114025-21-7; XVI, 117710-60-8; BOC-Cit-ONp, 56612-88-5; BOC-Pro-OH, 15761-39-4; BOC-Cys(Bzl)-OH, 5068-28-0; BOC-Asn-ONp, 4587-33-1; BOC-Val-OH, 13734-41-3; BOC-Phe-OH, 13734-34-4; BOC-D-Tyr(Et)-OH, 76757-92-1; 1-($CH_2COOC_6H_4$-p-NO_2)-1-(SCH_2Ph)-c-C_6H_{10}, 55154-81-9; BOC-Arg(Tos)-OH, 13836-37-8; BOC-Gln-ONp, 15387-45-8; BOC-Ile-OH, 13139-16-7; BOC-Tyr(Me)-OH, 53267-93-9; BOC-Tyr(Me)-Ile-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2, 117710-62-0; H-Tyr(Me)-Ile-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2·TFA, 117710-65-3; H-Leu-NH_2·HCl, 10466-61-2; BOC-Tyr(Me)-Ile-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2, 117710-66-4; $PhCH_2SC(CH_3)_2CH_2COOC_6H_4$-p-$NO_2$, 7536-40-5; oxytocin, 50-56-6.

Structure–Activity Relationships of Novel Vasopressin Antagonists Containing C-Terminal Diaminoalkanes and (Aminoalkyl)guanidines[1]

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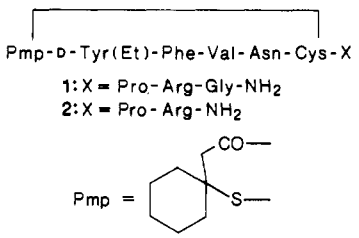
We report the synthesis and biological activity of a series of analogues of the vasopressin antagonists [Pmp[1],D-Tyr(Et)[2],Val[4]]arginine-vasopressin (1) and [Pmp[1],D-Tyr(Et)[2],Val[4],desGly[9]]arginine-vasopressin (2), where part or all of the tripeptide tail has been replaced by a simple alkyldiamine [$NH(CH_2)_nNH_2$] or (aminoalkyl)guanidine [$NH(CH_2)_nNHC(=NH)NH_2$] in order to examine the effects that variation of the length and orientation of the tripeptide tail have on renal vasopressin (V_2) receptor antagonist activity. The results show that the entire tripeptide tail (Pro-Arg-Gly-NH_2) can be replaced by an alkyldiamine or an (aminoalkyl)guanidine, compounds **15** and **16**, respectively, indicating that there is no orientational requirement for the basic functional group coming off the cyclic hexapeptide ring. Also, there seems to be an "optimal" distance between the basic functional group and the hexapeptide ring since receptor affinity of the antagonists begins to fall off when the basic functional group is too close (compound **13**) or extends too far (compounds **8–10**) from the hexapeptide ring. These results suggest all that is necessary for retention of antagonist affinity and potency is a basic functional group, amine or guanidine, extended an optimal distance from the hexapeptide ring.

In a preliminary communication,[1] we outlined the effects of removing parts of the tripeptide tail of the potent vasopressin antagonists [Pmp[1],D-Tyr(Et)[2],Val[4]]arginine-vasopressin (**1**)[2,3] and [Pmp[1],D-Tyr(Et)[2],Val[4],desGly[9]]arginine-vasopressin (**2**)[4,5] and replacing them with simple diaminoalkanes. In this paper we examine in greater



detail the effects that variation of the length and orientation of the tripeptide tail have on renal vasopressin (V_2) receptor antagonist activity.

When this study was initiated, little was known concerning the contribution of the tripeptide tail (Pro-Arg-Gly-NH_2) to the renal vasopressin (V_2) antagonist pharmacophore. The model proposed by Walter et al.[6] for the V_2 agonist pharmacophore emphasized the important role of the complete tripeptide tail in the binding of AVP to the renal V_2 receptor. The full tripeptide tail was also a common feature in many of the early V_2 antagonists.[3] However, more recent studies have shown that the tripeptide tail of V_2 antagonists can be significantly modified without affecting receptor affinity or antagonist potency.[4,5,7,8] Indeed, it has been shown that the C-terminal glycine can be deleted[4,5] and the proline at position 7 can be freely substituted for[7] or even deleted[8] all with retention

Scheme I. Synthesis of Mono-Boc-diamines[a]

path A

$H_2N(CH_2)_nNH_2 \xrightarrow{(a)}$ Boc-$NH(CH_2)_nNH_2$

path B

$H_2NCH_2CH_2OH \xrightarrow{b}$ Boc-$NHCH_2CH_2OH \xrightarrow{c}$

Boc-$NHCH_2CH_2N_3 \xrightarrow{d}$ Boc-$NHCH_2CH_2NH_2$

[a] (a) $(Boc)_2O$, CH_2Cl_2; (b) $(Boc)_2O$, Et_3N, CH_2Cl_2; (c) Ph_3P, DEAD, HN_3, THF/toluene; (d) Ra-Ni, $H_2NNH_2·H_2O$, EtOH.

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‡ Department of Pharmacology.
§ Department of Molecular Pharmacology.

(1) For a preliminary communication of this work, see: Huffman, W. F.; Ali, F. A.; Bryan, W. M.; Callahan, J. F.; Moore, M. L.; Silvestri, J. S.; Yim, N. C. F.; Kinter, L. B.; McDonald, J. E.; Ashton-Shue, D.; Stassen, F. L.; Heckman, G. D.; Schmidt, D. B.; Sulat, L. *J. Med. Chem.* **1985**, *28*, 1759.
(2) Abbreviations of amino acids follow the recommendations of the IUPAC-IUB Commision on Biochemical Nomenclature: *Eur. J. Biochem.* **1984**, *158*, 9. All optically active amino acids are assumed to be L unless otherwise specified. The following additional abbreviations are used: Pmp, β-thio-β,β-pentamethylenepropionic acid; AVP, [Arg[8]]vasopressin; LVP, [Lys[8]]vasopressin.
(3) (a) Manning, M.; Lammek, B.; Kruszynski, M.; Seto, J.; Sawyer, W. H. *J. Med. Chem.* **1982**, *25*, 408. (b) Manning, M.; Olma, A.; Klis, W. A.; Kolodziejczyk, A. M.; Seto, J.; Sawyer, W. H. *J. Med. Chem.* **1982**, *25*, 45 and references cited therein.
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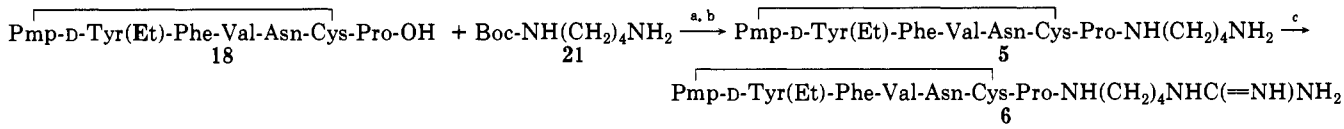
Scheme II. Representative Synthesis of Modified Tail Antagonists[a]



[a] DCC, HOBt, DMF; (b) TFA, 0 °C; (c) *O*-methylisourea, aqueous NaOH.

of high receptor affinity. This suggested that in antagonists, the major pharmacophore element of the tail was the amine or guanidine side chain of a lysine or arginine residue, but there was very little data further defining the optimal distance between this basic functional group and the cyclic hexapeptide ring or their optimal relative orientation.

In order to define the optimal distance between the terminal basic functional group and the hexapeptide ring as well as its relative orientation, we made a series of analogues that varied the position of the basic functional group on the tail relative to the hexapeptide ring. We first replaced the Lys-NH_2 in compound **3** with a simple alkyldiamine (compound **7**) and replaced the Arg-NH_2 in compound **2** with an (aminoalkyl)guanidine (compound **6**) of similar length to examine the effects of removal of the terminal carboxamide. The Lys-NH_2 in compound **12** was replaced with an alkyldiamine of similar length (compound **15**) to observe the effects of the removal of all conformational constraints in the tail. We also explored the optimal distance of the basic functional group from the hexapeptide ring by varying the length of the alkyl spacers in analogues **4–10** and in the desproline analogues **13–16**. Finally, we eliminated the rotation of several of the bonds in the alkyl spacer by the introduction of a phenyl ring (compounds **17** and **18**).

Peptide Synthesis

The mono-*tert*-butyloxycarbonyl (Boc) diamines, except for mono-Boc-diaminoethane, were prepared by treatment of the corresponding diamine with di-*tert*-butyl dicarbonate in methylene chloride followed by extractive workup (Scheme I, path A).[9] Mono-Boc-diaminoethane was prepared from *N*-Boc-aminoethanol by the conversion of the alcohol to an azide [diethyl azodicarboxylate (DEAD), triphenylphosphine, HN_3] and then the reduction of the azide to the desired amine using Raney nickel (Scheme I, path B).[10] The proline acid peptide **19** was prepared by solid-phase peptide synthesis[11] on chloromethylated resin. Coupling reactions were performed by using *N,N'*-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) except for the final coupling of the 4-MeBzl-Pmp,[12] where the HOBt was replaced with 4-dimethylaminopyridine (DMAP). The peptide was cleaved from the resin, with removal of protecting groups, by using anhydrous hydrogen fluoride and anisole. The resulting disulfhydryl peptide was oxidatively cyclized with $K_3[Fe(CN)_6]$[13] and the crude cyclic peptide was purified by flash chromatography on C-18 reverse phase silica gel. The cysteine acid peptide **20** was prepared in an analogous fashion. The diaminoalkyl analogues **4**, **5**, **7–9**, **14**, **15**, **17**, and **18** were prepared by coupling the respective peptide acids **19** and **20** to the corresponding mono-Boc-diaminoalkanes using DCC/HOBt in dimethylformamide. Analogue **10** was prepared by the direct coupling of 1,8-diaminooctane with the proline acid peptide **19** using DCC/HOBt in dimethylformamide. The Boc-protected peptides were then treated without purification with trifluoroacetic acid (TFA) at 0 °C to give the desired products. The final products were purified in most cases by ion exchange chromatography (BioRex 70) followed by preparative reverse phase HPLC. The guanidino analogues **6** and **16** were synthesized from the corresponding amino peptides, **5** and **15**, respectively, using *O*-methylisourea in basic aqueous solution[14] followed by purification by preparative reverse phase HPLC (Scheme II).

Bioassay Methods

Peptides **1–20** were tested in vitro as previously described[15] for vasopressin V_2 receptor binding and antagonist activity on pig renal medullary membrane preparations. Affinity for the receptors was determined by competition with tritiated LVP and is expressed as K_{bind}. The antagonist activity for analogues **1–20** was determined by their ability to inhibit LVP-stimulated adenylate cyclase in the same preparation and is expressed as the inhibition constant K_i. The antagonist activity for selective analogues was also determined by their ability to inhibit LVP-stimulated adenylate cyclase in preparations of human renal medullary membrane preparations. The in vivo evaluation for the antagonists **1–20** was carried out by using a hydropenic rat bioassay,[16] which is outlined below. Groups of male rats were deprived of food and water overnight to establish a stable hydropenic state, characterized by an elevated plasma vasopressin level and a urine osmolality $\simeq$ 1500 mOsm/kg H_2O. The rats, in groups of four, were administered antagonist at varying doses and urine volume and osmolality were determined. Potency was expressed as the effective dose (ED_{300}, μg/kg) required to dilute urine osmolality from hydropenic levels to osmolality levels of 300 mOsm/kg H_2O.

Results

The renal V_2 receptor affinity in pig and antagonist activities in pig and human of peptides **1–20** are shown in

(6) Walter, R.; Smith, C.; Mehta, P.; Boonjarern, S.; Arruda, J.; Kurtzman, N. *Disturbances in Body Fluid Osmolality*; Andreoli, T., Grantham, J., Rector, F., Jr., Eds.; American Physiological Society: Bethesda, 1977; pp 1–36.
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(9) Hansen, J. B.; Nielsen, M. C.; Ehrbar, U.; Buchardt, O. *Synthesis* **1982**, 404.
(10) (a) Mitsunobu, O. *Synthesis* **1981**, 1. (b) Loibner, H.; Zbiral, E. *Helv. Chim. Acta* **1976**, *59*, 2100.
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(13) Hope, D. V.; Murti, V. V. S.; duVigneaud, V. *J. Biol. Chem.* **1962**, *237*, 1563.
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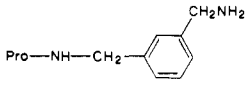
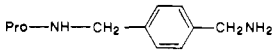
(15) Stassen, F.; Erickson, R.; Huffman, W.; Stefankiewicz, J.; Sulat, L.; Wiebelhaus, V. *J. Pharmacol. Exp. Ther.* **1982**, *223*, 50.
(16) Kinter, L.; Huffman, W.; Wiebelhaus, V.; Stassen, F. *Diuretics: Chemistry, Pharmacology and Clinical Applications*; Puschett, J. Ed.; Elsevier: New York, 1984; pp 72–81.

Table I. Activity of Vasopressin Antagonists

Pmp-D-Tyr(Et)-Phe-Val-Asn-Cys-X

compd no.	X	pig K_{bind},[a] nM	pig K_i,[b] nM	human K_i,[c] nM	rat ED_{300},[d] μg/kg
1	Pro-Arg-Gly-NH_2	12 (7)	5.6 (3)	4.2 (5)	11 (7)
2	Pro-Arg-NH_2	14 (7)	4.4 (4)	3.6 (15)	9.8 (28)
3	Pro-Lys-NH_2	37	9.4	NT	22 (2)
4	Pro-NH-$(CH_2)_2$-NH_2	10 (2)	4.7	4.8 (2)	108 (2)
5	Pro-NH-$(CH_2)_4$-NH_2	12 (3)	10 (3)	7.6 (2)	19 (2)
6	Pro-NH-$(CH_2)_4$-NH-C(=NH)NH_2	16	6.0	3.7 (3)	20 (5)
7	Pro-NH-$(CH_2)_5$-NH_2	30 (2)	8.3 (3)	NT	27 (2)
8	Pro-NH-$(CH_2)_6$-NH_2	51 (2)	28 (2)	NT	NT
9	Pro-NH-$(CH_2)_7$-NH_2	190 (3)	44 (2)	NT	>181
10	Pro-NH-$(CH_2)_8$-NH_2	310	NT[f]	NT	NT
11	Arg-NH_2	10 (2)	2.9 (2)	1.5 (3)	58 (3)
12	Lys-NH_2	26 (2)	5.8	3.3 (2)	59 (3)
13	NH-$(CH_2)_2$-NH_2[e]	68	NT	NT	NT
14	NH-$(CH_2)_4$-NH_2	13	27	NT	221
15	NH-$(CH_2)_5$-NH_2	19	6.8	6.6	94.5
16	NH-$(CH_2)_5$-NH-C(=NH)NH_2	20	11	NT	186
17		61 (3)	11	NT	77
18		81	28	NT	205 (2)
19	Pro-OH	400	130	NT	>182
20	OH	1100 (2)	490	NT	>4706

[a] Inhibition constant for [^{3}H]LVP binding to medullary membranes of pig kidney. Average of (n) determination, each determination an average of tests performed in triplicate. [b] Inhibition constant for vasopressin-stimulated adenylate cyclase of medullary membranes of pig kidney. Average of (n) determination, each determination an average of tests performed in triplicate. [c] Inhibition constant for vasopressin-stimulated adenylate cyclase of medullary membranes of human kidney. Average of (n) determination, each determination an average of tests performed in triplicate. [d] Dose required to lower urine osmolality to 300 mOsm/kg. Average of (n) determinations, each determination an average of four rats. [e] See ref 16. [f] NT = analogue not tested in this assay.

Table I. It can be seen that replacement of the terminal lysine amide in **3** or the terminal arginine amide in **2** with a diaminoalkane or an aminoguanidinoalkane of equivalent chain length, peptides **7** and **6**, respectively, gave analogues that display almost identical receptor affinity and antagonist activity both in vivo and in vitro. Reducing the length of the methylene chain to two or four carbon units gave peptides **4** and **5**, whose receptor affinity and in vitro antagonist activity were similar to the reference antagonist **3**. However, increasing the length of the chain to up to eight methylene units, compounds **8–10**, revealed a trend of decreasing receptor affinity that appears to be directly related to the length of the alkyl spacer unit. An attempt to somewhat restrict tail mobility by replacing part of the alkyl spacer with an aromatic ring gave analogues **17** and **18**, whose receptor affinity and in vitro antagonist activity did not differ significantly from analogues with an alkyl spacer of similar length.

Replacement of the terminal carboxamide of the Lys-NH_2 antagonist **12** with a diaminoalkane of equivalent chain length, peptide **14**, resulted in an analogue that displayed similar receptor affinity and in vitro antagonist activity. Shortening the alkyl spacer by one carbon, compound **14**, had little effect on receptor affinity but shortening the spacer to two carbons, compound **13**,[17] resulted in reduced receptor affinity.

In several cases, the in vitro antagonist activity (K_i) was also measured in human renal medullary membrane preparation. In all cases, the results in human tissue were analogous to those obtained in the pig.

The in vivo antagonist activity, as measured by the hydropenic rat assay, did vary to a greater extent with variations in the length of the antagonist tail as well as with the introduction of an aromatic spacer group. However, all the analogues that were tested retained significant in vivo antagonist activity.

Discussion

The results summarized above provide substantial new information concerning the role of the tripeptide tail in the V_2 receptor antagonist pharmacophore while reiterating some of the conclusions that have been made previously.[4,5,7,8] There is no evidence for an orientational requirement for the basic functional group coming off of the cyclic hexapeptide ring in these antagonists and even the removal of proline fails to significantly affect the antagonist activity. The length of the tail containing the basic functional group appears to be somewhat flexible, although there seems to be an "optimal" distance from the hexapeptide ring. When the basic functional group is too close to the hexapeptide ring, e.g. compound **13**, or extends too far from the hexapeptide ring, e.g. compounds **8–10**, the receptor affinity of the antagonists begins to fall off. This is in strong contrast to vasopressin agonists where the basic functional group in the tripeptide tail is thought to be fixed in a much more defined spacial orientation in the receptor-bound conformation.[6]

In vasopressin agonists, there seems to be three pharmacophore elements associated with the tripeptide tail.[18] These include a hydrophobic interaction with the proline at position 7,[19] a requirement of a basic amino acid in the

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L configuration at position 8,[15] and a glycinamide residue at position 9.[8,20] Also, the conformational constraint of an *N*-methyl amino acid at position 7 is necessary to correctly orient the tail with respect to the cyclic hexapeptide ring.[21] Variations in any one of these positions results in significant loss of agonist potency.[15,19-21] For vasopressin antagonists, however, all that is necessary for retention of antagonist affinity and potency is a basic functional group, amine or guanidine, extended an optimal distance from the hexapeptide ring. The fact that stereochemical constraints are not necessary and that even the proline can be totally eliminated without affecting antagonist affinity definitely shows that the tail portion of the antagonist pharmacophore is quite different from that of the agonist. There does seem to be a specific binding pocket for the basic functional group on the tail in these vasopressin antagonists, however, since lengthening or shortening the tail beyond a certain point begins to reduce antagonist affinity. It could be that the tail is constrained by the interaction of the cyclic hexapeptide with the receptor in such a way that it can only bind in one orientation, rendering other constraints in the tail superfluous. Conversely, the tail may bind to a site close enough to the cyclic hexapeptide ring so that it must fold back on itself to make the site accessible. In that case, a constraint close to the ring will have little affect on the entropy loss associated with binding while analogues with the basic functional group located too far from the hexapeptide would have to decrease entropy in order to bind, thus lowering their affinity.[7] Whatever explanation is used, it is clear that vasopressin antagonists in this series bind the V_2 receptor in a manner different from vasopressin agonists.

The in vivo antagonist activity, as measured by the hydropenic rat assay, showed greater variability with modifications in the tail. However, all the analogues tested retained significant antagonist activity. These anomalous results may reflect metabolic as well as pharmacokinetic differences, but at this time there is insufficient data to substantiate these or any other explanations.

In conclusion, we have shown that the structural and conformational requirements for the tail vary significantly for vasopressin V_2 agonists and antagonists. This is yet another example of how vasopressin agonists and antagonists present distinct pharmacophores to the renal V_2 receptor.

Experimental Section

Thin layer chromatography (TLC) was performed on silica gel plates (E. Merck) by using the following systems: (A) 1-butanol–acetic acid–water (BAW) 4:1:1; (B) BAW 4:1:5 (upper layer); (C) 1-butanol–acetic acid–water–ethyl acetate (BAWE) 1:1:1:1. The plates were visualized by using 10% bleach/1% starch–KI. High performance liquid chromatography (HPLC) was performed on 5-μm reverse phase ODS columns (Beckman or Jones) using either a Spectra-Physics 8700, a Waters Associates, or a Beckman series 342 liquid chromatograph with ultraviolet detection at 220 nm. The mobile phases were (D) 0.05 M aqueous KH_2PO_4 and acetonitrile, (E) 0.25% aqueous trifluoroacetic acid and 0.25% trifluoroacetic acid in acetonitrile, and (F) 0.1% aqueous trifluoroacetic acid and 0.1% trifluoroacetic acid in acetonitrile. All peptides were shown to be a single homogeneous peak both by isocratic and gradient HPLC. For amino acid analysis, peptides were hydrolyzed either in HCl/propionic acid (1:1) at 130 °C for 2 h or in HCl/trifluoroacetic acid (2:1) containing 0.005% w/v phenol at 160 °C for 1 h. Cysteine was determined separately as cysteic acid. Cysteine-containing samples were first oxidized with performic acid at 0 °C for 4 h followed by lyophilization. The resulting sample was then hydrolyzed in 6 N HCl at 110 °C for 24 h. Amino acid analyses were performed on a Kontron Liquimat III or a DIONEX T600 analyzer. Proton NMR were obtained on a Varian EM-390 spectrometer at 90 MHz by using tetramethylsilane (TMS) as an internal standard. Fast atom bombardment (FAB) mass spectra were obtained as a VG ZAB high resolution mass spectrometer and chemical ionization (CI) mass spectra were obtained on a Finnigan Model 3300 mass spectrometer. All amino acids were obtained from commercial sources except for β-[(4-methylbenzyl)thio]-β,β-pentamethylenepropionic acid (4-MeBzl-Pmp)[12] and Boc-*O*-ethyl-D-tyrosine,[22] which were prepared by literature methods.

Pmp-D-Tyr(Et)-Phe-Val-Asn-Cys-Pro-OH (19). Boc-proline-Merrifield resin was made by coupling Boc-proline to Merrifield resins using the cesium salt method.[23] The synthesis of **19** was carried out on a Beckman 990B peptide synthesizer using the following protocol: 3 equiv of the amino acid was dissolved in the appropriate solvents [the Boc derivatives of 4-MeBzl-Cys, Val, and Phe in methylene chloride, Asn in dimethylformamide, D-Tyr(Et) in 1:1 methylene chloride/dimethylformamide, and 4-MeBzl-Pmp in methylene chloride] and coupled by using an equimolar amount of dicyclohexylcarbodiimide and 1-hydroxybenzotriazole except for the coupling of 4-MeBzl-Pmp where 1 equiv of (dimethylamino)pyridine was used as a catalyst. The extent of coupling was determined by qualitative ninhydrin analyses and the couplings were repeated when necessary. The Boc-protecting group was removed by using 1:1 trifluoroacetic acid/methylene chloride and the free amine was generated by using 5% diisopropylethylamine in methylene chloride. One millimole of Boc-Pro-Merrifield yielded 2.24 g of peptide resin after the final coupling.

One-half (1.1 g, 0.5 mmol) of the peptide resin was stirred with anisole (3 mL) in anhydrous hydrogen fluoride (20 mL) for 60 min at 0 °C. The hydrogen fluoride was removed under reduced pressure and the residue washed with ether (discarded) and then with dimethylformamide (3 × 10 mL), 20% aqueous acetic acid (3 × 10 mL), and 0.3 N ammonium hydroxide (3 × 10 mL). The filtrate was added to degassed water (2 L) and the pH adjusted to 7.1 with concentrated ammonium hydroxide. A 0.01 M solution of potassium ferricyanide was added dropwise with stirring until a faint yellow color persisted (41 mL). The solution was then stirred with 30 g of AG-3 × 4 Bio-Rad ion exchange resin (Cl^- form) for 15 min and filtered over an additional 30 g of resin. The combined resin was washed with 20% aqueous acetic acid (4 × 200 mL) and the filtrate passed through a column (5 × 10 cm) of reverse phase C-18 silica gel. The column was then washed with water (350 mL) and the peptide eluted with 1:1 acetonitrile/water containing 0.25% trifluoroacetic acid to yield after lyophillization 189 mg of **19**, which was used without purification for the synthesis of the modified tail vasopressin antagonists. An analytical sample was further purified by reverse phase HPLC (5-μm ODS, 10 × 250 mm column, isocratic elution with 60:40 0.25% aqueous TFA:0.25% in acetonitrile) to give pure peptide **19**: AA analysis, Asp 1.00, Pro 1.04, Cys 1.00 (determined separately), Val 0.98, Tyr 0.88, Phe 0.97; mass spectrum (FAB), m/z $(M + H)^+$ 924, $(M - H)^-$ 922; isocratic reverse phase HPLC (method F 60:40), $k' = 5.93$; TLC (silica gel), R_f(A) = 0.52, R_f(C) = 0.77.

Pmp-D-Tyr(Et)-Phe-Val-Asn-Cys-OH (20). The peptide **20** was synthesized in an analogous fashion to the Pro acid peptide

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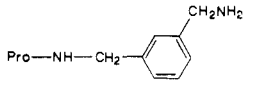
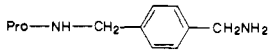
(24) Both compounds **21** and **23** compare favorably to identical compounds prepared previously by a similar method (ref 9). The ^1H NMR data of all the alkyl mono-Boc diamines show two sets of resonances between 2.5 and 3.3 ppm, one downfield of the other, which are characteristic of the two protons α to the amido group and the two protons α to the amino group, respectively.

Table II. Physical Properties of Peptide Antagonists

Pmp-D-Tyr(Et)-Phe-Val-Asn-Cys-X

compd no.	X	mol for	M_r	FAB-MS, m/z $(M + H)^+$	yield,[a,b] %	system[d] (R_f) TLC (silica gel)	isocratic RP-HPLC method,[e] K'
4	Pro-NH-$(CH_2)_2$-NH_2	$C_{47}H_{67}N_9O_9S_2$	965.45	966	42	A (0.53), C (0.66)	F, 4.28 (40)[f]
5	Pro-NH-$(CH_2)_4$-NH_2	$C_{49}H_{71}N_9O_9S_2$	993.48	994	36	C (0.60)	D, 3.58 (40)
6	Pro-NH-$(CH_2)_4$-NH-C(=NH)NH_2	$C_{50}H_{73}N_{11}O_9S_2$	1035.50	1036	64[c]	A (0.45), C (0.47)	D, 8.00 (40)
7	Pro-NH-$(CH_2)_5$-NH_2	$C_{50}H_{73}N_9O_9S_2$	1007.50	1008	30	C (0.61)	E, 4.81 (40)
8	Pro-NH-$(CH_2)_6$-NH_2	$C_{51}H_{75}N_9O_9S_2$	1021.51	1022	32	B (0.28), C (0.59)	F, 7.62 (40)
9	Pro-NH$(CH_2)_7$-NH_2	$C_{52}H_{77}N_9O_9S_2$	1035.53	1036	16	A (0.57), C (0.62)	F, 3.87 (40)
10	Pro-NH-$(CH_2)_8$-NH_2	$C_{53}H_{79}N_9O_9S_2$	1049.54	1050	12	A (0.38), C (0.53)	F, 4.66 (50)
14	NH-$(CH_2)_4$-NH_2	$C_{44}H_{64}N_8O_8S_2$	896.43	897	25	A (0.46), C (0.80)	F, 5.22 (40)
15	NH-$(CH_2)_5$-NH_2	$C_{45}H_{66}N_8O_8S_2$	910.44	911	13	A (0.48), C (0.49)	D, 7.79 (40)
16	NH-$(CH_2)_5$-NH-C(=NH)NH_2	$C_{46}H_{68}N_{10}O_8S_2$	952.47	953	24[c]	A (0.37), C (0.53)	F, 2.35 (50)
17		$C_{53}H_{71}N_9O_9S_2$	1041.48	1042	44	B (0.38), C (0.65)	F, 3.60 (45)
18		$C_{53}H_{71}N_9O_9S_2$	1041.48	1042	18	B (0.36), C (0.65)	F, 3.29 (45)

[a] All yields based on starting peptide acid and were not optimized. [b] All peptides were shown to be a single homogeneous peak by both isocratic and gradient HPLC. [c] Yield based on starting peptide **5** and **15** respectively. [d] System A, BAW, 4:1:1; system B, BAW, 4:1:5 (upper layer); system C, BAWE, 1:1:1:1. [e] Method D, 0.05 M aqueous KH_2PO_4 and acetonitrile; method E, 0.25% aqueous TFA and 0.25% TFA in acetonitrile; method F, 0.1% TFA and 0.1% TFA in acetonitrile. [f] Percentage acetonitrile.

19. Starting with 0.5 mol of Boc-Cys (4-MeBzl)-Merrifield resin, there was obtained after purification by reverse phase HPLC (5-μm ODS, 10 × 250 mm column, isocratic elution with 60:40 0.25% aqueous TFA:0.25% TFA in acetonitrile) 65 mg of pure Cys acid peptide **20**: AA analysis, Asp 1.00, Cys 0.95 (determined separately), Val 0.96, Tyr 0.98, Phe 0.94; mass spectrum (FAB), m/z $(M + H)^+$ 827, $(M - H)^-$ 825; isocratic reverse phase HPLC. (method F, 60:40): $K' = 6.38$; TLC (silica gel), R_f(A) = 0.55, R_f(C) = 0.76.

Mono-Boc-1,4-diaminobutane (21). A solution of 1,4-diaminobutane (10 mL, 99.5 mmol) in methylene chloride (70 mL) was treated with di-*tert*-butyl dicarbonate (7.24 g, 33.2 mmol) at 0 °C and the resulting solution stirred overnite at room temperature. The reaction mixture was diluted with chloroform (75 mL), washed with 5% aqueous sodium carbonate, dried over $MgSO_4$, and evaporated. The residue was dissolved in a minimum of 1 N aqueous HCl (10 mL) and washed 2 times with ether. The aqueous layer was the then made basic (pH = 10) with 2 N aqueous NaOH and extracted with ethyl acetate. The ethyl acetate extracts were dried over $MgSO_4$ and evaporated to give 821 mg of homogeneous **21**, which was used without further purification:[24] ^{1}H NMR ($CDCl_3$) δ 1.40 (br s, 15 H), 2.85–2.55 (br s, 2 H), 3.30–2.97 (m, 2 H), 5.53–5.03 (br s, 1 H); mass spectrum (CI), m/z $(M + H)^+$ 189.

Mono-Boc-1,5-diaminopentane (22). Starting with 1,5-diaminopentane (14.0 mL, 120 mmol), synthesis using the same procedure as for **21** gave 1.6 g of mono-Boc-1,5-diaminopentane (**22**): ^{1}H NMR ($CDCl_3$) δ 1.43 (br, s, 17 H), 2.83–2.53 (m, 2 H), 3.10–2.90 (m, 2 H), 5.10–4.80 (br s, 1 H); mass spectrum (CI), m/z $(M + Cl)^-$ 237.

Mono-Boc-1,6-diaminohexane (23). Starting with 1,6-diaminohexane (10.0 g, 86 mmol), synthesis using the same procedure as for **21** gave 1.49 g of mono-Boc-1,6-diaminohexane (**23**):[24] ^{1}H NMR ($CDCl_3$) δ 1.43 (br s, 17 H), 2.53–2.25 (br s, 2 H), 2.87–2.53 (m, 2 H), 3.30–2.87 (m, 2 H), 4.88–4.52 (br s, 1 H); mass spectrum (CI), m/z $(M + H)^+$ 217.

Mono-Boc-1,7-diaminoheptane (24). Starting with 1,7-diaminoheptane (13.0 g, 100 mmol), synthesis using the same procedure as for **21** gave 350 mg of mono-Boc-1,7-diaminoheptane (**24**): ^{1}H NMR ($CDCl_3$) δ 1.70–1.15 (m, 21 H), 2.80–2.48 (m, 2 H), 3.23–2.83 (m, 2 H), 5.47–5.17 (m, 1 H); mass spectrum (CI), m/z $(M + H)^+$ 231.

Mono-Boc-1,3-bis(aminomethyl)benzene (25). Starting with 1,3-bis(aminomethyl)benzene (15 mL, 114 mmol), synthesis using the same procedure as for **21** gave 1.81 g of mono-Boc-1,3-bis-(aminomethyl)benzene (**25**): ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9 H), 1.55 (br s, 2 H), 3.80 (s, 2 H), 4.27 (d, 2 H, J = 6 Hz), 5.47–5.12 (br s, 1 H), 7.20 (br s, 4 H); mass spectrum (CI), m/z $(M + H)^+$ 237.

Mono-Boc-1,4-bis(aminomethyl)benzene (26). Starting with 1,4-bis(aminomethyl)benzene (10 g, 73.4 mmol), synthesis using the same procedure as for **21** gave 1.1 g of mono-Boc-1,4-bis-(aminomethyl)benzene (**26**): ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9 H), 1.61 (s, 2 H), 3.82 (s, 2 H), 4.27 (d, 2 H, J = 6 Hz), 5.17–4.82 (br s, 1 H), 7.23 (s, 4 H); mass spectrum (CI), m/z $(M + H)^+$ 237.

Mono-Boc-1,2-diaminoethane (27). Ethanolamine (5 mL, 82.8 mmol) was dissolved in methylene chloride (200 mL) and was treated at room temperature with triethylamine (11.6 mL, 82.8 mmol) and di-*tert*-butyl dicarbonate for 18 h. The reaction mixture was then washed with saturated NaCl solution and 1 N aqueous HCl, dried over $MgSO_4$, and evaporated to give 11.6 g of 1-Boc-aminoethan-2-ol: ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9 H, 3.43–3.12 (m, 2 H), 3.88–3.50 (m, 2 H), 5.57–5.23 (br s, 1 H). The 1-Boc-aminoethan-2-ol (1.0 g, 6.2 mmol) from above in tetrahydrofuran was treated first with triphenylphosphine (1.79 g, 6.82 mmol) and diethyl azodicarboxylate (1.07 mL, 6.82 mmol) at 0 °C, followed after 5 min by 11.6 mL of 1.6 N HN_3 in toluene.[10] The reaction mixture was allowed to warm to room temperature and was stirred for 18 h. The solvent was removed under reduced pressure and the residue purified by flash chromatography (silica gel, 20% ethyl acetate/hexane) to give 550 mg of azide. The azide (200 mg, 1.08 mmol) was dissolved in ethanol (2 mL) and treated with Raney Ni (50 mg) and hydrazine monohydrate (54 μL) at room temperature for 3 h. The catalyst was removed by filtration and evaporation of the filtrate gave 50 mg of mono-Boc-1,2-Diaminoethane (**27**): ^{1}H NMR ($CDCl_3$) δ 1.47 (s, 9 H), 3.05–2.68 (br s, 2 H), 3.37–3.05 (m, 2 H), 5.31–4.95 (br s, 1 H): mass spectrum (CI), m/z $(M + H)^+$ 161.

Pmp-D-Tyr(Et)-Phe-Val-Asn-Cys-Pro-NH-$(CH_2)_4$-NH_2 (5). A solution of **19** (48.6 mg, 0.05 mmol) and mono-Boc-1,4-diaminobutane (**21**) (30 mg, 0.16 mmol) in dimethylformamide (500 μL) was treated with dicyclohexylcarbodiimide (16 mg, 0.08 mmol) and 1-hydroxybenzotriazole hydrate (11 mg, 0.08 mmol) and was stirred at room temperature for 114 h. The dimethylformamide was then removed under vacuum. The residue was treated with trifluoroacetic acid at 0 °C for 2 h. After this time, the trifluoroacetic acid was removed under reduced pressure and the residue in 1% aqueous acetic acid was passed through a BioRex 70 (H^+ form) ion exchange column. The basic products were washed off the ion exchange column with pyridine acetate buffer (water/pyridine/acetic acid, 66:30:4) and evaporated. Final purification by preparative reverse HPLC (5-μm ODS 10 × 250 mm column, isocratic elution with 60:40 0.25% aqueous TFA/0.25% TFA in acetonitrile) gave 19 mg (36%) of **5**. Peptides **4**, **7–9**, **14**, **15**, **17**, and **18** were synthesized in a similar fashion.

Peptide 10 was prepared by coupling unprotected 1,8-diaminooctane to the proline acid peptide 19 using DCC/HOBt in dimethylformamide. Each of the final peptide products were purified by preparative reverse phase HPLC on 5-μm ODS. Their physical properties are listed in Table II.

Pmp-D-Tyr(Et)-Phe-Val-Asn-Cys-Pro-NH-$(CH_2)_4$-NH-C(=NH)NH_2 (6). *O*-Methylisourea hydrogen sulfate (88 mg, 0.51 mmol) was dissolved in water (3 mL) and the pH of the solution was adjusted to 10 by using 3 N aqueous NaOH. The peptide 5 (8.3 mg, 0.0084 mmol) in water (2 mL) was added at 0 °C, the pH was again adjusted to 10, and the reaction mixture was kept at 0 °C until the reaction was shown to be complete by HPLC. The solution was then adjusted to pH 4.5 with 1% aqueous acetic acid and evaporated. The residue was purified by preparative reverse phase HPLC (5-μm ODS, 10 × 250 mm column, isocratic elution with 60:40 0.25% aqueous TFA/0.25% TFA in acetonitrile) to give 5.5 mg (64%) of pure 6. The peptide 16 was prepared in an analogous fashion from the terminal amino peptide 15.

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Registry No. 1, 80148-24-9; 2, 90332-82-4; 3, 98612-53-4; 4, 117861-32-2; 5, 99733-17-2; 6, 99733-16-1; 7, 98612-54-5; 8, 117861-33-3; 9, 99733-19-4; 10, 117861-34-4; 11, 94497-37-7; 12, 98612-56-7; 13, 117861-35-5; 14, 117861-36-6; 15, 98612-57-8; 16, 117861-37-7; 17, 108467-93-2; 18, 108467-94-3; 19, 98612-55-6; 20, 98612-58-9; 21, 68076-36-8; 22, 51644-96-3; 23, 51857-17-1; 24, 99733-18-3; 25, 108467-99-8; 26, 108468-00-4; 27, 57260-73-8; BOC-Cys(4-MeBzl)-OH, 61925-77-7; BOC-Val-OH, 13734-41-3; BOC-Phe-OH, 13734-34-4; BOC-Asn-OH, 7536-55-2; BOC-D-Tyr(Et)-OH, 76757-92-1; $H_2N(CH_2)_4NH_2$, 110-60-1; $H_2N(CH_2)_5NH_2$, 462-94-2; $H_2N(CH_2)_6NH_2$, 124-09-4; $H_2N(CH_2)_7NH_2$, 646-19-5; $H_2NCH_2C_6H_4$-*m*-CH_2NH_2, 1477-55-0; $H_2NCH_2C_6H_4$-*p*-CH_2NH_2, 539-48-0; $H_2NCH_2CH_2OH$, 141-43-5; BOC-$NHCH_2CH_2OH$, 26690-80-2; $H_2N(CH_2)_8NH_2$, 373-44-4; HN=C(OMe)-NH_2·$^1/_2H_2SO_4$, 52328-05-9; BOC-$NHCH_2CH_2N_3$, 117861-38-8.

Potential Antitumor Agents. 57. 2-Phenylquinoline-8-carboxamides as "Minimal" DNA-Intercalating Antitumor Agents with in Vivo Solid Tumor Activity

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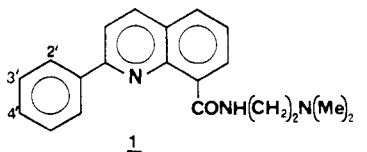
A series of phenyl-substituted derivatives of the "minimal" DNA-intercalating agent *N*-[2-(dimethylamino)ethyl]-2-phenylquinoline-8-carboxamide (1) have been synthesized and evaluated for in vivo antitumor activity, in a continuing search for active compounds of this class with the lowest possible DNA association constants. Substitution on the 2′-position of the phenyl ring gave compounds of lower DNA binding ability that did not intercalate DNA, indicating that it is necessary for the phenyl ring to be essentially coplanar with the quinoline for intercalative binding. An extensive series of 4′-substituted derivatives was evaluated, but there was no overall relationship between biological activity and substituent lipophilic or electronic properties. However, several compounds showed good solid tumor activity, with the 4′-aza derivative 18 being clearly superior to the parent compound, effecting about 50% cures in both leukemia and solid tumor models.

The evaluation of DNA-intercalating ligands as broad-spectrum agents with the ability to act against remotely sited solid tumors in vivo is an active area of anticancer drug development, and several new agents of this type are now in clinical trial.[1] Recent results from this laboratory[2-5] have shown that, while intercalative binding is an absolute requirement for antitumor activity, there may be additional advantages in compounds that interact with DNA in this fashion but have the lowest possible association constants. Such compounds, with a higher proportion of unbound drug available at equilibrium, which may permit better distribution in vivo, generally show a broader spectrum of activity than structurally related compounds with higher binding constants.[2]

An important goal of recent work in this laboratory has therefore been to delineate the minimum chromophore requirement for intercalative binding, in a search for compounds of this class with the lowest possible association constants. A systematic study[5] of the isomeric phenylquinoline-8-carboxamides showed that, while the mode of binding depended critically on the placement of the appended phenyl ring, several of the compounds did intercalate DNA and had in vivo antitumor activity. In particular, the 2-isomer *N*-[(2-dimethylamino)ethyl]-2-phenylquinoline-8-carboxamide (1) possesses excellent broad-spectrum activity, providing a high proportion of cures against the remotely sited Lewis lung carcinoma.[5]



1

In this paper we present structure–activity relationships for substitution of the phenyl ring of 1 at the three available positions with groups of varying steric, electronic, and lipophilic properties. One aim of this work was the identification of compounds with superior activity to that of the parent 1. Additionally, the hypothesis[5] that the phenyl ring has to lie coplanar with the quinoline in order for the compounds to intercalate DNA was tested by studying the mode of binding of compounds substituted at the ortho position of the phenyl ring, which cannot easily assume such a coplanar conformation.

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