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Hybrid peptide-small molecule oxytocin analogs are potent and selective agonists of the oxytocin receptor

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ABSTRACT

Oxytocin (OT) is a peptide hormone agonist of the oxytocin receptor (OTR) that has been proposed as a therapeutic to treat a number of social and emotional disorders in addition to its current clinical use to induce labor and treat postpartum bleeding. OT is administered intravenously and intranasally rather than orally, in part because its low passive permeability causes low oral bioavailability. Non-peptidic OTR agonists have also been reported, but none with the exquisite potency of the peptide based agonists. In this report, we describe the OTR agonist activity and exposed polarity of a set of truncated OT analogs as well as hybrid peptide-small molecule analogs of OT. Examples of both truncated analogs and peptide-small molecule hybrid analogs are potent and selective OTR agonists. Hybrid agonist **13**, which is 232 Da smaller than OT, still retains subnanomolar potency, full agonist activity, and selectivity over V1a. While these compounds were designed to address the low permeability of OT and other full length analogs, we found that reduction in molecular weight and the removal or replacement of the three amino acid tail of OT did not have a significant effect on passive permeability.

Oxytocin (OT) **1** modulates social and emotional behaviors and has thus been proposed as a therapeutic for schizophrenia,[1] post-traumatic stress disorder (PTSD),[2] and autism spectrum disorder (ASD).[3,4] OT is an agonist of the oxytocin receptor (OTR), and while it is routinely administered to patients intravenously to induce labor (Pitocin), its therapeutic potential is limited by its lack of receptor-selectivity. OT is also a potent agonist of the vasopressin family of receptors, which gives it the potential for deleterious side effects, for example cardiovascular issues associated with V_{1a} agonism.[5] Few clinically tested synthetic variants of the natural hormone have been generated (see Fig. 1); carbetocin (carba-1-[Tyr(Me)^2]dOT, **2**) has been approved in several countries for the treatment of postpartum bleeding,[6,7] demoxytocin (1-(3-mercaptopropanoic acid)OT or [Mpa^1]OT, **3**) is marketed in some European countries for induction of labor and lactation support,[8] and FE 202,767 (carba-1-[4-FBzlGly^7]dOT, **4**) is in clinical development for the treatment of preterm mothers requiring lactation support.[9] Because of poor oral bioavailability due in part to the low passive permeability of peptidic chemical matter, these analogs of OT are administered either intravenously or intranasally. Oral delivery of OT analogs is hindered by their size, the number of hydrogen bond donors (HBDs), and the number of polar side chains, all of which contribute to poor passive permeability across biological membranes. There are reports of non-peptidic OTR agonists, such as WAY-267,464 (**5**) and Ferring compound **6**[10,11]; while these compounds are not peptides, they have high molecular weight and are also not permeable or orally bioavailable. Nonetheless, these types of non-peptidic OTR agonists have shown efficacy in mouse *in vivo* anxiety models and in a rat model of uterine contraction.

OT is a cyclic peptide with a six amino acid macrocycle and three amino acid tail. There have been reports of cyclic hexapeptides with moderate levels of passive membrane permeability and measureable bioavailability, due to extensive intramolecular hydrogen bonding and lack of polar side chains.[12–15] OT is also a cyclic hexapeptide with an intramolecular hydrogen bonding network[16–18]; however, OT contains several polar side chains and a three amino acid tail, both of which likely contribute to its poor permeability. In seeking to understand the importance of the three amino acid tail to both the passive permeability and the OT agonist effects at the OTR, we synthesized a set of truncated OT analogs, and measured their agonist effects at OTR and V_{1a}. We also synthesized a set of peptide hybrids, which replace the peptidic tail portion of OT with non-peptidic moieties selected through overlays of OT and **6**. For both data sets we determined EPSA, a chromatographic measure of the exposed polarity of a compound that has been correlated to levels of passive permeability of peptides.[19]

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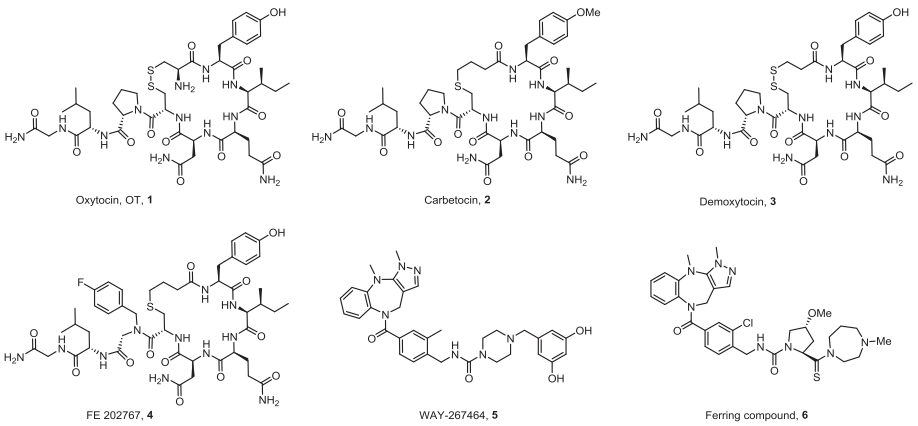


Fig. 1. Structures of peptidic and non-peptidic OTR agonists.

Human liver microsomal (HLM) stability data was collected to monitor the effect of different tail substitutions on metabolic stability.

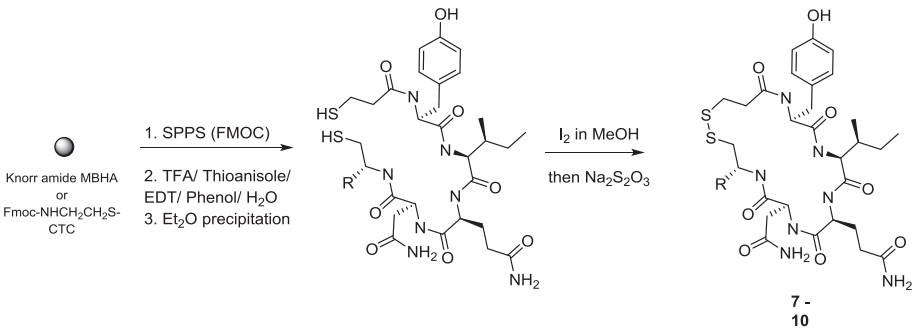
We used EPSA as an indirect chromatographic method of measuring exposed polarity as a function of retention time to estimate whether the truncated OT analogs have improved permeability as it has been suggested. The lower the measured EPSA, the less polarity is exposed in the compound, and therefore the more likely the compound is to be permeable. Goetz et al. studied the correlation between EPSA and passive cell permeability using RRCK (Ralph Russ canine kidney cell assay)[20] on a set of 814 cyclic peptides and found that a compound with an EPSA value below 80 has a much greater chance of being permeable, as measured by RRCK $>1 \times 10^{-6}$ cm/s (72%), than a compound with a higher EPSA.[21]

Truncated OT analogs are synthesized starting with Knorr amide 4-methylbenzhydrylamine (MBHA) resin or Fmoc-NH_2NH_2-S-chlorotrityl chloride (CTC) resin and building the linear peptide using standard FMOC solid phase peptide synthesis (SPPS) methods. The linear peptides are cleaved from the resin and deprotected with a mixture of TFA, thioanisole, EDT, phenol and water and then are precipitated from ether. After acidification, the peptide is then cyclized by addition of iodide in methanol, quenched with sodium thiosulfate, then purified by reverse phase HPLC purification (see Scheme 1).

It has been demonstrated in the literature that demoxytocin **3**, the OT analog which lacks the *N*-terminal amine, is equipotent to OT[24,25] but with a lower overall polarity as measured by EPSA.[22] Therefore we made the truncated analogs **7** through **10** in Table 1 without the *N*-terminal amine. The structures of these compounds along with their OTR agonist activity and percent agonist effect, V_{1a} agonist activity, EPSA and HLM are reported in Table 1. Removal of just the C-terminal Gly[9] resulted in a drop in both EC_{50} and agonist effect at OTR, but compound **7** is still low single digit nanomolar (1.8 nM) and a high partial agonist (82%). Moreover, **7** has no measured agonist effect at the V_{1a} receptor, suggesting that the Gly[9] makes an important contribution to V_{1a} activity. This is in agreement with previously reported data for the related 9-decarboxyamido OT, which has also been shown to have no effect at the V_{1a} receptor.[26] Indeed, all compounds synthesized for this report had a similar lack of agonist activity at the V_{1a} receptor. Compound **8**, the Gly[9]Leu[8] truncation, has comparable potency, activity, and selectivity to compound **7**. Some erosion of potency is seen with the truncation of the entire three amino acid tail Gly[9]-Leu[8]Pro[7], but compound **9** only loses twofold activity when compared to **7** and **8**. Removal of the entire tail including the C-terminal portion of Cys[6] (**10**) results in a significant loss in potency and percent effect at the OTR receptor, suggesting that some substitution in that position is necessary for low nanomolar activity.

In general, reduction in molecular weight and number of HBDs should improve the permeability in a set of compounds. Compared to demoxytocin **3**, fully truncated analog **10** has a lower molecular weight by 310 Da and six fewer HBDs, but it has only a few units lower EPSA measurement than the fully elaborated **3**. The EPSA of compound **10** is the lowest measured in the truncated set, but given the difference in molecular weight and HBDs between **3** and **10**, there is surprisingly little difference in their apparent polarity.

The measured activity of the truncated analogs **7** through **10** shows that the three amino acid OT tail is not necessary for OTR agonist activity. Examination of the structure of OT and the non-peptidic OTR agonist **6** shows some structural similarities between the OT tail and the tail portion of **6** (Fig. 2a). Molecular overlay of the OT tail and the proline containing portion of the Ferring compound shows that alignment is possible (Fig. 2b), suggesting the



Scheme 1. Synthesis of truncated OT analogs **7**–**10** through FMOC SPPS. Peptides were synthesized at Chinese Peptide Company (Hangzhou, China).