Synthesis of Novel Aminopropylguanidine Derivatives with Potential Antihypertensive Activity

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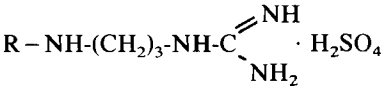
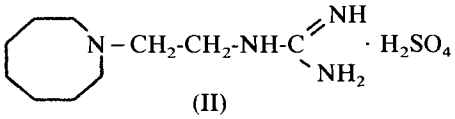
INTRODUCTION

An examination of the structures of many commercially available antihypertensive drugs reveals a common functionality in the guanidine group. This group is present in compounds representing diverse classes of antihypertensives, including adrenergic neuron blockers (*guanethidine*), central α_2 agonists (*clonidine, guanabenz, guanfacine*), α_1 antagonists (*prazosin*), vasodilators (*minoxidil*), and diuretics (*amiloride, triamterene*). This widespread nature of the guanidine functionality is well documented in the literature (1) and led us to test a series of guanidine-containing compounds which were prepared as intermediates in an unrelated enzyme-inhibitor study.

It was previously found that compounds with the general structure (I) were potent antihypertensive agents with significant pharmacological differences from guanethidine (II), their closest structural relative available commercially (2).



(I)



(II)

In that study, cyclic hydrophobic groups substituted for R in (I) appeared to be the most potent antihypertensives within the series [54% reduction in mean arterial pressure (MAP) at 2.0 mg/kg], while long aliphatic chains appeared to elicit the maximum antihypertensive effect (83% reduction in MAP at 10.0 mg/kg). In continuation of the study, various analogues of structure (I) were synthesized by varying either the R substituent or changing the secondary amine to an ether functionality. None of the tested compounds is referenced in the literature.

MATERIALS AND METHODS

Melting points were determined on a Fisher-Johns Uni-Melt apparatus and are reported uncorrected. Microanalyses were performed by Atlantic Microlab, Inc., Norcross, GA. Analytical results obtained for all compounds were within ± 0.4% of the theoretical values unless otherwise stated. NMR spectra were recorded on a Varian XR-200 or VXR-300 spectrometer with tetramethylsilane (TMS) as the internal reference. Infrared (IR) spectra were determined from Nujol mulls or liquid films on a Perkin-Elmer 1430 spectrophotometer. Reactions were routinely followed by thin-layer chromatography (TLC) with Whatman MK6F silica gel plates; species were identified by UV absorption at 254 nm and iodine development.

SYNTHESIS

The synthetic procedure for compounds 4a - 4d is shown in Scheme 1. Scheme 2 summarizes the synthesis of compound 8. The use of S-methylisothiourea to introduce the guanidine functionality is a well-known procedure and was, in fact, utilized in the original synthesis of guanethidine (3). NMR assignments for each compound were made by comparison with the NMR spectrum of their respective precursor.

N-[3-(1-Adamantylamino)-1-propyl]-guanidine sulfate 4a (4)

To a solution of 1-adamantylamine (5.0 g, 0.033 mol) 1a in 50 ml of acrylonitrile was added 0.5 ml of water and the mixture was heated under reflux overnight. The excess acrylonitrile was evaporated and the residue was distilled under reduced pressure to produce 6.1 g (95% yield) of 3-(1-adamantylamino)-propionitrile 2a as a viscous oil [bp 165 °C/0.7 mm Hg].

To a 50 ml ethereal suspension of $LiAlH_4$ (2.3 g, 0.066 mol) in an ice bath was added 2a (6.2 g, 0.030 mol) dropwise with stirring. The reaction mixture was stirred at room temperature for 3 hr and the reaction was quenched with sequential dropwise additions of 4 ml of water, 3 ml of 5N NaOH, and 14 ml of water. The mixture was then filtered under suction and the residue was washed with ether. The ether layers were combined and dried over $MgSO_4$. After the ether was evaporated, N-(1-adamantyl)-1,3-propanediamine 3a (4) was utilized without further purification.

To a solution of 3a (10.4 g, 0.0500 mol) in 50 ml of water was added S-methylisothiourea hemisulfate (7.0 g, 0.025 mol) at room temperature. The mixture was heated at 50 °C for 5 hr under a continuous nitrogen purge. The exiting gases were passed through two flasks, each containing a saturated ethanolic solution of KOH to trap the CH_3SH byproduct. The mixture was then concentrated and a suitable amount of acetone was added. The precipitate was filtered and recrys-

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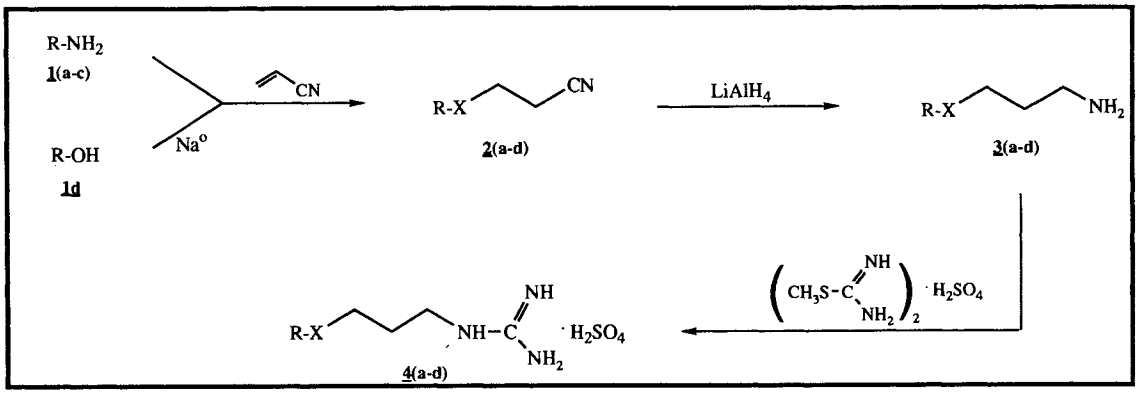


Scheme 1

tallized from water to give 17.6 g (96% yield based on 3a) of title compound 4a as white crystals [mp 287-8 °C (d)]; ^{1}H NMR (D_2O) δ 1.43 - 2.15 (m, 17H, 1-adamantyl and CH_2), δ 2.95 (m, 2H, -CH_2-guanidine), δ 3.15 (m, 2H, -CH_2-amine); IR (Nujol) 1680, 1645 cm^{-1} (guanidinium), 2420-2600 cm^{-1} (+N-H); Anal. ($C_{14}H_{26}N_4 \cdot H_2SO_4 \cdot H_2O$, 366.47 g/mol). Calculated: *C* 45.88, *H* 8.25, *N* 15.29. Found: *C* 45.89, *H* 8.28, *N* 15.34.

N-[3-(2-Adanantylamino)-1-propyl]-guanidine sulfate 4b

This compound was prepared in a similar manner to that of 4a. Recrystallization from water produced 17.2 g (94% yield based on 3b) of title compound 4b as white crystals [mp 284-5 °C (d)]; ^{1}H NMR (D_2O) δ 1.43-2.03 (m, 16H, 2-adamantyl and CH_2), δ 2.95 (m, 2H, -CH_2-guanidine), δ 3.12 (m, 2H, -CH_2-amine), δ 3.25 (s, 1H, 2-adamantyl); IR (Nujol) 1695, 1645 cm^{-1} (guanidinium), 2420-2600 cm^{-1} (+N-H); Anal. ($C_{14}H_{26}N_4 \cdot H_2SO_4 \cdot H_2O$, 366.47 g/mol). Calculated: *C* 45.88, *H* 8.25, *N* 15.29. Found: *C* 45.89, *H* 8.28, *N* 15.34.

N-[3-(2-Adamantylamino)-1-propyl]-guanidine sulfate 4b

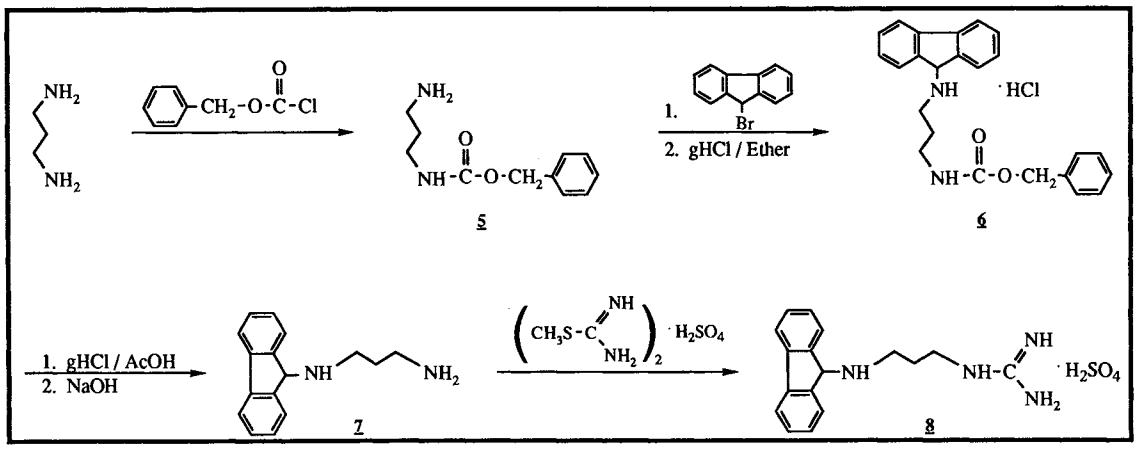
This compound was prepared in a similar manner to that of 4a. Recrystallization from water produced 17.2 g (94% yield based on 3b) of title compound 4b as white crystals [mp 284-5 °C (d)]; ^{1}H NMR (D_2O) δ 1.43-2.03 (m, 16H, 2-adamantyl and CH_2), δ 2.95 (m, 2H, -CH_2-guanidine), δ 3.12 (m, 2H, -CH_2-amine), δ 3.25 (s, 1H, 2-adamantyl); IR (Nujol) 1695, 1645 cm^{-1} (guanidinium), 2420-2600 cm^{-1} (+N-H); Anal. ($C_{14}H_{26}N_4 \cdot H_2SO_4 \cdot H_2O$, 366.47 g/mol). Calculated: *C* 45.88, *H* 8.25, *N* 15.29. Found: *C* 46.74, *H* 8.25, *N* 15.47.

N-[3-(2-Norbornanemethylamino)-1-propyl]-guanidine sulfate 4c

This compound was prepared in a similar manner to that of 4a. Recrystallization from ethanol-water produced 15.0 g (93% yield from 3c) of title compound 4c as white crystals [mp 273-4 °C (d)]; ^{1}H NMR (D_2O) δ 0.75 - 2.20 (m, 13H, 2-norbornane and CH_2), δ 2.57 - 2.84 (m, 1H, 2-norbornane), δ 2.92 (m, 3H, -CH_2-guanidine and 2-norbornane), δ 3.12 (t, 2H, -CH_2-amine); IR (Nujol) 1695, 1645 cm^{-1} (guanidinium), 2420-2600 cm^{-1} (+N-H); Anal. ($C_{12}H_{24}N_4 \cdot H_2SO_4$, 322.42 g/mol). Calculated: *C* 44.70, *H* 8.13, *N* 17.38. Found *C* 44.03, *H* 8.17, *N* 17.15.

n-Hexyloxypropylamino guanidine sulfate 4d

To a solution of sodium (0.023 g, 0.0010 mol) in n-hexanol (33.7 g, 0.330 mol) was added acrylonitrile (17.5 g, 0.426 mol) with stirring over 30 min and subsequently heated under reflux for 18 hr. A small quantity of brown solid was then filtered and n-hexyloxypropionitrile 2d was isolated by distillation under reduced pressure (b.p. 115.5 °C/9 mm Hg) (4,5). n-Hexyloxypropylamine 3d (6-8) was synthesized by



Scheme 2

the reduction of 2d with $LiAlH_4$ in ether. The title compound 4d was prepared by reacting 3d with S-methylisothiourea hemisulfate in a similar manner to that for preparation of 4a. Recrystallization from water-acetone produced 8.0 g (62% yield from 3d) of title compound 4d as white crystals [mp 105-7 °C]; ^1H NMR (D_2O) δ 0.72 (t, 3H, $-CH_3$), δ 1.15 (m, 6H, hexane), δ 1.42 (m, 2H, hexane), δ 1.62 (m, 2H, $-CH_2$), δ 3.13 (t, 2H, $-CH_2$-guanidine), δ 3.40 (m, 4H, $-CH_2$-O-CH_2); IR (Nujol) 1670, 1630 cm^{-1} (guanidinium), 1110 cm^{-1} (C-O); **Anal.** ($C_{10}H_{23}N_3O \cdot \frac{1}{2}H_2SO_4 \cdot \frac{1}{2}H_2O$, 259.36 g/mol). Calculated: *C* 46.31, *H* 9.91, *N* 16.20. Found: *C* 46.09, *H* 9.51, *N* 16.03.

N-(Benzyloxycarbonyl)-1,3-propanediamine 5

Propane-1,3-diamine (19.3 g, 0.260 mol) was dissolved in water (50 ml) containing bromocresol green. Methanesulfonic acid (46 g, 0.48 mol) in water (50 ml) was added to the solution until a blue to pale yellow color transition was achieved (pH~3.8). After this solution was diluted with ethanol (140 ml), it was vigorously stirred and treated with alternating solutions of benzylchloroformate (CBz) (39 g, 0.23 mol) in dimethoxyethane (50 ml) and 50% w/v aqueous potassium acetate (100 ml) at 20 °C. After the additions were complete, the mixture was stirred at room temperature for 1 hr and the volatile solvents were removed at low temperature under vacuum. The residue was shaken with 500 ml of water and filtered to remove small quantities of the bis-substituted derivative. The filtrate was washed with benzene (3 × 150 ml), treated with excess 40% aqueous NaOH, and extracted with benzene (300 ml). The organic layer was washed with saturated aqueous NaCl (200 ml), dried with Na_2SO_4, and evaporated under vacuum. N-(Benzyloxycarbonyl)-1,3-propanediamine 5 was isolated as a viscous oil (27 g, 57% yield based on CBz) (9).

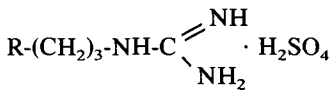
N-(Benzylocycarbonyl)N'-(9-fluorenyl)-1,3-propanediamine hydrochloride 6

A solution of 9-bromofluorene (0.61 g, 0.0025 mol) and 5 (1.4 g, 0.0067 mol) in 10 ml of dry benzene was refluxed for 2 hr. The cooled reaction mixture was diluted with 20 ml ether and N-(benzyloxycarbonyl)-1,3-propanediamine hydrobromide was removed by filtration. Ether saturated with dry HCl gas was added to the filtrate and the precipitate was filtered and washed with ether to give 1.0 g (83% yield based on 9-bromofluorene) of title compound 6 [mp 194-7 °C].

N-(9-Fluorenyl)-propane-1,3-diamine 7

Dry HCl gas was bubbled into a hot (75 °C) solution of 6 (1.0 g, 0.0024 mol) in 10 ml of glacial acetic acid for 2 hr and allowed to stand at room temperature overnight. Ether (20 ml) was added and the resulting precipitate was filtered and washed with ether to produce [N-(9-fluorenyl)-1,3-propanediamine dihydrochloride], mp 228-230 °C. A solution of this compound (0.31 g, 0.0010 mol in 5 ml of water) was adjusted to pH 12 using 2N NaOH and extracted with chloroform (3 × 5 ml). The organic layer was washed with 5 ml of water, 5 ml of saturated NaCl, and dried over anhydrous Na_2SO_4. Filtration and removal of chloroform under vacuum

Table 1. Yield and Melting Point of the Compounds Tested for Antihypertensive Activity



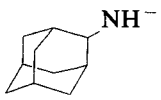
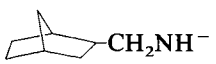
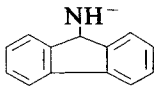
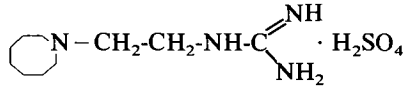
Compound #	R	Yield	M.P. (°C)
4a		96%	287-8 (d)
4b		94%	284-5 (d)
4c		93%	273-4 (d)
4d	CH_3-$(CH_2)_5$-O-	62%	105-7
8		76%	276-7
Guanethidine			249-51

Table II. Dose Response Relationships and Duration of Action of Tested Compounds

Dose/Time[a]	% Change in MAP[b]					
	Guanethidine	4a	4b	4c	4d	8
Initial MAP[c]	157 ± 8	155 ± 9	145 ± 13	158 ± 19	160 ± 5	158 ± 8
0.1 mg/kg	−9.2 ± 3.0	−4.3 ± 2.0	−7.0 ± 3.8	8.2 ± 14.1	−1.0 ± 1.7	15.7 ± 6.0
0.3 mg/kg	−13.3 ± 1.6	−6.6 ± 3.5	−4.7 ± 2.2	6.3 ± 12.6	4.8 ± 5.8	21.7 ± 5.7
1.0 mg/kg	−17.3 ± 7.5	−9.7 ± 3.4	−11.7 ± 8.4	7.7 ± 17.7	19.1 ± 10.1	26.4 ± 5.0
3.0 mg/kg	−11.0 ± 17.3	−32.6 ± 5.6	−32.2 ± 6.7	−39.0 ± 11.9	20.5 ± 5.6	18.8 ± 9.1
10.0 mg/kg	−22.4 ± 9.0	−42.9 ± 5.5	−38.7 ± 9.3	−45.2 ± 8.8	22.2 ± 12.1	16.4 ± 10.2
15 min	−16.9 ± 7.3	−16.1 ± 8.5	−7.1 ± 4.0	−3.3 ± 12.1	−4.6 ± 8.9	4.2 ± 1.0
30 min	−19.0 ± 5.7	−11.2 ± 8.3	—[d]	—[d]	−14.3 ± 12.6	4.8 ± 1.8
45 min	−18.0 ± 5.8	−9.5 ± 8.3	—	—	−25.6 ± 15.8	4.4 ± 6.7
60 min	−19.2 ± 5.5	−4.6 ± 6.6	—	—	−31.6 ± 19.1	4.9 ± 2.5

[a] Time following administration of the 10.0 mg/kg dose.
[b] Mean ± standard deviation.
[c] (mm Hg).
[d] MAP not observed during these times.

afforded 0.57 g (89% yield from 6) of title compound 7 [mp 73-5 °C].

N-[3-(9-Fluorenylamino)-1-propyl]-guanidine sulfate 8

Compound 8 was prepared by reacting 7 (0.47 g, 0.0015 mol) with S-methylisothiourea hemisulfate (0.21 g, 0.0015 mol) in a manner similar to that for 4a. Recrystallization from water gave 0.43 g (76% yield from 7) of title compound 8 [mp 276-7 °C]; ^{1}H NMR (D_2O) δ 1.8 (m, 2H, $-CH_2$), δ 2.79 (t, 2H, $-CH_2$-guanidine), δ 3.08 (t, 2H, $-CH_2$-amine), δ 5.62 (s, 1H, fluorenyl), δ 7.50 - 7.91 (m, 8H, fluorenyl); IR (Nujol) 1680, 1660, 1620 cm^{-1} (guanidinium), 2420-2600 cm^{-1} (+N-H); **Anal.** ($C_{17}H_{20}N_4O \cdot H_2SO_4$, 378.45 g/mol). Calculated: *C* 53.95, *H* 5.86, *N* 14.81. Found: *C* 53.69, *H* 5.92, *N* 14.69.

PHARMACOLOGY

Male spontaneously hypertensive rats (SHR; 16-17 weeks of age; Harlan Sprague-Dawley, Indianapolis, IN) were used in the study (n = 3 for each tested compound and the controls). Data from all control animals were grouped together. Initial MAP for the control animals was 150 ± 26 mm Hg (mean ± SD). Initial MAP's for the rats used in testing compounds are given in Table II. Initial heart rates for all 18 animals were 349 ± 29 beats per minute (mean ± SD).

The rats were anesthetized with an intraperitoneal injection of ketamine (120 mg/kg) plus acepromazine (12 mg/kg). The abdominal aorta and inferior vena cava were catheterized with PE50 tubing filled with heparinized saline (100 units/ml) via the left femoral artery and vein respectively. The catheters were tunneled subcutaneously up the back of the animal and exteriorized at the nape of the neck between the scapulae. The arterial catheter was used for measurement of arterial blood pressure and heart rate and the venous catheter was used for injection of drugs. The animals were allowed a 24 hour recovery period from the surgery before studies were conducted on the antihypertensive agents of interest.

Experiments were performed in conscious, unrestrained rats in their home cages. The catheters were connected to a 2-channel swivel and tether system. From this swivel, the arterial catheter was connected to a Gould P23ID pressure

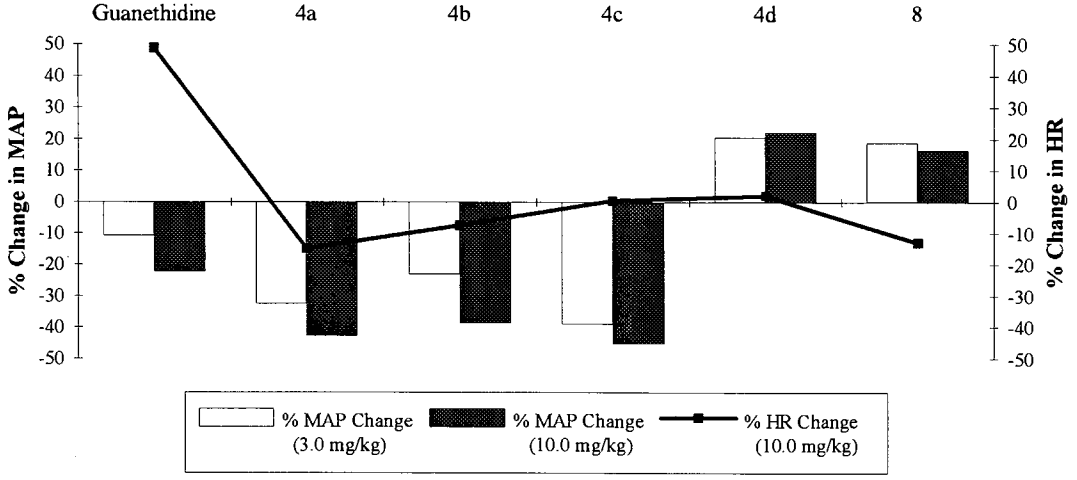


Fig. 1. Acute cardiovascular effects of the tested compounds.

transducer and the venous catheter connected to a 1 cc tuberculin syringe. Changes in mean arterial pressure and heart rate in response to the antihypertensive compounds were recorded on a Grass model 7D polygraph. Bolus injections of the antihypertensive agents were administered in volumes ranging from 2.5 to 250 µl (dose range of 0.1 to 10.0 mg/kg) using Hamilton microliter syringes. The doses were spaced at least 3 minutes apart, which allowed the acute effects of each dose to be measured.

RESULTS AND DISCUSSION

The general procedure in Scheme 1 is that of Ueda and Ishizaki (10). It was used successfully to synthesize the novel compounds 4a, 4b, and 4c in high yield (>93%) and the known compound 4d in 62% yield. Compound 8 was not amenable to the procedure, but was synthesized in good yield (76%) by different methods (9-11) as shown in Scheme 2. The positive control, guanethidine, was chosen because of its structural similarity to the synthesized compounds (Table I). The negative control was 0.9% saline.

These compounds were designed and synthesized to continue the study of Finch *et al* (2). The alkyl substituents in 4a - 4c produced compounds with high antihypertensive activity (Table II). These compounds were similar to those previously tested (2) and confirmed that alkylaminopropylguanidines have antihypertensive properties. The high degree of hydrophobicity of the adamantyl groups did not seem to augment the activity of the guanidine derivatives, but rather possessed intermediate potency in the series of alkylaminopropylguanidines. This is consistent with the parabolic relationship of hydrophobicity and pharmacological activity (12). Compounds 4a - 4c lowered MAP with minimal effects on heart rate, in marked contrast to the acute effects of guanethidine (Figure 1), although their duration of action was shorter.

The pharmacological results from compound 4d (Table II) revealed a significant structure-activity characteristic. The compound raised MAP at each of the doses, up to a maximum of a 20% increase. However, MAP was lowered at 15 minutes following the 10.0 mg/kg dose and was lowered 32% at one hour. The same compound with an amino linkage was previously shown (2) to acutely lower MAP, to a maximum of an 80% decrease. This compound also exhibited a 34% lowered MAP at one hour. This suggests that the amino linkage is necessary for acute antihypertensive activity, but can be altered to design compounds which have an improved profile for chronic administration.

Compound 8 also raises MAP, which indicates that aromatic substituents do not retain antihypertensive activity. This effect has subsided by 15 minutes following the final dose and retains the baseline MAP for at least one hour.

In conclusion, it appears that propylguanidine derivatives substituted with alkylamino groups exhibit the greatest acute antihypertensive activity. Altering of the alkyl group to an aromatic may produce compounds with hypertensive, rather than antihypertensive, activity. The linkage between the hydrophobic substituent and the propylguanidine functionality modifies the acute effects on MAP, but appears not to influence the chronic effects.

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