Novel Hypotensive Agents from *Verbesina caracasana*. 2. Synthesis and Pharmacology of Caracasanamide[1]

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Caracasanamide, one of the hypotensive agents isolated from *Verbesina caracasana*, is a mixture of (*Z*)-**1a** and (*E*)-**1b** forms of 1-[(3,4-dimethoxycinnamoyl)amino]-4-[(3-methyl-2-butenyl)-guanidino]butane.[1] The structure of (*E*)-caracasanamide (**1b**) was confirmed by high-yielding synthesis starting from *N,N'*-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea. The water-soluble *Z*-form of **1a**, assayed by iv route in anesthetized rats at doses ranging from 50 to 1600 μg/kg body weight, was found to decrease blood pressure, to increase cardiac inotropism, respiratory frequency, and tidal volume, and to induce a very slight and not significant tachycardia. Higher doses determined respiratory depression and, in some cases, consequent cardiac arrest. The compound was shown to affect cardiovascular function by acting at the vascular level in inducing arterial vasodilation, by determining sympathetic hypotone through central neurogenic mechanisms, and by interacting with the cardiac β_1-adrenoreceptors. The respiratory effects were independent of the cardiovascular ones. In lowering blood pressure, the compound was more potent than guanethidine and not less potent than reserpine and papaverine. (*Z*)-Caracasanamide may therefore be useful in the treatment of arterial hypertension of moderate degree.

Introduction

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.[2,3] As a result, guanethidine,[4] guanabenz,[5] and guanfacine[6] have been introduced in antihypertensive drug therapy. Guanethidine is unable to cross the blood–brain barrier, thus inducing arterial hypotension only by peripheral mechanisms, which include depletion of noradrenaline in the postganglionic adrenergic endings.[7,8] On the other hand, 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.[9,10]

A new (arylamino)guanidine derivative with a hypotensive effect, pinacidil (P 1134), has been recently synthesized.[11] Like guanethidine, pinacidil is devoid of actions in the central nervous system, and its ability to lower blood pressure depends on arterial vasodilation not related to changes of the peripheral adrenergic nerve activity.[12,13]

Short *et al.* studied 84 synthetic guanidine derivatives in order to find a relationship between their sympathetic blocking activity and chemical structure.[14,15] It was noted that the nitrogen of the guanidine nucleus, to which the side chain is linked, must also bear a hydrogen for the compound to have pharmacological activity. Small variations in the structure of the side chain decreased the activity of these compounds.

Conversely, the introduction of a second basic nitrogen into the side chain in the form of a piperazine nucleus has yielded one of the most active compounds of the series, whereas introduction of a third nitrogen caused loss of activity. Fielden *et al.* studied the relationship between structure of some aralkylguanidines and their adrenergic nerve blocking activity.[16] They found that small changes in structure led to wide variations of activity. The adrenergic blocking activity of *N*-aralkylguanidines was dependent on the nature of the alkyl side chain, the substitution of the aromatic nucleus, and the stereochemistry of the optical isomers. These isomers showed a similar activity in the rabbit nerve-jejunum preparation, but only the (−) isomers showed adrenergic blocking activity when assayed on the nictitating membrane preparation, in which the (+) isomers antagonized the blocking action of the (−) ones. Such findings suggested that these guanidine derivatives were provided with a sympathetic blocking activity related to specific chemical properties.

A crude methanol extract of the Venezuelan plant *Verbesina caracasana* Fries, intravenously administered to mice, was found to induce biological effects such as erection of hair, initial stimulation, and subsequent blockade of breathing. Experiments on male dogs showed that the crude extract of the plant (0.5–4.0 mg/kg body weight by iv route, ratio 2.0) was able to reduce mean blood pressure (BP) in a dose-related manner; the maximum effect was observed at the dose of 2.0 mg/kg (−58 ± 4% with respect to the basal BP values; mean +SE; n = 6) and did not change significantly under barbiturate or chloralose anesthesia.[17]

Biologically controlled purification, culminating in silica gel chromatography, yielded a series of active compounds, the least polar of which ($C_{21}H_{32}N_4O_2$) was named caracasanamide and assigned the structure 1-[(3,4-dimethoxycinnamoyl)amino]-4-[(3-methyl-2-butenyl)guanidino]-butane.[1] The compound was a mixture of the (*E*)- and

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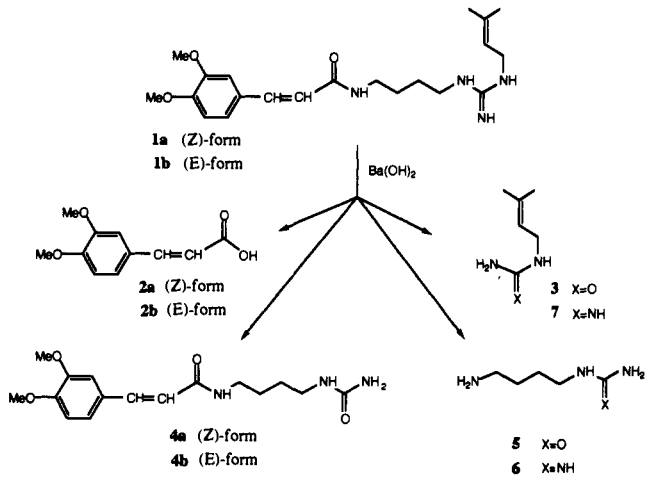
Scheme I



(*Z*)-forms (^{1}H NMR evidence). The higher solubility in water of the latter provided a basis for the separation.

In this paper the pharmacological profile of the water-soluble (*Z*)-form (**1a**) and the synthesis of the (*E*)-form of caracasanamide are reported.

Results and Discussion

Structure. Hydrolysis with $Ba(OH)_2$ of caracasanamide[1] afforded a series of products (**2–5**), summarized in Scheme I. Compounds **2a** and **2b** were (*Z*)- and (*E*)-3,4-dimethoxycinnamic acids, respectively, as expected by the presence in caracasanamide of both (*Z*)- and (*E*)-forms. Also, compounds **4a** and **4b** constituted a pair of (*Z*)- and (*E*)-isomers, as revealed by ^{1}H- and ^{13}C-NMR spectra which were very similar to those of caracasanamide except for signals due to the γ,γ-dimethylallyl chain.

The most abundant and crystalline isomer was assigned the structure **4a** on the basis of spectral data.

3-Methylbut-2-enylurea (**3**) and 4-amino-1-ureidobutane (**5**, as monohydrogensulfate) were finally isolated from the reaction mixture and identified by comparison of the spectral data with those of agmatine (**6**) and galegine[18] (**7**), respectively.

The presence of the guanidine group in caracasanamide was supported by the absorbance at 1655 cm^{-1} in the infrared, the ^{13}C-NMR signal due to the C=NH carbon (δ 155), and by consideration of the hydrolysis products. Taken together with the ^{1}H- and ^{13}C-NMR spectra of **1a**, **2a**, **3**, **4a**, and **5**, compared in Table I, these data established the structure of (*Z*)-caracasanamide as **1a**, occurring along with the (*E*)-isomer **1b**.

Synthesis

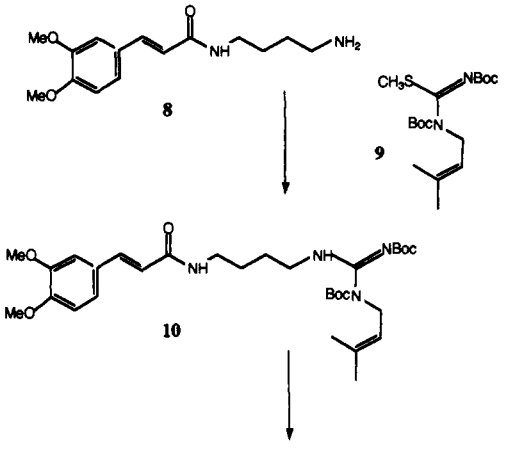
The first approach to the synthesis of (*E*)-caracasanamide (**1b**) involved the acylation of the commercially available 1,4-diaminobutane with 3,4-dimethoxycinnamoyl chloride (Scheme II).[1] The reaction, carried out under different experimental conditions, always gave a mixture of products, the most abundant being the symmetrically disubstituted 1,4-bis(3,4-dimethoxycinnamoylamino)butane. In order to minimize this diacylation reaction, 1,4-diaminobutane was treated with the less reactive acylating agent 3,4-dimethoxycinnamamide, following a thermal transamidation procedure recently reported[19] as a method of choice for the selective monoacylation of α,ω-diaminoalkanes.

Table I. ^{1}H- and ^{13}C-NMR Data of (*Z*)-Caracasanamide and Its Hydrolysis Products[a]

	1a	2a	3	4a	5
H_2-1, H_2-4	3.5–2.9			3.4–2.9	3.3–2.8
H_2-2, H_2-3	1.6–1.3			1.6–1.2	2.0–1.6
H-2′		7.60		7.21	
H-5′	7.15-6.95	7.18		6.75	
H-6′		6.85		6.97	
H-α	5.98		5.80		5.80
H-β	6.78		6.80		6.56
H_2-1″	3.74		3.72		
H-2″	5.18		5.22		
H_3-4″	1.76		1.72		
H_3-5″			1.58		
OMe	3.85, 3.82	3.90, 3.89		3.96, 3.83	
C-1	40.5[b]			39.2[b]	39.9[b]
C-2	27.2[c]			26.2[c]	26.8[c]
C-3	27.7[c]			27.0[c]	26.4[c]
C-4	39.8[b]			38.8[b]	39.8[b]
C-1′	129.3	128.4		127.7	
C-2′	111.6	111.0		109.7	
C-3′	150.8	148.7		148.8	
C-4′	152.1	150.4		150.3	
C-5′	112.9	113.8		111.0	
C-6′	122.6	125.1		121.7	
OMe	56.5	56.3/56.2		55.8/55.7	
C-α	141.7	143.0		140.2	
C-β	119.4	118.4		118.4	
C=O	169.1	169.9		167.2	
C=X[d]	157.4		159.3	160.2	161.3
C-1″	42.3		38.3		
C-2″	123.3		121.1		
C-3″	138.2		135.7		
C-4″	25.8		25.6		
C-5″	18.1		17.8		

[a] Solvents: (D_2O), **1a**, **5**; $CDCl_3$–CD_3OD, **2a**, **4a**; $CDCl_3$, **3**. TMS as internal reference. The signals showed the appropriate integrate intensity. ^{1}H NMR multiplicities (Hz): $J_{2',6'}$ = 2 Hz; $J_{5',6'}$ = 8 Hz, $J_{\alpha,\beta}$ = 13 Hz, $J_{1'',2''}$ = 7. Due to the presence of the amide and guanido groups some signals are doubled; only the signal with major intensity is reported. [b,c] In the same column may be interchanged. [d] X = NH; **1a**, **3**. X = O; **4a**, **5**.

Scheme II



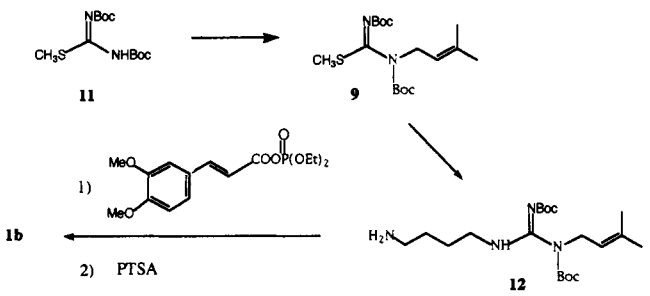
However, only a small amount of monoacylated product was obtained. We have therefore developed a synthesis starting from the guanidine moiety, as outlined in Scheme III. *N,N′*-Bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea (**11**), a valuable precursor of protected guanidine derivatives, was prepared by a modification of the literature method.[20] Alkylation of **11** with 4-bromo-2-methyl-2-butene in the presence of sodium hydride in dry DMF at room temperature cleanly afforded **9**, which was reacted with 1,4-diaminobutane (2.6 mol equiv) in THF. Under these experimental conditions the displacement of the

Table II. Changes[a] in Systolic and Diastolic Blood Pressure, Heart Rate, and Maximum Rate of Rise of the Left Ventricular Isovolumetric Pressure (dP/dt) Following iv Administration of (Z)-Caracasanamide (1a) in Anesthetized Rats

	blood pressure							
	systolic		diastolic		heart rate		dP/dt	
1a (μg/kg)	mmHg	%	mmHg	%	beats/min	%	mmHg/s	%
50	−4 ± 2	−5 ± 1	−5 ± 1	−8 ± 2	+8 ± 3	+2 ± 1	+52 ± 8	+1 ± 0.5
100	−6 ± 2	−8 ± 2	−6 ± 2	−9 ± 1	+10 ± 1	+2 ± 1	+141 ± 25	+3 ± 1
200	−11 ± 3	−14 ± 2	−10 ± 2	−16 ± 3	+13 ± 2	+3 ± 2	+1284 ± 78	+25 ± 3
400	−15 ± 4	−18 ± 4	−14 ± 3	−23 ± 2	+14 ± 5	+3 ± 1	+1712 ± 107	+33 ± 5
800	−17 ± 3	−21 ± 3	−15 ± 3	−23 ± 3	+18 ± 4	+4 ± 1	+2140 ± 263	+41 ± 6
1600	−21 ± 6	−26 ± 5	−18 ± 4	−29 ± 5	+22 ± 3	+5 ± 1	+2996 ± 104	+57 ± 6
3200	−25 ± 5	−29 ± 4	−29 ± 4	−47 ± 3	+25 ± 8	+5 ± 2	+2984 ± 362	+55 ± 4
6400[b]	−34 ± 4	−40 ± 3	−31 ± 2	−48 ± 4	+20 ± 4	+5 ± 1	+3074 ± 441	+58 ± 6
base line	86 ± 3		65 ± 3		475 ± 6		+5411 ± 257	

[a] Values are means ± SE (n = 10). [b] n = 6.

Scheme III



methylthio group of 9 proceeded smoothly to give 12 in fairly good yield, and no diguanidinobutane derivative was detected in the reaction mixture. Subsequent acylation of 12 with (E)-3,4-dimethoxycinnamoyl chloride did not provide good results even at low reaction temperatures.

However, when (E)-3,4-dimethoxycinnamic acid was activated as a mixed anhydride with diethyl chlorophosphate, the selective introduction of the dimethoxycinnamoyl moiety at the primary amino group was achieved. Simple chromatographic purification afforded 10 in 70% yield. Removal of the *tert*-butoxycarbonyl groups with an excess of trifluoroacetic acid at room temperature gave a complex mixture, in which the deprotected product was present only in a small amount. Deprotection of 10 was best performed with a catalytic amount of *p*-toluenesulfonic acid in refluxing 1,4-dioxane. Column chromatography on alumina then furnished a product (60% yield), which was proved to be (E)-caracassanamide (1b) by comparison of its spectral and chromatographic data with those of an authentic sample.

We also attempted to prepare (Z)-3,4-dimethoxycinnamic acid in order to synthesize 1a in an analogous fashion. Although a literature report[21] claims the Z form of the acid to be obtained *via* partial hydrogenation of the corresponding (3,4-dimethoxyphenyl)propiolic acid in the presence of the Lindlar catalyst, we obtained only a mixture of completely and partially hydrogenated compounds. These results are in agreement with those recently obtained by other authors[22] in the stereoselective hydrogenation of phenylpropiolic acid derivatives.

This straightforward and high-yielding synthesis of 1b represents a basis for the preparation of analogous products of potential pharmacological interest. Such studies are in progress in our laboratory.

Pharmacology

The cardiovascular and respiratory effects of (Z)-caracasanamide are reported in Table II. The drug reduced systolic and diastolic blood pressure (BP) in a dose-related manner; the hypotensive response was associated to a dose-dependent increase of dP/dt.

Table III. Changes[a] in Respiratory Frequency and Tidal Volume Following iv Administration of (Z)-Caracasanamide (1a) in Anesthetized Rats

	respiratory frequency		tidal volume	
1a (μg/kg)	beats/min	%	(μL)	%
50	+6 ± 2	+6 ± 1	+1030 ± 252	+17 ± 4
100	+9 ± 4	+8 ± 3	+1539 ± 374	+25 ± 7
200	+12 ± 2	+12 ± 2	+2092 ± 650	+34 ± 6
400	+16 ± 3	+16 ± 4	+2220 ± 260	+37 ± 6
800	+25 ± 6	+24 ± 5	+3134 ± 351	+51 ± 8
1600	+23 ± 8	+21 ± 5	+2501 ± 448	+41 ± 7
3200	−41 ± 5	−39 ± 4	−4318 ± 522	−70 ± 8
6400[b]	−87 ± 9	−80 ± 6	−5286 ± 630	−86 ± 9
base line	109 ± 9		6131 ± 348	

[a] Values are means ± SE (n = 10). [b] n = 6.

Heart rate (HR) was slightly increased by (Z)-caracasanamide, without significant differences within the tested dose range of the drug. Submaximal effects of (Z)-caracasanamide in diastolic BP and dP/dt were observed with doses of 3200 and 1600 μg/kg, respectively. On the other hand, respiratory frequency (RF) and tidal volume (TV) increased up to the dose of 1600 μg/kg, with a maximal effect at the dose of 800 μg/kg. However, a great reduction of both RF and TV was observed when (Z)-caracasanamide was administered at the doses of 3200 and 6400 μg/kg.

In this respect, four of the 10 rats treated with the dose of 6400 μg/kg died because of respiratory blockage, which developed within a period of 4–5 min starting from administration of the drug. The effects of (Z)-caracasanamide on respiratory function reported in Table III concern the six rats that survived to the dose of 6400 μg/kg; these rats needed up to 10 min for restoration of the values of RF and TV observed before the administration of the drug. The hypotensive effect of (Z)-caracasanamide began 4–5 s, starting from its iv administration, and increased progressively, reaching a maximum at the maximal increase of TV. The drug-induced hypotension lasted, in the dose interval 100–1600 μg/kg, from 85 ± 10 s to 131 ± 14 s (n = 10). dP/dt increased gradually with a maximum in coincidence with the maximal effect of the drug on BP and TV and returned to the values preceding administration within the same times of restoration of the BP values. The slight tachycardic response to (Z)-caracasanamide developed during the course of the effects on BP and dP/dt. However, in the dose interval 1600–6400 μg/kg, a bradycardic response to the drug was observed alongside the maximal effects of this compound on BP,

Table IV. Cardiovascular Responses[a] to iv Doses of (*Z*)-Caracasanamide (1a) Alone (Control) and after iv Administration of Adrenoreceptor-Blocking Drugs, Hexamethonium, or Spinalization under Vagotomy in Anesthetized Rats

treatment	1a (μg/kg)	blood pressure (mmHg) systolic	blood pressure (mmHg) diastolic	heart rate (beats/min)	dP/dt (mmHg/s)
control	100	−7 ± 2	−8 ± 1	+13 ± 2	+227 ± 16
	200	−13 ± 1-	−12 ± 1	+17 ± 4	+1154 ± 42
	800	−19 ± 3	−16 ± 3	+19 ± 3	+2428 ± 174
	1600	−22 ± 3	−18 ± 3	+21 ± 2	+2823 ± 211
	3200	−28 ± 3	−29 ± 3	+25 ± 3	+2909 ± 159
	6400	−34 ± 6	−31 ± 5	+29 ± 5	+3317 ± 398
phentolamine (2 mg/kg)	800	−22 ± 3	−19 ± 4	+17 ± 6	+2503 ± 204
	3200	−37 ± 6	−39 ± 7	+24 ± 8	+2627 ± 209
propranolol (3 mg/kg)	800	−18 ± 6	−13 ± 7	+6 ± 2[b]	+415 ± 51[b]
	1600	−25 ± 3	−20 ± 2	+7 ± 1[b]	+520 ± 47[b]
	3200	−30 ± 4	−26 ± 5	+9 ± 1[b]	+760 ± 64[b]
	6400	−35 ± 4	−28 ± 6	+11 ± 3[b]	+853 ± 70[b]
hexamethonium (5 mg/kg)	800	−5 ± 1[b]	−6 ± 2[b]	+5 ± 3[b]	+890 ± 45[b]
	1600	−8 ± 2[b]	−9 ± 2[b]	+10 ± 3[b]	+1008 ± 91[b]
	3200	−11 ± 6[b]	−17 ± 1[b]	+9 ± 5[b]	+1124 ± 470[b]
	6400	−13 ± 4[b]	−20 ± 2[b]	+12 ± 4[b]	+1447 ± 126[b]
spinalization under vagotomy	100	0	0	0	0
	200	0	0	0	0
	3200	−8 ± 5[b]	−12 ± 3[b]	+3 ± 1[b]	+680 ± 71[b]

[a] Values are means ± SE (n = 6 in each group). [b] $p < 0.05$ (compared with the control mean).

Table V. Cardiovascular Responses[a] to (*Z*)-Caracasanamide (1a) and Several Antihypertensive or Vasodilating Drugs Administered by iv Route in Anesthetized Rats

drug	blood pressure (mmHg) systolic	blood pressure (mmHg) diastolic	heart rate (beats/min)	dP/dt (mmHg/s)
(*Z*)-caracasanamide (4.12 μM/kg)	−21 ± 6	−18 ± 4	+22 ± 3	+2996 ± 104
guanethidine (25 μM/kg)	−27 ± 5	−19 ± 3	−18 ± 6	−863 ± 160
clonidine (0.108 μM/kg)	−15 ± 4	−9 ± 1	−40 ± 8	−871 ± 74
hexamethonium (12 μM/kg)	−44 ± 2	−34 ± 2	−48 ± 9	−4040 ± 812
reserpine (8 μM/kg)	−31 ± 6	−24 ± 5	−47 ± 9	−1580 ± 134
papaverine (5 μM/kg)	−23 ± 3	−18 ± 3	−21 ± 5	−604 ± 82
histamine (0.044 μM/kg)	−30 ± 3	−28 ± 3	−7 ± 1	−1314 ± 180

[a] Values are means ± SE (n = 5 for each drug).

dP/dt, and TV. RF and TV increased at the same time after administration of 100–1600 μg/kg of the drug; the latency times for observing these effects ranged from 37 ± 6 s to 21 ± 5 s (n = 10). RF and TV reached their maximal values within another 15 s and, up to the dose of 1600 μg/kg, returned to the predrug values within 71 ± 8 s (lowest dose) to 112 ± 13 s (highest dose) starting from the reached maximal effect (n = 8). At the doses of 200 and 6400 μg/kg, (*Z*)-caracasanamide reduced RF and TV within 7 ± 2 s and 5 ± 3 s, respectively (n = 6). Four of the rats treated with 6400 μg/kg of the drug developed an irreversible respiratory blockade from administration of the drug. Subsequent to this blockade, there were bradycardia, ventricular extrasystolia, ventricular fibrillation, and final cardiac arrest. On the other hand, in the rats which survived to the dose of 6400 μg/kg of the drug, there was reduction of BP, HR, and dP/dt, with ventricular extrasystolia; these cardiovascular parameters gradually reached the predrug values along with the progressive restoration of the respiratory function.

Table IV shows the cardiovascular effects obtained with selected iv doses of (*Z*)-caracasanamide in rats treated with phentolamine (an $\alpha_{1,2}$-adrenoreceptor reversible antagonist), propranolol (a $\beta_{1,2}$-adrenoreceptor reversible antagonist), or hexamethonium (a ganglioplegic drug blocking the "nicotinic" cholinergic receptors) or when subjected to spinalization under vagotomy. The doses of phentolamine and propranolol were preliminarly assessed in order to produce a 50% reduction in the effects of norepinephrine (1 μg/kg, by iv route) and isoprenaline (0.625 μg/kg, by iv route) on mean BP. The dose of hexamethonium used was able to reduce the mean BP of about 15 mmHg. In these experimental conditions, phentolamine did not change the effects of (*Z*)-caracasanamide on BP, HR, and dP/dt, and propranolol did not affect the hypotensive responses to the drug. However, the stimulatory effects of (*Z*)-caracasanamide on HR and dP/dt were greatly opposed by propranolol. On the other hand, neither hexamethonium nor spinalization under vagotomy markedly antagonized the effects of the drug on BP, HR, and dP/dt. In particular, doses of the drug at 100 and 200 μg/kg were completely ineffective in changing the cardiovascular parameters when tested after transection of both vagi nerves and spinal cord.

Bilateral occlusion of the common carotid arteries (BCO) was performed[23] in five of the 10 rats used for assessing the dose–effect relationship for the drug, both before and after (20 s) iv administration of the drug (800 μg/kg). The BCO-induced increase of BP, HR, and dP/dt appeared to be consistently reduced by (*Z*)-caracasanamide.

As compared with some antihypertensive or vasodilatory drugs, (*Z*)-caracasanamide was more potent than guanethidine in lowering BP but less potent than clonidine and histamine. On the other hand, the hypotensive effects of the drug were similar to those induced by comparable doses of papaverine and reserpine (Table V). The drug-induced hypotension lasted more than that following administration of guanethidine, histamine, and papaverine.

In conclusion, (*Z*)-caracasanamide is a hypotensive drug that increases remarkably cardiac inotropism. The drug also has stimulating respiratory effects, consisting in

increase of the minute volume due to increase of both RF and TV. All these effects can be observed in the rat following iv administration of doses ranging from 50 to 1600 μg/kg. Moreover, at doses of 3200 μg/kg or higher administered by iv injection, the drug induces respiratory depression or blockade with consequent bradycardia, ventricular arrhythmias, and possible cardiac arrest.

The hypotensive effect of (*Z*)-caracasanamide precedes the other cardiovascular and respiratory actions. The few seconds required for observing the drug-induced arterial hypotension indicate that the drug acts at the vascular level determining arterial vasodilation and reduction of the total peripheral resistance. Therefore, the subsequent gradual increase of dP/dt and HR, reaching maximal values concomitantly to the maximal hypotensive effect, may be explained by a drug-induced baroreflex hyporeactivity.[24] In fact, the stimulation of vascular baroreceptors by arterial pulse is known to reduce, also through an enhanced vagal parasympathetic activity, both HR and stroke volume, with consequent decrease of cardiac output and BP. Conversely, a reduced baroreceptor stimulation leads to a higher sympathetic activity increasing HR, stroke volume, and peripheral vascular resistance.[25] In this way, the baroreceptor reflex system represents a mechanism of homeostatic regulation of the BP values that is triggered by changes of systemic BP.

On the basis of these considerations, the reduced neurogenic discharge from the baroreceptor areas may also explain the reduced cardiovascular responses to BCO observed in the rats treated with the drug.[24,25] The inability of phentolamine and propranolol to change, respectively, cardiovascular and vascular responses to caracasanamide shows that the guanidine compound does not interact with the cardiovascular $\alpha_{1,2}$-adrenoreceptors and the vascular β_2-adrenoreceptors.[26] On the other hand, considering the cardiovascular responses to the drug during either ganglionic blockade or spinalization under vagotomy, there is no doubt that (*Z*)-caracasanamide decreases sympathetic nerve outflow by acting on central sympathetic pathways. Therefore, the hypotensive effect of the drug appears to involve a peripheral vascular component and, subsequently, central neurogenic mechanisms. Further studies will be able to clarify levels of action and neuropharmacological effects of (*Z*)-caracasanamide within the central nervous system.

The observed effects of the drug on the heart show, considering the antagonistic properties of propranolol, that (*Z*)-caracasanamide interacts with cardiac β_1-adrenoreceptors and/or intracellular β_1-receptors-dependent biochemical pathways and/or baroreflex and central sympathetic mechanisms. In fact, like clonidine and α-methyldopa, propranolol induces arterial hypotension by also enhancing baroreflex responsiveness[24,25,27] or sensitivity and by reducing sympathetic tone by central mechanisms. Thus, when administered after propranolol, the drug is likely to be provided with lower effects in reducing both baroreflex reactivity and sympathetic tone. In this respect, the inability of propranolol to change the hypotensive response to (*Z*)-caracasanamide indicates, besides the lack of significant interactions of the drug with the vascular β_2-adrenoreceptors, that this response is prevalently related to the peripheral vasodilatatory effect. Moreover, it may also be thought that, at the cardiac level, the drug-induced sympathetic hypotone is overcome by a reduced baroreflex activity and/or by an increased β_1-adrenoreceptor reactivity. These mechanisms may explain why caracasanamide effects arterial hypotension even during increase of both dP/dt and HR.

Notably, (*Z*)-caracasanamide is more potent than guanethidine in lowering BP and as potent as reserpine and papaverine. Although provided with hypotensive effects lower than those of clonidine, hexamethonium, and histamine, (*Z*)-caracasanamide does not decrease (at nontoxic doses) cardiac inotropism and chronotropism in contrast with all the hypotensive drugs mentioned. The longer duration of the hypotensive effect of (*Z*)-caracasanamide, as compared to that of drugs (like guanethidine, papaverine, and histamine) acting by peripheral vasodilatatory mechanisms,[7,8] may be further evidence that this drug acts by both peripheral and central mechanisms.

The biphasism of the respiratory effects of (*Z*)-caracasanamide is not related to the cardiovascular actions of the drug. In fact, it decreases BP and increases dP/dt and HR in the presence of either stimulation or depression of RF and TV. The relatively long latency time for observing the gradual increase of RF and TV is likely to depend on the processes regulating passage and distribution of the drug into the central nervous system. However, the present data do not allow us to hypothesize on the mechanisms by which caracasanamide stimulates, and then depresses, the central respiratory pathways. This leads to the cardiac arrhythmias described along with possible cardiac arrest.

Preliminary experiments showed (*E*)-caracasanamide (**1b**) to be equiactive with the (*Z*)-form (**1a**) in affecting both cardiovascular and respiratory parameters.

Caracasanamide is the first compound of a series of six guanidine derivatives, isolated from *Verbesina caracasana*, for which a pharmacological profile has been defined. It will be possible to establish a structure–activity relationship among all these compounds when the pharmacological assays for the remaining five drugs have been completed.[28,29] The present study indicates that caracasanamide may be a hypotensive drug of low-mild potency, devoid of significant tachycardic effects, provided with central and peripheral mechanisms of action in affecting cardiovascular function, and with stimulating respiratory effects when administered at nontoxic doses.

Experimental Section

General. Melting points were determined with a Kofler apparatus and are uncorrected. The NMR spectra were determined with a Varian XL300, using tetramethylsilane as an internal standard and the following solvents: D_2O **1a**, **5**; $CDCl_3$–CD_3OD (3:1), **1b**, **2a**, **4a**; $CDCl_3$, **3**. UV and IR spectra were determined with a Perkin-Elmer 237 and a Perkin-Elmer Lambda 5 instrument, respectively. Electron impact (EIMS), chemical ionization (CIMS), and high-resolution (HRMS) mass spectra were determined on a VG 7070EQ spectrometer.

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

Extraction and Fractionation. The leaves (5 kg) were extracted in a Sohxlet extractor with MeOH. The extract was concentrated *in vacuo*, added to H_2O, and washed (×3) with EtOAc. The pooled organic extracts were washed with water, and the two aqueous solutions were collected, concentrated, and lyophilized. The residue from the lyophilization was suspended in MeOH, and the precipitate was discarded. The solvent was removed by evaporation, the residue was again suspended in a small volume of MeOH, and the precipitate was discarded. The crude extract (26 g) obtained by several operations like this was fractionated on silica gel with $CHCl_3$/MeOH mixtures. Extended

chromatography of the less polar fractions gave caracasanamide (0.5 g) as a mixture of (*Z*)- and (*E*)-forms. Pure (*Z*)-caracasanamide was obtained by dissolving the mixture in water, in which (*E*)-caracasanamide was less soluble.

(*Z*)-1-[(3,4-Dimethoxycinnamoyl)amino]-4-[(3-methylbut-2-enyl)guanidino]butane (1a): foam; UV (MeOH) λ_{max} 235, 291, 316 nm; IR ($CHCl_3$) ν_{max} 3320, 3220, 1655, 1638, 1515, 1260, 1205, 1138, 1020; ^{1}H- and ^{13}C-NMR in Table I; HRMS found M^+ 388.2472, $C_{21}H_{32}O_3N_4$ requires 388.2474; EIMS *m/e* (rel int) 388 $[M]^+$ (15), 387 (12), 207 (45), 206 (32), 191 $[ArCH{=}CHCO]^+$ (100), 163 $[ArCH{=}CH]^+$ (25), 133 (10), 91 (11), 82 (45), 70 (78), 69 $[C_5H_9]^+$ (55), 43 (30), 41 $[C_3H_5]^+$ (100); m* 139.1 (191 → 163), 24.4 (69 → 41).

Hydrolysis of Caracasanamide. A solution of caracasanamide ((*Z*)- and (*E*)-forms, 600 mg) in MeOH (25 mL) was added to a saturated solution of $Ba(OH)_2$ in H_2O and left under reflux for 2 h. After evaporation of the MeOH the aqueous solution was acidified with H_2SO_4 and extracted with EtOAc (×3). The residue of the pooled organic extracts gave by chromatography on Si gel with $CHCl_3$/MeOH (95:5) a mixture (200 mg) of **2a/2b**, which was separated by preparative TLC in the same solvent system. The lyophilizate of the aqueous solution, on chromatography on Sephadex LH-20, afforded a mixture (200 mg) of compounds **3** and **4a/4b** (which were successively separated by Si gel chromatography with $CHCl_3$/MeOH mixtures) and compound **5** (90 mg).

(*Z*)-Dimethoxycinnamic acid (2a): mp 99–101 °C; ^{1}H- and ^{13}C-NMR in Table I. Identical with an authentic sample. **(*E*)-Dimethoxycinnamic acid (2b)**: mp 179–80 °C. Identical with an authentic sample.

3-Methylbut-2-enylurea (3): mp 116-7 °C (CH_2Cl_2/hexane); IR (KBr) ν_{max} 3450, 3360, 1650 (amide I band), 1570 (amide II band), 840 840 cm^{-1}; ^{1}H- and ^{13}C-NMR in Table I; HRMS *m/e* (rel int) 128 (M^+, 47) [found 128.0953, calcd for $C_6H_{12}N_2O$ 128.0950], 113 (M – Me, 42) [found 113.0716, calcd for $C_5H_9N_2O$ 113.0715], 84 (M – $CONH_2$, 36) [found (84.0813, calcd for $C_5H_{10}N$ 84.0813], 73 (M – C_4H_7, 32), 70 (113 – CONH, 100) [found 70.0660, calcd for C_4H_8N 70.0657], 69 (M – $NHCONH_2$, 10) [found 69.0708, calcd for C_5H_9 69.0704], 68 (31), 67 (32), 44 ($CONH_2$, 13), 43 (CONH and C_3H_7l, 18 and 6), 41 (37); m* 99.8 (128 → 113), 55.1 (128 → 84), 43.4 (113 → 70), 41.6 (128 → 73), 26.5 (73 → 44), 24.4 (69 + 41).

(*E*)-4-[(3,4-Dimethoxycinnamoyl)amino]butylurea (4). The mixture **4a/4b** gave, on crystallization, the pure (*Z*)-form **4a**: mp 200–1 °C (MeOH); UV (MeOH) λ_{max} nm (log ϵ) 236 (4.11), 292 (4.05), 312 (3.82); IR (KBr) ν_{max} cm^{-1} 3450, 3360, 3265, 1650 sh, 1640, 1545, 1515, 1492, 1482, 1334, 1290, 1260, 1238, 1208, 1129, 1115, 1022, 970, 840, 796; ^{1}H- and ^{13}C-NMR in Table I; HRMS found 321.1688, $C_{16}H_{23}N_3O_4$ requires 321.1673; EIMS *m/z* (rel int) 321 $[M]^+$ (6), 304 $[M - NH_3]^+$ (8), 278 $[M - HCNO]^+$ (6), 261 $[M - H_2NCONH_2]^+$ (6), 191 $[ArCH{=}CHCO]^+$ (100), 163 $[ArCH{=}CH]^+$ (26), 133 (16), 113 [304 – $ArCH{=}CHCO]^+$ (18), 70 (94), 44 (23), 43 (67). The (*E*)-form **4b** was present only in traces in the mixture and was evidenced by the signals of the olefinic protons in the ^{1}H NMR spectrum at δ 7.40 (1H, d, J = 16 Hz) and 6.32 (1H, d, J = 16 Hz).

1-Amino-4-ureidobutane (5): mp 102–3 °C (MeOH); ^{1}H- and ^{13}C-NMR in Table I; EIMS *m/z* (rel int) 149 $[M + H_2O]^+$ (1), 114 $[M - NH_3]^+$ (8), 70 (3), 69 (5), 60 $[H_2NCONH_2]^+$ (100); HRMS found 114.0801, $C_5H_{10}N_2O$ requires 114.0793; found 70.0663, C_4H_9N requires 70.0657; found 69.0578, C_4H_7N requires 69.0578; found 60.0351, CH_4N_2O requires 60.0324; CIMS *m/z* (rel int) 132 $[M + H]^+$ (2), 115 $[M - NH_3]^+$ (15), 105 (12), 102 (14), 101 (11), 99 (27), 98 (12), 87 (19), 85 (100), 83 (78).

Synthesis. *N,N'*-Bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea (11). A mixture of *S*-methylthioisourea sulfate (13.92 g, 50 mmol) and di-*tert*-butyl dicarbonate (43.65 g, 200 mmol) in a biphasic system of CH_2Cl_2 (200 mL) and saturated aqueous $NaHCO_3$ was vigorously stirred overnight at room temperature. Layers were separated, and the aqueous phase was extracted with CH_2Cl_2. The combined organic solution was washed with water, dried (Na_2SO_4), and concentrated. Chromatography on silica gel ($CHCl_3$) afforded a white solid, which was triturated with petroleum ether and filtered to give 18.6 g of **11**: mp 126-128 °C (lit.[19] 122–123 °C). Analytical and spectral data are in agreement with those reported previously.[19]

***N,N'*-Bis(*tert*-butoxycarbonyl)-*N*-(γ,γ-dimethylallyl)-*S*-methylisothiourea (9)**. NaH (97%, 0.72 g, 29 mmol) was added portionwise under N_2 to a solution of **11** (7.0 g, 24 mmol) in dry DMF (25 mL). When the evolution of hydrogen ceased, 4-bromo-2-methyl-2-butene (3.93 g, 26.4 mmol) was slowly added. The mixture was stirred for 3 h at room temperature, carefully quenched with water, and extracted with diethyl ether. The organic layer was washed with water, dried (Na_2SO_4), and concentrated to provide an oil, which was chromatographed on silica gel (hexane/EtOAc (9:1)) to yield 6.4 g (74%) of **9**: oil; IR ($CHCl_3$) ν_{max} 1720, 1620 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.26 (1H, br t, J = 6.5 Hz, H-2), 4.12 (2H, d, J = 6.5 Hz, H_2-1), 2.36 (3H, s, S-Me), 1.71, 1.66 (3H each, br s, H_3-4, H_3-5, 1.50, 1.46 (9H each, s, BOC); FAB-MS (TDEG + Gly) *m/z* 359 (M^+ + 1). Anal. ($C_{17}H_{30}N_2O_4S$) C, H, N, S.

4-[*N*²,*N*³-Bis(*tert*-butoxycarbonyl)-*N*³-(γ,γ-dimethylallyl)-guanidino]-1-aminobutane (12). A solution of **9** (3.0 g, 8.4 mmol) in THF (20 mL) was dropped into a solution of 1,4-diaminobutane (1.93 g, 21.9 mmol) in 2% aqueous THF (50 mL). After the solution was stirred for 1 h at 50 °C, the solvent was removed under reduced pressure and the residue was partitioned between $CHCl_3$ and 10% aqueous $NaHCO_3$. The organic phase was dried (Na_2SO_4) and concentrated. Column chromatography on silica gel ($CHCl_3$/Et_3N (95:5)) gave 2.3 g (69%) of **12** as a clear oil: IR ($CHCl_3$) ν_{max} 3260, 1720, 1630 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.15 (1H, br t, J = 7 Hz, H-2'), 4.20 (2H, d, J = 7 Hz, H_2-1'), 3.20, 2.68 (2H each, t, J = 6.5 Hz, H_2-1, H_2-4), 1.7–1.3 (4H, br m, H_2-2, H_2-3), 1.70, 1.66 (3H each, br s, H_3-4', H_3-5'), 1.50, 1.46 (9H each, s, BOC); FAB-MS (TDEG + Gly) *m/z* 399 (M^+ + 1). Anal. ($C_{20}H_{38}N_4O_4$) C, H,N.

(*E*)-4-[*N*²,*N*³-Bis(*tert*-butoxycarbonyl)-*N*³-(γ,γ-dimethylallyl)guanidino]-1-[(3,4-dimethoxycinnamoyl)amino]butane (10). A solution of diethyl chlorophosphate (380 mg, 2.2 mmol) in dry THF (5 mL) was slowly added under N_2 to a solution of (*E*)-3,4-dimethoxycinnamic acid (375 mg, 1.8 mmol) and Et_3N (0.56 mL, 4 mmol) in dry THF (15 mL). After the solution was stirred at room temperature for 2 h, the precipitated salt was filtered off and the clear solution was added by dropping to a solution of **12** (1.0 g, 2.5 mmol) and Et_3N (0.56 mL, 4 mmol) in CH_2Cl_2 (20 mL) under N_2. The reaction mixture was stirred at room temperature for 2 h and then concentrated to a small volume and diluted with CH_2Cl_2 and 10% aqueous Na_2CO_3. The organic solution was washed with water, dried (Na_2SO_4), and evaporated to dryness. The crude residue was chromatographed on silica gel (EtOAc) to give 742 mg (70%) of **10**: viscous oil; IR ($CHCl_3$) ν_{max} 3300, 1710, 1630 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.54 (1H, d, J = 16 Hz, H-α), 7.06 (1H, d, J = 8 Hz, H-5'), 7.01 (1H, br s, -2'), 6.83 (1H, br d, J = 8 Hz, H-6'), 6.28 (1H, d, J = 16 Hz, H-β), 5.97 (1H, t, ex w/D_2O, CONH), 5.17 (1H, br t, J = 7 Hz, H-2''), 4.22 (2H, d, J = 7 Hz, H_2-1''), 3.88 (6H, s, 2 × OMe), 3.38, 3.24 (2H each, br m, H_2-1, H_2-4), 1.8–1.5 (4H, br m, H_2-2, H_2-3), 1.68, 1.65 (3H each, br s, H_3-4'', H_3-5''), 1.47, 1.44 (9H each, s, BOC); FAB-MS (TDEG-Gly) *m/z* 589 (M^+ + 1). Anal. ($C_{31}H_{48}N_4O_7$) C, H, N.

(*E*)-1-[(3,4-Dimethoxycinnamoyl)amino]-4-[*N*³-(γ,γ-dimethylallyl)guanidino]butane (1b). A solution of **12** (130 mg, 0.22 mmol) and *p*-toluenesulfonic acid monohydrate (10 mg) in dry 1,4-dioxane (10 mL) was refluxed under N_2 for 3 h. The cooled mixture was made basic (pH = 10) by adding 10% methanolic KOH, and volatiles were removed under vacuum at 30 °C. The crude residue was purified by column chromatography on alumina (10% CH_3OH/CH_2Cl_2) to afford 52 mg (60%) of **1b**: foam; ^{1}H NMR ($CDCl_3$–CD_3OD (3:1) δ 7.48 (1H, d, J = 16 Hz, H-α), 7.12 (1H, d, J = 2 Hz, H-2'), 7.09 (1H, dd, J = 2 Hz, H-5'), 6.84 (1H, d, J = 8 Hz, H-6'), 6.58 (1H, d, J = 16 Hz, H-β), 5.19 (1H, br t, J = 6 Hz, H-2''), 3.85, 3.82 (3H each, s, 2 × OMe), 3.75 (2H, d, J = 6 Hz, H_2-1''), 3.34, 3.22 (2H each, br m, H_2-1, H_2-4), 1.72, 1.66 (3H each, brs, H_3-4'', H_3-5''), 1.65-1.55 (4H, br m, H_2-2, H_2-3); APT ^{13}C NMR ($CDCl_3$–CD_3OD (3:1)) δ 167.4 (C=O), 155.3 (C=NH), 150.0 (C-4'), 148.6 (C-3'), 140.0 (C-α), 137.2 (C-3''), 127.6 (C-1'), 121.6 (C-6'), 118.3 (C-β), 117.7 (C-2''), 110.6 (C-5'), 109.4 (C-2'), 55.3 (2 × OMe), 40.6, 39.0 (C-1, C-4), 38.1 (C-1''), 25.8 (C-4''), 25.3, 24.9 (C-2, C-3), 17.2 (C-5''). Spectral (UV, IR, and EIMS) and chromatographic data were identical with those of an authentic sample of caracasanamide.

Pharmacology. Animals. Adult male Wistar rats, weighing 284 ± 2 g (mean + SE; n = 75), were housed in stainless steel

cages and fed a standard laboratory diet. The rats received "ad libitum" deionized drinking water and were kept undisturbed for 20 days in controlled conditions of dampness, light, temperature, and noise.

Cardiovascular and Respiratory Determinations. The rats were anesthetized with 10% (w/v) ethylurethane (1 mL/kg body weight), which was dissolved in 0.9% NaCl solution (saline) and administered with a single ip injection. The trachea was cannulated to allow spontaneous breathing. Polyethylene catheters (PE 20 tubing) were placed in the left femoral artery for recording aortic blood pressure (BP) and into the right femoral vein for drug administration. A calibrated 3F catheter-tip pressure transducer (Millar Instruments, Houston, TX), inserted in the right common carotid artery and advanced in the left ventricle, was used for determining the maximum rate of rise of the left ventricular isovolumetric pressure (dP/dt), an index of cardiac inotropism.[16,30] Systolic and diastolic BP was measured by a P23Db Statham pressure transducer (Statham Medical Instruments, Los Angeles, CA) and averaged electronically. Heart rate (HR) was obtained by a 9875B Beckman cardiotachometer coupler (Beckman Instruments, Inc., Schiller Park, IL), which was triggered by the *R*-peak of the lead II electrocardiogram. A Biotronex derivative computer (Model BL622; Biotronex Laboratories, Inc., Kensington, MA) was used for determining dP/dt, by differentiating the pulsatile BP registered in the left ventricle.[31] The computer was adjusted to minimize the expression of preload and afterload, according to Crawford *et al.*[32] and Davidson *et al.*[33] The unit of measurement of dP/dt was mmHg/s. BP, HR, and dP/dt were continuously monitored by means of a Beckman RM Dynograph recorder. The body temperature of the animals was kept constant at 37 °C by using an electrically heated table. Each rat received by iv route 1 mL of 0.9% saline solution containing 100 USP of sodium heparin. Respiration was monitored by means of a pneumotachograph adapted to a Biotronex BL 620 integrator to yield the full respiratory wave. Respiratory frequency (RF) and tidal volume (TV) were assessed under spontaneous breathing by connecting the tracheal cannula to the pneumotachograph.[34] RF and TV were monitored polygraphically along with the cardiovascular parameters. After completion of the surgical procedure, the rats were kept undisturbed for 60 min to allow for the stabilization of all cardiovascular and respiratory parameters.

Protocol. Ten rats were used to determine the dose-response relationship for (*Z*)-caracasanamide. In this respect, saline solutions of the compound were prepared daily and injected by iv route in a volume of 50 μL. The doses of the drug ranged from 50 to 6400 μg/kg body weight (ratio 2.0). In this respect, lethal $dose_{50}$ (LD_{50}) for (*Z*)-caracasanamide, administered intraperitoneally in male Swiss mice, was found to be 57.0 ± 3.6 mg/kg (mean + SE; $n = 24$).

Thirty rats were randomly divided into five groups in order to test the effects of various doses of the drug, injected by iv route, before and after (a) iv administration of phentolamine (2 mg/kg), propranolol (3 mg/kg), or hexamethonium (5 mg/kg) and (b) transection of the spinal cord carried out between the first and the second cervical vertebras (spinalization) under bilateral vagotomy at the neck below the nodose ganglion. After spinalization, the respiration was maintained through a respiratory pump, which was regulated in order to assure the minute volume calculated for each rat in basal conditions (i.e., preceding administration of the first dose of the drug).

Thirty-five rats were randomly divided into seven groups in order to compare on molar basis the cardiovascular effects of some antihypertensive or vasodilating drugs with those of (*Z*)-caracasanamide. These rats received, by iv injection or infusion under the above experimental conditions, (*Z*)-caracasanamide (4.12 mM/kg body weight), guanethidine (25 mM/kg), clonidine (0.108 mM/kg), hexamethonium (12 mM,kg), reserpine (8 mM/kg), papaverine (5 mM/kg), or histamine (0.044 mM/kg). All drugs were dissolved in saline solution, and all doses were expressed in terms of free bases. The control administration of solvent alone caused insignificant changes in both cardiovascular and respiratory parameters. Peak effects were considered for each assay. Each of the consecutive tests was not made until the parameters had returned to the values preceding the previous administration of (*Z*)-caracasanamide and had stabilized.

Statistics. Data were expressed as means ± SE and compared by analysis of variance.[35] Only a P value less than 0.05 was considered to be significant.

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