C, H, N.

***N*^{3}-Prenylagmatine (2).** Treatment of **13** (125 mg) with anion-exchange resin gave G3 (**2**; 59 mg), identical (^{1}H, ^{13}C NMR, FABMS) with the natural sample.

***N*,*N*′-Bis(tert-butoxycarbonyl)-N-(γ,γ-dimethylallyl)-guanidine (14).** To a solution of **11** (1.0 g, 2.8 mmol) in EtOH (30 mL) was added a 25% NH_4OH solution (14 mL) with stirring at room temperature over 7 h in small portions. The reaction mixture was evaporated, and the residue was partitioned between CH_2Cl_2 and water. The organic layer was washed with water, dried (Na_2SO_4), and evaporated to give **14** (560 mg, 61%) as an oil. Anal. ($C_{16}H_{29}N_3O_4$) C, H, N.

***N*-(γ,γ-Dimethylallyl)guanidine Acetate (15).** To a solution of **14** (262 mg, 0.8 mmol) in dry 1,4-dioxane (20 mL) was added methanesulfonic acid (52 mL, 0.8 mmol) at 0 °C with stirring. The reaction mixture was held at reflux for 20 h and evaporated. The residue by chromatography on silica gel (CH_2Cl_2/MeOH, 7:3) gave **15** (168 mg, 90% yield) as an oil. Anal. ($C_8H_{17}N_3O_2$) C, H, N.

Galegine (Prenylagmatine, 7). Treatment of **15** (168 mg) with anion-exchange resin gave G7 (**7**; 108 mg), identical (^{1}H, ^{13}C NMR, FABMS) with the natural sample.

Compounds 8b−e. The compounds were obtained as oils following the same procedure reported for **8a** and using the appropriate α,ω-diaminoalkane. Yields:

1-amino-4-[*N*^{2},*N*^{3}-bis(*tert*-butoxycarbonyl)guanidino]-ethane **(8b)**, 48%; 1-amino-4-[*N*^{2},*N*^{3}-bis(*tert*-butoxycarbonyl)-guanidino]propane **(8c)**, 76%; 1-amino-4-[*N*^{2},*N*^{3}-bis(*tert*-butoxycarbonyl)guanidino]pentane **(8d)**, 67%; 1-amino-4-[*N*^{2},*N*^{3}-bis(*tert*-butoxycarbonyl)guanidino]hexane **(8e)**, 57%.

Compounds 9b−e. The compounds were obtained as viscous oils starting from **8b−e** and following the procedure described for **9a**. Yields:

(*E*)-4-[*N*^{2},*N*^{3}-bis(*tert*-butoxycarbonyl)guanidino]-1-[(3,4-dimethoxycinnamoyl)amino]ethane **(9b)**, 98%;

(*E*)-4-[N^2,N^3-bis(*tert*-butoxycarbonyl)guanidino]-1-[(3,4-dimethoxycinnamoyl)amino]propane **(9c)**, 87%;

(*E*)-4-[N^2,N^3-bis(*tert*-butoxycarbonyl)guanidino]-1[(3,4-dimethoxycinnamoyl)amino]pentane **(9d)**, 68%;

(*E*)-4-[N^2,N^3-bis(*tert*-butoxycarbonyl)guanidino]-1-[(3,4-dimethoxycinnamoyl)amino]hexane **(9e)**, 80%.

Compounds 10b−e. Following the same procedure described for **10a**, the title compounds were prepared.

(*E*)-1-[(3,4-Dimethoxycinnamoyl)amino]-2-guanidinoetane Mesylate (10b): yield 74%; mp 148−151 °C; ^{1}H NMR (C_5D_5N) δ 9.35 (1H, t, J = 6 Hz, NHCO), 9.30 (1H, br t, J = 5 Hz, NH), 8.04 (1H, d, J = 15.5 Hz, H-α), 7.30 (1H, d, J = 2 Hz, H-2′), 7.23 (1H, dd, J = 8 and 2 Hz, H-6′), 7.12 (1H, d, J = 15.5 Hz, H-*β*), 6.86 (1H, d, J = 8 Hz, H-5′), 3.84 (2H, q, J = 6 Hz, 1-CH_2N), 3.76, 3.72 (3H each, s, 2 × OMe), 3.72 (2H, q, J = 5.5 Hz, 2-CH_2N), 3.12 (3H, s, Me); ^{13}C NMR δ 167.68 (s, CO), 159.03 (s, C═NH), 150.09, 149.19 (s each, C-3′, C-4′), 140.50 (d, C-α), 128.69 (s, C-1′), 122.20, 120.28 (d each, C-6′, C-*β*), 112.09 (d, C-5′), 110.83 (d, C-3′), 55.75 (q, OMe), 42.14 (t, C-1), 40.29 (q, Me), 39.06 (t, C-2); FABMS (TDEG/Gly) m/z 293 (M^+ + 1). Anal. ($C_{15}H_{24}N_4O_6S$) C, H, N.

(*E*)-1-[(3,4-Dimethoxycinnamoyl)amino]-3-guanidinopropane Mesylate (10c): yield 47%; mp 170−173 °C; ^{1}H NMR (C_5D_5N) δ 9.02 (1H, br t, J = 5 Hz, NH), 9.00 (1H, t, J = 6.5 Hz, NHCO), 8.04 (1H, d, J = 15.5 Hz, H-α), 7.31 (1H, d, J = 2 Hz, H-2′), 7.22 (1H, dd, J = 8 and 2 Hz, H-6′), 7.14 (1H, (1H, d, J = 15.5 Hz, H-*β*), 6.86 (1H, d, J = 8 Hz, H-5′), 3.79, 3.73 (3H each, s, 2 × OMe), 3.72 (2H, q, J = 6 Hz, 1-CH_2N), 3.58 (2H, q, J = 6 Hz, 4-CH_2N), 3.12 (3H, s, Me), 2.05 (2H, m, J = 6 Hz, CH_2); ^{13}C NMR δ 167.17 (s, CO), 158.61 (s, C═NH), 150.08, 149.18 (s each, C-3′, C-4′), 140.07 (d, C-α), 128.91 (s, C-1′), 122.23, 120.75 (d each, C-6′, C-*β*), 112.12 (d, C-5′), 110.75 (d, C-3′), 55.79 (q, OMe), 40.37 (q, Me), 40.05 (t, C-1), 37.24 (t, C-3), 29.49 (t, C-2); FABMS (TDEG/Gly) m/z 307 (M^+ + 1). Anal. ($C_{16}H_{26}N_4O_6S$) C, H, N.

(*E*)-1-[(3,4-Dimethoxycinnamoyl)amino]-5-guanidinopentane Mesylate (10d): yield 57%; mp 193−196 °C; ^{1}H NMR (C_5D_5N) δ 8.95 (1H, br t, J = 6 Hz, NH), 8.79 (1H, t, J = 7 Hz, NHCO), 8.12 (1H, d, J = 15.5 Hz, H-α), 7.50 (1H, d, J = 2 Hz, H-2′), 7.25 (1H, dd, J = 8 and 2 Hz, H-6′), 7.39 (1H, (1H, d, J = 15.5 Hz, H-*β*), 6.86 (1H, d, J = 8 Hz, H-5′), 3.84, 3.72 (3H each, s, 2 × OMe), 3.62 (2H, q, J = 6 Hz, 1-CH_2N), 3.34 (2H, q, J = 6 Hz, 5-CH_2N), 3.11 (3H, s, Me), 1.64 (4H, m, J = 6 Hz, 2- CH_2,4-CH_2), 1.58 (2H, br m, 3-CH_2); ^{13}C NMR δ 166.81 (s, CO), 158.61 (s, C═NH), 150.08, 149.18 (s each, C-3′, C-4′), 139.75 (d, C-α), 129.16 (s, C-1′), 122.32, 121.36 (d each, C-6′, C-*β*), 112.02 (d, C-5′), 110.57 (d, C-3′), 55.84, 55.73 (q each, OMe), 41.49 (t, C-1), 40.41 (q, Me), 39.00 (t, C-5), 29.20, 28.13 (t each, C-2, C-4), 24.09 (t, C-3); FABMS (TDEG/Gly) m/z 335 (M^+ + 1). Anal. ($C_{18}H_{30}N_4O_6S$) C, H, N.

(*E*)-1-[(3,4-Dimethoxycinnamoyl)amino]-6-guanidinohexane Mesylate (10e): yield 72%; mp 150−153 °C; ^{1}H NMR (C_5D_5N) δ 9.11 (1H, br t, J = 6 Hz, NH), 8.75 (1H, t, J = 7 Hz, NHCO), 8.12 (1H, d, J = 15.5 Hz, H-α), 7.51 (1H, d, J = 2 Hz, H-2′), 7.38 (1H, (1H, d, J = 15.5 Hz, H-*β*), 7.23 (1H, dd, J = 8 and 2 Hz, H-6′), 6.86 (1H, d, J = 8 Hz, H-5′), 3.83, 3.71 (3H each, s, 2 × OMe), 3.58 (2H, br q, J = 6 Hz, 1-CH_2N), 3.32 (2H, br q, J = 6 Hz, 5-CH_2N), 3.10 (3H, s, Me), 1.62 (4H, m, 2-CH_2,5-CH_2), 1.55 (4H, br m, 3- CH_2, 4-CH_2); ^{13}C NMR δ 166.76 (s, CO), 158.62 (s, C═NH), 150.68, 149.16 (s each, C-3′, C-4′), 139.68 (d, C-α), 129.18 (s, C-1′), 122.35, 121.48 (d each, C-6′, C-*β*), 111.98 (d, C-5′), 110.66 (d, C-3′), 55.82, 55.76 (q each, OMe), 41.42 (t, C-1), 40.28 (q, Me), 39.16 (t, C-6), 29.20, 28.13 (t each, C-2, C-5), 24.02, 23.89 (t each, C-3, C-4); FABMS (TDEG/Gly) m/z 349 (M^+ + 1). Anal. ($C_{19}H_{32}N_4O_6S$) C, H, N.

4-[N^2,N^3-Bis(tert-butoxycarbonyl)guanidino]-1-[(4-methoxybenzoyl)amino]butane (16). Compound **16** was prepared as a white amorphous solid from 4-methoxybenzoic acid following the same procedure described for **9a**; yield 54%.

1-[(4-Methoxybenzoyl)amino]-4-guanidinobutane Mesylate (16a). Compound **16a** was prepared as described for **10a** in 59% yield: pale yellow glass; IR ($CHCl_3$) ν_{max} 3240, 1730 cm^{-1}; ^{1}H NMR (CD_3OD) δ 7.87 (2H, d, J = 8.5 Hz, H-2′, H-6′), 6.88 (2H, d, J = 8.5 Hz, H-3′, H-5′), 3.79 (3H, s, OMe), 3.42, 3.25 (2H each, br t, J = 6 Hz, 2 CH_2N), 2.72 (3H, s, Me), 1.64 (4H, m, 2 × CH_2); ^{13}C NMR δ 168.93 (s, CO), 162.08 (s, C-4′), 158.78 (s, C═NH), 125.91 (s, C-1′), 133.04 (d each, C-2′, C-6′), 115.27 (d, C-3′, C-5′), 56.48 (q, OMe), 42.25 (t, C-1), 39.63 (q, Me), 38.89 (t, C-4) 27.90, 27.78 (t each, C-2, C-3); FABMS (TDEG/Gly) m/z 265 (M^+ + 1). Anal. ($C_{14}H_{24}N_4O_5S$) C, H, N.

Pharmacology. Animals. Adult male Wistar rats (average mass 280 ± 5 g, mean ± SE; n = 56) were provided by the Catholic University farm, Rome, Italy. Maintenance, feeding, and general treatment of the animals were as previously described.[20,22]

Cardiovascular Determinations and Respiratory Monitoring. Animals were anesthetized with 10% (w/v) ethylurethane (1 mL/100 g of body mass), which was dissolved in 0.9% NaCl solution (saline) and administered with a single intraperitoneal (ip) injection. The trachea was cannulated to allow spontaneous breathing. Polyethylene catheters, containing sodium heparin (100 US pharmacopeia U/mL), were inserted into the left femoral artery for recording aortic BP and into the right femoral vein for drug administration. Cardiac inotropism was evaluated as the maximum rate of rise of the left ventricular isovolumetric pressure (dP/dt), which was obtained by means of a calibrated 3F catheter-tip pressure transducer (Millar Instruments, Houston, TX) inserted into the left common carotid artery and advanced into the left ventricle.[29,30] A P23Db pressure transducer (Statham Medical Instruments, Los Angeles, CA) was used for measuring systolic and diastolic BPs, which were averaged electronically.[31] HR was obtained by a 9875B Beckman cardiotachometer coupler (Beckman Instruments, Inc., Shiller Park, IL), which was triggered by the R-peak of the lead II electrocardiogram.[32] The pulsatile BP registered in the left ventricle was differentiated through a BL 622 derivative computer (Biotronex Laboratories, Inc., Kensington, MA) for determining positive and negative dP/dt (measured in mmHg/s), as previously described.[26,30] All cardiovascular parameters were continuously monitored by means of a polygraphic recorder (Mangoni Elettronica, Pisa, Italy), which was interfaced to a computer system giving the cardiovascular measures in real time.[26] A light-heated table was employed to mantain a constant 37 °C body temperature of the animals. They were heparinized, as previously described,[27,29] and respiration was also monitored by using a pneumotachograph adapted to a Biotronex BL 620 integrator to yield the full respiratory wave. In this regard, the tracheal cannula was connected to the pneumotachograph to assess RF and TV under spontaneous breathing.[28,30] Also RF and TV were continuously monitored polygraphically. After completion of the surgical procedures, the rats received no treatment for 60 min to allow for the stabilization of the cardiovascular and respiratory parameters.

Protocol. Eight rats were used to determine the dose−response relationship for each of the tested compounds [(*E*)-G5, (*Z*)-G5, **16a**, **10b**−**e**]. Saline solutions of these compounds were prepared daily and injected in a volume of 50 μL; the doses ranged from 50 to 12800 μg/kg of body mass (ratio 2.0) and were expressed in terms of free bases. The control administration of the solvent alone did not change the cardiovascular and respiratory parameters. Peak effects were considered for each assay. Each of the consecutive tests was not performed until the parameters had returned to the values preceding the first administration and had stabilized.

Fifty-two rats were used to compare, on a molar basis, the mean BP responses to some antihypertensive or vasodilating drugs with those to (*E*)-G5, (*Z*)-G5 (**10a**), **16a,** and **10b**−**e** (n = 4 for each drug). These rats received, by iv injection under the above experimental conditions, a selected dose (4.12 μM/kg) of each of the guanidine compounds or a dose of guanethidine (25 μM/kg), clonidine (0.108 μM/kg), hexamethonium (12 μM/kg), reserpine (8 μM/kg), papaverine (5 μM/kg), or histamine (0.044 μM/kg). All drugs were dissolved in saline solution, and all doses were expressed in terms of free bases. General experimental conditions were as above.

Statistics. Data were expressed as means ± SE. Dose−response relationships of the guanidine compounds were

processed by regression analysis followed by analysis of variance for testing differences among them (at levels of $P < 0.05$).[33]

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Supporting Information Available: ^{1}H NMR, IR, and FABMS spectral data of the synthetic intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

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