Journal of Medicinal Chemistry

Volume 29, Number 12 December 1986

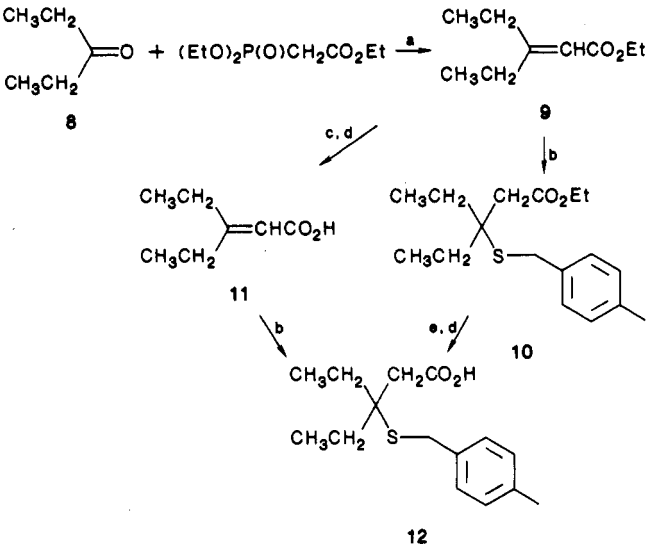
Communications to the Editor

Potent Antagonists of Vasopressin Antidiuretic Activity That Lack the β,β-Cyclopentamethylene-β-mercaptopropionic Acid Substitution at Position 1

Sir:

It was first shown by du Vigneaud[1-4] and subsequently by others[5-7] that antagonists of the neurohypophyseal hormones oxytocin and vasopressin could be obtained by substitution of a β,β-dialkyl-β-mercaptopropionic acid for the cysteine at position 1. The alkyl groups used have included methyl (dPen), ethyl (Et_2Mpr), and cyclopentamethylene (Pmp). While potent antagonists of the pressor (or V_1) activity of vasopressin have been obtained with the dPen and Et_2Mpr substitutions, these analogues have always proven to be weak agonists of the vasopressin antidiuretic (or V_2) activity in vivo.[8,9,14] The first potent in vivo V_2 antagonists, reported by Manning and Sawyer, contained the Pmp substitution.[10,11] While we have demonstrated the activity of analogues with differing ring size of the β,β-cycloalkyl moiety,[12] V_2 activity has always seemed to require such a cyclic structure at position 1.[13,14] We report here the first examples of potent vasopressin V_2 antagonists that do not possess a β,β-cycloalkyl moiety at position 1. In addition, we report a convenient and facile synthesis of S-protected β,β-dialkyl-β-mercaptopropionic acids suitable for use in peptide synthesis.

Scheme I[a]



[a] (a) 1 equiv of NaH, toluene; (b) 1 equiv of *p*-methylbenzyl mercaptan, excess piperidine, reflux; (c) 5% K_2CO_3, water–MeOH (1:1); (d) H_3PO_4; (e) 5% K_2CO_3, water–MeOH–THF (1:1:1).

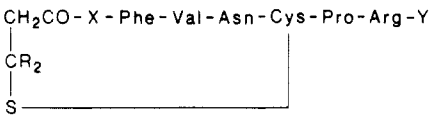
The activity of analogues synthesized is given in Table I. The binding affinity (K_{bind}) and adenylate cyclase activation (K_{act}) or inhibition (K_i) were measured in a pig renal medullary membrane preparation and represent V_2 receptor interactions.[15] The in vivo activity is measured as the dose of antagonist necessary to lower urine osmolality to 300 mOsm/kg in a hydropenic rat preparation (ED_{300}).[16] There are several important points to be mentioned. The dPen[1] analogue **1** is a partial agonist in vitro and is not a V_2 antagonist in vivo. The Et_2Mpr^1 analogues **2**, **4**, and **6** are full antagonists both in vitro and in vivo and are very similar in potency to their corresponding Pmp[1] analogues **3**, **5**, and **7**. Note also that the analogues with D-Tyr(Et) at position 2 are about 10-fold more potent than the corresponding L-Tyr(Et) analogues. Finally, note that the activity of the des-Gly^9 analogues **4** and **5** is comparable to that of the corresponding nonapeptides **2** and **3**.

Manning et al. have recently reported the activity of [Et_2Mpr^1,$Tyr(Et)^2$, Val^4]-AVP, which in their hands is a

(1) Schultz, H.; du Vigneaud, V. *J. Med. Chem.* **1966**, *9*, 647.
(2) Vavrek, R. J.; Ferger, M.F.; Ashled, A. G.; Rich, D. H.; Blomquist, A. T.; du Vigneaud, V. *J. Med. Chem.* **1972**, *15*, 123.
(3) Nestor, J. J., Jr., Ferger, M. F.; du Vigneaud, V. *J. Med. Chem.* **1975**, *18*, 284.
(4) Dyckes, D. F.; Nestor, J. J., Jr.; Ferger, M. F.; du Vigneaud, V. *J. Med. Chem.* **1974**, *17*, 250.
(5) Manning, M.; Lowbridge, J.; Stier, C. T., Jr.; Haldar, J.; Sawyer, W. H. *J. Med. Chem.* **1977**, *20*, 1228.
(6) Lowbridge, J.; Manning, M.; Haldar, J.; Sawyer, W. H. *J. Med. Chem.* **1978**, *21* 313.
(7) Bankowski, K.; Manning, M.; Seto, J.; Haldar, J.; Sawyer, W. H. *Int. J. Peptide Prot. Res.* **1980**, *16*, 382.
(8) Bankowski, K.; Manning, M.; Haldar, J.; Sawyer, W. H. *J. Med. Chem.* **1978**, *21*, 850.
(9) Manning, M.; Lammek, B.; Kruszynski, M.; Seto, J.; Sawyer, W. H. *J. Med. Chem.* **1982**, *25*, 408.
(10) Manning, M.; Lammek, B.; Kolodziejczyk, A.; Seto, J.; Sawyer, W. H. *J. Med. Chem.* **1981**, *24*, 701.
(11) Sawyer, W. H.; Pang, P. K. T.; Sent, J.; McEnroe, M.; Lammek, B.; Manning, M. *Science (Washington, D.C.)* **1981**, *212*, 49.
(12) Yim, N. C. F.; Ali, F. E.; Bryan, W. M.; Huffman, W. F.; Moore, M.; Silvestri, J.; Erickson, R.; Heckman, G.; Kinter, L.; McDonald, J.; Schmidt, D.; Shue, D.; Stassen, F.; Stefankiewicz, J.; Sulat, L. *Peptides, Structure and Function*, Proceedings of Ninth American Peptide Symposium; Deber, C. M., Hruby, V. J., Kopple, K. D., Eds.; Pierce Chemical Co.: Rockford, IL, 1985; pp 615–618.
(13) Manning, M.; Sawyer, W. H. *Prog. Brain Res.*, **1983**, *60*, 367.
(14) Manning, M.; Lammek, B.; Bankowski, K.; Seto, J.; Sawyer, W. H. *J. Med. Chem.* **1985**, *28*, 1485.
(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.

0022-2623/86/1829-2425$01.50/0

Table I. Biological Activity of Vasopressin Antagonist Analogues Modified at Position 1



compd	R	X	Y	K_{bind},[a] nM	K_i,[b] nM	K_{act}[c] nM	ED_{300},[d] (μg/kg)
1	CH_3	D-Tyr(Et)	Gly-NH_2	39 (2)	[e]	79	na
2	CH_2CH_3	D-Tyr(Et)	Gly-NH_2	35	5.3 (2)		23 (3)
3	$(CH_2)_5$	D-Tyr(Et)	Gly-NH_2	12 (9)	6.4 (9)		11 (7)
4	CH_2CH_3	D-Tyr(Et)	NH_2	30	8.9		12 (3)
5	$(CH_2)_5$	D-Tyr(Et)	NH_2	12 (9)	3.9 (8)		10 (26)
6	CH_2CH_3	Tyr(Et)	NH_2	450 (2)	nd		120
7	$(CH_2)_5$	Tyr(Et)	NH_2	280	nd		300

[a] Binding affinity measured by competition with tritiated LVP; each determination represents the average of triplicate assays; the number in parentheses is the number of determinations. [b] Inhibition of LVP-sensitive adenylate cyclase; each determination represents the average of triplicate assays; the number in parentheses is the number of determinations. [c] Activation of adenylate cyclase; each determination represents the average of triplicate assays. [d] Dose to lower urine osmolality to 300 mOsm/kg; each determination is the average for four rats; the number in parentheses is the number of determinations. [e] Partial agonist with a relative V_{max} of 14%.

potent V_1 antagonist but completely devoid of V_2 antagonist activity in vivo.[14] In fact, they report it to be a weak V_2 agonist. Our compound **6**, which differs only in being the des-Gly[9] analogue, is a fairly potent V_2 antagonist in vivo. It is unlikely that this difference could be due to the des-Gly modification. [Pmp[1],Tyr(Et)[2],Val[4]]-AVP exhibits a K_{bind} of 87 nM and an ED_{300} of 58 μg/kg, only a factor of 3 better than compound **7**, implying that the des-Gly modification is well-tolerated in analogues with L-Tyr(Et) at position 2. In addition, the fact that the activities of the Et_2Mpr analogues **2** and **4** are essentially the same as that of the corresponding Pmp analogues **3** and **5** suggests that the structure–activity relationship with respect to the des-Gly modification is the same in both the Pmp and Et_2Mpr series of analogues. We can only surmise that this discrepancy is due to differences in the bioassay for antagonists. We have previously noted that the rank order of antagonist potency we generate differs from the one generated by Manning and Sawyer.[16]

The synthesis of the protected β,β-diethyl-β-mercaptopropionic acid is shown in Scheme I. The diethylacrylate **9** is conveniently prepared from the corresponding ketone **8** by using the Wadsworth–Emmons reaction.[17] Michael addition of *p*-methylbenzyl mercaptan to **9** yields the S-protected mercaptopropionic acid **10**. We have shown previously in the synthesis of Pmp that using a catalytic amount of sodium hydride as base greatly improves the yield of the Michael adduct.[18] In this case, however, use of such a strong base significantly increases rearrangement of the double bond of **9** to give the unreactive β,γ-isomer. The reaction proceeds cleanly, however, with use of weaker base piperidine.[2] Saponification of the Michael adduct **10** yields the desired free acid **12**. This is accompanied by a significant amount of retro-Michael elimination of *p*-methylbenzyl mercaptan to give the diethylacrylic acid **11** as a contaminant. While the retro-Michael reaction can be suppressed to a degree by using 5% potassium carbonate in a 1:1:1 methanol–tetrahydrofuran–water mixture, it cannot be eliminated entirely. In order to circumvent this problem, we investigated the possibility of performing the Michael addition directly on the acrylic acid.[1] The diethylacrylic acid **11** is easily prepared from **9** by saponification. It cleanly undergoes Michael addition with *p*-methylbenzyl mercaptan with use of piperidine as the base, as before, to yield the desired S-protected mercaptopropionic acid **12**. We have used this method to prepared a number of mono- and disubstituted β-mercaptopropionic acids and find it to be a convenient and generally applicable procedure.

Registry No. **1**, 104532-36-7; **2**, 104532-37-8; **3**, 80148-24-9; **4**, 104532-38-9; **5**, 90332-82-4; **6**, 104532-39-0; **7**, 90332-81-3; **8**, 96-22-0; **9**, 15249-93-1; **11**, 79930-59-9; **12**, 104532-41-4; Me_2CO, 67-64-1; $Me_2C{=}CHCO_2Et$, 638-10-8; $Me_2C{=}CHCO_2H$, 541-47-9; *p*-$Me_2C(CH_2CO_2H)SCH_2C_6H_4Me$, 104532-40-3; $(EtO)_2P(O)CH_2CO_2Et$, 867-13-0; cyclohexanone, 108-94-1; ethyl cyclohexylideneacetate, 1552-92-7; cyclohexylideneacetic acid, 1552-91-6; 1-[*p*-tolylmethylthio]cyclohexaneacetic acid, 87242-91-9; adenylate cyclase, 9012-42-4.

Nelson C. F. Yim, Michael L. Moore*
William F. Huffman, Heidi G. Bryan, Hsin-Lan Chang
Department of Peptide Chemistry

Lewis B. Kinter, Richard Edwards, Frans L. Stassen
Dulcie Schmidt, Grace Heckman
Departments of Pharmacology and Molecular Pharmacology
Smith Kline & French Laboratories
Swedeland, Pennsylvania 19479
Received May 27, 1986

(17) Wadsorth, W. S., Jr.; Emmons, W. D. *J. Am. Chem. Soc.* **1961**, *83*, 1733. *Organic Syntheses*; Baumgarten, H., Ed.; Wiley: New York, 1973; Collect. Vol. V, pp 547–549.
(18) Yim, N. C. F.; Huffman, W. F. *Int. J. Peptide Prot. Res.* **1983**, *21*, 568.