Bioorganic & Medicinal Chemistry Letters, Vol.3, No.2, pp. 337-340, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00

POTENT, NON-PEPTIDIC OXYTOCIN RECEPTOR ANTAGONISTS FROM A NATURAL SOURCE

Gino M. Salituro[a]*, Douglas J. Pettibone[b], Bradley V. Clineschmidt[b], Joanne M. Williamson[c], and Deborah L. Zink[a]

Departments of Natural Products Chemistry[a] and Membrane Biochemistry & Biophysics[c]
Merck Research Laboratories, P.O. Box 2000, Rahway, NJ, 07065

and

Department of New Lead Pharmacology[b]
Merck Research Laboratories, Sumneytown Pike, West Point, PA, 19486

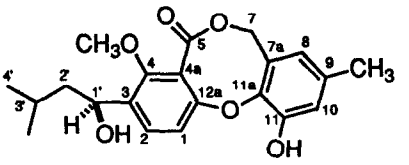
(*Received in USA* 29 *October* 1992)

Abstract: Penicillide, a previously described natural product, and several related compounds have been discovered to be antagonists of the peptide hormone oxytocin. A few simple derivatives of the compound were isolated and/or synthesized and its absolute stereochemistry was determined.

Due to the generically poor pharmacokinetic properties of peptides, considerable effort has been allocated to the discovery of non-peptide ligands for peptide receptors.[1] The biochemical screening of natural products represents a propitious potential source of peptide mimics; the CCK antagonist asperlicin representing the most significant success from this field thus far.[2]

The peptide hormone oxytocin plays a pivotal role in the initiation of labor and selective antagonists of oxytocin have potential as treatment for preterm labor.[3] The antagonists of oxytocin described to date include mainly peptidic analogs of oxytocin or the structurally related hormone arginine vasopressin and a class of cyclic hexapeptides isolated from *Streptomyces silvensis*.[4] Recently a class of spiroindene oxytocin antagonists were disclosed.[5] This report describes a set of non-peptidic oxytocin antagonists based on the natural product penicillide.

During the course of our screening program for oxytocin antagonists, fermentation extracts from *Taloromyces flavus* (ATCC 74110) were discovered to give specific, dose-dependent inhibition of the binding of oxytocin to its receptor. Bioassay[4] guided fractionation led to the isolation of two active compounds as well as two structurally related but biologically inactive compounds. The major active component, penicillide (**1**), was purified from a methanol extract of the fermentation by sequential extraction followed by silica gel chromatography and ultimately RP-18 HPLC. The minor active compound (**2**) and the two inactive components (**3**) and (**4**) were isolated in small quantities from the silica gel side cuts by RP-18 HPLC.



1

Compound (1) was shown to be the known fungal metabolite penicillide by comparison of its UV, IR, MS, ^{1}H NMR, and ^{13}C NMR spectra to those reported previously.[6,7] Similarly, metabolite (2) was readily identified spectroscopically as a monoacetylated derivative equivalent to purpactin A[7] which was previously described as an inhibitor of acyl-CoA cholesterol acyltransferase. Aldehyde (3) was established to be identical to purpactin C'[7] by the same criteria. An oxidized metabolite, ketone (4), had been previously described as a product from the Jones oxidation of penicillide[6] but had not been fully characterized. The structure of 4 was supported by ^{1}H and ^{13}C NMR as well as HREI-MS and IR spectra.

The absolute stereochemistry of penicillide was established by employing Trost's O-methylmandelate methodology.[8] The (*S*)- and (*R*)-O-methylmandelate esters of 5 were prepared using the acyl chlorides of (*S*)- and (*R*)-O-methylmandelic acid and their ^{1}H NMR spectra were compared to that for acetate (7). The chemical shifts of the relevant protons are shown in Table 1. The aromatic protons of the (*S*)-ester were shifted upfield relative to (7), while protons H_c, H_d, and H_e were shifted upfield in the (*R*)-ester relative to 7. As demonstrated by Figure 1, these results are consistent only with the (*S*) stereochemistry for the natural product since in that case the effected protons are shifted upfield due to the anisotropic shielding effect induced by the eclipsing mandelate phenyl group.

Figure 1. Extended Newman projections for the (*S*)- and (*R*)-O-methylmandelate esters of (*S*)-penicillide monomethyl ether (5).

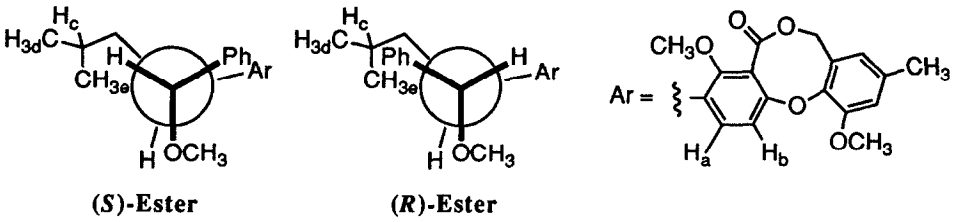
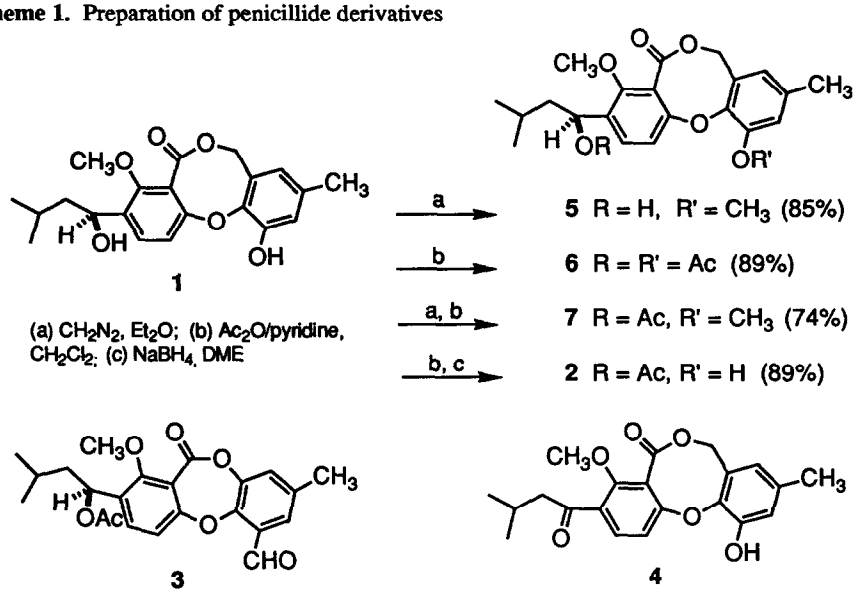


Table 1. Proton chemical shifts (δ) of the acetate (7), (*S*)-ester, and (*R*)-ester of 5.

Proton	7	(*S*)-ester	(*R*)-ester
H_a	7.42	**6.78**	7.40
H_b	6.95	**6.61**	6.89
H_c	1.63	1.59	**1.26**
H_d, H_e	0.94, 0.94	0.91, 0.92	**0.73, 0.76**

As indicated in Table 2, the acetylated natural component (2) was approximately 10 fold more potent than the parent compound while the oxidized metabolite (4) had little or no *in vitro* activity. In order to obtain larger quantities of the more active compound and to further investigate the emerging SAR of these antagonists several simple derivatives were prepared, Scheme 1. The major component, penicillide, (1) was readily converted to the more active derivative (2) by forming the diacetate (6) followed by selective, reductive hydrolysis of the phenolic acetate with sodium borohydride in dimethoxyethane.[9] The monomethyl ether (5) was prepared by diazomethane treatment of penicillide and could easily be acetylated under standard conditions to provide 7.

Scheme 1. Preparation of penicillide derivatives



All of these compounds were tested for their ability to inhibit the binding of oxytocin to its receptor in rat uterine tissue.[4] Clearly acetylation of the secondary hydroxyl group produces the most notable impact on activity, improving potency 5 - 10 fold. However oxidation of the C-1' position virtually eliminates all binding activity. Blocking of the phenolic hydroxyl by forming an acetate has little effect on potency whereas methylation at this site has a slight deleterious effect on potency.

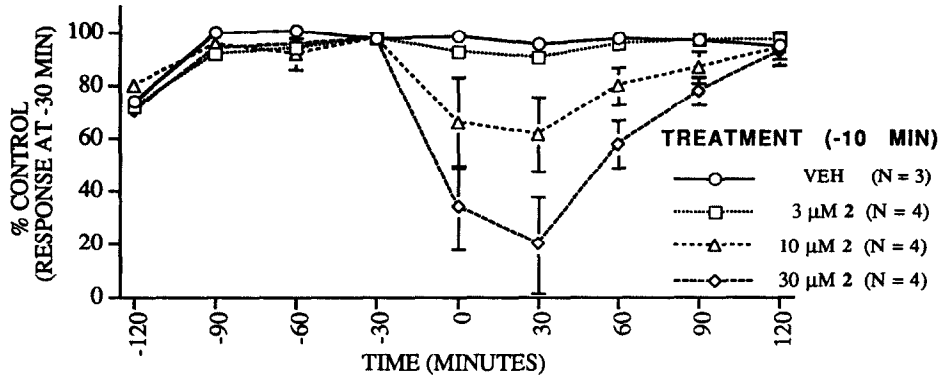
Table 2. Oxytocin (OT) and arginine vasopressin (AVP) binding inhibition by penicillide derivatives

Compound	IC_{50} OT (μM)	IC_{50} AVP-V_1 (μM)
1*	67	--
2*	8.4	>100
3*	>100	--
4*	>100	--
5**	>100	--
6**	5.0	--
7**	21	>100

* Natural products, ** Semisynthetic derivatives
Standard errors are ± 10 - 30% of the mean.

Penicillide monoacetate (**2**) acts as a μM inhibitor of oxytocin binding and shows at least 10 fold selectivity with respect to the arginine vasopressin receptor V_1 subtype (Table 2). This compound was also shown (Figure 2) to inhibit the contractile response of the isolated rat uterus to repeated exposures of 10nM oxytocin *in vitro* [4] with a potency predicted from the IC_{50} in the binding assay. Significantly lesser antagonism was observed in the same system to bradykinin and $PGF_{2\alpha}$ stimulated contractions.[10] The monoacetate therefore is acting as a relatively specific antagonist of oxytocin activity.

Figure 2. Effect of 2 on the contraction of isolated rat uterus induced at 30 min intervals by repeated challenges with oxytocin (10nM)



These compounds represent one of very few examples of non-peptidyl antagonists of peptide hormone activity from microbial sources and validates the usefulness of natural product screening as a provenience for new medicinal lead compounds. Efforts continue to further define the activity of this class of compounds and to discover new natural product regulators of receptor signalling.

References and Notes

1. Morgan, B. A.; Gainor, J. A. *Annual Reports in Medicinal Chemistry*; Allen R. C., Ed.; Academic Press: San Diego, 1989; Vol 24, p. 243.

2. Goetz, M. A.; Lopez, M.; Monaghan, R. L.; Chang, R. S. L.; Lotti, V. J.; Chen, T. B. *J. Antibiot.* **1985**, *38*, 1634.

3. Pritchard, J. A.; MacDonald, P. C.; Gant, N. F. *Williams Obstetrics*, 17th ed.; Appleton-Century-Crofts: Norwalk, 1985; p. 295.

4. Pettibone, D. J.; Clineschmidt, B. V.; Anderson, P. S.; Freidinger, R. M.; Lundell, G. F.; Koupal, L. P.; Schwartz, C. D.; Williamson, J. M.; Goetz, M. A.; Hensens, O. D.; Liesch, J. M.; Springer, J. P. *Endocrinology*, **1989**, *125*, 217.

5. Evans, B. E.; Leighton, J. L.; Rittle, K. E.;Gilbert, K. F.; Lundell, G. F.; Gould, N. P.; Hobbs, D. W.; Dipardo, R. M.; Veber, D. F.; Pettibone, D. J.; Clineschmidt, B. V.; Anderson P. S.; Freidinger, R. M. *J. Med. Chem.* in press.

6. Sassa, T.; Niwa, G.; Unno, H.; Ikeda, M.; Miura, Y. *Tetrahedron Lett.* **1974**, 3941.

7. Nishida, H.; Tomoda, H.; Cao, J.; Okuda, S.; Omura, S. *J. Antibiot.* **1991**, *44*, 144.

8. Trost, B. M.; Belletire, J. L.; Godleski, S.; McDougal, P. G.; Balkovec, J. M.; Baldwin, J. J.; Christy, M. E.; Ponticello, G. S.; Varga, S. L.; Springer, J. P. *J. Org. Chem.* **1986**, *51*, 2370.

9. Quick, J.; Crelling, J. K. *J. Org. Chem.* **1978**, *43*, 155.

10. Clineschmidt, B. V.; unpublished results