Atrial Natriuretic Peptide Receptor Modulators: Effects of Disubstituted Quinazolines on Receptor Binding and in Vitro Biological Activity

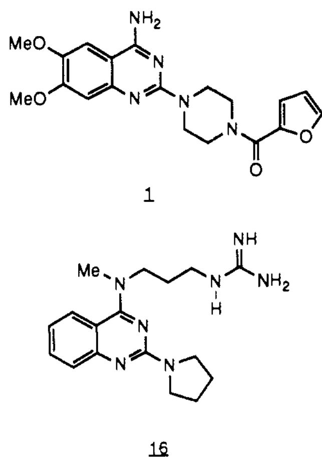
Melvin J. Yu,* Jefferson R. McCowan, Mitchell I. Steinberg, Sally A. Wiest, Virginia L. Wyss, and J. S. Horng

Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285. Received February 2, 1989

Prazosin (25 μM) was found to increase ^{125}I-labeled rat atrial natriuretic peptide ([^{125}I]rANP) receptor binding by 50% (SC_{50}) in bovine adrenal zona glomerulosa membranes. A series of 2,4-disubstituted quinazolines was prepared in order to identify more potent analogues for additional in vitro testing. Compound **7** (*N*-[3-[[2-(diethylamino)-4-quinazolinyl]amino]propyl]guanidine dinitrate) from this series (3 μM) significantly decreased the EC_{50} for rANP-mediated inhibition of ACTH-stimulated aldosterone synthesis in rat adrenal glomerulosa cells. At a higher concentration (20 μM), compound **7** had no effect on particulate guanylate cyclase from rabbit glomeruli in either the presence or absence of rANP.

Atrial natriuretic peptide (ANP) possesses potent natriuretic, diuretic, and vasorelaxant properties and can inhibit both basal and ACTH-stimulated aldosterone biosynthesis in vitro.[1] The predominant circulating form is a 28 amino acid peptide[2] whose effects are mediated through specific high-affinity receptors located in the cell membranes of target tissues.[1] A nonpeptide, low molecular weight ANP mimetic might help clarify the physiological and pathophysiological role of this hormone and possibly provide a proteolytically stable agent for treating cardiac and renal failure.[3] However, since the rational design of a nonpeptide ANP mimetic appeared formidable, our goal instead focused on compounds that alter ANP receptor binding properties as an alternate approach for potentiating the biological actions of this peptide.

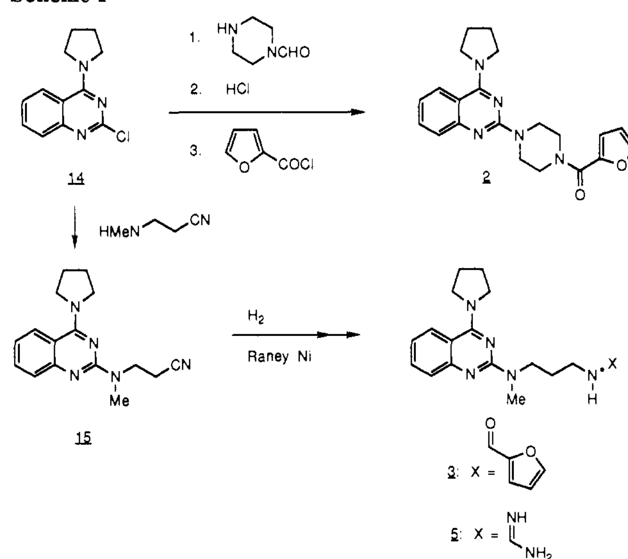
Through general screening, we found that prazosin, **1**, caused a concentration-dependent and reversible enhancement in specific [^{125}I]rANP[4] binding with receptors in bovine adrenal zona glomerulosa membranes.[5] Since



other α-adrenergic agonists or antagonists did not produce a similar effect, the stimulation of ANP binding by prazosin is probably unrelated to α-adrenergic receptor interactions.[6] A structure-activity study was therefore initiated to identify more potent analogues for in vitro studies. This paper details our efforts in this area.

Chemistry. The compounds listed in Table I were synthesized by the routes shown in Schemes I and II. Compound **2** was obtained by refluxing a mixture of **14** with piperazinecarboxaldehyde followed by acid hydrolysis and acylation with 2-furoyl chloride. Propanediamine derivatives **3** and **5** were prepared by heating 3-(methylamino)propionitrile with **14** in refluxing 2-methylbutanol, hydrogenating the resulting nitrile over Raney nickel,[7] and

Scheme I



then either acylating with 2-furoyl chloride to give **3** or refluxing with 3,5-dimethylpyrazole-1-carboxamidine nitrate[8] in dioxane-water to furnish **5**. The synthesis of **16**, a structural isomer of **5**, was also investigated. However, in our hands, the hydrogenation and guanylation of the intermediate nitrile only led to an intractable mixture of products. Consequently, we employed an alternate strategy for preparing analogues related to **16** (Scheme II). Compound **17**[9] was treated with the appropriate alkane-

* Address correspondence to: Dr. Melvin J. Yu, MC705, 88/1, Lilly Corporate Center, Indianapolis, IN 46285.

(1) For recent reviews on ANP, see: (a) Genest, J.; Cantin, M. *Rev. Physiol. Biochem. Pharmacol.* **1988**, *110*, 1. (b) Baxter, J. D.; Lewicki, J. A.; Gardner, D. G. *Biotechnology* **1988**, *6*, 529. (c) Espiner, E. A.; Nicholls, M. G. *Clin. Endocrinol. (Oxford)* **1987**, *26*, 637. (d) Lang, R. E.; Unger, T.; Ganten, D. *J. Hypertens.* **1987**, *5*, 255. (e) Zeidel, M. L.; Brenner, B. M. *Semin. Nephrol.* **1987**, *7*, 91. (f) Genest, J.; Cantin, M. *Circulation* **1987**, *75* (*Suppl I*), I-118.

(2) Gutkowska, J.; Genest, J.; Thibault, G.; Garcia, R.; Larochelle, P.; Cusson, J. R.; Kuchel, O.; Hamet, P.; De Lean, A.; Cantin, M. *Clin. Endocrinol. Metab.* **1987**, *16*, 183.

(3) Nicholls, M. G.; Richards, A. M. *Clin. Endocrinol. Metab.* **1987**, *16*, 199.

(4) Rat ANP(102-126) was used in all of our receptor-binding and in vitro assays.

(5) A portion of this work has been presented previously: Horng, J. S.; Steinberg, M. I.; Wyss, V. L.; Wiest, S. A.; Schirmer, E. W.; McCowan, J. R.; Yu, M. *FASEB J.* **1989**, *3*, A733.

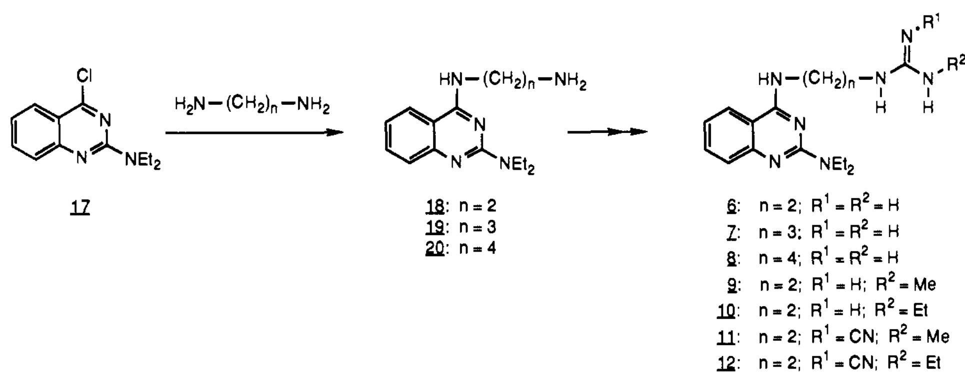
(6) The following α-adrenergic agents (20 μM) did not enhance [^{125}I]rANP receptor binding in bovine adrenal zona glomerulosa membranes: α_1 agonists—methoxamine and cirazoline; α_1 antagonists—clozapine; α_2 agonists—clonidine; α_2 antagonists—yohimbine.

(7) Manoury, P. M.; Binet, J. L.; Dumas, A. P.; Lefevre-Borg, F.; Cavero, I. *J. Med. Chem.* **1986**, *29*, 19.

(8) Habeeb, A. F. S. A. *Biochem. Biophys. Acta* **1959**, *34*, 294.

0022-2623/90/1833-0348$02.50/0 © 1989 American Chemical Society

Scheme II



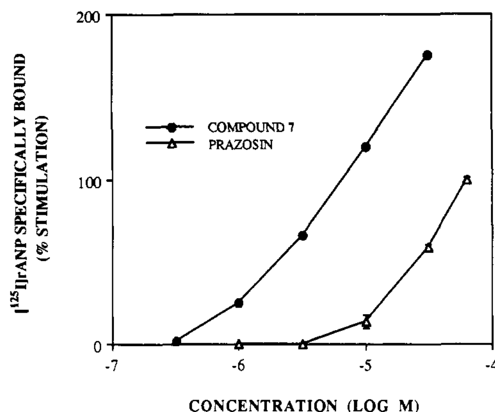


Figure 1. Effect of prazosin and 7 on [^{125}I]rANP binding to bovine adrenal zona glomerulosa membranes. Each point represents the mean ± SE of three determinations.

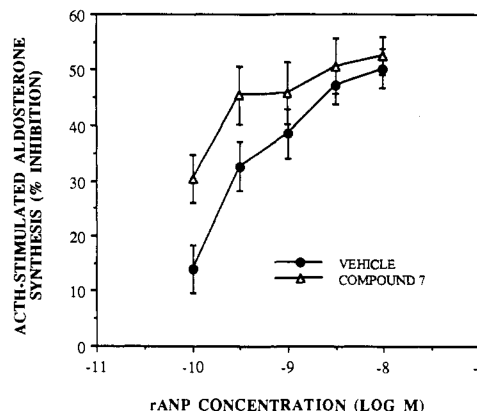


Figure 2. Effect of 7 (3 μM) on inhibition of ACTH-stimulated aldosterone synthesis by rANP in rat adrenal glomerulosa. Maximal stimulation of aldosterone synthesis in the presence of ACTH (1 nM) was 8.5 ± 0.9 ng/mL per 2 h. The EC_{50} for rANP inhibition of aldosterone synthesis was 0.31 ± 0.06 nM for vehicle and 0.12 ± 0.03 nM for 7 ($p < 0.002$). Each point represents the mean ± SE of 10 experiments.

diamine either neat or in THF and then guanylated to produce the quinazoline analogues 6–8. The intermediate amine 18 upon treatment with either methyl or ethyl isothiocyanate and methyl iodide followed by ammonia provided alkylguanidines 9 and 10. Similarly, stirring 18 with dimethyl *N*-cyanodithioiminocarbonate and then refluxing with either methyl- or ethylamine led to the corresponding cyanoguanidine analogues 11 and 12.

Receptor-Binding Study. The effect of prazosin, 1, on rANP binding with bovine adrenal zona glomerulosa (BAZG) membranes is shown in Figure 1. The concentration of this drug required to increase specific [^{125}I]rANP binding by 50% (SC_{50}) is 25 μM. Scatchard analysis of rANP receptor binding in the presence of prazosin (30 μM) revealed an increase in receptor affinity from a K_d of 251 ± 24 to 97.4 ± 8.3 pM without a significant change in receptor density (1922 ± 109 fmol/mg for control vs 1705 ± 48 fmol/mg in the presence of prazosin).

A number of substituted quinazolines and quinolines were subsequently screened but they were found to exhibit only weak stimulatory activity.[10] Although individually these initially screened compounds were uninteresting, collectively they suggested that prazosin's activity in the rANP binding assay might be improved by two simple functional group modifications; first, the R-2 amino group could be replaced with a pyrrolidine ring and, second, the 6,7-dimethoxy groups could be removed altogether. Thus, compound 2 was prepared and found to be modestly more potent than prazosin (Table I). Bioisosteric replacement[7] of the piperazine ring to give compound 3 had little effect on activity despite the increased conformational flexibility expected for an alkyl chain. Similarly, the furoic acid moiety of 2 could be entirely eliminated to give the amine 4 without loss of activity. As a result, we replaced the furoic acid group of 3 with a highly basic substituent to furnish guanidinium analogue 5. This compound was found to have an SC_{50} of 1.5 μM, a 16-fold improvement in potency over our original lead.

We also wanted to investigate the structurally isomeric series of analogues which have the R-1 and R-2 substituents interchanged and to establish the optimal distance separating the guanidinium group from the heterocyclic nucleus. Since compound 16 could not be obtained in pure form, the related analogues 6–12 were examined. Surprisingly, the potency of compounds 6–8 were comparable with that of 5. Additional alkyl substitution on the guanidine moiety had a small but detrimental effect on potency whereas the corresponding cyanoguanidines were essentially inactive.

In Vitro Studies. In order to establish if enhanced ANP receptor binding by our series would be functionally manifest, compound 7 was chosen for in vitro studies. The effect of increasing concentration of rANP on aldosterone synthesis in isolated rat adrenal glomerulosa cells stimulated by 1 nM ACTH is shown in Figure 2. In the presence of compound 7 (3 μM), the concentration-response curve for rANP inhibition of aldosterone synthesis was shifted to the left, significantly decreasing the EC_{50} of rANP from 0.31 nM ± 0.06 nM to 0.12 ± 0.03 nM ($p < 0.002$). Basal aldosterone synthesis both in the presence and absence of ACTH was unaffected by 7 (3 μM) (Table II). On the other hand, compound 13 (10 μM), an ana-

(9) Scarborough, H. C.; Lawes, B. C.; Minielli, J. L.; Compton, J. L. *J. Org. Chem.* **1962**, *27*, 957.
(10) Data not shown.

Table I. Chemical and Receptor Binding Data

no.	R_1	R_2	salt	mp, °C	formula[a]	SC_{50},[b] μM
1	prazosin[c]					25
2				170–171	$C_{21}H_{23}N_5O_2$	7.0
3				147–149	$C_{21}H_{25}N_5O_2$	5.6
4			$2HNO_3$	200–201	$C_{16}H_{23}N_7O_6$	4.2
5			$2HNO_3$	167–168 dec	$C_{17}H_{27}N_9O_6$	1.5
6	Et_2N		$2HNO_3$, $0.5H_2O$	224–227 dec	$C_{15}H_{25}N_9O_6 \cdot 0.5H_2O$	2.4
7	Et_2N		$2HNO_3$	186.5–187 dec	$C_{16}H_{27}N_9O_6$	1.8
8	Et_2N		$2HNO_3$	150–151 dec	$C_{17}H_{29}N_9O_6$	2.2
9	Et_2N		2HCl	242–245	$C_{16}H_{27}Cl_2N_7$	4.5
10	Et_2N		$2HNO_3$	152–154	$C_{17}H_{29}N_9O_6$	7.4
11	Et_2N			187–188	$C_{17}H_{24}N_8$	+25%[d]
12	Et_2N			174–176	$C_{16}H_{26}N_8$	+12%
13	Et_2N	OMe	1.5 oxalate	166–166.5	$C_{16}H_{20}N_3O_7$	NA[e]

[a] Satisfactory C, H, and N elemental analyses (±0.4%) were obtained for all new compounds. [b] The SC_{50} was determined by linear regression and log-probit analyses of the binding data. [c] A sample of prazosin was obtained from Pfizer. [d] Percent increase of specific [^{125}I]rANP receptor binding at 20 μM. [e] Not active at 20 μM.

logue that did not potentiate rANP receptor binding, was ineffective in potentiating rANP inhibition of aldosterone production (Figure 3). This compound likewise lacked any effect on basal aldosterone synthesis.

The possibility for receptor heterogeniety and the uncertainty regarding cGMP's role in ANP inhibition of aldosterone synthesis[11] prompted us to examine if **7** similarly potentiates ANP activation of particulate guanylate cyclase (PGC). The effect of compound **7** on PGC from rabbit glomeruli both in the presence and absence of rANP is shown in Figure 4. Although at 20 μM **7** stimulated rANP binding by 156% (see Figure 1), it did not affect either basal or rANP-induced PGC activity in this tissue.

Table II. Effect of Compounds 7 and 13 on Basal and ACTH-Stimulated Aldosterone Synthesis in the Absence of rANP

	aldosterone concentration, ng/mL per 2 h[a]			
	control	7 (3 μM)	control	13 (10 μM)
basal	1.0 ± 0.1	0.9 ± 0.2[b]	1.8 ± 0.5	1.4 ± 0.5[b]
1 nM ACTH	8.5 ± 0.9	7.7 ± 0.7[b]	17.4 ± 2.5	17.8 ± 2.4[b]

[a] Mean ± SE, n = 7–10. [b] Not significantly different from control using paired sample t test.

(11) Elliott, M. E.; Goodfriend, T. L. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1986**, *45*, 2376.

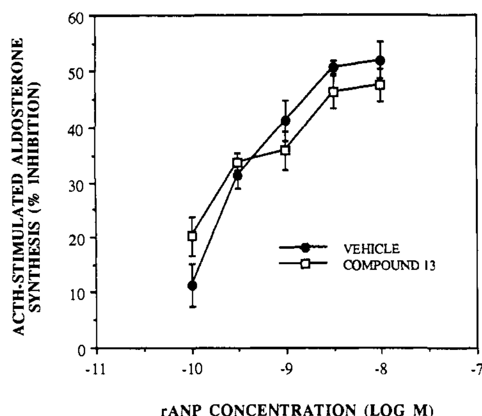


Figure 3. Effect of 13 (10 μM) on inhibition of ACTH-stimulated aldosterone synthesis by rANP in rat adrenal glomerulosa. Maximal stimulation of aldosterone synthesis in the presence of ACTH (1 nM) was 17.4 ± 2.5 ng/mL per 2 h. The EC_{50} for rANP inhibition of aldosterone synthesis was 0.24 ± 0.04 nM for vehicle and 0.18 ± 0.06 nM for 13; not statistically significant ($p < 0.05$). Each point represents the mean ± SE of 10 experiments.

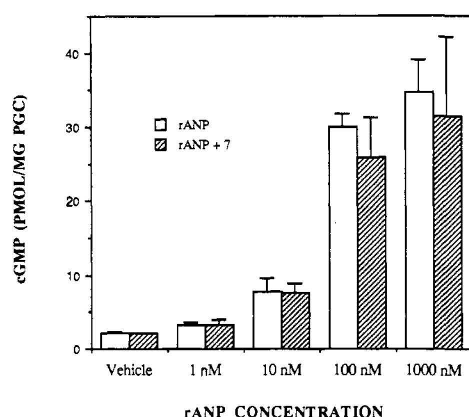


Figure 4. Effect of 7 (20 μM) on particulate guanylate cyclase from rabbit glomeruli in the presence and absence of rANP. Each value represents the mean ± SE of three experiments. In each experiment the individual values were determined in quintuplicate and averaged. Differences between the two groups were not significant at all concentrations of rANP tested.

Discussion. The cellular mechanism underlying ANP inhibition of aldosterone release is presently uncertain.[11,12] Whereas ANP reportedly activates PGC[13] and increases cGMP[14] levels in the adrenal gland and in isolated adrenal cells, the addition of cGMP analogues such as 8-bromo-cGMP does not inhibit basal or angiotensin II stimulated aldosterone synthesis in bovine adrenal glomerulosa cells.[11,15] Thus, the importance of cGMP as a second messenger in ANP inhibition of aldosterone synthesis is not clear.[16] The dissociation between receptor binding and PGC stimulation for truncated ANP analogues[17] suggests that, in certain responsive tissues, a proportion of the ANP receptors may not be associated with guanylate cyclase activity.[12] A recent receptor characterization study by Takayanagi et al. with bovine adrenal cortex[18] supports the presence of guanylate cyclase coupled and noncoupled ANP receptors in the BAZG and raises the possibility that the intracellular action of ANP in this tissue may be mediated through both receptor subtypes. On the other hand, characterization and affinity cross-linking studies in the same tissue by Meloche et al.[19,20] point to a single protein displaying characteristics consistent with only the guanylate cyclase coupled ANP receptor.

During the course of our synthetic efforts, De Lean[21] reported that amiloride, guanabenz, and clonidine[22] also enhanced ANP binding with BAZG membrane ANP receptors. The enhanced binding in the presence of amiloride was due to an increase in both the number of high affinity receptor sites and their affinity. A later report by Meloche et al. suggested that the functional heterogeniety of ANP receptors in the BAZG could be explained by an amiloride-sensitive high affinity complex.[19] The purified ANP receptor from this tissue, which also displayed high guanylate cyclase activity, retained its sensitivity to amiloride modulation, suggesting that the guanylate cyclase coupled ANP receptor contains an amiloride-sensitive allosteric binding site.[20] The compounds reported by De Lean that enhance ANP receptor binding possess a guanidinium group as a common structural feature. While not a requirement for activity in our series, the more potent quinazoline analogues do contain this functional group, suggesting that the interactions between all of these agents and the ANP receptor may be similar at the molecular level.

Although our aldosterone results are congruent with those reported by De Lean for amiloride,[21] the precise interaction between compound 7 and the ANP receptor(s) in the adrenal glomerulosa remains to be elucidated. One possible interpretation of the data is that compound 7 similarly interacts with an allosteric site on the ANP receptor, resulting in enhanced receptor binding and potentiation of rANP inhibition of ACTH-stimulated aldosterone synthesis. The failure of 7 to potentiate rANP's effect on PGC from rabbit glomeruli, however, raises the possibility that the effects of this compound may be independent of PGC. It is unclear whether compound 7 acts through guanylate cyclase uncoupled ANP receptors or if it selectively alters the ANP receptor binding site properties of the guanylate cyclase coupled ANP receptor such that enhanced ANP binding is not functionally manifest in ANP-mediated PGC activity. Additional work will be needed to distinguish between these two possibilities. Since the physiological role of the guanylate cyclase uncoupled ANP receptor is presently unknown,[23] studies with

(12) (a) Jacobs, J. W.; Vlasuk, G. P.; Rosenblatt, M. *Clin. Enodocrinol. Metab.* **1987**, *16*, 63. (b) Leitman, D. C.; Murad, F. *Clin. Endocrinol. Metab.* **1987**, *16*, 79.
(13) Waldman, S. A.; Rapoport, R. M.; Fiscus, R. R.; Murad, F. *Biochim. Biophys. Acta* **1985**, *845*, 298.
(14) (a) Jaiswal, N.; Paul, A. K.; Jaiswal, R. K.; Sharma, R. K. *FEBS Lett.* **1986**, *199*, 121. (b) Matsuoka, H.; Ishii, M.; Sugimoto, T.; Hirata, Y.; Sugimoto, T.; Kangawa, K.; Matsuo, H. *Biochem. Biophys. Res. Commun.* **1985**, *127*, 1052.
(15) Aguilera, G.; Kigoshi, T.; Baukal, A.; Hausdorff, W.; Catt, K. J. *J. Cardiovasc. Pharmacol.* **1986**, *8*, 1291.
(16) See, for example: Matsuoka, H.; Ishii, M.; Hirata, Y.; Atarashi, K.; Sugimoto, T.; Kangawa, K.; Matsuo, H. *Am. J. Physiol.* **1987**, *252*, E643.
(17) Leitman, D. C.; Murad, F. *Biochem. Biophys. Acta* **1986**, *885*, 74.
(18) Takayanagi, R.; Snajdar, R. M.; Imada, T.; Tamura, M.; Pandey, K. N.; Misono, K. S.; Inagami, T. *Biochem. Biophys. Res. Commun.* **1987**, *144*, 244.
(19) Meloche, S.; Ong, H.; De Lean, A. *J. Biol. Chem.* **1987**, *262*, 10252.
(20) Meloche, S.; McNicoll, N.; Liu, B.; Ong, H.; De Lean, A. *Biochemistry* **1988**, *27*, 8151.
(21) De Lean, A. *Life Sci.* **1986**, *39*, 1109.
(22) Clonidine in our hands did not enhance ANP receptor binding at the concentrations tested (0.2, 2.0, 20 μM). This is consistent with De Lean's data, which suggests that concentrations of clonidine greater than 20 μM would be required to significantly enhance ANP binding to BAZG membrane receptors.

compound 7 and ANP analogues[24] which selectively bind to this receptor subpopulation may be of interest. Moreover, studies with purified receptors in the presence of compound 7 might shed additional light on the nature of the presumed allosteric site with respect to the guanylate cyclase coupled and noncoupled ANP receptor subtypes. Thus, 7 and its structurally related analogues may represent a useful tool for studying the interactions of ANP with its receptor.

Experimental Section

Methods. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. The ^{1}H NMR spectra were recorded with a GE QE-300 and were consistent with the assigned structure. Mass spectra were recorded with a CEC 21-110 (EI) or with a Varian-MAT 731 (FD) spectrometer. Microanalytical data were provided by the Physical Chemistry Department of Lilly Research Laboratories. Where analyses are indicated only by symbols of the elements, results obtained were within ±0.4% of the theoretical values.

2-(1-Piperazinyl)-4-(1-pyrrolidinyl)quinazoline Dinitrate (4). A mixture of chloroquinazoline 14 (1.00 g, 4.3 mmol), 1-piperazinecarboxaldehyde (0.49 g, 4.3 mmol), Et_3N (0.65 g, 6.4 mmol), and DMF (20 mL) was heated to 110 °C for 1 h. The solvent was evaporated under reduced pressure and the resulting solid was dissolved in a mixture of EtOH (30 mL) and 6 N HCl (30 mL). After refluxing for 2 h, the reaction mixture was concentrated under reduced pressure and the residue was dissolved in CH_2Cl_2, washed with 1 N NaOH, and dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the crude product was dissolved in hot $EtOH/CH_2Cl_2$. After cooling, the suspension was filtered, concentrated, and passed through a short SiO_2 column (gradient elution from 1% $MeOH/CHCl_3$ to 50% $MeOH/CHCl_3$) to provide 0.59 g (48% overall yield) of the desired free-base product. Recrystallization of the dinitrate salt from MeOH/EtOH provided analytically pure 4, mp 200–201 °C. Anal. ($C_{16}H_{23}N_7O_6$) C, H, N.

1-(2-Furanylcarbonyl)-4-[4-(1-pyrrolidinyl)-2-quinazolinyl]piperazine (2). A mixture of 4 (1.00 g, 3.5 mmol), Et_3N (0.53 g, 5.3 mmol), furoyl chloride (0.46 g, 3.5 mmol), and CH_2Cl_2 (20 mL) was stirred at room temperature for 2 h. The solvent was evaporated under reduced pressure and the residue was partitioned between CH_2Cl_2 and H_2O. The organic phase was separated, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting solid was purified by flash chromatography on SiO_2 (1% $MeOH/CHCl_3$) and recrystallized from MeOH to give 0.96 g (72%) of 2, mp 170–171 °C. Anal. ($C_{21}H_{23}N_5O_2$) C, H, N.

3-[Methyl[4-(1-pyrrolidinyl)-2-quinazolinyl]amino]propanenitrile (15). A mixture of 14 (3.96 g, 16.9 mmol) and freshly distilled 3-(methylamino)propionitrile (3.5 mL g, 37 mmol) in 2-methyl-1-butanol (40 mL) was refluxed under N_2 for 5 h. The reaction mixture was allowed to cool and was concentrated under reduced pressure. The resulting solid residue was dissolved in CH_2Cl_2, washed with H_2O, and dried over Na_2SO_4. Concentration under reduced pressure and recrystallization from EtOAc/hexanes afforded 3.00 g (63%) of 15, mp 106–108 °C. Anal. ($C_{16}H_{19}N_5$) C, H, N.

***N*-[3-[Methyl[4-(1-pyrrolidinyl)-2-quinazolinyl]amino]propyl]-2-furancarboxamide (3).** A solution of 15 (2.49 g, 8.85 mmol) in EtOH (100 mL) and NH_3 (25 mL) was hydrogenated over 1.25 g of Raney nickel at 70 °C for 3 h at an initial H_2 pressure of 1000 psi. The reaction mixture was cooled, filtered, and concentrated under reduced pressure to leave 3.24 g of a green foam that was dissolved in a mixture of Et_3N (2.4 mL, 17 mmol) and CH_2Cl_2 (50 mL). Freshly distilled furoyl chloride (1.23 mL, 12.5 mmol) was added dropwise under N_2 at 0 °C. After the addition was complete, the reaction mixture was warmed to room temperature and stirred for 18 h. The mixture was washed with H_2O, dried over Na_2SO_4, and concentrated under reduced pressure. Recrystallization from EtOAc/hexanes provided 2.31 g (69% yield for two steps) of 3, mp 147–149 °C. Anal. ($C_{21}H_{25}N_5O_2$) C, H, N.

***N*-[3-[Methyl[4-(1-pyrrolidinyl)-2-quinazolinyl]amino]propyl]guanidine Dinitrate (5).** A solution of 15 (3.32 g, 11.8 mmol) was hydrogenated as described above. Following solvent evaporation, the crude oily product was purified by oxalate salt formation and NaOH treatment to give 2.67 g of the free base as a viscous, pale yellow oil. This purified amine was added to a mixture of 3,5-dimethylpyrazole-1-carboxamidine nitrate (3.76 g, 18.7 mmol), H_2O (10 mL), and dioxane (30 mL). After stirring under reflux for 24 h, the reaction mixture was cooled and the dioxane was evaporated under reduced pressure. The aqueous residue was diluted with H_2O and washed with CH_2Cl_2 (1×) and *n*-BuOH (3×). The organic layers were discarded, and the aqueous phase was lyophilized. Recrystallization from MeOH/EtOH furnished 2.6 g (49% yield for the two steps) of 5, mp 167–168 °C dec. Anal. ($C_{17}H_{27}N_9O_6$) C, H, N.

***N*-[2-(Diethylamino)-4-quinazolinyl]-1,2-ethanediamine (18).** To a solution of 17 (2.75 g, 10.6 mmol) in THF (50 mL) at 0 °C was added ethylenediamine (1.5 mL, 22 mmol) all at once. The reaction mixture was stirred for 5 min and then refluxed under a drying tube for 24 h. The resulting cloudy yellow reaction mixture was allowed to cool and was concentrated under reduced pressure. The residue was shaken with aqueous 1 N NaOH and extracted with CH_2Cl_2 (2×). The combined organic layers were dried over Na_2SO_4, filtered through Celite, and concentrated under reduced pressure. The resulting oil was purified by filtration through SiO_2 (EtOAc followed by 20% $MeOH/CH_2Cl_2$) to give 2.12 g (70%) of 18 that was sufficiently pure for the subsequent reaction. An analytical sample was obtained by oxalate salt formation followed by recrystallization from H_2O/MeOH, mp 224–227 °C dec. Anal. ($C_{16}H_{23}N_5O_4$) C, H, N. Alternatively, the CH_2Cl_2 layer from the extraction step was concentrated under reduced pressure to approximately one-half of the original volume. Hexanes were added and the solvent evaporation was slowly continued until crystallization occurred to give 18, mp 96–97 °C. Anal. ($C_{14}H_{21}N_5$) C, H, N.

***N*-[2-[[2-(Diethylamino)-4-quinazolinyl]amino]ethyl]guanidine Dinitrate Hemihydrate (6).** A mixture of 18 (2.1 g, 8.1 mmol), 3,5-dimethylpyrazole-1-carboxamidine nitrate (2.45 g, 12.2 mmol), H_2O (10 mL), and dioxane (30 mL) was stirred under reflux for 24 h. The reaction mixture was cooled and concentrated under reduced pressure. The resulting solid was dissolved in H_2O and washed sequentially with CH_2Cl_2 and *n*-BuOH. The organic layers were discarded, and the aqueous phase was lyophilized to leave 1.69 g (49%). Recrystallization from EtOH/*i*-PrOH provided 6 in analytically pure form, mp 161–162 °C dec (sealed tube). Anal. ($C_{15}H_{25}N_9O_6\cdot{}^1/_2H_2O$) C, H, N.

***N*-[2-[[2-(Diethylamino)-4-quinazolinyl]amino]propyl]guanidine Dinitrate (7).** Compound 17 (5.61 g, 23.8 mmol) upon treatment with 1,3-propanediamine (4.0 mL, 48 mmol) was converted to 19 in a fashion analogous to that of 18. The crude product was purified by oxalate salt formation and recrystallized from H_2O/MeOH to give 5.21 g (60%) of 19 (oxalate salt), mp 207–209 °C. This material (2.50 g, 9.1 mmol) upon NaOH treatment furnished 1.95 g of the free base, which was guanylated as described for 6 to give 0.88 g (28%) of dinitrate salt 7 after recrystallization first from EtOH/*i*-PrOH and then from EtOH, mp 186.5–187 °C dec. Anal. ($C_{16}H_{27}N_9O_6$) C, H, N.

***N*-[2-[[2-(Diethylamino)-4-quinazolinyl]amino]butyl]guanidine Dinitrate (8).** Neat 1,4-diaminobutane (3.35 g, 14.2 mmol) was added to neat 17 all at once at 0 °C. The resulting mixture was stirred at 50 °C for 1 h and then at 80 °C for 1.5 h. After cooling, the mixture was diluted with CH_2Cl_2, washed with aqueous 1 N NaOH and brine, dried over Na_2SO_4, and concentrated under reduced pressure to a yellow oil. Flash chromatography on SiO_2 (sequentially EtOAc, 10% $MeOH/CH_2Cl_2$, 20% $MeOH/CH_2Cl_2$) provided 3.1 g of 20 as a pale yellow oil that crystallized from Et_2O/hexanes, mp 59.5–62 °C. Guanylation of 20 (3.0 g, 10.4 mmol) as described for 6 furnished 2.10 g (44%) of the desired dinitrate salt 8 after recrystallization from

(23) The guanylate cyclase uncoupled receptors are reportedly biologically silent and do not mediate any of the known renal effects of ANP. Maack, T.; Suzuki, M.; Almeida, F. A.; Nussenzveig, D.; Scarborough, R. M.; McEnroe, G. A.; Lewicki, J. A. *Science* 1987, *238*, 675.

(24) (a) Vlasuk, G. P.; Arcuri, K. E.; Ciccarone, T. M.; Nutt, R. F. *FEBS Lett.* 1988, *228*, 290. (b) Olins, G. M.; Patton, D. R.; Bovy, P. R.; Mehta, P. P. *J. Biol. Chem.* 1988, *263*, 10989.

EtOH/*i*-PrOH, mp 150–151 °C. Anal. ($C_{17}H_{29}N_9O_6$) C, H, N.

***N*-[2-[[2-(Diethylamino)-4-quinazolinyl]amino]ethyl]-*N'*-methylguanidine Dihydrochloride (9).** To a solution of 18 (2.0 g, 7.7 mmol) in dry DMF (20 mL) was added a solution of methyl isothiocyanate (5.6 g, 7.7 mmol). After refluxing for 15 min, the solvent was evaporated under reduced pressure. The resulting residue was recrystallized from acetonitrile to give 1.76 g of a solid that was dissolved in THF (30 mL) and cooled to 0 °C. Methyl iodide (0.75 g, 5.3 mmol) was added, the reaction mixture was stirred for 1 h under reflux, allowed to cool, and finally concentrated under reduced pressure. The resulting product was dissolved in EtOH (15 mL) and anhydrous NH_3 (5 mL) and then heated to 100 °C for 1 h in a sealed tube. After cooling and solvent evaporation, the residue was passed through an Ag2-X8 anion-exchange resin (chloride form, 200 mesh) with 1:1 H_2O/EtOH. The EtOH was removed under reduced pressure and the remaining aqueous solution was lyophilized to give 1.89 g (63%) of the desired dihydrochloride salt **9**. An analytical sample was obtained by recrystallization from EtOH/*i*-PrOH, mp 242–245 °C. Anal. ($C_{16}H_{27}N_7Cl_2$) C, H, N.

***N*-[2-[[2-(Diethylamino)-4-quinazolinyl]amino]ethyl]-*N'*-ethylguanidine Dinitrate (10).** In a fashion analogous to that of **9**, compound **19** (2.3 g, 8.8 mmol) was converted to 1.3 g (32%) of **10**, mp 152–154 °C, after filtration through the anion-exchange resin (nitrate form) and recrystallization from EtOH/*i*-PrOH. Anal. ($C_{17}H_{29}N_9O_6$) C, H, N.

***N''*-Cyano-*N*-[2-[[2-(diethylamino)-4-quinazolinyl]-amino]ethyl]-*N'*-methylguanidine (11).** To a solution of 18 (1.8 g, 6.9 mmol) in EtOH (25 mL) was added a solution of dimethyl *N*-cyanodithioiminocarbonate (1.0 g, 6.8 mmol) in EtOH (20 mL). The resulting suspension was stirred overnight and the precipitated solid was collected by suction filtration to provide 2.08 g (84%) of the desired *N*-cyano-*S*-methylthiourea intermediate, which was divided into two halves. One-half (1.0 g, 2.8 mmol) was treated with 40% aqueous $MeNH_2$ (5 mL) and EtOH (15 mL) and then refluxed until a homogeneous solution was obtained (1 h). The reaction mixture was allowed to cool and was concentrated under reduced pressure. Recrystallization from EtOH provided 0.90 g (95%) of **11**, mp 187–188 °C. Anal. ($C_{17}H_{24}N_8$) C, H, N.

***N''*-Cyano-*N*-[2-[[2-(diethylamino)-4-quinazolinyl]-amino]ethyl]-*N'*-ethylguanidine (12).** The second half of the above *N*-cyano-*S*-methylthiourea intermediate (1.0 g, 2.8 mmol) was similarly treated with 70% aqueous $EtNH_2$ (5 mL) and EtOH (15 mL) except that it was heated in a sealed tube at 125 °C until a homogenous solution was obtained (45 min). The reaction mixture was allowed to cool and then concentrated under reduced pressure. Recrystallization from EtOH gave 0.78 g (79%) of **12**, mp 174–176 °C. Anal. ($C_{18}H_{26}N_8$) C, H, N.

***N,N*-Diethyl-4-methoxy-2-quinazolineamine Ethanedioate (13).** A mixture of **17** (1.00 g, 4.24 mmol), NaOMe (460 mg, 8.50 mmol), and MeOH (40 mL) was stirred under reflux for 4 h. After cooling, the solvent was evaporated under reduced pressure and the residue was partitioned between H_2O and Et_2O. The organic layer was separated, washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to leave 0.92 g of a yellow oil. Oxalate salt formation and recrystallization from EtOH/*i*-PrOH afforded 1.27 g (82%) of **13**, mp 166–166.5 °C. Anal. ($C_{16}H_{20}N_3O_7$) C, H, N.

In Vitro Assays. The receptor binding assay was performed in bovine adrenal zonal glomerulosa membranes with [^{125}I]rANP as previously described.[25]

PGC activity was measured as described.[26,27] Glomeruli from male New Zealand white rabbits were placed in ice-cold saline and the capsule was removed. The remaining tissue was homogenized in 20 volumes of Tris-HCl buffer (50 mM), pH 8.0; EDTA (1 mM); sucrose (250 mM); and dithiothreitol (1 mM) at 4 °C and then centrifuged at 1500*g* for 10 min at 4 °C. The supernatant was recentrifuged at 105,000*g* for 1 h and the resulting pellet was suspended in Tris-HCl (50 mM) at pH 7.4. The reaction was initiated by adding the enzyme to a mixture of Tris-HCl (50 mM), creatine phosphate (15 mM), creatine phosphokinase (200 μg/mL), GTP (1 mM), $MgCl_2$ (4 mM), theophylline (10 mM), and 3-isobutyl-1-methylxanthine (2 mM) and was incubated at 37 °C in the presence/absence of rANP and drug. The reaction was terminated after 10 min by adding ice-cold 50 mM NaOAc buffer, pH 4.0 (0.9 mL), and heating for 3 min at 95 °C. The cGMP formation was determined by RIA (BT-340, Biomedical Technologies, Inc.). Under the experimental conditions, rANP selectively activated PGC without affecting soluble guanylate cyclase, consistent with the observation of several laboratories.[28]

Aldosterone synthesis was studied in isolated rat adrenal glomerulosa cell suspensions prepared by a modification of the method described by Catt.[29] Both right and left adrenal glands from male Sprague–Dawley rats were cleaned of fat and the capsule with the attached adrenal glomerulosa cell layer was washed with ice-cold, zero K^+ medium 199 (M199), minced, and incubated with collagenase (1 mg/mL), bovine serum albumin (BSA) (2 mg/mL), and M199 under 95% air/5% CO_2. Glomerulosa cells were then mechanically dispersed in M199 containing soybean trypsin inhibitor (0.1 mg/mL), DNAase (0.05 mg/mL), and BSA (2 mg/mL). The solution was filtered through 30-μm platinum mesh and centrifuged at 150*g* for 10 min. The pellet was immediately resuspended in M199 (30 mL) containing KCl (3.5 mM) and BSA (2 mg/mL), yielding a final concentration of approximately 30 000 cells/mL. The incubation was begun by adding 1-mL aliquots of the cell suspension to vials containing 10 μL of each of the following (final concentration): ACTH (1 nM); rANP (10^{-10}–10^{-8} M); **7** (3 μM), **13** (10 μM), or distilled H_2O (vehicle). The vials were equilibrated with the air/CO_2 mixture, capped, and shaken for 2 h at 37 °C. The suspensions were then centrifuged at 3500*g* and the aldosterone concentration was determined in the supernatant by a direct solid-state RIA (Diagnostic Products, Inc.). Appropriate dilutions with the resuspension buffer were made to ensure that the measurements fell on the linear portion of the standard curve (5–800 pg of aldosterone/tube). The EC_{50} for rANP inhibition of ACTH-stimulated aldosterone synthesis was calculated using a four parameter logistic model (NLIN, SAS institute). Significance of the difference between EC_{50}'s was determined by Student's *t* test for two means.

Acknowledgment. We thank Dr. Charles Paget and Marie Reamer for their help in the initial general screening of compounds. We would also like to thank Jack Campbell for performing the Raney nickel hydrogenation reactions and E. Schirmer for preparing the [^{125}I]rANP.

Registry No. 1, 19216-56-9; 2, 123264-76-6; 3, 123264-77-7; 4, 123264-78-8; 4·2HNO$_3$, 123264-88-0; 5, 123264-79-9; 5·2HNO$_3$, 123264-89-1; 6, 123264-80-2; 6·2HNO$_3$, 123264-90-4; 7, 123264-81-3; 7·2HNO$_3$, 123264-91-5; 8, 123264-82-4; 8·2HNO$_3$, 123264-92-6; 9, 123264-83-5; 9·2HCl, 123264-93-7; 10, 123264-84-6; 10·2HNO$_3$, 123264-94-8; 11, 123264-85-7; 11 *N*-cyano-*S*-methylthiourea intermediate, 123265-01-0; 12, 123264-86-8; 13, 123264-87-9; 13·$^3/_2$oxalate, 123264-95-9; 14, 1804-50-8; 15, 123264-96-0; 17, 78490-71-8; 18, 123264-97-1; 18·oxalate, 123264-98-2; 19, 123264-99-3; 19·oxalate, 123265-00-9; ACTH, 9002-60-2; 1-piperazinecarboxaldehyde, 7755-92-2; 3-(methylamino)propionitrile, 693-05-0; furoyl chloride, 3187-94-8; 3,5-dimethylpyrazole-1-carboxamidine nitrate, 38184-47-3; 1,3-propanediamine, 109-76-2; 1,4-diaminobutane, 110-60-1; methyl isothiocyanate, 556-61-6; dimethyl *N*-cyanodithioiminocarbonate, 10191-60-3; aldosterone, 52-39-1; atriopeptin, 85637-73-6; ethylenediamine, 107-15-3.

(25) (a) De Lean, A.; Racz, K.; Gutkowska, J.; Nguyen, T.-T.; Cantin, M.; Genest, J. *Endocrinology* **1984**, *115*, 1636. (b) Horng, J. S.; Shields, J. E.; Murphy, P. J. *Pharmacologist* **1985**, *27*, 145.
(26) Ezekiel, M.; Ohlstein, E. H. *Pharmacologist* **1986**, *28*, 193.
(27) Garbers, D. L.; Murad, F. *Adv. Cyclic Nucleotide Res.* **1979**, *10*, 57.
(28) (a) Inui, K.; Saito, H.; Matsukawa, Y.; Nakao, K.; Morii, N.; Imura, H. Shimokura, M.; Kiso, Y.; Hori, R. *Biochem. Biophys. Res. Commun.* **1985**, *132*, 253. (b) Ohlstein, E. H.; Berkowitz, B. A. *Hypertension* **1985**, *7*, 306. (c) Waldman, S. A.; Rapoport, R. M.; Murad, F. *J. Biol. Chem.* **1984**, *259*, 14332. (d) Winquist, R. J.; Faison, E. P.; Waldman, S. A.; Schwartz, K.; Murad, F.; Rapoport, R. M. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 7661.
(29) Fujita, K.; Aguilera, G.; Catt, K. J. *J. Biol. Chem.* **1979**, *254*, 8567.