Over One Hundred Peptide-Activated G Protein-Coupled Receptors Recognize Ligands with Turn Structure

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1. GPCRs: Importance and Occurrence

G protein-coupled receptors (GPCRs) are seven transmembrane helical bundle proteins (Figure 1) found on the surface of all cells.[1−3] They mediate cellular responses to a diverse range of extracellular stimuli, including both endogenous chemical signals and exogenous environmental agents (e.g. light, amino acids, peptides, proteins, small organic molecules such as amines and lipids, nucleosides, nucleotides, metal ions, and pharmaceuticals). Once activated by an extracellular signal, GPCRs activate heterotrimeric G proteins that interact promiscuously with multiple receptors including guanine nucleotides GTP and GDP, and with the GPCR itself at the

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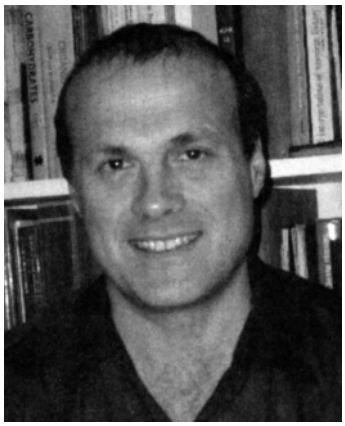
Photograph and biography for Joel Tyndall can be found on p 974.

Dr. Bernhard Pfeiffer obtained his diploma in chemistry from the University of Basel in 1997 and received his Ph.D. in 2002 from the University of Zurich under the guidance of Prof. J. A. Robinson, working in the field of new protein epitope mimetics. He then spent a year as a research fellow at the Institute of Molecular Bioscience at the University of Queensland under the supervision of Prof. D. Fairlie before taking up his current position in late 2004 as a postdoctoral fellow in the group of Prof. K.-H. Altmann at the Institute of Pharmaceutical Science of the Federal Institute of Technology Zurich (ETH). E-mail: bernhard.pfeiffer@pharma.ethz.ch.



Dr. Giovanni Abbenante received his B.Sc. (Hons) degree from the Department of Chemistry at the University of Adelaide and his Ph.D. degree on the synthesis of $GABA_b$ receptor antagonists from Flinders University in 1992. He undertook postdoctoral studies on the synthesis of ladderanes at the Central Queensland University and on HIV-1 protease inhibitors at the University of Queensland. He is currently a Senior Research Officer in the Institute for Molecular Bioscience with interests in drug design and in the synthesis of protease inhibitors, macrocycles, receptor antagonists, and peptidomimetics. E-mail: j.abbenante@imb.uq.edu.au.

Photograph and biography for David P. Fairlie can be found on p 974.

large third cytoplasmic loop. GPCR activation thereby triggers intracellular signal transduction cascades via numerous intracellular messengers, producing cellular changes that characterize or initiate physiological processes.

Sequencing of the human genome has so far revealed between 700 and 1000 human genes that encode G protein-coupled receptors and, although many more may be added to this list, this is already

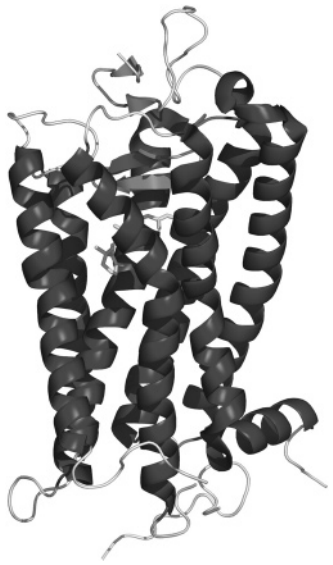


Figure 1. G protein-coupled receptor (GPCR) from bovine rhodopsin.

the largest group of membrane-spanning surface receptors on human cells.[4−6] Around 60% of known GPCRs are thought to be olfactory or sensory receptors associated with smell, taste, vision, etc, leaving at least 300−400 GPCRs that are nonsensory in function, including ~175 "orphan" receptors for which an endogenous ligand is still to be identified.[7−9] Almost half of all registered pharmaceuticals today exert therapeutic effects by binding to GPCRs,[10] yet they target only ~30 GPCRs and only a few of those are peptide-activated GPCRs.

2. GPCRs: Classification

GPCRs generally consist of a single polypeptide chain of 400−3000 residues that form a variable extracellular N-terminus (7−3000 amino acids), seven transmembrane α-helices (20−27 residues), an intracellular C-terminus (12−400 amino acids), three endo- and three exo-loops (5−250 amino acids). Except for the highly conserved 7TM helical region, all these domains vary considerably between GPCRs, indicative of their diverse structures and functions. There is a small positive correlation between the length of the N-terminus of a GPCR and the size of its extracellular ligand,[11] consistent with the N-terminus being the principal binding site for most large polypeptides and glycoprotein hormones. Notable exceptions are the 500−600 residue N-terminal segments of neurotransmitter receptors, which bind small ligands (e.g. Ca^{2+}, mGluR, GABA).

Historically, GPCRs have been grouped into three classes, but a number of receptors have since been found to lie outside these classes. "Class A" is the largest group of GPCRs, most of which have short N-terminal domains and highly conserved transmembrane regions. These GPCRs are structurally related to rhodopsin or adrenergic receptors, and bind to many amine, purine, and peptide ligands. As many as 900 olfactory receptors may belong to this class. Some members that have long N-terminal domains tend to bind to glycoprotein hormones (e.g. LHR, FSHR, TSHR). Most current pharmaceuticals that

target GPCRs regulate the biogenic monoamine-stimulated neurotransmitter-binding receptors.[12,13]

"Class B" is the secretin-like receptors that tend to have six conserved cysteines and a hormone-binding domain in their long N-terminus. These receptors bind large peptide ligands such as glucagon, corticotropin releasing hormone, parathyroid hormone, vasoactive intestinal peptide (VIP), growth hormone releasing hormone, calcitonin, gastric inhibitory polypeptide, and adenylate cyclase activating polypeptide receptor (PACAP). Recently a group of over 30 human GPCRs of this class have been reported with long N-termini (of up to 2000−3000 residues) forming mucin-like stalks with many Ser/Thr glycosylation sites.[14]

"Class C" are the neurotransmitter receptors with long N-terminal tails (500−600 residues), comprising a separately folded ligand-binding domain, and no trans-membrane homology with other GPCR families.[15] This family includes the Ca^{2+} receptor, GABA receptors, and metabotropic glutamate receptors (mGluRs). There are at least 8 mGluRs, all binding the neurotransmitter Glu in the long extracellular N-terminal domain via a "Venus-flytrap" mechanism, involving ligand capture by an open, functionally inactive, form of the receptor which, upon closing, becomes functionally active and leads to signal transduction.

In addition to these rhodopsin-like, glutamate-like, and secretin-like classes of GPCRs, there are at least two other families, the adhesion- and frizzled/TAS2-GPCRs which do not fit into the above classes and are described in more detail elsewhere.[5,16]

Whereas most current pharmaceuticals bind within the hydrophobic transmembrane regions of GPCRs, most extracellular protein/peptide ligands are thought to bind mainly to the extracellular loops or N-terminal domain of GPCRs. Since almost all of the peptide/protein- binding GPCRs are implicated in disease pathology, there is a need to better understand these protein−protein interactions if such GPCRs are to be more effectively targeted by new pharmaceuticals. The pharmaceutical industry has taken a long time to pursue these protein-binding GPCRs, in part because of a perception that such protein−protein interactions are complex and involve large surface areas that need to be competed for, or mimicked by, molecules that would need to be much larger than traditional pharmaceuticals. The availability of more detailed information on protein−protein interactions has led to the realization that small molecule agonists/antagonist can indeed be effectively designed to bind to peptide-activated GPCRs.

3. GPCR-Binding Peptide Ligand Structures and Activities

Because only a three-dimensional structure of one GPCR (light-activated bovine rhodopsin) is currently known,[17] and only for its inactive state, the development of pharmaceuticals that can regulate this important class of regulatory proteins is still heavily reliant on ligand-based drug design. While much is already being accomplished with "privileged" structures as scaffolds for building GPCR ligands,[2,18−20] it is still often not much more than a random process that could benefit substantially from more information on requirements for ligand binding to GPCRs. We therefore set out in this article to gather structural evidence on the preferred conformations of known GPCR-binding proteins and peptides. We have inspected ~120 GPCRs for which there are known peptide/protein ligands, and herein summarize available information that supports a common (perhaps universal) pattern of shape recognition by this important class of receptors. We suggest that a general "turn" motif is adopted by these ligands in solution and is likely associated with binding to, and mediating bioactivity through, GPCRs.

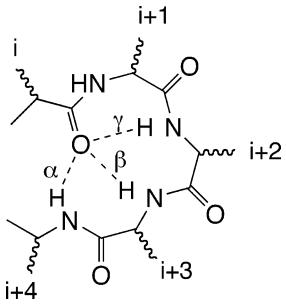


Figure 2. Distinction between general α-, β-, and γ-turns.

A peptide turn may be defined (Figure 2) by 3 residues (γ-turn), 4 residues (β-turn), 5 residues (α-turn) that can respectively form 7-, 10- and 13-membered hydrogen bonded rings. When linked consecutively, multiple β-turns are frequently described as 3_{10}-helices, multiple α-turns define α-helices. Recognition of a turn conformation normally only involves interactions between side chain residues of the ligand with the receptor, and thus the peptide turn can be considered to be a scaffold, which could potentially be either conformationally constrained or entirely replaced by an alternative rigid non-peptidic scaffold designed to support moieties that may mimic peptide side chains.

Four main lines of evidence are presented herein for recognition by GPCRs of turn conformations in peptide/protein ligands. Although structures of the GPCRs and their ligand complexes are not yet available, structures have been determined for some ligands unbound to the receptor and many such molecules do show a preference for a turn structure in solution for the bioactive region of the ligand sequence. Second, in some cases small synthetic peptides that display turn structures are agonists or antagonists of individual GPCRs, the induction of structure often correlating with ligand affinity or agonist/antagonist potency. A third line of evidence originates from the high affinity/potency of cyclic peptide ligands, which stabilize turn conformations, supporting the likely involvement of such conformations in recognition of native ligands. Finally, a large number of synthetic non-peptidic and peptidomimetic ligands have been developed as GPCR agonists or antagonists. In many cases they are mimetics of peptide turns though have not always been reported as such. They are more extensively reviewed elsewhere,[21−32] and only a few examples are shown here

in support of the importance of ligand turn conformation for binding to specific examples of GPCRs.

The information on ligand turn structures assembled in this article may help in the generic design of GPCR-binding scaffolds and ligands, encourage proteomics approaches to the further characterization of post-translational GPCR ligand and receptor modifications (e.g. glycosylation, phosphorylation, acetylation, sulfation) that cannot be detected through gene expression analyses, and catalyze further development of clinically useful agonists and antagonists for GPCRs, both those receptors described herein and those which remain to be discovered.

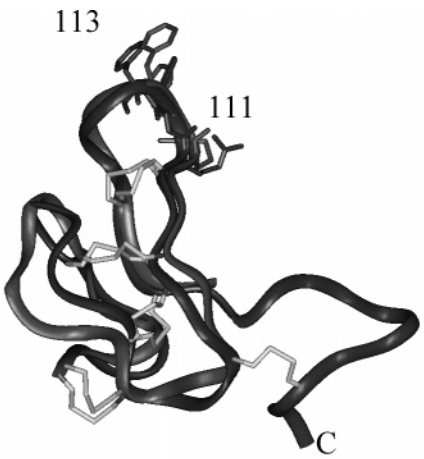
4. Mammalian GPCR-Binding Peptide Hormones

4.1. Adrenomedullin

Adrenomedullin[33] (AM, Accession No. P35318) is a hypertensive 52 residue, disulfide bridged (16−21) peptide YRQSMNNFQGLR$_{12}$SFGC$_{16}$RFGTC$_{21}$TVQKLAHQIYQFTDKDKDNVAPRSKISPQGY-NH$_2$. It is a member of a family of peptide hormones that includes calcitonin, α- and β-calcitonin gene related peptide, and amylin. Adrenomedullin is widely distributed in humans and possesses a remarkable array of actions, including regulating cellular growth and differentiation, modulation of hormone secretion, antimicrobial activity,[34] and potent vasodilatory and hypertensive effects.[35,36] Like its counterparts, AM receptors (AMR) are made up of a GPCR calcitonin receptor like receptor (CRLR) and a receptor-activity-modifying protein (RAMP). The combination of CRLR and RAMP 2 or 3 which reconstitutes AM receptors to show the pharmacological characteristics of the native receptors is reviewed elsewhere.[37] It has been shown that the disulfide bridged cyclic hexapeptide turn component and the C-terminal amide are necessary for biological activity, while removal of the N-terminal 12 residues does not reduce potency.[38] One study also identified the shorter bovine AM(11−26) as a biologically active ring-constrained endogenous peptide.[38] No structural studies have been published yet but, based on homology with its neighbors, it is believed to contain a helical region beyond the cyclic portion of the peptide in the C-terminal region.

4.2. Agouti Protein and Agouti-Related Peptide

Agouti protein (Accession No. P42127; pdb 1hyk) and Agouti-related protein (AGRP; Accession No. O00253; pdb 1mrO) are 131 and 132 residue proteins, respectively, that act as endogenous antagonists at melanocortin receptors (MCR) and competitively antagonize α-melanocyte-stimulating hormone (α-MSH),[39,40] which is discussed in more detail below. Murine agouti protein is normally expressed in hair follicles and is associated with pigmentation.[41] Agouti is a potent melanocortin antagonist at MC1-R and MC4-R (Ki 2.6 and 54 nM respectively), a weak antagonist at MC3-R and MC5-R (Ki 190 and 12,000 nM respectively). AGRP is related to the regulation of feeding behavior and metabolism and it is also linked to obesity and diabetes. AGRP is equipotent at central melanocortin receptors, MC3-R and MC4-R (binding affinity ∼1 nM).[39,41] The cysteine rich C-terminal regions of both proteins (Agouti 48 residues; AGRP 46 residues) have been shown to be as potent as the full length proteins.[41] The NMR structure of the C-terminal domain of AGRF (87−132) has been determined and shows the five disulfide bonds characteristic of the cysteine knot, **1**.[40,42] The first 34 residues of the peptide adopt a three-stranded antiparallel β-sheet with the latter two strands forming a β-hairpin. Within this hairpin are the three residues essential for receptor binding RFF (111−113).[43] A second structure of an AGRP truncated analogue (87−120, C105A) is also shown superimposed, **1**, and displays similar receptor binding and activity to the longer AGRP (87−132).[44] A lactam bridged cyclic antagonist, Tyr-c[Glu-Arg-Phe-Phe-Asn-Ala-Phe-Dpr]-Tyr-NH$_2$, **2**, based on hAGRP(109−118) is a turn mimetic that acts as an antagonist to MC1-R and MC4-R [pA$_2$ 5.9 (Ki 1.2 μM) and 6.9 respectively] but has no activity at MC3-R or MC5-R.[45]



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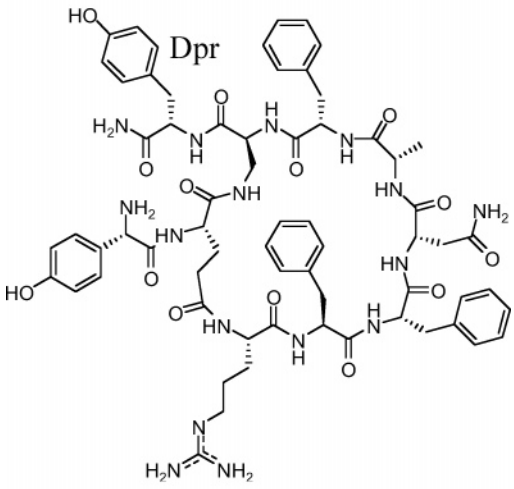

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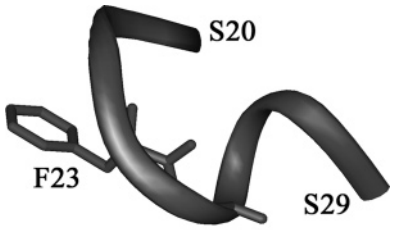
4.3. Amylin

Amylin[37] (AMY; islet amyloid polypeptide; diabetes-associated peptide, DAP; insulinoma amyloid peptide; Accession No. P10997; pdb 1kuw) is a 37-residue peptide (KC$_2$NTATC$_7$ATQRLANFLVHSS$_{20}$NNF$_{23}$G$_{24}$AILSS$_{29}$TNVGSNTY) with one disulfide bond and is

closely related to CGRP and adrenomedullin. It is associated with meal ingestion, potently inhibiting gastric emptying and gastric acid secretion[46] and selectively inhibiting insulin-stimulated glucose utilization and glycogen deposition in muscle.[47] It has also been isolated from amyloid deposits of human insulinoma and the pancreas of type II diabetes patients.[48] Amylin receptors can be reconstituted via expression of a calcitonin receptor (CTR) and receptor-activity-modifying protein 3 (RAMP3).[49] The selectivity of the receptors within the family depends on which CTR or CRLR is coupled to which RAMP. Much of the research into amylin has focused on its amyloidogenic behavior and not on its normal biological action. However, one study of amylin (20−29) in micelles shows the dominant conformation to be a distorted type I β-turn centered on F23 and G24, **3**.[50] This region for the CGRP family has been associated with a hinge and is reportedly necessary for binding (see below).[51]



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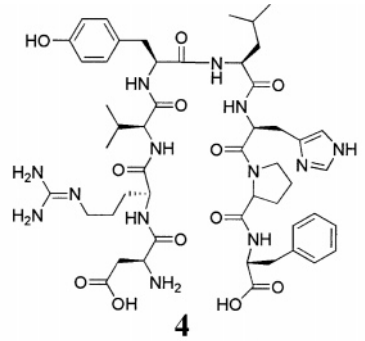
4.4. Apelins

Apelin peptides (Accession No. Q9ULZ1) are endogenous ligands for human APJ receptors (previously orphan receptors),[52,53] which have some structural similarities with chemokine receptors and 35% sequence identity with angiotensin AT1 receptor. They are derived from the C-terminus of the apelin precursor and range in length from 13 to 36 amino acids ($LVQP_4RGSRNGP_{11}GP_{13}WQGGRRKFRRQRP_{26}$-$RLSHKGP_{33}MP_{35}F$). Apelins are expressed in a wide range of human tissues including heart, brain, spinal cord and inhibit adenyl cyclase, elicit positive ionotopic responses, activate extracellular regulated kinases and the MAP kinase cascade, suppress cytokine production from immune cells, regulate cardiac contractility, reduce blood pressure and flow, and a number of cardiovascular functions.[54,55] APJ has also been shown to be a co-receptor for HIV and SIV, apelin specifically inhibiting cellular entry of HIV-1.[56] We are not aware of specific structures for apelins reported to date, but note 6 turn-favoring proline residues and 16 helix-favoring residues (Arg, Lys, His, Leu, Gln) in the sequence.[57]

4.5. Angiotensins

Angiotensin I ($DRVY_4IH_6PF_8HL$, AI) is progressively hydrolyzed to angiotensin II ($DRVY_4IH_6PF_8$, AII), angiotensin III (RVYIHPF, AIII), and angiotensin IV (VYIHPF, AIV). Angiotensins (Precursor Accession No. P01019 (human); pdb 1n9u, 1n9v) are intrinsically involved in maintenance of blood pressure via the renin-angiotensin system. They bind to three known receptors (AT1, AT2, AT4).[58] AT1 subtypes are expressed in liver, kidney, heart and other organs, while AT2 is mainly expressed in fetal tissues. AT2 subtypes have the characteristics of a GPCR,[59] but AT4 may not be a GPCR.[58] Compared with AIII, AII has higher affinity for AT1 but lower affinity for AT2, and AI has even lower affinity for both receptors,[58] while angiotensin peptides shorter than AIII do not activate AT1 or AT2. Extensive structural studies have been carried out on AII presenting many differing conformations.[60,61] These studies include the determination of AII bound to a monoclonal antibody in a compact hairpin structure.[62] More recent studies of AII have shown it to be disordered in water, but its backbone adopts a folded hairpin turn in phospholipid micelles, **4**.[63,64] Another



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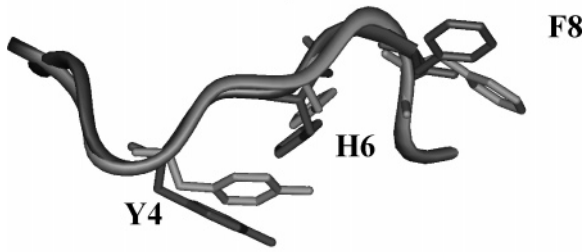

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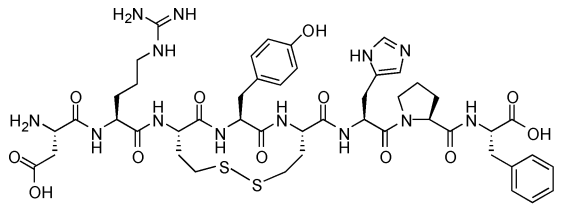
study of the structures of AI and AII in solution, illustrate two turn conformations at either terminus for AI and a similar turn at the N-terminus of AII, **5**, indicating the bioactive conformation is a turn.[60] The bioactive conformation has also been characterized by a charge relay system involving the tyrosine hydroxyl (Y4), histidine imidazole (H6) and the phenylalanine carboxylate (F8) with the specific spatial arrangement of the side chains of these three aromatic side chains being important.[65]

Cyclic octapeptide analogues of AII that adopt an inverse γ-turn, **6**, have high affinity for rat uterine membranes (IC_{50} 2.1 nM),[66] and those with a γ-turn mimetic, **7**, are equipotent with AT1 (IC_{50} 2.0 nM).[67] Compound **8** selectively inhibits the AT2 receptor (AT1, Ki >10000nM; AT2, Ki 3.0 nM) and consists of a benzodiazepine-based γ-turn scaffold, supporting the idea that a turn conformation of Angiotensin II may bind the AT2 receptor.[68] There are currently seven drugs that mimic the action of angiotensins: losartan, valsartan, telmisartan, irbesartan, candesartan, olmersartan, and eprosartan.



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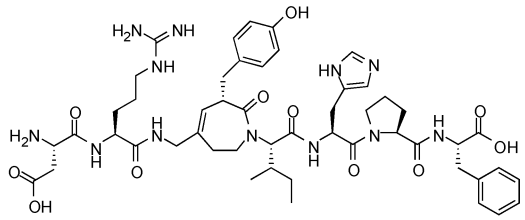

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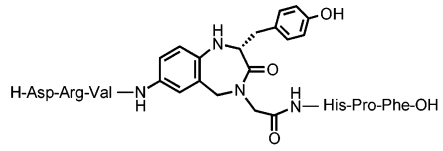

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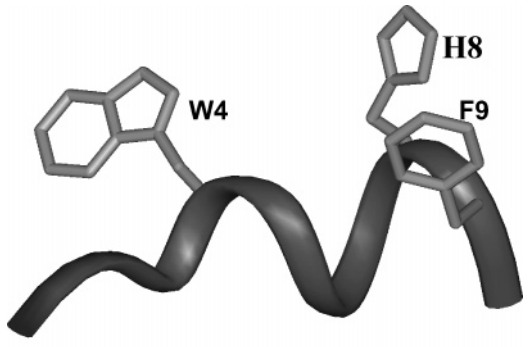
4.6. Bombesin, Neuromedin B, and Gastrin Releasing Peptide

Bombesin (Accession No. 21591 (frog); pEQRLGN-$QW_8AVGH_{12}L_{13}M$-NH_2) is a tetradecapeptide that increases blood glucose and acts at specific brain nuclei to inhibit food intake. Neuromedin B (Precursor Accession No. 08949 (human); pdb 1c98, 1c9a; $GNLW_4ATGH_8F_9M$-NH_2) and the gastrin releasing peptide (Accession No. P07492 (human); VPLPAGGG-TVLTKM-YPRGNHWAVGHLM-NH_2) are bombesin-like peptides of the CNS and GI tract implicated in control of food intake, smooth muscle contraction, and thermoregulation. They are also produced in several tumor cell lines. All three hormones bind to bombesin receptors, BB1−4, with different selectivity.[69] Structure−activity relationships suggest that W8, H12 and L13 residues (W4, H8, F9 in neuromedin B) are important for receptor binding.[70] NMR studies[71,72] showed that bombesin is a random coil in water, but α-helical at its C-terminus in aqueous TFE. Helicity is also evident via IR, CD, fluorescence, and molecular dynamics studies for bombesin and neuromedin B.[73,74] A recent NMR study of neuromedin B in aqueous TFE and SDS micelles, **9**, illustrates this helicity.[75] Substitution of Val-Gly in the potent agonist (D-Phe)QWAVGHLL-NH_2 with several turn mimetics has led to potent antagonists and agonists with high affinity for GRP/BN receptors (IC_{50} 1−5 nM).[76]



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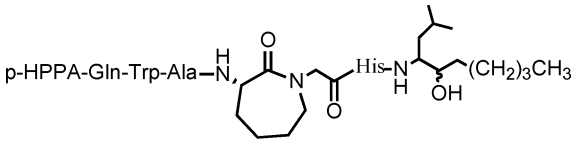
Substitution of Val-Gly in the potent agonist (D-Phe)QWAVGHLL-NH_2 with several turn mimetics has led to potent antagonists (eg. **10**, K_i 3.8 nM) and agonists (eg. **11**, K_i 1.8 nM, EC_{50} 0.05 nM) with high affinity for GRP/BN receptors.[76] In addition, based



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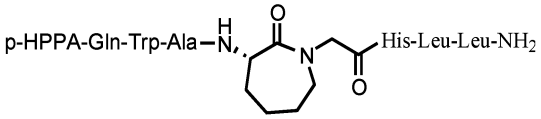

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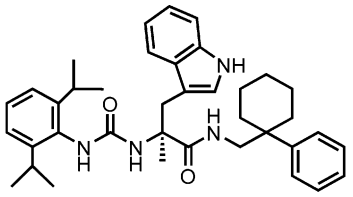
on a proposed γ-turn conformation of AcBN(7−14) and essential amino acid side chains (Trp-8, Leu-13, Val-10) in the peptide sequence, a series of non-peptide antagonists were developed culminating in compound **12**.[77] A series of constrained cyclic peptide derivatives have also been described (eg. **13** IC_{50} 4 μM), lending further support to a turn conformation of the ligand being responsible for activating these receptors.[78]



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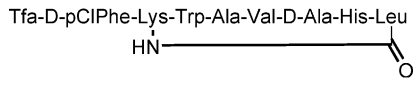

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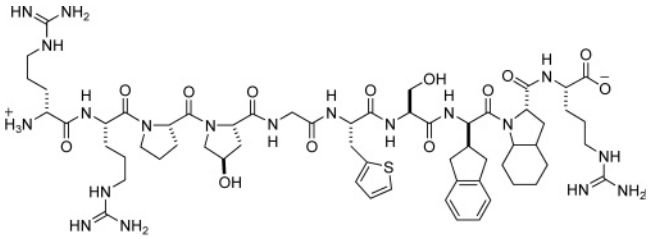
4.7. Bradykinin

Bradykinin (human BK; Precursor Accession No. P01042) is a nine-residue peptide ($RP_2PGF_5S_6P_7F_8R_9$) released from the plasma precursor kininogen during inflammation and tissue injury. This and related kinins (Lys-BK (kallidin), BK(1−8), and Lys-BK(1−8) with the C-terminal Arg removed) possess a wide range of pharmacological actions. They are important mediators of pain and inflammation and control blood flow. They bind to two main types of receptor, B1 and B2.[79] Conformational analysis of BK in SDS shows a

β-turn centering on S6-P-F-R9,[80] although recent studies of BK derivatives suggest that a type II β-turn at positions 2−5 is important for activity.[81] Structural studies using NMR, molecular modeling and CD on the BK antagonist B-9340, **14**, (pIC50-(B1): 8.1; pIC50(B2): 9.8) show two β-turns, **15**.[82]



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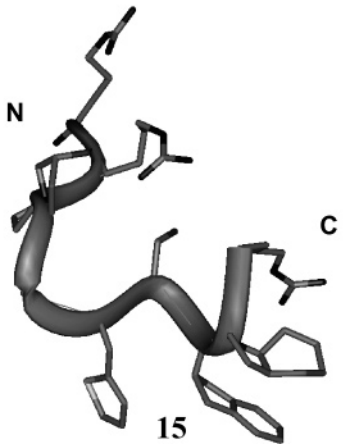

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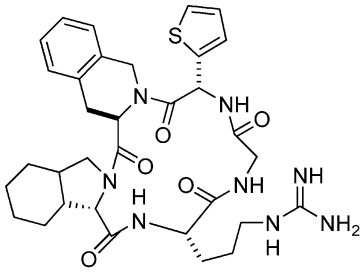
Numerous bradykinin agonists and antagonists, including many cyclic compounds, reveal the presence of a β-turn from residues 6−9 for agonists and an additional β-turn (type II) from residues 2 to 5 for antagonists.[83] This information has led to both agonists and antagonists with non-peptidic structures.[84]



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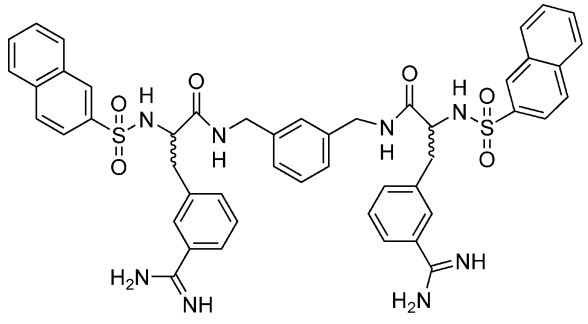

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Particular examples which further support the recognition of β-turns at bradykinin receptors include cyclic peptide **16**, a bradykinin receptor antagonist (pA_2 7.4) that displays type II′ β-turn structure in solution,[85] and the bis-benzamidine **17**.[86] Compound **17** was discovered from a 3D pharmacophore model of the bradykinin binding site, based on conformational data of linear and cyclic bradykinin antagonists, as well as a non-peptide antagonist discovered from random screening.

4.8. Calcitonin

Calcitonin (CT; Accession No. P01258 (human); pdb 1bku, 1byv, 1bzb) is a 32 residue, disulfide-bridged peptide (C_1GNLSTC_7MLGTYTQDFN$_{17}$KFHT$_{21}$FPQTAIGVGAP) produced in the thyroid gland of mammals. It functions as a calcium regulator *in vivo* and recombinant CT (Miacalcin) is in clinical use for the treatment of Paget's bone disease, osteoporosis, and hypercalcemia of malignancy.[87,88] It forms part of a hormone family with CGRP, amylin and adrenomedullin. The three-dimensional structures of eel, **18**, salmon, and human CT have all been investigated by NMR techniques that identified an amphipathic α-helix as the common structure.[89−91] The length of the helical region varies as a consequence of sequence and species, extending from residues 6−9 to residues 16−22, followed by a nominally unstructured region. It was shown that the human CT adopts a type I β-turn, at residues F16−F19, acting as a cap to stabilize the C-terminus of the helix.[90] Further studies have identified potent, conformationally constrained calcitonin analogues, **19**,[33,92] in which a β-turn conformation is stabilized by a bridge across residues 17−21 and increases *in vivo* hypocalcaemic potency 5−10 times over that of hCT.

18

CGNLSTCMLGTYTQDFDKFHKFPQTAIGVGAP

19

4.9. Calcitonin Gene-Related Peptide

Calcitonin gene-related peptide (CGRP I, P06881; CGRP II, P10092) is a neuropeptide with vasodilatory activity and is expressed from the gene coding for calcitonin.[87] There are two peptides, AC_2DT-ATC$_7$V$_8$THRLAGLLSR$_{18}$SGGV$_{22}$VKNNF$_{27}$VPTNVG-SKAF-NH_2 (I) and AC_2NTATC$_7$V$_8$THRLAG-LLSR$_{18}$-

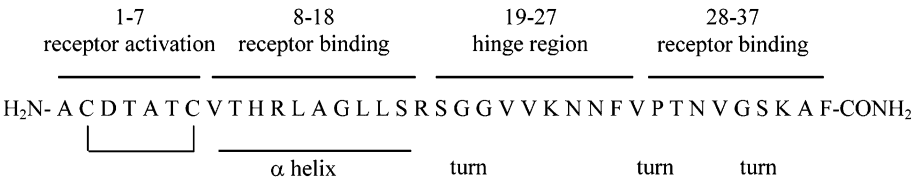


Figure 3. Structural components of CGRP I. (Reproduced with permission from ref 51. Copyright 2002 Portland Press.)

$SGGM_{22}VKSNF_{27}VPTNVGSKAF\text{-}NH_2$ (II), both with one disulfide bridge. They share the disulfide bonded cyclic constraint at their N-termini and possess an amphipathic α-helix and a C-terminal amide.[51] CGRP interacts with a heterodimeric receptor consisting of a calcitonin receptor-like receptor (CRLR) and a single-pass transmembrane protein, receptor-activity-modifying protein 1 (RAMP1). The truncated CGRP(8−37) is an antagonist to the CGRP receptor (K_i 3 nM),[93] indicating the importance of this cycle for activation. A recent review summarized CGRP, its receptors, and structural evidence for an α-helix between residues V8 and R18, a β- or γ-turn from S19 to V22, followed by a largely unstructured region (Figure 3).[51]

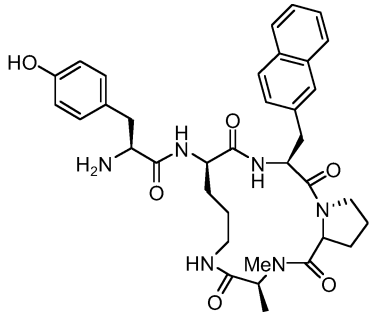
Affinity and structural data have also been reported for truncated CGRP(27-37), which is thought to be important for receptor binding. By systematically modifying the decapeptide FVPTNVGSEAF-NH_2 (IC_{50} 3 μM) it was possible to obtain antagonists **20** (K_i 36.5 nM) and **21** (K_i 6.56 nM) with similar receptor affinity as human α(CGRP(8−37) (K_i 3.5 nM). ^1H NMR structural analysis of these two peptides showed that both peptides displayed a β-turn structure centered at Pro-29, while **20** had α-helical structure at residues 32−35 and **21** showed a γ-turn in this region of the peptide.[94]

20 β-turn; Helical-turn
Phe-Val-Pro^{29}-Thr-Asp^{31}-Val-Gly-Ala^{34}-Phe^{35}-Ala-Phe-NH_2

21 Phe-Val-Pro^{29}-Thr-Asp^{31}-Val-Gly-Pro^{34}-Phe^{35}-Ala-Phe-NH_2
β-turn; γ-turn

4.10. β-Casomorphin

β-Casomorphins (Precursor β-Casein Accession No. P05814 (human)) are short acyclic peptides derived from the milk protein β-casein (e.g. bovine β-casomorphin (1−11) YPFPGPIPNSL, human (1−4 amide) YPFV-NH_2) where the first three residues, YPF, are highly conserved.[95−97] The β-casomorphins are selective for μ opioid receptors (MOR). Many other peptides derived from milk proteins are known to have opioid receptor activity and include the casoxins (derived from α- and κ-casein), lactorphins (derived from α- and β-lactalbumin) and lactoferroxins (derived from lactoferrin). The majority of these short peptides contain residues that resemble the opioid message sequence (YGGF). The cyclic β-casomorphin-5 derivative, Tyr-c[-D-Orn-2-Nal-D-Pro-NMe-Ala], **22**, has been shown to be a potent and selective μ-opioid receptor agonist (IC_{50} 35 nM).[98] In addition, the MOR selective antagonist *cyclo*-D-Phe-[Cys-Tyr-D-Trp-Lys-Thr-Pen]-Thr-NH_2, **23** (MOR, IC_{50} 1.2nM; DOR, IC_{50} 9324nM), displays a type II′ β-turn by ^1H NMR spectra in DMSO,[99] again suggesting that turn conformations are recognized by this receptor.



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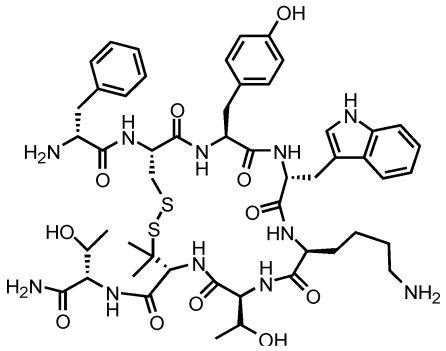

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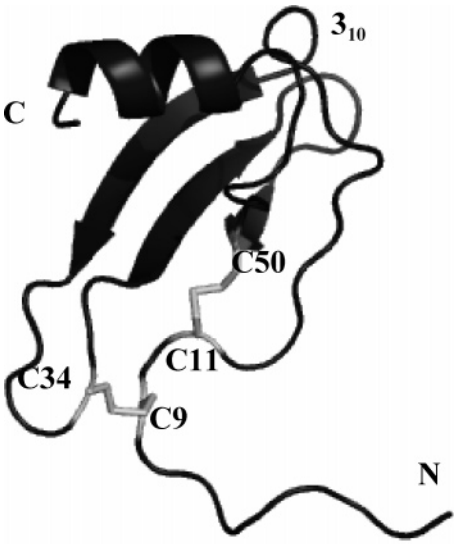
4.11. Chemokines

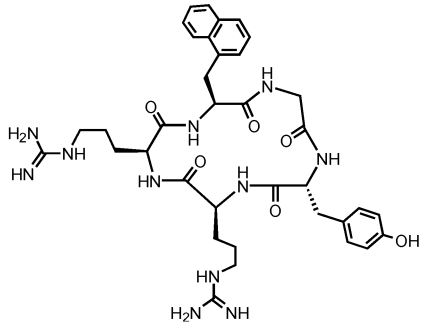
There are at least 44 identified chemokines[100−102] (e.g. CXCL12, stromal cell-derived factor 1 (SDF-1); pre-B cell growth stimulating factor (PBSF) (hIRH); SDF-1α; SDF-1β Accession No. P48061; pdb 1a15, 1qg7, 1sdf, 2sdf) divided into four subfamilies based on the arrangement of N-terminal cysteine residues (CC, CXC, CX3C, and C). There are at least 19 chemokine receptors (CXCR1−6, CCR1−11, CX3CR1, and XCR1) with ligands identified so far as CXCL1−16, CCL1−28, CX3CL1, and XCL1. CXC chemokines can be further classified according to the presence (or absence) of an ELR motif, where CXC chemokines containing the ELR motif activate human neutrophils *in vitro*. Apart from their classical function in cell migration, chemokines are involved in signaling in cells, hematopoiesis and immune responses, and diverse disease models as autoimmunity, allergy, asthma, cancer, diabetes, sepsis and HIV.[103−105] The overall structural fold of chemokines (e.g. CXCL12, **24**) is well conserved and consists of a three stranded antiparallel β-sheet with an α-helix at the C-terminus overlying the β-sheet. The N-terminus is generally

disordered but important for activation. An extended loop region leading into a 3_{10}-helix immediately prior to the β-sheet follows this. It is generally thought that at least the N-terminus is required for activation, if not the C-terminus as well. While specific residues are of importance in many individual cases, it is unclear as to whether a specific turn conformation is recognized.



24

Perhaps the most compelling evidence to date that suggests at least some chemokine receptors recognize turns in their ligands, comes from the CXCR4 antagonist **25**. This pentapeptide (IC_{50} 4nM, EC_{50} 38nM) displays a conformation in DMSO consisting of a type II′ β-turn and a γ-turn. The compound was designed based on the solution structure of the 14 residue peptide antagonist T140 (IC_{50} 4nM, EC_{50} 60nM) **26** by restricting the residues that were



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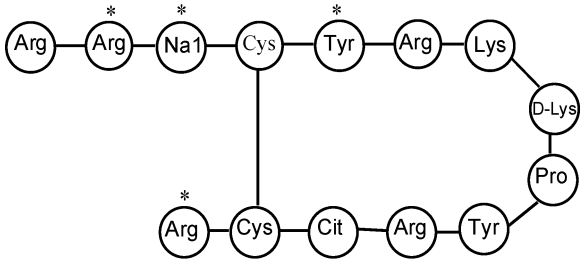


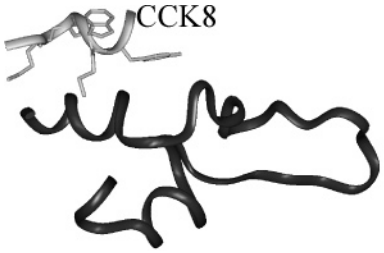
26

essential for binding (*) using cyclic pentapeptide libraries.[106]

4.12. Cholecystokinin and Gastrin

Cholecystokinin (CCK; Precursor Accession No. P06307 (human); pdb 1d6g) was originally described as a 33 residue peptide, but is now known to exist in a variety of bioactive forms such as CCK-22, CCK-33, CCK-39, CCK-58, sulfated and unsulfated CCK-8 [$DY(SO_3H)MGW_5MDF_8$-NH_2] and CCK-4, CCK-5, CCK-7, all derived from a 115 residue precursor (prepro-CCK). Gastrin [pEGPWLEEEEEAY(SO_3H)-GWMDF-NH_2; Precursor Accession No. P01350 (human)] closely resembles CCK, sharing the same C-terminal pentapeptide, the bioactive section, but differs in the position of sulfation. Physiological functions of these peptides include stimulation of pancreatic enzyme secretion and gall bladder contraction, delay of gastric emptying, and satiety.[107] CCK is currently used as a diagnostic for gall bladder and pancreatic disorders. There are two GPCRs for CCK-peptides, CCK1-R ("alimentary" or CCKA) and CCK2-R ("brain" or CCKB), the receptor for gastrin.

Numerous structural studies have been carried out on CCK, particularly CCK-8,[108−110] which has β- and γ-turns centered on GWMD and MDF-NH_2 respectively. The NMR-derived solution structure of the C-terminal octapeptide of CCK8 in complex with the N-terminal portion of the CCKA receptor (1−47), **27**, shows the helix-like conformation of CCK-8, **28**, binding to this portion of the receptor.[111] A compari-



N-terminal of CCKA receptor

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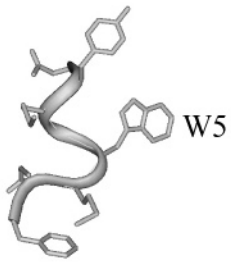

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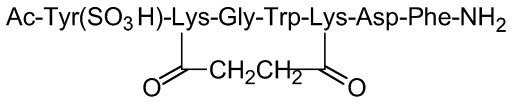
son between CCK-8 and CCK-58 showed that the longer peptide had a different conformation in the C-terminal eight residues, which may explain their differences in receptor binding and activation.[112] A recent study showed CCK-15 to adopt a similar helical conformation to CCK-8 in aqueous SDS solvent.[113] A large number of β-turn-mimicking cyclic

peptides and peptidomimetics based on CCK-8 and CCK-4 have shown high affinity and efficacy at CCK1-R and CCK2-R receptors, some with high receptor selectivity. Examples are cyclic analogue **29**, a turn-mimicking agonist at CCKB (IC_{50} 1.3 nM; 10,000-fold selectivity),[114] **30** which has a type II′ β-turn constraint (CCK_1, IC_{50} 4.7nM; CCK_2, IC_{50} >10000 nM),[115] and **31** which is an α-methyl tryptophan derivative of tetrapeptide CCK-4 (CCK_1, IC_{50} 4300 nM; CCK_2, IC_{50} 1.7 nM).[116]



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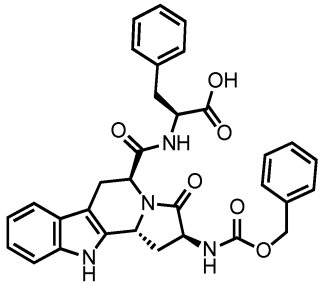

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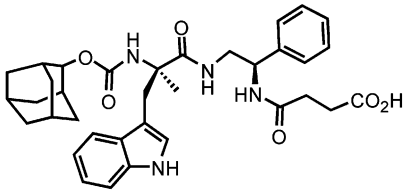

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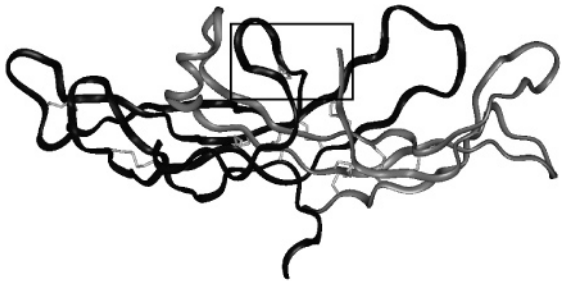
4.13. Chorionic Gonadotropin

Chorionic gonadotropin[117] (hCG; Accession No. P01233, Q8WXL1; pdb 1hcn, 1hrp, 1qfw, 1xul; α subunit pdb 1dz7, 1e9j, 1hd4) is a member of the family of glycoprotein hormones associated with human fertility which also includes FSH, LH and TSH. hCG is a placental hormone and one of its primary functions is to stimulate the production of progesterone until the placenta can produce hCG itself. Recombinant hCG is currently available clinically for use in fertility, replacing human urinary preparations of hCG (under Chorex and Novarel).[118] The presence of hCG in urine has also been utilized in pregnancy testing as it is the first practical indicator of fertilized ovum implantation. HCG, **32**, is a heterodimeric protein with an α subunit of 92 residues (grey) conserved within the family, and a β subunit of 145 residues (black).[119] The overall structure of hCG is a cysteine knot motif at a core of extended hairpin loops, and is highly conserved within this cysteine-knot growth factor family.[120] C-terminal residues 88−92 of the α subunit and residues 94−114 of the β subunit (boxed) are deemed important for receptor binding. hCG binds to the LH/CG receptor, which possesses a large N-terminal leucine-rich repeat domain, the structure of which has been modeled bound to hCG, **33** (dark).[121]



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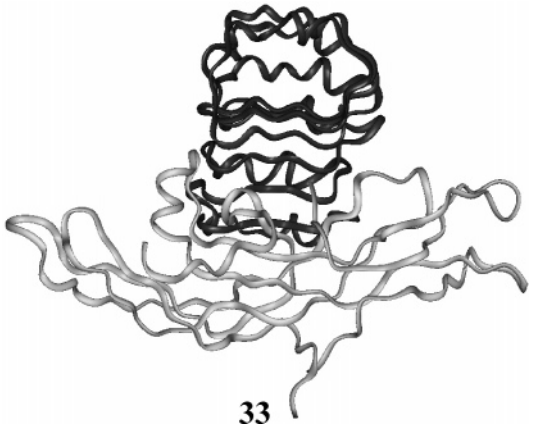

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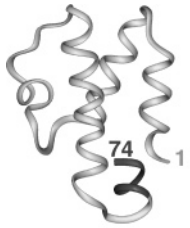
4.14. Complement Factor C5a

C5a (C5a anaphylatoxin; Precursor Accession No. P01031) is a chemoattractant pro-inflammatory hormone with 74 amino acids arranged in a helix bundle, **34**.[122] It interacts with at least two GPCRs (CD88 and



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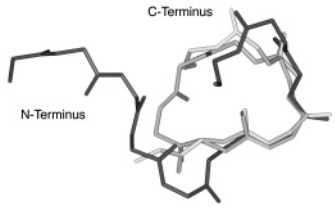

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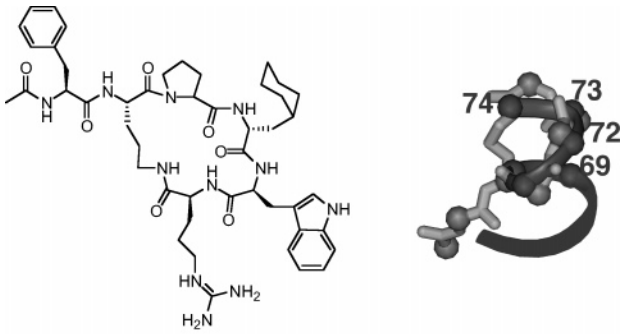
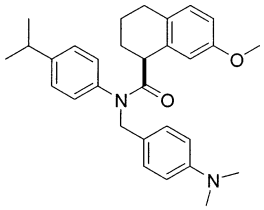
C5L2) on human monocytes and mast cells through high affinity N-terminal helical and low affinity C-terminal loop regions. Short peptides derived from the C-terminal loop (ISHKDMQLGR) are alone capable of activating the receptor. Superimposition of NMR structures, **35**, for a C-terminal decapeptide analogue of C5a (dark) and acyclic/cyclic hexapeptide antagonists[123] (light) reveal a common turn motif. A consensus turn mimetic **36** (restrained by an 18 membered ring, 5 trans-amide bonds, Pro, D-cyclohexylalanine, & 2 transannular H-bonds) was designed to reproduce this turn motif and structural mimicry (**37** = **36** on bolded **34**) led to potent antagonism (IC_{50} 20 nM) of C5a receptors on human monocytes and neutrophils.[123−125] Compound **36**

(known as 3D53) is an orally active anti-inflammatory drug at ≤1 mg/kg/day in rats.[123,126−128]



36 **37**

A number of orally active non-peptidic antagonists of human C5a antagonists have since been made, including **38** which is reportedly 7 times more potent than **36** against neutrophils.[129]



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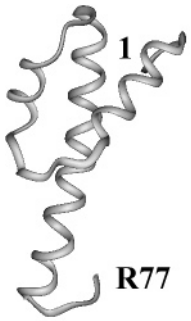
4.15. Complement Factor C3a

There are significant differences between the *in vivo* pro-inflammatory properties of C5a and the anaphylatoxin C3a (C3a anaphylatoxin, Precursor Accession No. P01024),[130,131] its crystal structure showing a 77-residue helix bundle, **39**.[132] However,



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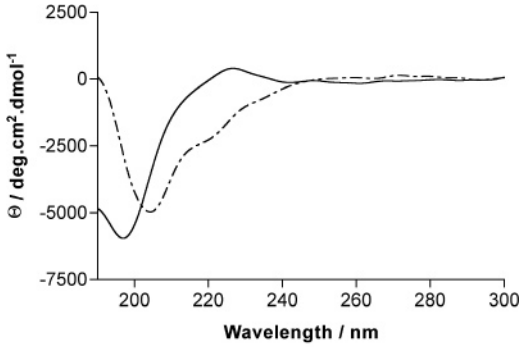
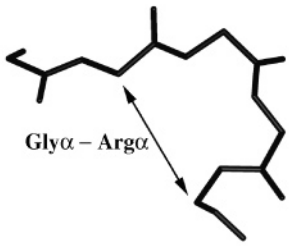
activity is similarly localized in its 10-residue C-terminal loop (ARASHLGLAR$_{77}$). Genetic deletion of the C3a receptor, 5% as prevalent on human neutrophils as C5a receptors, protects against changes in lung physiology after allergen challenge,[133,134] and human asthmatics develop significant levels of C3a.[135,136] C3a reportedly stimulates or inhibits release of TNFα, IL1β, or IL-6 from immune cells depending on conditions, and there is some evidence that C3a and C5a antagonize one another even though they selectively act on different GPCRs. CD (below, dashed line) and NMR studies (**40**) reveal that the 15-residue peptide WWGKKYRASKLGLAR, a C3a super-agonist,[137] has a C-terminal turn conformation in aqueous 0.1 M SDS solvent (Beyer, Stoermer, Fairlie. Unpublished work).





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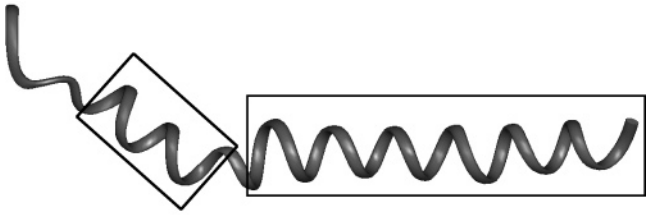
4.16. Corticotropin Releasing Factor

Corticotropin releasing factor (corticoliberin, CRF; corticotropin releasing hormone, CRH; Accession No. P06850; pdb 1go9, 1goe) is a 41 amino acid peptide (SEEPP$_5$ISL$_8$DLTFHLLR$_{16}$EVLE$_{20}$MARAEQLAQQ-AHSNRKLMEII-NH$_2$) and a member of a larger family of related peptides that includes the urotensins, urocortins, and sauvagine. CRF is associated with stress responses and acts via the CRH receptors (CRH1 (Ki 3.3 nM), CRH2α, CRH2β, CRH2γ as well as CRF-binding protein, not a GPCR).[138] Residues 1−4 are not required for binding. Residues 5−8 are important for activation, and residues 12−41 are mostly responsible for binding (boxed regions below). The smallest peptide with corresponding CRH activity is CRH(5−41) indicating that the entire C-terminus is required for activity. Numerous studies have indicated an amphipathic α-helical structure for the peptide and for constrained peptide derivatives.[139] In particular the solution structure of human CRF shows a well defined α-helix between residues 6−36.[140]

Interestingly, the peptide antagonist CRH(9−41) is often referred to as α-helical and studies as early as 1983 indicated that the bioactive conformation is α-helical.[141] The structure of a human CRF analogue, [D-Phe12, Aib15]-CRF, **41**, illustrates the helical



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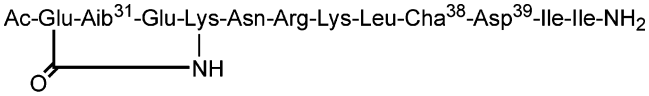
nature of this peptide with two main α-helical portions from I6 to R16 and from E20 to I40 (boxed) separated by a kink.[142] Numerous researchers have developed lactam-bridged N-terminally truncated antagonists to stabilize this helical conformation, the smallest being a 12 residue lactam bridged peptide **42**, which displays potent affinity at the CRF receptor (K_i 5.5 nM) and is active *in vivo* due to stability imparted by the α-aminobutyric acid residue at position 31.[143,144] See also Urocortins.

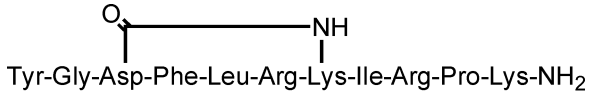


42

4.17. Dynorphin A

Dynorphin A[145] (Accession No. P01213) is the 17 residue ($YGG_3FLRRIR_9PKLKW_{14}DNQ_{17}$) κ opioid receptor agonist (KOR, OP2) derived from pro-dynorphin and ligands for this receptor may have analgesic properties like morphine without side effects. Opioid peptide hormones are characterized by an N-terminal message sequence YGGF followed by an address sequence. Among conflicting NMR studies on Dynorphin A (1−7), an α-helical conformation has been identified between G3-R9 with a β-turn from W14 to Q17 in a micelle environment.[146] A cyclic dynorphin A analogue (cyclo-(5,11)-[$YGGFC_5RRIRPC_{11}$-NH_2]) showed similar potency and selectivity as the native form for the κ opioid receptor.[147] Molecular dynamics calculations supported cis−trans isomerization about the R9−P10 amide bond and β-turn structures around R7−P10 and C5 and R8 for the cis and trans isomers respectively, although others[148] suggest an R7−K13 loop centered on P10. There is a consensus that truncated peptide 1−11 is sufficient to produce activity.

Structure−activity data for many constrained analogues of Dynorphin A[149] tend to suggest an α-helical structure of the message sequence (YGGF), while an address sequence is required for activation of the KOR receptor. This is supported by **43**, which



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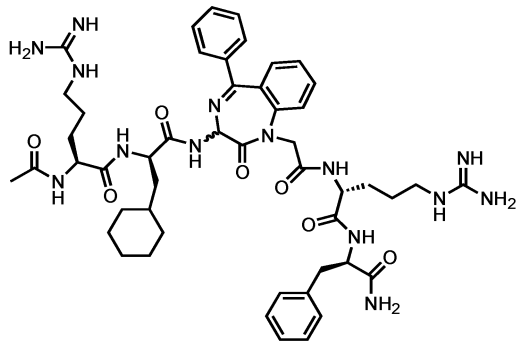
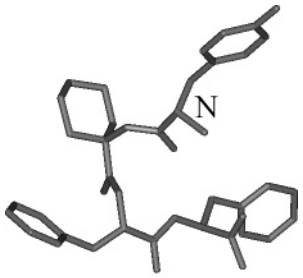


44

is a potent and selective cyclic [*i*, *i*+4] lactam analogue of Dyn A (KOR, IC_{50} 25 nM; MOR, IC_{50} 740 nM; DOR, IC_{50} 1710 nM),[150] lactam constraints being well-known to induce α-helicity in peptides. Further evidence is provided by **44**, discovered by screening synthetic combinatorial libraries incorporating β-turn inducing constraints in short peptide sequences, which is selective for KOR (KOR, K_i 60 nM; MOR, K_i 2700 nM; DOR, K_i 6700 nM).[151]

4.18. Endomorphin

Endomorphins 1 ($YPWF\text{-}NH_2$) and 2 ($YPFF\text{-}NH_2$) (CCSD: LENKPH11) are highly selective endogenous μ opioid receptor (MOR) agonist peptides (K_i 0.36 nM and 0.69 nM, respectively) with at least 4000-fold selectivity over other opioid receptors.[152,153] Originally isolated from mammalian brain cortex,[154] these two peptides are associated with regulation of gastrointestinal motility, manifestation of anti-nociception, and effects on vascular systems and memory.[152] The structures for endomorphins and analogues[155−158] indicate a mixture of conformations ranging from a β-turn **45**, similar to Leu-Enkephalin,[155] an extended



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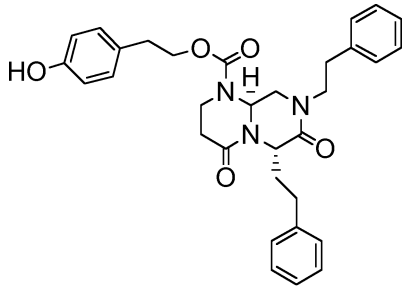
conformation for *trans*-Endomorphin1, and a turn conformation for the cis isomer about the Tyr-Pro amide bond.[156−158] Evidence in support of a β-turn as the biologically active conformation comes from **46**, which possesses a β-turn mimicking scaffold and has nanomolar affinity as an agonist for MOR, and from constrained peptides **47** and **48**. These are derivatives of morphiceptin (H-Tyr-Pro-Phe-Pro-NH2), a specific agonist of MOR (IC_{50} 550 nM). Substitution of Phe by a D-amino acid increases potency 5-fold (**47**, IC_{50} 109 nM)[159] while the thiazolidine constraint in **48** strongly suggests that a cis-conformation of the Tyr-pro amide bond is required for receptor activation.[160]

4.19. β-Endorphin

β-Endorphin (β-lipotropin C-fragment, Accession No. P01189) is a natural, 31 amino acid, opioid peptide ($YGGF_4MTSEKSQTP_{13}LVTLFKNAIIKN\text{-}AY_{27}KKGE$).[145,153] It is derived from the C-terminal fragment (237−267) of pro-opiomelanocortin (POMC) and is the largest natural opioid hormone.

It contains the classical message domain sequence (YGGF or FGGF) characteristic of opioid peptides followed by the address domain. β-Endorphin has been suggested to interact with μ, δ and κ receptors (MOR, DOR, KOR) with minimal selectivity. More recent studies discuss its interaction with a putative



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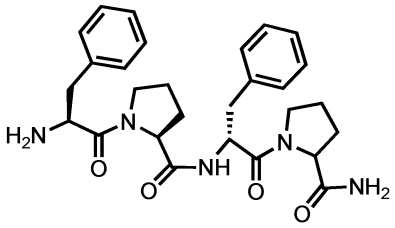

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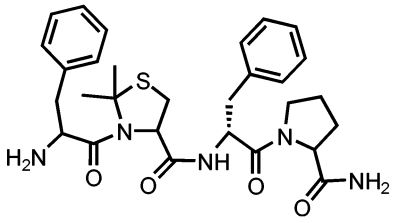
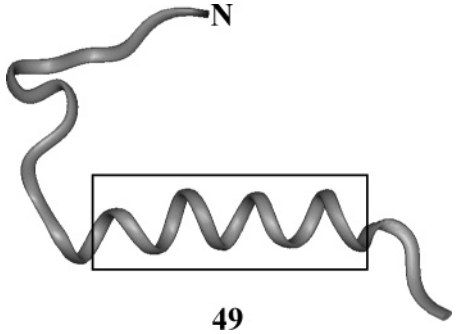


48

ϵ opioid receptor.[153,161] The NMR structure of β-endorphin, **49**, shows a strong tendency to form an α-helical structure in the address domain (P13 to Y27, boxed) while the N- and C-terminal domains exist in a random coil conformation.[161] (The coordinates of β-endorphin were kindly provided by Dr. Teodorico Tancredi.[161])



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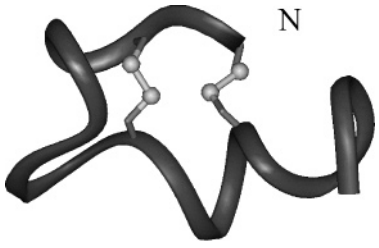
4.20. Endothelins

Endothelins (ET-1, Precursor Accession No. P05305 (human), pdb 1edn, 1edp, 1v6r; ET-2, Precursor Accession No. P20800 (human); ET-3 (Precursor Accession No. P14138 (human); and others, pdb 1srb, 3cmh, 6cmh) are 21 residue peptides cross-linked by two disulfide bonds. They interact with two known receptors, ETA and ETB. ET-1 and ET-2 show higher affinity for ETA than ET-3, while all three have similar affinity for ETB. A third receptor ETC, reported to be ET-3 specific, has been cloned but no mammalian homologue has yet been identified. Endothelin antagonists show utility in congestive heart failure, stroke, kidney failure, asthma, pain and cancer.[162] Bosentan (Tracleer) is used clinically to treat pulmonary arterial hypertension, and several other antagonists are in clinical trials.[162,163] X-ray structures (**50**),[164] and NMR structures[165,166] as well



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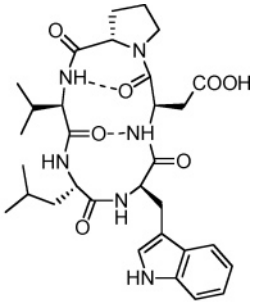
as structure−activation relationships,[167] have shown that ET-1 [$C_1SC_3SSLMDKE_{10}C_{11}VYF_{14}C_{15}HLD_{18}$-$IIW_{21}$] adopts a helical structure with all key residues (E10, F14, D18, W21) on the same helical surface.[168] Modeling studies support interactions between those residues and ETA.[169] NMR studies show that ET-2 [$C_1SC_3SSWLDK_9EC_{11}VYFC_{15}HL_{17}DIIW$] and ET-3 [$C_1TC_3FTYKDK_9EC_{11}VYYC_{15}HLDIIW$] adopt helical structure between residues 9−17 and 9−15 respectively, similar to ET-1.[170,171]

In addition to the endogenous endothelin hormones, several snake venom toxins, called sarafotoxins, have been identified with similar helical structures as the human endothelins (e.g. SRTb, **51**).[172] Furthermore, cyclic molecules such as BQ123 (IC_{50} 22nM), **52**, are potent endothelin antagonists that appear to mimic the turn structure of the endothelins.[173]

51



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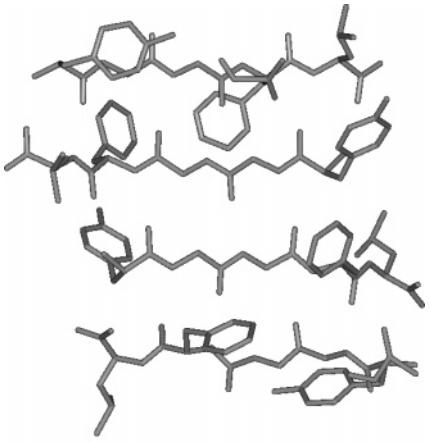
4.21. [Met]-Enkephalin and [Leu]-Enkephalin

[Met]-Enkephalin and [Leu]-enkephalin (YGGFM/L, respectively; Accession No. P01210; CCD Code: BIXNIF10, FABJIB, GEWWAG, LENKPH11) are natural δ opioid receptor (DOR) agonists with morphine like activity. They also bind to the μ receptors, but with lower affinity. Opioid peptide hormones are characterized by the initial N-terminal message sequence of YGGF followed by the address sequence, in this case simply M5 or L5. The presence of two

contiguous glycine residues confers flexibility to the linear peptide. Indeed X-ray structures have shown [Leu]-Enkephalin to adopt a β-sheet conformation **53**,[174,175] as well as a β-turn, **54**.[176,177] In fact various types of β-turns (types I, I′, II′, and III) have been identified by many structural studies.[178−182] It is generally accepted that the biologically active conformation of enkephalins is the β-turn, supported by structural evidence of cyclic enkephalin analogues possessing opioid activity, e.g. **55** (K_i 0.79 nM).[183−185]



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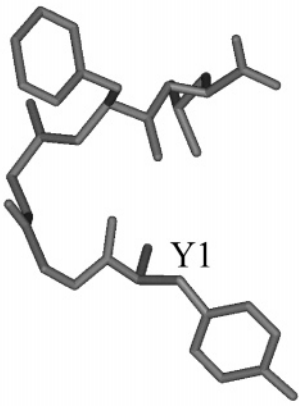

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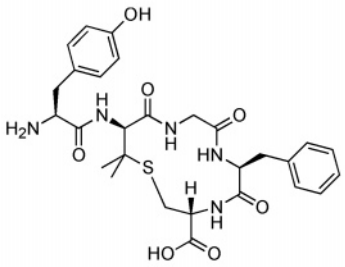

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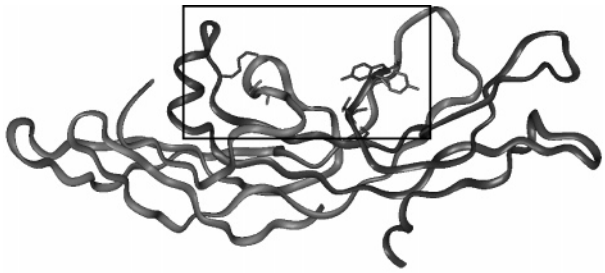
4.22. Follicle-Stimulating Hormone

Follicle-stimulating hormone[117,186] (FSH; follitropin; 111 residue protein; Accession No. P01225; pdb 1fl7) is a pituitary hormone and a member of the family of glycoprotein hormones associated with human fertility. These hormones are heterodimeric each consisting of a common α subunit (92 residues) and a unique β subunit. FSH facilitates ovarian folliculogenesis and is essential for Sertoli cell proliferation and maintenance of sperm quality in the testis. Currently, recombinant FSH is used clinically in ovarian stimulation (under the names Puregon and Gonal F).[187] FSH binds to the FSH receptor, which contains a long N-terminal region, consisting of a Leucine-rich repeat region (residue 54−254) responsible for recognition and binding (see hCG).[117] The glycoprotein hormones are structurally unique, compared with other peptide hormones. Residues required for receptor binding have been identified, but those required for activation have not been fully identified. The structure of FSH, **56**, and other glycoproteins is similar to that of cysteine-knot growth factors.[186] Residues of known importance for hFSH receptor binding are K51, S85, T86, Y88, and Y89 at the C-terminus of the alpha unit, and D93 toward the C-terminus of the β unit, all of which are located on the concave surface of the molecule (**57**, boxed). The structure of human FSH bound to the



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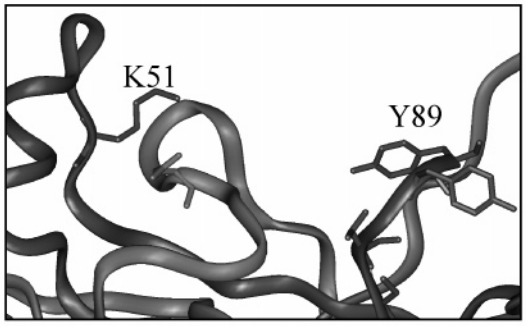


57

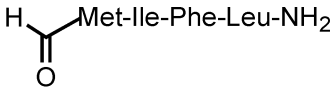
extracellular binding domain of its receptor, released just prior to this publication, highlights key interations between hormone and receptor (1xwd).[413]

4.23. *N*-Formyl Peptides

N-Formyl peptides are short (3−4 residues) N-terminal formylated peptides, expressed by bacteria and humans. They are chemoattractants for neutrophils and macrophages, binding to a family of GPCR receptors that includes the formyl-peptide receptor (FPR) (IC_{50} 0.1−1 nM), and its variants FPRL1 (FPR-like 1) (IC_{50} 1 μM) and FPRL2.[188] The best known and most studied *N*-formyl peptide is *N*-formyl-methionine-leucyl-phenylalanine (fMLF). Crystallographic studies on fMLF bound to an immunoglobulin light chain dimer (Bence−Jones protein)

suggested that the peptide adopts a conformation resembling a wedge-shaped cavity, its formyl group forming a hydrogen bond with the phenolic hydroxyl group of a tyrosine at the base of the cavity.[189] NMR studies of fMLF and analogues in solution suggest an extended β-sheet-like structure at position 2.[190,191] However, recently a nonformyl peptide and full antagonist for hFPR, Phe-D-Leu-Phe-D-Leu-Phe, was reported to have a β-turn conformation.[192]

Conformational studies on a series of peptide agonists suggest that a β-turn conformation might activate these receptors. Tetrapeptide **58** (K_i 2.8 nM) is a very active agonist that forms a highly ordered β-turn in TFE and 1:1 TFE/water. In contrast tetrapeptide **59** (K_i = 2500 nM) has a weakly ordered



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59

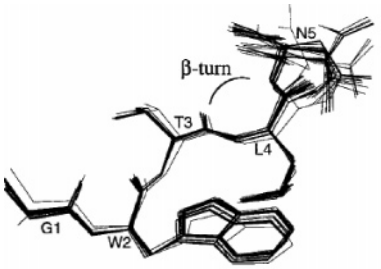
solution structure and exhibits weak agonism.[193] Other non-formyl ligands have been found to bind to FPR-like domains from HIV-1 envelope protein, such as the HIV-1 inhibitory peptide TP20/DP178 (IC_{50} 0.5 μM (FPR)) and TP21/DP07 (IC_{50} 0.1 μM (FPR), IC_{50} 50 nM (FPRL1)).[194] Although there is not much structural information available for those HIV-1 inhibitory peptides, it was reported that enhancement of α-helicity lead to increased affinity for human mAb 2F5.[195]

4.24. Galanin and Galanin-like Peptide

Galanin (Precursor Accession No. P22466 (human)) is a 30 residue neuro-endocrine peptide hormone (G_1-WTLN$_5$SA$_7$GYL$_{10}$LGPHAVGNHRSFSD$_{24}$KNG$_{27}$-LTS) with several physiological functions, the control of pain thresholds, appetite and physiological effects of insulin, acetylcholine, somatostatin and others.[196] Three galanin receptor subtypes have been cloned (GalR1, R2, R3).[197] Galanin-like peptide (GALP; Accession No. Q9UBC7 (human)) is a related 60-residue endogenous ligand (APAHRGRGGWTLN-S A G Y L L G P V L H L P Q M G D Q D G K R E T A-LEILDLWKAIDGLPYSHPPQPS), a neuropeptidic hormone isolated from porcine hypothalamus.[198] A recent NMR study of galanin in SDS showed β-turns at positions 1−5 and 7−10, **60** and **61**, and an inverse γ-turn at residues 24−27, **62**.[199] (Structures **60**−**62** are reprinted with permission from ref 199. Copyright 1998 American Chemical Society.) These findings differ from earlier studies that suggested an α-helix in TFE[200] and a nascent helix in water.[201] However both studies present dominant turn conformations.

4.25. Ghrelin

Ghrelin[202] (GHRL; growth hormone secretogue, GHS; growth hormone releasing peptide; motilin-



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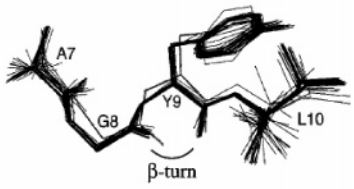

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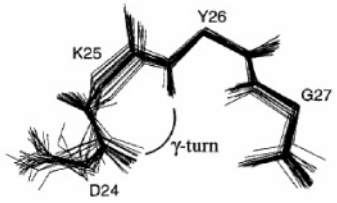

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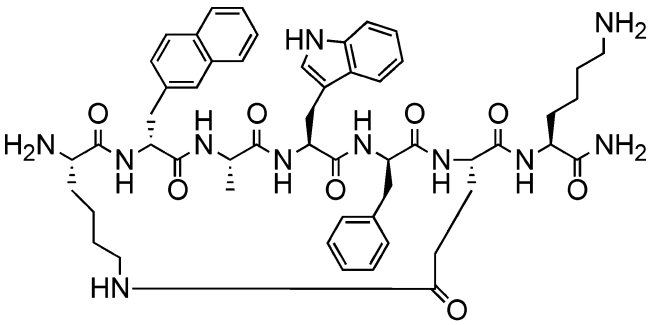
related peptide; Accession No. Q9UBU3) was recently identified as the endogenous 28-residue peptide (GSS*FLSPEHQRVQQRKESKKPPAKLQPR, S* *n*-octanoylated Ser3), which binds to the orphan growth hormone secretagogue receptor (GHS-R).[203] GHS-R is known to bind other artificial growth hormone secretagogues such as GHRP-6 and hexarelin.[204] Ghrelin is involved in the release of growth hormone (GH) from pituitary somatotrophs independently of GHRH and has been implicated in the regulation of food intake. It is present in oxyntic mucosa of the stomach and in other areas including the kidney, pituitary, small intestine, pancreas and brain, and in especially high concentrations in human plasma.[203,204] Ghrelin exhibits vasodilator activity in human vascular tissue, reverses endothelin-induced contractions, reduces arterial blood pressure and dilates blood vessels, and exerts a number of other beneficial cardiovascular properties that may be



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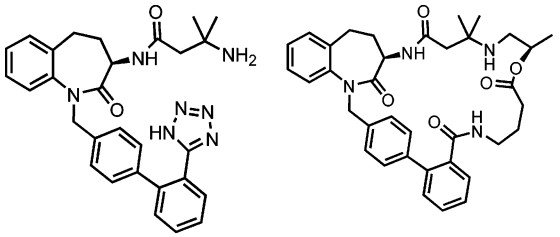
related to growth hormone-dependent mechanisms as it has strong growth hormone releasing properties.[205] The active core of Ghrelin is the first four residues (GSS*F), which are required for agonist potency[206] but alone exhibit little structure. A cyclic hexapeptide analogue of GHRP-2, **63**, shows 10-fold higher potency (EC_{50} 0.43 nM) than GHRP-6 (His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2). Its NMR structure shows "nested hairpin turns", initiated by D-Lys1 and Ala3.[207]

Molecular modeling studies of GHRP-6 have suggested that the active conformation of this peptide is a turn, which brings the head and tail of the peptide into close proximity to one another.[208] Overlays of this folded conformation of GHRP-6 and the low energy conformers of non-peptide L-692−429 **64** have suggested that **64** can mimic the major pharmacophoric elements of GHRP-6 in this turn conformation.[209] These studies gave rise to compound **65** (ED_{50} 21nM), a restrained analogue of **64** (ED_{50} 60nM),[210] which is three times more potent than **64**, consistent with this conformation being recognized by the receptor.



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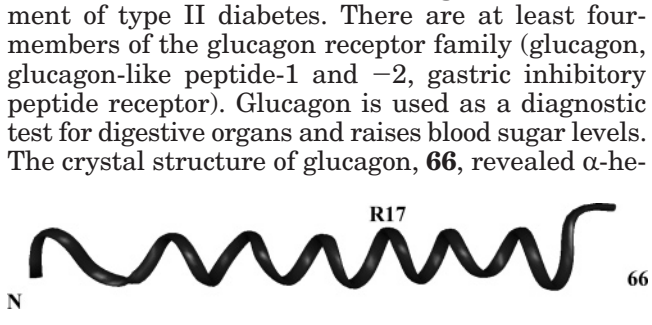
4.26. Glucagon

Glucagon[211] (Accession No. P01275; pdb 1bh0, 1gcn, 1kx6, 1nau) is a 29 amino acid peptide member of the glucagon superfamily that includes secretin, vasoactive intestinal peptide, and gastric inhibitory peptide (HSQGTFTSDYSKYLDSR$_{17}$R$_{18}$AQD$_{21}$FVQWLMNT). It was originally isolated as a hyperglycaemic factor originating in the pancreas. Glucagon counteracts insulin in the control of glucose metabolism mainly via stimulation of glycogenolysis and gluconeogenesis from lactate, pyruvate, glycerol and certain amino acids. Glucagon is the primary mediator of the overproduction of glucose and ketone bodies in diabetes and is an attractive target for the treatment of type II diabetes. There are at least four-members of the glucagon receptor family (glucagon, glucagon-like peptide-1 and −2, gastric inhibitory peptide receptor). Glucagon is used as a diagnostic test for digestive organs and raises blood sugar levels. The crystal structure of glucagon, **66**, revealed α-helicity,[212] supported in a lipid environment by NMR analysis,[213] and structure−function studies[214] illustrated the importance of charged residues and salt bridges at positions 17, 18, and 21. The crystal structure of [K17, K18, E21]-glucagon, a superagonist (K_i 0.3 nM, K_i 1.5 nM), also shows an increase in potency due to increased α-helicity. β-Turn and α-helical constraints have also been incorporated into the ligand, with those stabilizing β-turns having weaker affinity (IC_{50} 84 nM, **67**; 562 nM, **68**) than glucagon (IC_{50} 1.5 nM) or the respective reduced peptides, while α-helix inducing constraints have higher affinity (IC_{50} 0.2 nM, **69**; 0.24 nM, **70**).[215]



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H-S-Q-C-T-F-T-S-C-Y-S-K-Y-L-D-S-R-R-A-Q-D-F-VQ-W-L-M-N-T **67**

H-S-Q-G-T-F-T-S-D-Y-S-K-Y-C-D-S-R-R-C-Q-D-F-VQ-W-L-M-N-T **68**

H-S-Q-G-K-F-T-S-E-Y-S-K-Y-L-D-S-R-R-A-Q-D-F-VQ-W-L-M-N-T **69**

H-S-Q-G-T-F-T-S-D-Y-S-K-Y-L-D-S-K-R-A-Q-E-F-VQ-W-L-M-N-T **70**

4.27. Glucagon-like Peptides 1 and 2

Glucagon-like peptides 1 and 2[211,216,217] (GLP-1 and GLP-2; Accession No. P01275; pdb 1d0r, 1jrj) are 30 and 33 residues in length respectively, both of which are derived from the single precursor proglucagon (GLP-1, HAEGTFT$_7$SDVSSYLEGQAAKEFIAWLVK$_{28}$GR-NH_2; GLP-2, HADGSFSDEMNTILDNLAARDFINWLIQTKITD). They are members of the glucagon superfamily and are homologous to glucagon. GLP-1 is an important glucoincretin peptide hormone that can potentiate glucose-induced insulin secretion, stimulate the biological synthesis of insulin, and inhibit glucagon secretion. This makes it a potential drug lead for type II diabetes (diabetes melitus).[218] GLP-2 is involved in the regulation of the function and proliferation of gut epithelial mucosa. It has been shown to promote nutrient absorption and inhibit gastric acid secretion and gut motility. Structural studies indicate an α-helical structure for GLP-1, **71**, extending from residues 7 to 28 with a disordered N-terminal region.[219] Studies of exendin-4, **72**, a potent agonist of the GLP-1 receptor currently in late stage clinical trials for type 2 diabetes (exenatide),[220] has shown that the mutation from G16E (GLP-1 to Exendin-4) reduces flexibility and further stabilizes the helical conformation.[221]

71

72

4.28. Glucose-Dependent Insulinotropic Polypeptide

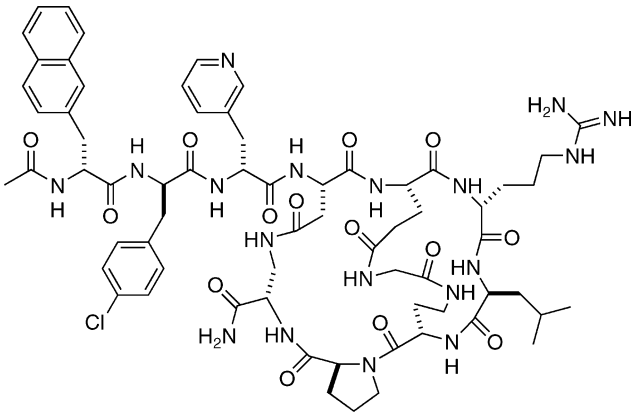
GIP (GIP; gastric inhibitory peptide; Accession No P09681) is classed as an incretin (regulator of insulin

secretion) and is a key intermediate, along with GLP-1, that stimulates insulin release in a glucose-dependent manner.[222] Abnormalities in GIP action has been linked to type II diabetes.[223] Recent studies also suggest GIP may link over-nutrition with obesity, linking GIP receptor antagonists to anti-obesity drugs.[224] It is a 42 residue peptide (YAEGTFISDY-SIAM$_{14}$DKIHQ$_{19}$QDFVNWLLAQK$_{30}$GKKNDWKH-NITQ) that targets the GIP receptor. The truncated peptide GIP(1−30)NH_2 has shown equivalent potency to its longer analogue.[225] Another study showed similar glucose lowering affects to GIP with GIP(1−14) or GIP(19−30)NH_2 thus supporting the developing hypothesis of two interaction sites on a single receptor.[222] Connection of these portions using helical peptide linkers gives 3−4-fold increased bioactivity and indicated an α-helical conformation for the C-terminal portion.[226]

4.29. Gonadotropin-Releasing Hormone

Gonadotropin-releasing hormones I and II (pEHW-SY$_5$GLR$_8$PG-NH_2 and pEHWSHGWYPG-NH_2; GnRH, gonadorelin and gonadoliberin; LHRH, luteinizing hormone-releasing hormone, luliberin; Accession No. GnRH I P01148; Accession No. GnRH II O43555) are 10 residue peptides which induce secretion of the pituitary gonadotropins follicle stimulating hormone (FSH) and luteinizing hormone (LH). GnRH II selectively binds to the GnRH type II receptor with the signaling mechanism distinctly different from that in the type I receptor. These two receptors provide the potential for differential FSH and LH secretion.[227] There are at least 6 peptidic compounds currently used in the clinic as GnRH agonists and antagonists. Goserelin,[228] Leuprorelin,[229,230] Buserelin,[231] and Triptorelin[232] are all agonists and are used in the treatment of prostate and breast cancer as well as endometriosis. Cetrolide[102,233] and Ganirelix[234] act as antagonists and are utilized in IVF treatments.[235]

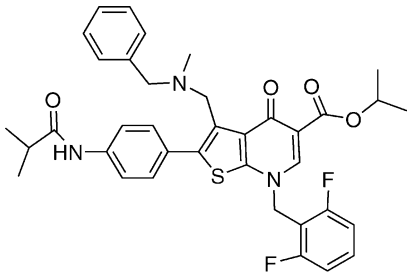
The NMR solution structure of Antide, **73**, an unnatural decapeptide antagonist (Ac-D-Nal-D-Cpa-D-Pal-Ser-Lys(Nic)-D-Lys(Nic)-Leu-Ilys-Pro-D-Ala-NH_2), in clinical trials for IVF, shows what the authors term a δ conformation which consists of a turn centered at residues 3 and 4 (D-Pal-Ser).[236] NMR studies of other GnRH analogues, with strong supporting evidence from computational studies, have indicated a type II β-turn conformation centered around residues YGLR.[237] Covalent constraints in the form of cyclic peptide analogues such as **74**, have



73

74

stabilized two type II and one type I′ β-turns resulting in high antagonist potency.[238] Based on mimicking this dominant β-turn conformation in Y$_5$GLR$_8$ of LHRH,[239,240] substituted 4-oxothieno[2,3-b]-pyridines were developed, leading eventually to various potent and orally active nonpeptidic antagonists (e.g. **75**) of the human LHRH receptor.[241,242]



75

Graphical representation of **73** kindly provided by Dr Giuseppe Digilio.[236]

4.30. Growth Hormone-Releasing Hormone

GHRH[243] (growth hormone-releasing factor, GRF; somatoliberin, somatocrinin, sermorelin; Accession No. P01286) is a 44-residue peptide produced in the hypothalamus and like its name suggests controls the release of growth hormone along with GH secretogues (GHS) and somatostatin. The hormone binds to a specific receptor (GHRH-R) found on somatotropes in the pituitary gland.[244] Abnormalities in this system can result in acromegaly, somatotroph hyperplasia, dwarfism, diabetes mellitus and hypoglycemia.[245] The fragment GHRH(1−29)-NH_2 has been shown to be the shortest fragment necessary for significant activity. The solution structures of two active poly(ethylene glycol) conjugates, Lys^{12}PEG-GRF(1−29), **76**, and Lys^{21}PEG-GRF(1−29), **77**, as well as hGRF(1−29)-NH_2 all show an overall α-helical conformation with some conformational flexibility located in the central region around residues 16−

18.[246] (The coordinates of **76** and **77** were kindly provided by Dr. Giuseppe Digilio.) Other structural

76

77

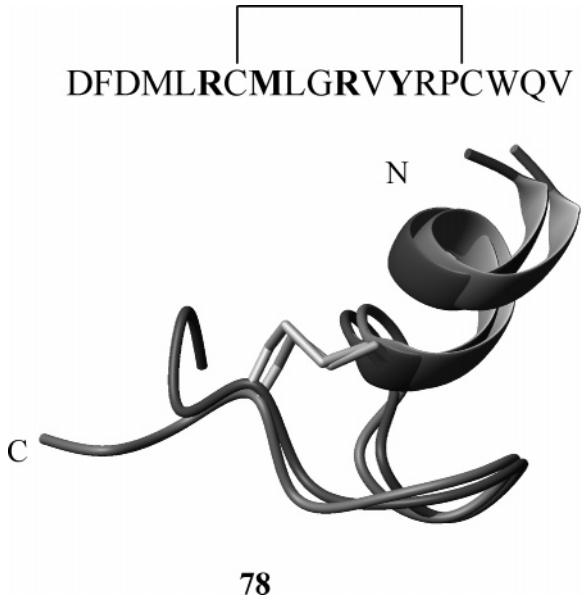
evidence for GHRH indicates two α-helical regions, residues 6−13 and 16−29, joined by a short segment of less well-defined structure.[247,248] SAR studies have shown that replacing Gly15 with helix stabilizing residues such as Ala and Aib increases potency of the peptide while helix destabilizing residues such as Sar abolish receptor binding affinity. Subsequent structural studies show increased helical conformations for the Ala and Aib derivatives and the opposite for the latter analogue. Side chain cyclization via lactam brides to stabilize the secondary structure has also produced higher potency analogues.[245,246]

4.31. Lutropin

Lutropin (luteinizing hormone, LH; Accession No. P01229) is a member of the glycoprotein hormone family and has the characteristic heterodimeric structure consisting of a conserved 92 residue α subunit and a unique 121 residue β subunit. Like the other members of the glycoprotein family, LH is associated with ovarian and testicular function and is secreted from the pituitary.[249] LH is the key hormone involved in the expulsion of the mature ovum and is regulated by GnRH and understood to also be regulated by oxytocin.[250] LH is also marketed as a recombinant product for use in the clinic. While the three-dimensional structure of LH has not been reported to date, it is very similar to that of hCG (gonadotropin) and FSH (follitropin) discussed above.

4.32. Melanin-Concentrating Hormone

Human melanin-concentrating hormone (hMCH; Precursor Accession No. P20382) is a 19 residue peptide ($DFDMLR_6C_7M_8L_9GR_{11}VY_{13}RPC_{16}WQV$) containing a 10 residue disulfide bridged cyclic peptide.[251] hMCH is involved in regulating food intake and obesity, hypothalamic/pituitary/adrenal activity, and energy balance. Two MCH-specific GPCRs are known, hMCH-2R having about 38% sequence identity with hMCH-1R. Mimics of hMCH and inhibitors appear to influence feeding behavior in rodents. Structure−activity studies suggest that residues R6, M8, R11, Y13 and the disulfide bond are important for activity,[252] and that the cyclic fragment hMCH6−16 alone has comparable activity to full length peptide for both receptors.[253] The structure of hMCH, **78**, has been determined[251] in water and 1:1 MeCN/



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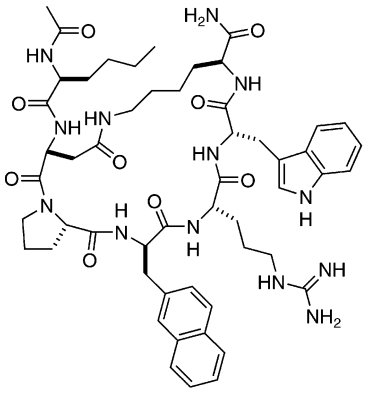
water by NMR spectroscopy and shows an N-terminal two-turn helix identified between residues 2 and 9, a stable fold between cysteine residues 7 and 16, and a well defined turn or loop within the cycle. (The coordinates and drawing of **78** were kindly provided by Dr. Michele Saviano.) These data strongly support the importance of a turn region for bioactivity.

4.33. Melanocortins and Corticotropin

The family of melanocortin peptides (melanotropins; MSH, α,β,γ-melanocyte stimulating hormones; Precursor Accession No. P01189) includes the three melanocyte-stimulating hormones α-MSH [$SYSMEH_6$-FRW_9GKPV], β-MSH [DEGPYRMEHFRWGSPPKD], and γ-MSH [YVMGHFRWDRF] and the adrenocorticotropin hormone (ACTH; Accession No. P01189) [SYSMEHFRWGKPVGKKRRPVKVYPNGAEDESAEAFPLEF] as well as Agouti and AGRP discussed above. All are derived from the corticotropin-lipotropin precursor or Pro-opiomelanocortin (POMC). These peptides are involved in a wide range of physiological functions, including pigmentation, steroidogenesis, sexual function, analgesia, inflammation, immunomodulation, cardiovascular regulation, neuromuscular regeneration and others. All four hormones bind to the five known melanocortin receptors, termed MC1 to MC5.[254] Studies of α-MSH and analogues showed a type I β-turn around the tetrapeptide, H6-F7-R8-W9, conserved in all natural melanocortins, which is important for biological activity.[255,256] Structure−activity studies of cyclic lactam analogues **79** (IC_{50}(*h*MC3) 0.19 nM) and **80** (IC_{50}(*h*MC4) 0.5 nM) led to the discovery of potent and selective antagonists for *h*MC3 and *h*MC4 which also adopt a β-turn conformation.[257−261] A β-turn is also mimicked by three side-chains of amino acids Phe, Arg, Trp (found



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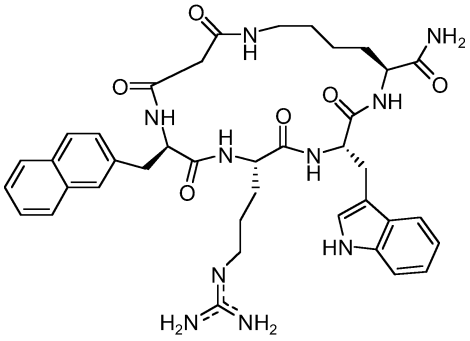

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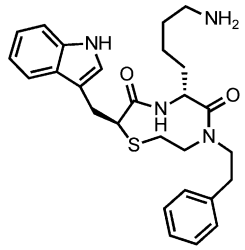
to be essential for activity in the native ligand) in **81** and **82**.[262] Compound **81** was a selective agonist (EC_{50} 63.4 μM) of the mouse MC1 receptor, but the activity of **82** (EC_{50} 42.5 μM) with no basic residue challenges the importance of Arg 8 of the native ligand for receptor activation.



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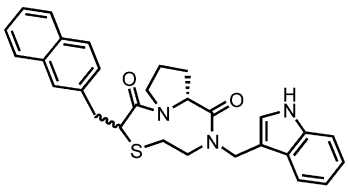

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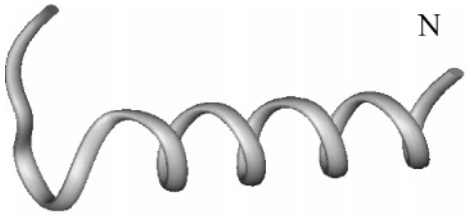
4.34. Motilin

Motilin (Accession No. P12872; pdb 1lbj) is a 22 residue peptide ($FVP_3IFT_6YGE_9LQRMQEKERNK_{20}$-GQ), the porcine sequence first characterized in 1973.[263] It is expressed in the gastrointestinal tract, particularly the small intestine, and stimulates gastric motility.[264] Only recently has the orphan receptor, GPR38 (MTL-R), been identified as the motilin receptor.[265] Erythromycin A, a macrolide antibiotic used for treating gram-positive bacterial infections, is understood to mediate its action through the MTL-R. A decomposition product of Erythromycin A is the causative agent of gastrointestinal distress.[264]

Numerous structural studies of motilin have reported a turn-like conformation at the N-terminus and a central α-helical core region.[266,267] A recent NMR solution structure in micelles shows a classical type I β-turn (Pro3-Thr6), followed by an ordered α-helix from Glu9-Lys20, **83**.[268] Based on the putative N-terminal turn structure of motilin, cyclic peptides were designed in order to mimic the conformation of motilin (1−7) (IC_{50} 4 μM, EC_{50} 135μM). Compound **84** was found to have similar affinity for MTL-R (IC_{50} 4 μM, pA_2 4.34) as the linear heptapeptide but was an antagonist.[269] This also suggests that an N-terminal turn might bind to the receptor.



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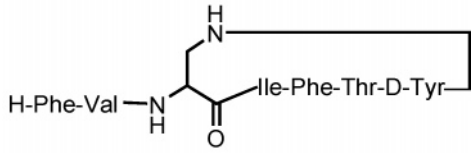

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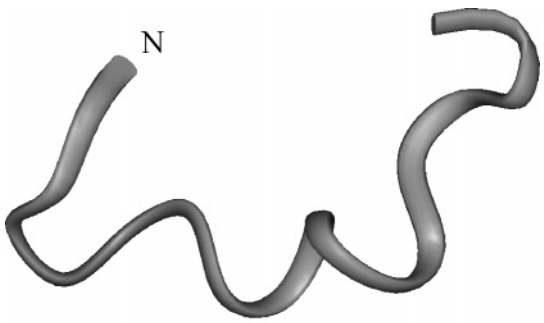
4.35. Neuropeptide AF and Neuropeptide FF

Neuropeptide AF ($AGEGLN_6SQFWSLAA_{14}PQRF$-$NH_2$) belongs to the peptide class FMRF-amide related peptides, as does the closely related neuropeptide FF ($FLFQPQRF$-NH_2). These peptides (NPAF, NPFF, FMRFamide related peptide; Accession No. O15130) are found widely within the central nervous system and have been linked with different functions, the most prominent being pain modulating and antiopiate effects.[270,271] The GPCR for these peptides is orphan receptor HLWAR77.[272,273] The structure of NPAF has been determined in two solvent systems and found to be primarily α-helical



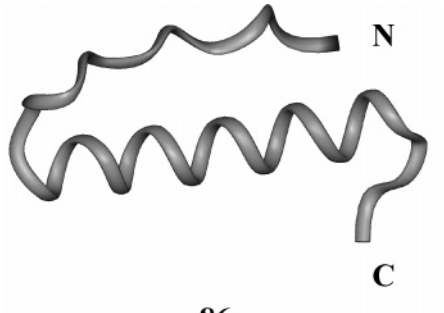
85

within the central region of the peptide from Asn6 to Ala14 in both solvents, **85**.[271] (The coordinates of **85** were kindly provided by Dr. George Kotovych.[271])

4.36. Neuropeptide Y, Peptide YY, and Pancreatic Polypeptide

Neuropeptide Y (NPY;[274] melanostatin, melanotropin-release-inhibiting factor; Precursor Accession No. P01303 (human); P29949 (frog); pdb 1f8p, 1fvn, 1d0w, 1d1e, 1d1f, 1qfa, 1ron) [YPSKPDNPGEDAPAEDMA-RYYSAL$_{24}$RHYI$_{28}$NLI$_{31}$T$_{32}$RQRY$_{36}$-NH$_2$] is a 36 residue peptide that is predominantly released by neurons as a neurotransmitter. Peptide YY (PYY; peptide tyrosine tyrosine; Precursor Accession No. P10082 (human)) [YPIKPEAPGEDASPEELNRYYASLRHY-LNLVTRQRY-NH$_2$] is released by intestinal endocrine cells, while pancreatic polypeptide (PP; Precursor Accession No. P01298 (human)) [A$_1$PLEPVYP$_8$GD-NATP$_{14}$EQMAQYAADLRRYINML$_{31}$TRPRY-NH$_2$] is found in pancreatic cells that do not store insulin, glucagon, or somatostatin. Physiological effects attributed to these peptides include stimulation of food intake and inhibition of anxiety and neurotransmitter release in the CNS and the periphery.[275] Six NPY receptors have been cloned, designated Y1, Y2, Y4, Y5, Y6, with evidence for putative receptor Y3 still being circumstantial. NPY and PYY show high agonist potency for Y1 (0.2 nM, 0.7 nM), Y2 (0.7 nM) and Y6, while PP shows agonist potency $<1\ \mu$M only for Y4 and Y5.[275]

The X-ray crystal structure of avian-PP, **86**, consists of an extended polyproline-like helix (1−8) and an α-helix (14−31) connected by a β-turn. NMR solution structures are also available for all three peptides.[276−280] The C-terminal helical region of NPY is responsible for biological activity.[281,282] Mimetics of the neuropeptide Y helical region are potent antagonists of NPY, and exhibit anti-hypertensive and neuromodulatory activity. An example is the helix in the cyclic NPY analogue **87**, Ac[A24,K28,-L31, E32]NPY(24−36), its helical structure being stabilized by a lactam bridge.[283]



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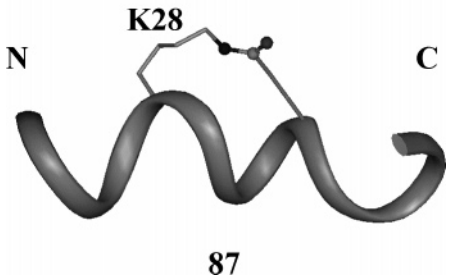

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Nonapeptides **88a** and **88b**, analogues of the C-terminus of peptide Y, further suggest that either α-helical or β-turn conformations are recognized by NPY receptors. The C-terminal amide **88a** shows

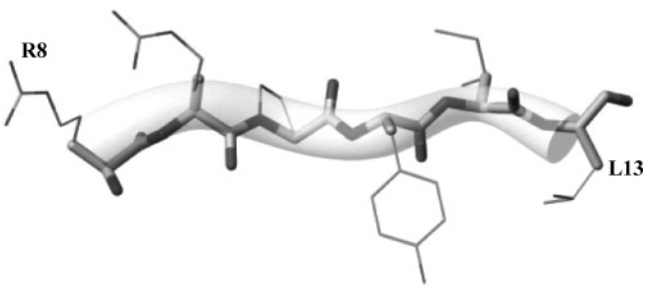
H-Ile-Asn-Pro-Ile-Tyr-Arg-Leu-Arg-Tyr-X

88 (**a,** X=NH$_2$; **b,** X=OMe)

high affinity at three receptor subtypes (Y1, $K_i = 5$ nM; Y2, K_i 11.3 nM; Y4, K_i 5.8 nM; Y5, $K_i > 1000$) and its ^{1}H NMR structure displays a β-turn centered at Asn-Pro-Ile-Tyr. The methyl ester derivative **88b** (Y1, K_i 25.7 nM; Y2, K_i 1420 nM; Y4, K_i 2403 nM; Y5, K_i 7100 nM) is specific to the Y1 receptor and its structure consists of two β-turns, one centered at Asn-Pro-Ile-Tyr and the other at Ile-Tyr-Arg-Leu in TFE.[284,285]

4.37. Neurotensin and Neuromedin N

Neurotensin (Precursor Accession No. P30990 (human)) [QLYENKPR$_8$RPYIL$_{13}$] and its analogue neuromedin N (Precursor Accession No. P30990 (human)) [IPYIL] are regulatory peptides, found mainly in the gut where they stimulate pancreatic and biliary secretions,[286] and also in the brain where they are antipsychotic.[287] Neurotensin binds to three receptors (NTR-1, -2, -3) found widely in both the central and peripheral nervous system,[288] while neuromedin N only binds to the latter. NMR studies of neurotensin in aqueous[289] and SDS[290] media showed no defined structure. Structure−function studies indicated that residues 8−13 are sufficient to elicit binding and activation of the neurotensin receptor.[291,292] Recent molecular modeling and site-directed mutagenesis studies suggest that NT(8−13) adopts a type I β-turn in the receptor-bound conformation,[293] while a solid-state structure shows a β-strand conformation, **89**, for the C-terminal fragment NT(8−13) bound to the receptor.[294] (The structure of **89** is reprinted with permission from ref 294. Copyright 2003 National Academy of Sciences.) To our knowledge this is one of only a few receptor-bound structures of a GPCR ligand, and it should be emphasized that the result (a β-strand or extended ligand conformation) contradicts the general principle being put forward in this review, namely that GPCRs tend to recognize turn conformations.



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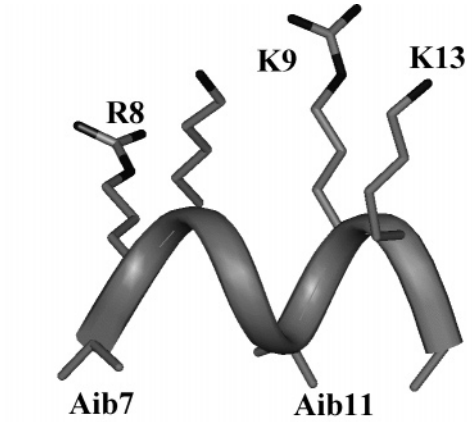
4.38. Nociceptin

Nociceptin (Orphan FQ; Accession No. Q13519) is a 17-residue peptide (FGGFTGAR$_8$K$_9$SAR$_{12}$K$_{13}$LANQ), similar in sequence to Dynorphin A, which interacts

with the opioid receptor like receptor 1 (ORLR-1). Despite this similarity, it displays no opioid activity. Activation of ORLR-1 by Nociceptin involves many physiological functions including hyperalgesia and anti-opioid effects in the brain and analgesia in the spinal cord.[295] Like other opioids the N-terminal residues (FGGF) make up the message domain. Like Dynorphin, Nociceptin shows little tendency to form a defined conformation in several solvents.[296] However nociceptin analogues containing the α-helix-inducing constraint Aib (Aib7, Aib11) are very potent ORLR1 agonists (Ki 0.05 nM, EC_{50} 0.08 nM),[297] supporting the notion that turns, possibly α-turns, **90**, are important for receptor binding. Hexapetide **91**, incorporating a β-turn inducing constraint at its center, has also been shown to be a potent and selective antagonist at the ORL1 receptor.[298]



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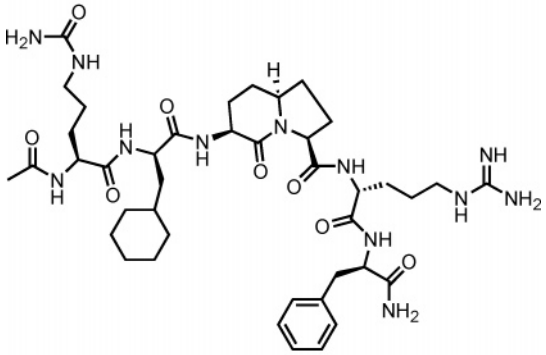

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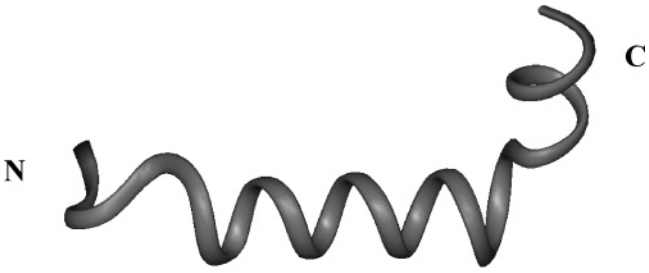
4.39. Orexin A and B

Neuropeptides Orexin A [pEPLPDC$_6$C$_7$RQKTC$_{12}$-SC$_{14}$RLYEL$_{19}$L$_{20}$HGAGNH$_{26}$AAGILTL$_{33}$-NH$_2$; disulfides 6−12, 7−14] and Orexin B (hypocretin 1 and hypocretin 2; Accession No. O43612 (human); pdb 1cq0) [RSGPPGL$_7$QGRLQRLLQASG$_{19}$NHAA$_{23}$-GILTM-NH$_2$], formed through proteolytic cleavage of prepro-orexin, bind two receptors OX1 and OX2 with little selectivity. They are involved in feeding behavior and energy homeostasis and are also understood to be involved the sleep/wake cycle.[299,300] The NMR solution structures of Orexin B in water, **92**, and in micelles, show two α-helices, L7-G19 and A23-M28. N-Terminal deletion studies indicate a minimum 19 residue C-terminal sequence of Orexin A is required for agonism.[301,302] Alanine scans revealed that residues H26-L33 are important for binding to OX1, and L19 and L20 are important for receptor affinity and/or conformation change. [Ala11, D-Leu15]-Orexin B is an agonist which is 400-fold more selective for the OX2 receptor,[303] where D−L15 corresponds to L20 of Orexin A, supporting the importance of C-terminal α-turn(s) for receptor binding.



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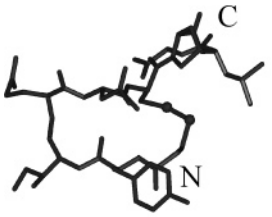
4.40. Oxytocin

Oxytocin (α-hypophamine, oxytocic hormone; Accession No. P01178 (human); pdb 1xy1, 1xy2, 1npo; CCD Code: DUPFAV) is a disulfide bridged nonapeptide pituitary hormone ($C_1Y_2IQN_5C_6PLG$-NH_2) synthesized in neurons in the hypothalamus. It is structurally related to vasopressin, which has only two differing residues I3F and L8R, and by binding to the oxytocin receptor (OT-R) it promotes uterine contraction, milk ejection, and behavioral functions.[304] Oxytocin has also been associated with the regulation of Lutropin (LH).[250] Crystal structures for the oxytocin deamino derivative, **93**,[305] and a complex with neurophysin,[304] a protein involved with hormone transport, both show oxytocin in a β-turn conformation (type II or III) at residues 2−5, **94**. These and numerous other cyclic antagonist analogues strongly suggest that the oxytocin receptor recognizes a β-turn conformation for oxytocin. The oxytocin antagonist Atosiban (Tractocile, **95**) became available in 2003



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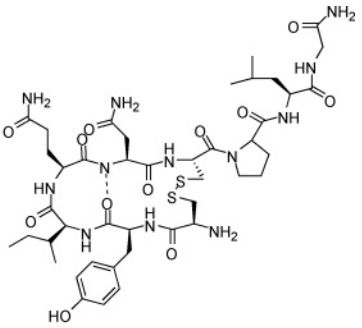

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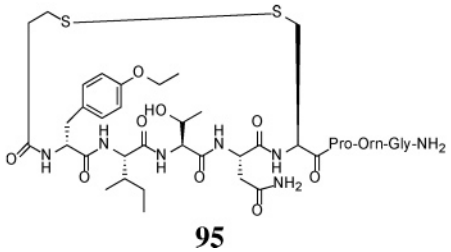

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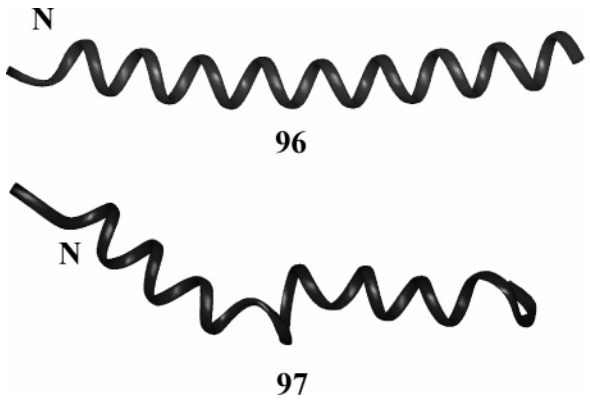
for delaying pre-term births,[306] while oxytocin itself is used clinically to induce labor and control bleeding.

4.41. Parathyroid Hormone

Parathyroid hormone (parathyrin, PTH; parathormone; Accession No. P01270; pdb 1bwx, 1et1, 1et2, 1fvy, 1hph, 1hpy, 1hth, 1zwa, 1zwb, 1zwc, 1zwd, 1zwe, 1zwf, 1zwg) is an 84 residue peptide secreted from the parathyroid gland. It is the major regulator of calcium homeostasis and acts via the kidney, bone and intestine. The predominant form isolated from human plasma is the N-terminal fragment hPTH-(1−37).[307] The hormone targets two receptors, PTHR1[308] and PTHR2.[309,310] Currently, Forteo (teriparatide), recombinant human parathyroid hormone (1−34), is used as a treatment for osteoporosis however it has been shown to induce osteosarcoma in rats.[311] More than a dozen structures have been published for this peptide. The crystal structure of hPTH(1−34), **96**, shows a slightly bent but well defined α-helix.[312] Solution structure analyses indicate a short N-terminal and a longer C-terminal helix as well as a loop region from residue 14−17, **97**.[313,314]



96

97

Conformationally constrained parathyroid hormone analogues incorporating lactam bridges between residues 18−22 (EC_{50} 0.29 nM), 18−22 and 26−30 (EC_{50} 0.13 nM), 13−17, 18−22 and 26−30 (EC_{50} 0.14 nM), all showed increased agonist activity compared with the parent hPTH(1−31) (EC_{50} 4.7 nM) and are shown to be helical.[315] Activity studies on the N-terminal fragment of PTH(1−14) revealed that a significant increase in signaling potency could be achieved by modifying the sequence, [$Ala^{3,10,12}$, Arg^{11}, Trp^{14}] rPTH(1−14)NH_2 (EC_{50} 0.6 nM) showing a 200-fold improvement over the native 14 residue peptide.[316] Recently NMR studies on (*i*-*i*+4) stabilized lactam derivatives of these peptides have shown that they exist in very well-defined helical conformations in DPC micelles, supporting the notion that an α-helix is the receptor binding conformation at this receptor.[317]

4.42. Pituitary Adenylate Cyclase Activating Peptide

PACAP (Accession No. P18509; pdb 1gea) (HSD_3-$GIFT_7DSYSR_{12}YRKQMAVKK_{21}YLA_{24}AVL_{27}GKRY$-KQRVKNK-$NH_2$), a 38-residue peptide hormone, was first isolated from ovine hypothalamic extracts based on its ability to stimulate cAMP in pituitary cells. PACAP has been found to have multiple effects including control of neurotransmitter release, increase of insulin, vaso- and broncho-dilation as well as the stimulation of cell multiplication and differentiation.[318] The shortened peptide, PACAP-27, truncated and amidated at Leu 27, is also capable of activity indicating the biologically active region. PACAP binds to three VPAC receptors, VPAC1 and VPAC2 which also bind VIP with similar affinity, as well as PAC1, the PACAP specific receptor.[319] Structural evidence has shown a core helical region, residues 12−24, with a turn-like structure at the N-terminus.[320,321]

A detailed structural analysis in 2001 presented the conformation of the full agonist, PACAP(1−21)-NH_2, **98**, bound to the PAC1 receptor and compared

98

this to the conformation of PACAP-27 in a micelle environment. The peptide forms a β-coil structure from residues 3−7, made up of consecutive type II′ (3−6) and type I (4−7) β-turns, creating an important hydrophobic patch necessary for receptor binding. The remaining C-terminal region, residues 8−21, forms an α-helix.[322] The difference between this conformation and the micelle bound peptide is limited to the seven N-terminal residues, where the micelle bound peptide was helical from residue 5−27. The C-terminal region has been shown to be important for binding but does not facilitate agonism, as truncated peptides act as a competitive antagonists.[323] This evidence strongly supports the recognition and activation of receptors by hormones in a turn conformation.

4.43. Prolactin-Releasing Peptide

The prolactin releasing peptides PrRP20 (TP_2DIN_5-$PAWYA_{10}$SRGIRPVGRF) and PrRP31 (SRTHRHS-MEIRTPDINPAWYASRGIRPVGRF) (Accession No. P81277) are two novel peptides derived from a common precursor isolated using an orphan receptor, Hgr3.[324] Their biological function was identified as the release of prolactin from pituitary cells, however a suggestion has been made of a potential role in the regulation of the CNS.[325,326] Structural studies have been carried out on PrRP20 and reveal an amphipathic α-helical structure for the 10 C-terminal residues.[327] There is also a weak tendency for the N-terminal residues Pro2-Asn5 to form a β-turn-like structure.

4.44. Protease-Activated Receptors

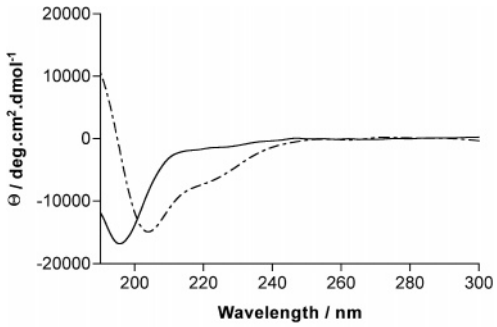
Protease activated receptors[328] (PARs-1,2,3,4; Accession Nos.: PAR1, P25116; PAR2, P55085; PAR3, O00254; PAR4, Q96RI0) are unusual GPCRs, activated by their own N-termini after removal of ~30

residues by serine proteases. The newly exposed N-terminus folds back and activates the GPCR. Four PAR receptors are known, each having a unique cleavage site, **99**, and a unique N-terminus that

```
PAR-1   ATNATLDPR↓SFLLRNPN
PAR-2   GTNRSSKGR↓SLIGKVDG
PAR-3   LAKPTLPIK↓TFRGAPPN
PAR-4       LPAPR↓GYPGQVCA
```

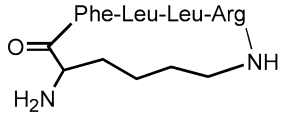
99

elicits distinctive pharmacology.[328] Short synthetic peptides corresponding to N-termini (**99**, bold sequences) can fully activate their respective receptors in the absence of proteases. The most studied receptor is PAR-1, activated by thrombin, where PAR-1 antagonists regulate platelet aggregation, clotting, wound healing, cardiovascular disorders, and cancer (angiogenesis). PAR-2 is activated by trypsin, tryptase, and other serine proteases, but not thrombin, and appears to be a target for inflammatory and proliferative diseases. PAR-2 peptides such as SLIGKV have recently been found to adopt a turn structure in non-aqueous solutions, as suggested by CD spectra in the membrane simulating solvent SDS (below, dashed line), and by NMR structural data, **100**, in the aprotic solvent DMSO-d_6 (Beyer, Flanagan, Stoermer, Fairlie. Unpublished).



100

Cyclic analogues **101**[329] and **102**[330]of SFLLR have been found to be equipotent in a functional assay for PAR-1. NMR solution structures suggest turn conformations, although an extended β-sheet structure is suggested from molecular modeling of linear analogues of SFLLR.[331]



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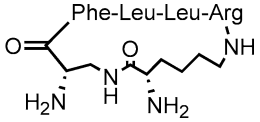

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4.45. Relaxins

Relaxins (RLX: relaxin 1, Accession No. P04808; relaxin 2, Accession No. P04090, pdb 6rlx; relaxin 3, Accession No. Q8WXF3) are unusual peptide hormones, which consist of two peptide chains linked by disulfide bonds. Their receptors have only recently been identified as the orphan leucine-rich repeat-containing GPCRs LGR7 and LGR8.[332] Relaxin itself has long been known as a reproductive hormone but its pleiotropic activities are now becoming more evident. RLX2 is the major stored and circulating form in humans, and this insulin-like growth factor is produced by the ovaries or placenta in pregnancy, facilitating growth of the cervix and uterus in preparation for birth. It is produced in the prostrate gland of males and is thought to increase the motility of sperm. The hormone also affects cardiovascular function and the regulation of blood pressure as well as stimulating the release of vasopressin and oxytocin from the posterior pituitary and is associated with the activation of the brain renin-angiotensin system.[333,334] The crystal structure of relaxin 2 (**103**) shows 2 disulfide linked chains of relaxin, (A chain, light, underneath; B chain, dark, above).[335] Several studies have shown that the two conserved arginine residues in the helical region of the B chain, R13 and R17, are important for receptor binding (see alignment below, relaxin 2 numbering).[336]

A Chain

```
Relaxin 1:    PYVALFEKCCLIGCTKRSLAKYC
Relaxin 2:  QLYSALANKCCHVGCTKRSLARFC
Relaxin 3:  DVLAGLSSSCCKWGCSKSEISSLC
```

B Chain

```
Relaxin 1:  VAAKWKDDVIKLCGRELVRAQIAICGMSTWS
Relaxin 2:    DSWMEEVIKLCGRELVRAQIAICGMSTWS
Relaxin 3     RAAPYGVRLCGREFIRAVIFTCGGSRW
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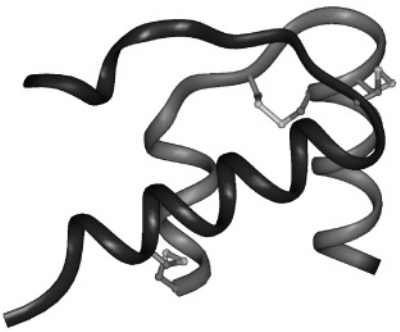


103

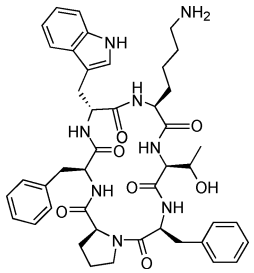
4.46. Secretin

Secretin (Accession No. P09683),[337] a 27-residue peptide hormone, $HSDGTFT_7SELSRL_{13}REGA_{17}$-$RLQRLLQG_{25}LV\text{-}NH_2$, was first discussed in 1902[338] as a messenger that mediates secretion of pancreatic juices. Secretin is also a member of the glucagon family of peptide hormones and their receptors (also called the secretin family) with the secretin receptor being the first member of class II to be cloned.[339] The

peptide is found in S cells in the upper small intestine and its major roles are stimulation of pancreatic and biliary bicarbonate and water secretion as well as inhibition of gastric emptying. Recombinant secretin has been approved for diagnosis of pancreatic exocrine dysfunction. Structure−activity analyses of the peptides from various species have shown the N-terminus involved in receptor selectivity, while the C-terminal region confers affinity and bioactivity.[211] This is consistent with the two step binding mode of activation within the glucagon/secretin family. Structural evidence for secretin provides evidence of two distinct helical regions (residues 7−13 and 17−25) connected by a half-turn (residues 14−16).[340] This structure is common within the hormone family.

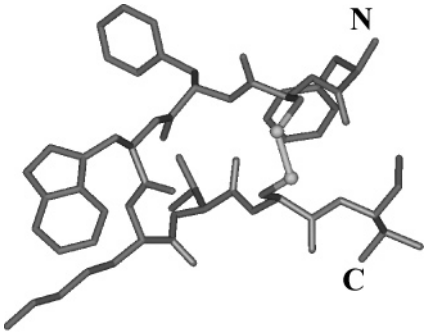
4.47. Somatostatin

Somatostatin (somatotropin release-inhibiting factor, SRIF; Precursor Accession No. P01166 (human)) is a 14 amino acid cyclic peptide (AG[C_3KNFFW$_8$-KTFTSC$_{14}$]) expressed in the CNS, GI tract and endocrine tissues. It plays an important role in neurotransmission and secretion, and may also control cell proliferation in normal and tumor tissues. Somatostatin binds to five GPCRs, sstR1−5 (IC_{50}-(sst2) 0.2 nM).[341] In cyclic hexapeptide analogues of somatostatin, **104**,[342] the central tetrapeptide Phe7-



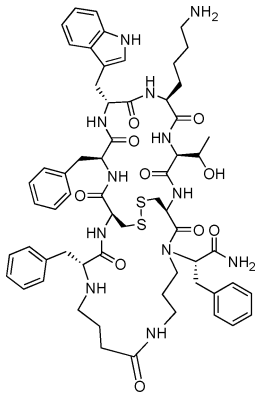
104

D-Trp8-Lys9-Thr10 contains the somatostatin pharmacophore. A potent analogue of somatostatin is the octapeptide sandostatin, currently used in the clinic (D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Tho, octreotide, IC_{50}(sst2) 0.6 nM) and the subject of extensive structural studies including NMR, **105**,[343] and X-ray



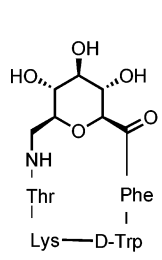
105

analysis.[344] These studies showed sandostatin to adopt a type II or type II′ β-turn conformation centered at D-Trp8-Lys9. A bicyclic compound **106** (IC_{50}(sst2) 3.7 nM) stabilizing a type II β-turn,[345] and a cyclic analogue (IC_{50}(sst2) 5.2 nM)[346] of somatostatin possessing a non-classical turn, were highly active. Further evidence of β-turn recognition is



106

provided by ligands **107** and **108**. The peptidomimetic **107** was designed from cyclic analogue **104** by replacing Phe-Pro with a glucose scaffold. The Phe-D-Trp-Lys-Thr portion adopted a type II′ β-turn by NMR studies and the compound was active in a radioligand binding assay (IC_{50} 150 nM).[347] A glucose scaffold has also been used to present amino acid side-chains of **104** in a β-turn conformation in **108** (IC_{50} 1.3 μM).[348]



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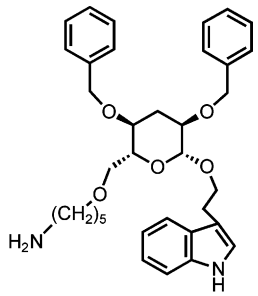
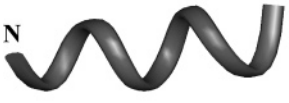


108

4.48. Tachykinins: Substance P, Neurokinin A, and Neurokinin B

Over 40 tachykinins are known for 3 receptor subtypes (NK_1, NK_2, NK_3). These receptors mediate neurotransmission in the CNS and periphery, smooth muscle contraction, immunological/inflammatory and other responses. Tachykinins isolated from mammalian tissues include substance P (Precursor Accession No. P20366 (human)) [RPKPQQFFGLM-NH_2], neurokinin A (α-neurokinin; substance K; neuromedin L; Precursor Accession No. P20366 (human); pdb 1n6t) [HKTDSFVGLM-NH_2] found in two elongated forms, K and γ,[349] and neurokinin B (β-neurokinin; neuromedin K; Precursor Accession No. Q9UHF0 (human); pdb 1mxq, 1my) [DMHDFFVGLM-NH_2]. NMR studies of substance P in micelles,[350,351]



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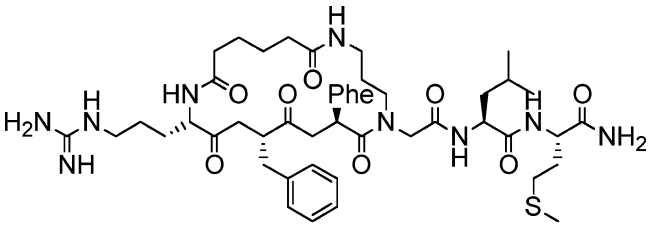
110

and neurokinins A[352] and B[353] in micelles and SDS respectively, suggest mid-sequence helical structure, thought to be important for receptor binding. Structures of non-mammalian tachykinins such as eledoisin, **109**, and kassinin, **110**, show helical turns in SDS.[354,355] Turns have been stabilized in various cyclic analogues of substance P, such as **111**, that are highly selective for NK-1 over NK-2 receptors.[356] Other cyclic peptides, such as bicyclic glycopeptide nepadutant, **112**,[357] are selective NK-2 antagonists (K_i 3 nM),[358] while pyrrolidine-based Trp-Phe mimetics were developed based on stabilizing βI/βII turn conformations.[359] A β-turn conformation of sugar



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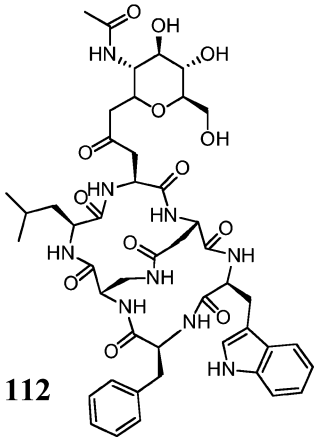
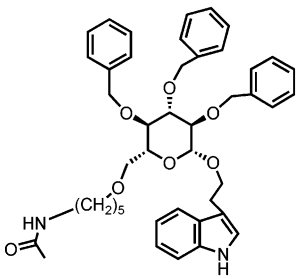


112

derivative **113**, has also been shown to have high affinity (IC_{50} 60 nM) for NK receptors.[360] Compound **113** is specific for NK receptors and does not interact with at least 50 different receptors, including somatostatin and β-adrenergic receptors. Interestingly this compound was a lead to the first cyclic peptide antagonist (**114**) of the NK1 receptor (IC_{50} 2 nM).[361] A small molecule NK1 antagonist, Aprepitant, is available for treating nausea caused by chemotherapy.



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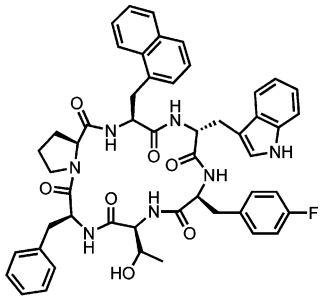


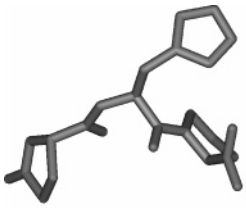
114

4.49. Thyrotropin

Thyroid-stimulating hormone (TSH; Accession No. P01222) is the fourth member of the glycoprotein hormone family (hCG, FSH, LH and TSH). The protein consists of an α and β subunit, the α subunit of 116 residues is identical to hCG, FSH and LH, while the β subunit of 112 residues is unique in sequence but adopts the characteristic cysteine knot of this family. TSH targets the TSH receptor and its major function is to maintain synthesis and secretion of thyroid hormones such as L-3,5,3′-triiodothyronine (T3) and L-thyroxine (T4).[362] Reduced TSH activity causes hypothalamic-pituitary hypothyroidism, tumors that secrete high levels of TSH cause hyperthyroidism,[363] and antibody agonists of TSH receptor have been found in patients with Graves disease.[364] Recombinant hTSH is a diagnostic and used in managing thyroid cancer. Like hCG, FSH and LH, it would appear that loops are involved in receptor activation.

4.50. Thyrotropin-Releasing Hormone

Thyrotropin releasing hormone (thyroliberin, TRH; protirelin; Accession No. P20396) is a three residue hormone (L-paraglutamyl-L-histidyl-L-prolinamide) produced in the hyperthalamus. It stimulates the synthesis and secretion of TSH[365,366]and is also associated with the release of other hormones including insulin, and with neurotransmitter and neuromodulatory functions.[367] Effects are mediated through binding to the TRH receptor. TRH or Protirelin (Relefact TRH) is used in the clinic as a diagnostic to test the response of the anterior pituitary. TRH exhibits its effects by binding to the TRH receptor. Numerous TRH analogues[368−371] have been studied as peptidomimetics of putative turn or Y-shaped conformations[372−374] of the native ligand **115**, such as constrained analogues **116**.[370,371] Epimers of **116**



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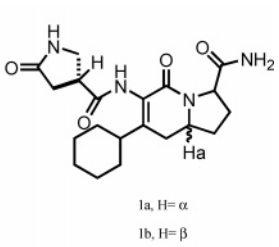


116

displayed substantially different binding and activation of the TRH-R (**116a**, K_i 290 μM, EC_{50} 44 μM; **116b**, K_i 1.9 μM, EC_{50} 91 nM), were critically dependent on stereochemistry of the turn-mimicking bicyclic ring, and show 3x better affinity for TRH-R than the less restrained cyclohexylalanine derivative of TRH (CyclohexyAla^2-TRH, K_i 6.5 μM, EC_{50} 430 nM).

4.51. Tuberoinfundibular Peptide

Tuberoinfundibular peptide (TIP39; Accession No. Q96A98) is a 39 residue peptide (SLALA$_5$DDAAFRERARLLAALER$_{22}$RHWL$_{26}$NSYMHKLLV$_{35}$LDAP) purified from the hypothalamus. It has been identi-

fied as the endogenous ligand for the parathyroid hormone receptor 2 (PTHR2), but lacks affinity for PTHR1.[310,375,376] PTHR2 is highly expressed in the central nervous system[375] and the interaction is thought to be involved in pain and pituitary function. The structure of TIP39,[377] **117**, has recently been characterized in a lipid environment and consists of two α-helical regions from A5 to R22 and L26 to V35. The N-terminal helix shares a high sequence as well as structural homology with PTH. (The coordinates of **117** were kindly provided by Dr. Dale Mierke.[377])

117

4.52. Urocortin, Urocortin II, and Urocortin III

Urocortins [Urocortin: Ucn; 40 residue peptide; Accession No. P55089. Urocortin II: UcnII; 41 residue peptide; stresscopin-related peptide; urocortin-related peptide; Accession No. Q96RP3. Urocortin III: UcnIII; 38 residue peptide; stresscopin; Accession No. Q969E3.] are members of the structurally homologous CRF (corticotropin releasing factor) family, which also includes amphibian sauvagine and fish urotensin I.[378,379] These peptide hormones target the CRF receptors with varying selectivity. CRF possesses mild selectivity for CRF1 over CRF2, and Ucn binds to both CRF1 and CRF2 whereas UcnII and UcnIII (and the human homologues stresscopin/stresscopin-related peptide) selectively bind to CRF2 receptors (2α, β, γ). Based on their homology to CRF,

hCrf SEEPPISLDLTF$_{12}$HLLREVLEM$_{21}$ARAEQLAQQ AHS NRKLMEII-NH$_2$

Ucn DNPSLSIDLTF$_{12}$HLLRTLLEL$_{21}$ARTQSQRER AEQ NRIIFDSV

Ucn II IVLSLDVPI$_{12}$GLLQILLEQ$_{21}$ARARAAREQ ATT NARILARVGHC

Ucn III FTLSLDVPT$_{12}$NIMNLLFNI$_{21}$AKAKNLRAQ AAA NAHLMAQI

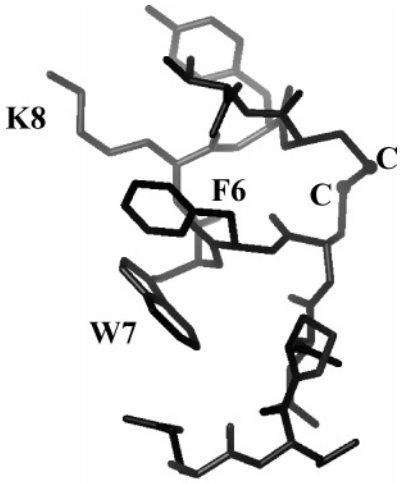
Ast* f$_{12}$HLLREVLEX$_{21}$ARAEQLAQE*AHK*NRKLXEI

X = Nle,

these peptide hormones are thought to maintain a helical conformation. Novel antagonists were derived from this family via deletions of the N-terminal 8−11 residues. One related antagonist, astressin, [cyclo(30−33)[D-Phe12,Nle21,Glu30*,Lys33*, Nle38]hCRF(12−41)] is assumed to adopt an α-helical conformation when interacting with receptors. In addition to cyclic constraints, CD studies indicate high levels of helicity (up to 94% in water) for both linear and cyclic analogues.[380]

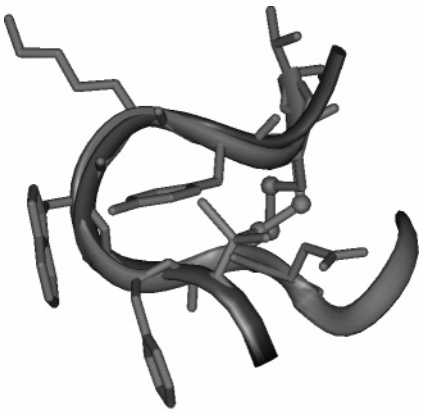
4.53. Urotensin II

Urotensin II (Accession No. O95399 (human)) is an 11 residue disulfide bridged cyclic peptide (ETPDC$_5$FWKYC$_{10}$V). It has been identified as the endogenous ligand for the orphan receptor GPR14.[381] The seven C-terminal residues (CFWKYCV) form a macrocycle and are deemed essential for biological activity [hU−II, K_i 0.8 nM; hU−II(4−11) K_i 0.25 nM].[382] Urotensin II is a vasoconstrictor and has been shown to be up to 2 orders of magnitude more potent than endothelin-1.[381,383] The NMR solution structure of the complete disulfide bridged cyclic peptide, **118**, (supplied



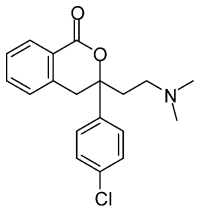
118

by Dr. T. Klabunde[384]) has been published by several groups.[384,385] The disulfide bridged cyclic portion shows a turn-like conformation, albeit a non-classical turn. Further studies[382,385] have generated potent Urotensin II analogues containing a disulfide bridge, **119** (K_i 0.2 nM), where the analogue is DXFWKYCV and X is β,β-dimethylcysteine superimposed on hU-II. (The coordinates of **119** were provided by Dr. A. Carotenuto.[382,385])



119

The solution structure of UT-II in SDS micelles[386] shows two major conformations, the most populated being a type II′ β-hairpin structure. Truncated agonist analogue **119** was exclusively a type II′ β-hairpin



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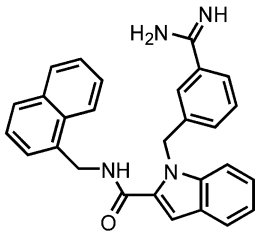


121

conformation in SDS micelles. A 3-point pharmacophore hypothesis of the most important residues required for receptor activation was proposed (Trp-7, Lys-8, Tyr-9) based on this β-turn. Interestingly, the pharmacophoric distances of the non-peptide agonist **120** (+)-enantiomer[387] were found to be within 0.5 Å to that found for **119**, suggesting that **120** (EC_{50} 300 nM) is a peptidomimetic of the receptor binding conformation of UT-II.

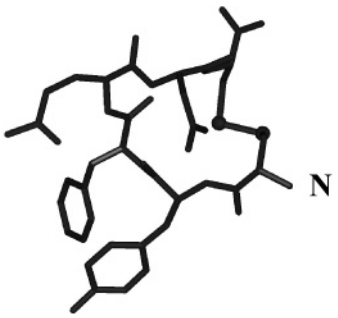
Another reported pharmacophore model, based on a β-turn conformation of UT-II, was used to screen libraries of inhibitors *in silico*. These efforts led to the discovery of a variety of antagonists of the urocortin receptor, including compound **121** (IC_{50} 400 nM).[384]

4.54. Vasoactive Intestinal Peptide

Vasoactive intestinal peptide (VIP; Accession No. P01282) is a C-terminally amidated 28-residue neuropeptide ($HSDAVFT_7DNYTRLRK_{15}QMAV_{19}KK-YLNSIL_{27}N\text{-}NH_2$) that is widely distributed in both the peripheral and central nervous systems. VIP has a large range of biological functions associated with the airways, digestive-tract, cardiovascular system, immune system, reproductive system, endocrine glands and the brain.[319] VIP binds two GPCRs, VPAC1 and VPAC2, with similar affinity. An investigation of key residues for receptor binding of VIP used an alanine scan to reveal a highly selective VPAC1 agonist, [$Ala^{11,22,28}$]VIP.[388] The structure of VIP, like those of related peptides in this family, consists of two helical regions between residues 7−15 and 19−27, while residues 16−18 possess no defined structure.[389] A peptide found in lizards, Helodermin, has high homology with VIP and PACAP and NMR studies show an α-helix from residues 9−23 in water.[390]

4.55. Vasopressin and Vasotocin

Vasopressin (β-hypophamine; [Arg8]-vasopressin; antidiuretic hormone; Accession No. P01185 (human); pdb 1jk4; CCD Code: DUNLON) [$C_1YFQ_4NC_6$-PRG-NH_2, disulfide 1−6)], is a mammalian neurohypophyseal hormone similar to oxytocin in structure and function, and vasotocin (Accession No. *P*23879 (fish)) [$C_1YIQNC_2PRG\text{-}NH_2$, disulfide 1−6] is found in lower order animals and plants. Vasopressin targets three receptors (V1a, V1b, V2) and a crystal structure of a cyclic derivative pressinoic acid, **122**,[391] while not showing any classical secondary structure, is similar in conformation to a β-turn. A recent structure for vasopressin **123**, complexed with hormone transport protein neurophysin, revealed a more classic β-turn (type I) shown below, **124**.[392] Desmopressin ([1-desamino,8-D-arginine]-vasopressin) a V2 agonist that exhibits an antidiuretic activity, has been used to treat diabetes insipidus as has vasopressin. Structural studies show that, in aqueous solution, it possesses an inverse γ-turn centered at Q4.[393]



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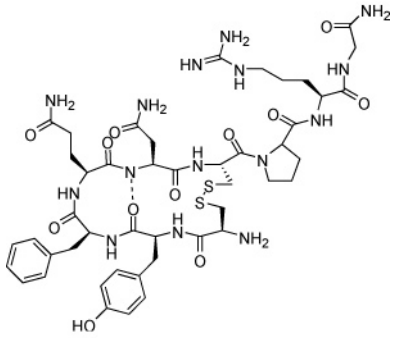

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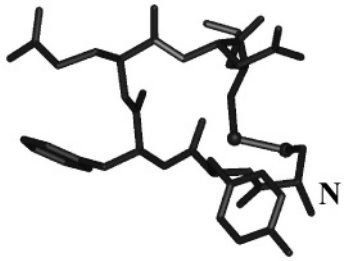

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5. Nonmammalian Peptide Hormones

This review has focused predominantly on mammalian peptide hormones that target GPCRs. Below are a few examples of ligands that target GPCRs in non-mammalian organisms.

5.1. Alpha Factors

Mating factors alpha (Accession No. P01149 *S. cerevisiae* alpha1, P32435 *S. cerevisiae* alpha2, P06648 *S. cerevisiae*) are 13 residue peptides (e.g. WHWLQL-$K_7P_8G_9$QPMY, *S. cerevisiae*) secreted as a trigger for the reproduction cycle in yeast. The system has been utilized extensively as a model system for short peptides binding to GPCRs with the yeast pheromones binding to the Ste2p receptor. Numerous studies of α factor and its analogues, including conformationally constrained peptides, have indicated that a β-turn exists from residues 7−10.[394−396] One particular study used a γ-lactam conformational constraint [3-(*R* or *S*)-amino-2-oxo-1-pyrrolidine-acetamido group] at positions 8 and 9 and found a γ-turn structure around the lactam group, as well as comparable activity to the native peptide.[395]

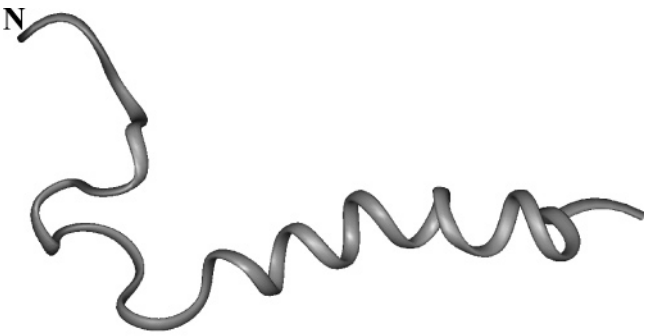
Table 1. Peptide Sequence Secondary Structure

peptide[a]	length	structure
allatostatin	6−18 residues	β-turn in active core[399]
proctolin (cockroach)	RYLPT	β-turn[400]/β-bulge[401]
AKH (locust)	$QLNFT_5PNW_8GT$	β-turn from res 5−8[402] helical (precursor)[403]
diuretic hormones	variable length and sequence	central helical region[404]
insect kinin	C-term ($FXXWG\text{-}NH_2$)	β-turn[405]
DCIN	$\rho ETF_3QYSHGW_9TN\text{-}NH_2$	turn[406]
corazonin	$\rho ETF_3QYSRGW_9TN\text{-}NH_2$	turn[406]

[a] AKH, adipokinetic hormone; DCIN, dark-color-inducing neurohormone.

5.2. Neuropeptide F

Neuropeptide F (NPF; Accession No. P41967; pdb 1k8v) is a 39 residue peptide originally isolated from the sheep tapeworm *Moniezia expansa* ($P_{-2}D_{-1}K_0D_1$-$FIVNPSDLVLDNK_{14}AALRDYLRQINEYFAII_{31}$-$GRPRF\text{-}NH_2$).[397] To date over 30 different peptides have been isolated from over 40 different flatworm species. NPF is similar in sequence to the vertebrate NPY family (above), in particular to the pancreatic polypeptides, where the C-terminal motif of RPRF is strictly conserved in amphibian and reptile PP, and almost conserved in the human sequence (RPRY).[398] Its origin links this peptide with a possible target for new antiparasitic drugs. The function of NPF is unknown. The NMR solution structure of NPF (**125**) shows an amphipathic α-helical region from residues K14 to I31, the remainder adopting random conformations.[398]



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There are many other ligands that have been investigated, including those listed in Table 1, that show evidence for turns.

6. Conclusions

G protein-coupled receptors (GPCRs) constitute the largest family of cellular receptors involved in signal transduction. There are ~700−1000 GPCRs presently documented, although some researchers predict that as many as 5000−6000 may exist.[407] Of the several hundred GPCRs known to be activated by peptide or protein hormones, only peptide-activated GPCRs for angiotensin, endothelin, oxytocin, neurokinin, and somatostatin have been successfully targeted by small molecule pharmaceuticals to date, although a number of GPCR-binding we have now examined ~120 GPCRs of all five known classes. The majority are members of the rhodopsin and secretin families.[5,408] Those classes bind peptides and proteins with remarkably similar structure−function relationships. Two major examples are those of the secretin or glucagon family and the glycoprotein hormones A. The former, the secretin family, consists of a medium sized peptide that has a general helical structure, commonly divided into two components which follows the commonly accepted two step GPCR activation. This ligand family includes calcitonin, CRLR, CRH, glucagon, GIP, GLP, GHRH, PACAP, PTH, secretin and VIP. The latter includes hCG, LH, FSH and TSH and consists of an almost identical structure, a two domain cysteine knot. One domain is identical in all four hormones, the other is unique in each hormone but maintains a similar structure with loop regions thought important for activation.

We have assembled for the first time the voluminous structural and/or activity evidence in support of a ligand turn shape being the likely recognition domain in GPCR-binding domains of all of these ligands. In the absence of structurally defined GPCR-ligand complexes, we have analyzed solution or solid-state structures for a variety of GPCR-binding ligands alone, including (i) peptide and protein hormone ligands, (ii) bioactive peptide fragments, (iii) cyclic peptides, often analogues of protein segments, and (iv) peptidomimetic antagonists with a conformational bias enforced by the presence of turn-inducing constraints.

GPCR-binding of endogenous proteins/peptides in which a β/γ-turn conformation is clearly associated with bioactivity, included bradykinin, C3a and C5a, endomorphin, enkephalin, melanocortin, oxerin, PARs, sandostatin, urotensin II, vassopressin. Proteins/peptides that bind to GPCRs and were reported to adopt helical turn conformations included bombesin, calcitonin, endorphin, endothelin, glucagon, glucagon-like, neuropeptide Y, nociceptin, oxerin, pancreatic peptide, parathyroid hormone, sarafotoxin.

We have also summarized examples of small cyclic peptidomimetics for naturally occurring GPCR-binding small peptides, including somatosatin (14 residues), melanocyte-stimulating hormone (13), oxytocin (9), vasopressin (9), substance P (11), bradykinin (9), opiod peptides such as enkephalins (5), dynorphins (11−17) and endorphins; and small fragments of proteins such as angiotensins, luteinizing release hormone (LHRH), endothelin, leukokinin, melanocortin, growth hormones, human parathyroid hormone, complement anaphylatoxins, PARs, and CCK. Most of these examples were β-turn mimetics that have quite potent antagonist activities at their cognate GPCRs.

A few examples of turn mimetics have also been presented, but these are reviewed elsewhere in much

more detail.[21−32] Those reviews summarize the growing collection of turn-inducing constraints that are now available for fixing α-, β-, and γ-turns in peptides and cyclic peptides and many such constraints have become useful as scaffolds for constructing nonpeptidic ligands for GPCRs. In particular the reverse β-turn has emerged as an important structural feature for incorporation into the design of GPCR antagonists. Although β-turns have been reported to play important roles in recognition phenomena, there is also now an emerging literature in support of γ-turns, and more recently α-turns (as found in α-helices), being key turn motifs that are recognized by GPCRs.

From this large volume of data for a structurally diverse collection of ~120 GPCR-binding ligands, we found herein that all ligands adopt either α-, β-, or γ-turns, with neurotensin the only example[294,409] to date of a GPCR-binding ligand that seemingly prefers an alternative ligand conformation (a β-strand). We emphasize this example, because the demonstrated recognition of a β-strand in that case is contradictory to the preponderance of circumstantial evidence that has been assembled herein in support of turn recognition by peptide-activated GPCRs in general. The preference of GPCRs for turn-shaped ligand motifs also contrasts with ligand preferences of proteolytic enzymes, which almost universally recognize the β-strand shape,[410−412] and with transcriptional receptors that recognize transcription factors mainly in alpha helical conformations. We think that these common patterns of ligand recognition by all or most members of a particular protein class may be much more common than currently documented. It is sensible that proteolytic enzymes would not recognize turns or helices, otherwise the latter folded structures would be indiscriminately degraded. Nature appears to have evolved GPCRs on cells to specifically recognize stable turn shapes that are not readily susceptible to proteolytic cleavage. This is an interesting contrast in conformational organization between ligands recognized by proteases and GPCRs and should be further studied in more detail.

A number of basic principles now appear to be common to all currently known peptide activated GPCRs. These include a multiplicity of ligands, a seven helical transmembrane receptor, receptor oligomerization, multiplicity of G protein signaling. We now highlight another important feature, a common ligand motif or shape for recognition by GPCRs. Herein we demonstrate what we believe to be a key paradigm for cell signaling via GPCRs, namely that peptide-activated GPCRs may recognize a broadly similar turn conformation or shape in their diverse peptidic ligand signals. This structural requirement, and the information assembled herein, may help toward a better general understanding of the interactions between peptide/protein ligands and GPCRs, and of the structural basis for GPCR-mediated signal transduction across cell membranes. The data also supports the view that the continued search for generic approaches to turn mimetics may be fruitful in the design and development of new antagonists/agonists of peptide/protein-binding GPCRs as new therapeutic leads for what is already a 30 billion dollar annual market.

7. Acknowledgment

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8. Abbreviations

Aib, α-aminoisobutyric acid; AKH, adipokinetic hormone; Cit, citrulline; Cpa, *p*-chlorophenylalanine; DCIN, dark-color-inducing neurohormone; GPCRs, G protein-coupled receptors; Lys(Nic)-Nϵ, nicotinoyllysine; Ilys-Nϵ, isopropyllysine; Dpr, diaminopropionic acid; Nal, β-(2-naphthyl)alanine; Nme, *N*-methyl; Nle, norleucine; Orn, ornithine; Pal, β-[pyridyl]alanine; pE, pyro glutamic acid; Pen, penicillamine; pHPPA, *p*-hydroxy-3-phenylproprionyl; Tfa, trifluoroacetyl; Tho, reduced threonine; Sar, sarcosine.

9. References

(1) Flower, D. R. *Biochim. Biophys. Acta* **1999**, *1422*, 207.
(2) Klabunde, T.; Hessler, G. *ChemBioChem* **2002**, *3*, 928.
(3) Kroeze, W. K.; Sheffler, D. J.; Roth, B. L. *J. Cell. Sci.* **2003**, *116*, 4867.
(4) Lander, E. S.; Linton, L. M.; Birren, B.; Nusbaum, C.; Zody, M. C.; Baldwin, J.; Devon, K.; Dewar, K.; Doyle, M.; FitzHugh, W.; Funke, R.; Gage, D.; Harris, K.; Heaford, A.; Howland, J.; Kann, L.; Lehoczky, J.; LeVine, R.; McEwan, P.; McKernan, K.; Meldrim, J.; Mesirov, J. P.; Miranda, C.; Morris, W.; Naylor, J.; Raymond, C.; Rosetti, M.; Santos, R.; Sheridan, A.; Sougnez, C.; Stange-Thomann, N.; Stojanovic, N.; Subramanian, A.; Wyman, D.; Rogers, J.; Sulston, J.; Ainscough, R.; Beck, S.; Bentley, D.; Burton, J.; Clee, C.; Carter, N.; Coulson, A.; Deadman, R.; Deloukas, P.; Dunham, A.; Dunham, I.; Durbin, R.; French, L.; Grafham, D.; Gregory, S.; Hubbard, T.; Humphray, S.; Hunt, A.; Jones, M.; Lloyd, C.; McMurray, A.; Matthews, L.; Mercer, S.; Milne, S.; Mullikin, J. C.; Mungall, A.; Plumb, R.; Ross, M.; Shownkeen, R.; Sims, S.; Waterston, R. H.; Wilson, R. K.; Hillier, L. W.; McPherson, J. D.; Marra, M. A.; Mardis, E. R.; Fulton, L. A.; Chinwalla, A. T.; Pepin, K. H.; Gish, W. R.; Chissoe, S. L.; Wendl, M. C.; Delehaunty, K. D.; Miner, T. L.; Delehaunty, A.; Kramer, J. B.; Cook, L. L.; Fulton, R. S.; Johnson, D. L.; Minx, P. J.; Clifton, S. W.; Hawkins, T.; Branscomb, E.; Predki, P.; Richardson, P.; Wenning, S.; Slezak, T.; Doggett, N.; Cheng, J. F.; Olsen, A.; Lucas, S.; Elkin, C.; Uberbacher, E.; Frazier, M. *Nature* **2001**, *409*, 860.
(5) Fredriksson, R.; Lagerstrom, M. C.; Lundin, L. G.; Schioth, H. B. *Mol. Pharmacol.* **2003**, *63*, 1256.
(6) Vassilatis, D. K.; Hohmann, J. G.; Zeng, H.; Li, F.; Ranchalis, J. E.; Mortrud, M. T.; Brown, A.; Rodriguez, S. S.; Weller, J. R.; Wright, A. C.; Bergmann, J. E.; Gaitanaris, G. A. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 4903.
(7) Venter, J. C.; Adams, M. D.; Myers, E. W.; Li, P. W.; Mural, R. J.; Sutton, G. G.; Smith, H. O.; Yandell, M.; Evans, C. A.; Holt, R. A.; Gocayne, J. D.; Amanatides, P.; Ballew, R. M.; Huson, D. H.; Wortman, J. R.; Zhang, Q.; Kodira, C. D.; Zheng, X. H.; Chen, L.; Skupski, M.; Subramanian, G.; Thomas, P. D.; Zhang, J.; Gabor Miklos, G. L.; Nelson, C.; Broder, S.; Clark, A. G.; Nadeau, J.; McKusick, V. A.; Zinder, N.; Levine, A. J.; Roberts, R. J.; Simon, M.; Slayman, C.; Hunkapiller, M.; Bolanos, R.; Delcher, A.; Dew, I.; Fasulo, D.; Flanigan, M.; Florea, L.; Halpern, A.; Hannenhalli, S.; Kravitz, S.; Levy, S.; Mobarry, C.; Reinert, K.; Remington, K.; Abu-Threideh, J.; Beasley, E.; Biddick, K.; Bonazzi, V.; Brandon, R.; Cargill, M.; Chandramouliswaran, I.; Charlab, R.; Chaturvedi, K.; Deng, Z.; Di Francesco, V.; Dunn, P.; Eilbeck, K.; Evangelista, C.; Gabrielian, A. E.; Gan, W.; Ge, W.; Gong, F.; Gu, Z.; Guan, P.; Heiman, T. J.; Higgins, M. E.; Ji, R. R.; Ke, Z.; Ketchum, K. A.; Lai, Z.; Lei, Y.; Li, Z.; Li, J.; Liang, Y.; Lin, X.; Lu, F.; Merkulov, G. V.; Milshina, N.; Moore, H. M.; Naik, A. K.; Narayan, V. A.; Neelam, B.; Nusskern, D.; Rusch, D. B.; Salzberg, S.; Shao, W.; Shue, B.; Sun, J.; Wang, Z.; Wang, A.; Wang, X.; Wang, J.; Wei, M.; Wides, R.; Xiao, C.; Yan, C. *Science* **2001**, *291*, 1304.
(8) Katugampola, S.; Davenport, A. *Trends Pharmacol. Sci.* **2003**, *24*, 30.
(9) Wise, A.; Jupe, S. C.; Rees, S. *Annu. Rev. Pharmacol. Toxicol.* **2004**, *44*, 43.

(10) Wilson, S.; Bergsma, D. *Drug Des. Discovery* **2000**, *17*, 105.
(11) Ji, T. H.; Murdoch, W. J.; Ji, I. *Endocrine J.* **1995**, *3*, 187.
(12) Betancur, C.; Azzi, M.; Rostene, W. *Trends Pharmacol. Sci.* **1997**, *18*, 372.
(13) Schwartz, T. W.; Gether, U.; Schambye, H. T.; Hjorth, S. A. *Curr. Pharm. Des.* **1995**, *1*, 325.
(14) Fredriksson, R.; Gloriam, D. E.; Hoglund, P. J.; Lagerstrom, M. C.; Schioth, H. B. *Biochem. Biophys. Res. Commun.* **2003**, *301*, 725.
(15) Conn, P. J.; Pin, J. P. *Annu. Rev. Pharmacol. Toxicol.* **1997**, *37*, 205.
(16) Perez, D. M. *Mol. Pharmacol.* **2003**, *63*, 1202.
(17) Palczewski, K.; Kumasaka, T.; Hori, T.; Behnke, C. A.; Motoshima, H.; Fox, B. A.; Le Trong, I.; Teller, D. C.; Okada, T.; Stenkamp, R. E.; Yamamoto, M.; Miyano, M. *Science* **2000**, *289*, 739.
(18) Bleicher, K. H.; Green, L. G.; Martin, R. E.; Rogers-Evans, M. *Curr. Opin. Chem. Biol* **2004**, *8*, 287.
(19) Muller, G. *Drug Discovery Today* **2003**, *8*, 681.
(20) Evans, B. E.; Rittle, K. E.; Bock, M. G.; DiPardo, R. M.; Freidinger, R. M.; Whitter, W. L.; Lundell, G. F.; Veber, D. F.; Anderson, P. S.; Chang, R. S.; et al. *J. Med. Chem.* **1988**, *31*, 2235.
(21) Fairlie, D. P.; Abbenante, G.; March, D. R. *Curr. Med. Chem.* **1995**, *2*, 654.
(22) Fairlie, D. P.; West, M. L.; Wong, A. K. *Curr. Med. Chem.* **1998**, *5*, 29.
(23) Andrews, M. J. I.; Tabor, A. B. *Tetrahedron* **1999**, *55*, 11711.
(24) Hruby, V. J.; al-Obeidi, F.; Kazmierski, W. *Biochem. J.* **1990**, *268*, 249.
(25) Hruby, V. J.; Sharma, S. D. *Curr. Opin. Biotechnol.* **1991**, *2*, 599.
(26) Stradley, S. J.; Rizo, J.; Bruch, M. D.; Stroup, A. N.; Gierasch, L. M. *Biopolymers* **1990**, *29*, 263.
(27) Toniolo, C. *Int. J. Pept. Protein Res.* **1990**, *35*, 287.
(28) Schiller, P. W. *Medicinal Chemistry for the 21st Century*; IUPAC/ Blackwell: London, 1992.
(29) Kemp, D. S. *Medicinal Chemistry for the 21st Century*; IUPAC/ Blackwell: London, 1992.
(30) Holzemann, G. *Kontakte (Darmstadt)* **1991**, *1*, 3.
(31) Holzemann, G. *Kontakte (Darmstadt)* **1991**, *2*, 55.
(32) Giannis, A. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1244.
(33) Kazantzis, A.; Waldner, M.; Taylor, J. W.; Kapurniotu, A. *Eur. J. Biochem.* **2002**, *269*, 780.
(34) Hinson, J. P.; Kapas, S.; Smith, D. M. *Endocr. Rev.* **2000**, *21*, 138.
(35) Kitamura, K.; Kangawa, K.; Kawamoto, M.; Ichiki, Y.; Nakamura, S.; Matsuo, H.; Eto, T. *Biochem. Biophys. Res. Commun.* **1993**, *192*, 553.
(36) Parkes, D. G. *Am. J. Physiol* **1995**, *268*, H2574.
(37) Poyner, D. R.; Sexton, P. M.; Marshall, I.; Smith, D. M.; Quirion, R.; Born, W.; Muff, R.; Fischer, J. A.; Foord, S. M. *Pharmacol. Rev.* **2002**, *54*, 233.
(38) Kitamura, K.; Matsui, E.; Kato, J.; Katoh, F.; Kita, T.; Tsuji, T.; Kangawa, K.; Eto, T. *Peptides* **2001**, *22*, 1713.
(39) Barsh, G. S.; Ollmann, M. M.; Wilson, B. D.; Miller, K. A.; Gunn, T. M. *Ann. N. Y. Acad. Sci.* **1999**, *885*, 143.
(40) Bolin, K. A.; Anderson, D. J.; Trulson, J. A.; Thompson, D. A.; Wilken, J.; Kent, S. B.; Gantz, I.; Millhauser, G. L. *FEBS Lett.* **1999**, *451*, 125.
(41) Dinulescu, D. M.; Cone, R. D. *J. Biol. Chem.* **2000**, *275*, 6695.
(42) McNulty, J. C.; Thompson, D. A.; Bolin, K. A.; Wilken, J.; Barsh, G. S.; Millhauser, G. L. *Biochemistry* **2001**, *40*, 15520.
(43) Tota, M. R.; Smith, T. S.; Mao, C.; MacNeil, T.; Mosley, R. T.; Van der Ploeg, L. H.; Fong, T. M. *Biochemistry* **1999**, *38*, 897.
(44) Jackson, P. J.; McNulty, J. C.; Yang, Y. K.; Thompson, D. A.; Chai, B.; Gantz, I.; Barsh, G. S.; Millhauser, G. L. *Biochemistry* **2002**, *41*, 7565.
(45) Thirumoorthy, R.; Holder, J. R.; Bauzo, R. M.; Richards, N. G.; Edison, A. S.; Haskell-Luevano, C. *J. Med. Chem.* **2001**, *44*, 4114.
(46) Hoppener, J. W.; Ahren, B.; Lips, C. J. *N. Engl. J. Med.* **2000**, *343*, 411.
(47) Nishi, M.; Sanke, T.; Nagamatsu, S.; Bell, G. I.; Steiner, D. F. *J. Biol. Chem.* **1990**, *265*, 4173.
(48) Cooper, G. J.; Willis, A. C.; Clark, A.; Turner, R. C.; Sim, R. B.; Reid, K. B. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 8628.
(49) Fischer, J. A.; Muff, R.; Born, W. *Biochem. Soc. Trans.* **2002**, *30*, 455.
(50) Mascioni, A.; Porcelli, F.; Ilangovan, U.; Ramamoorthy, A.; Veglia, G. *Biopolymers* **2003**, *69*, 29.
(51) Conner, A. C.; Hay, D. L.; Howitt, S. G.; Kilk, K.; Langel, U.; Wheatley, M.; Smith, D. M.; Poyner, D. R. *Biochem. Soc. Trans.* **2002**, *30*, 451.
(52) Tatemoto, K.; Hosoya, M.; Habata, Y.; Fujii, R.; Kakegawa, T.; Zou, M. X.; Kawamata, Y.; Fukusumi, S.; Hinuma, S.; Kitada, C.; Kurokawa, T.; Onda, H.; Fujino, M. *Biochem. Biophys. Res. Commun.* **1998**, *251*, 471.
(53) Maguire, J. J. *Curr. Opin. Pharmacol.* **2003**, *3*, 135.
(54) Szokodi, I.; Tavi, P.; Foldes, G.; Voutilainen-Myllyla, S.; Ilves, M.; Tokola, H.; Pikkarainen, S.; Piuhola, J.; Rysa, J.; Toth, M.; Ruskoaho, H. *Circ. Res.* **2002**, *91*, 434.
(55) Ishida, J.; Hashimoto, T.; Hashimoto, Y.; Nishiwaki, S.; Iguchi, T.; Harada, S.; Sugaya, T.; Matsuzaki, H.; Yamamoto, R.; Shiota, N.; Okunishi, H.; Kihara, M.; Umemura, S.; Sugiyama, F.; Yagami, K. I.; Kasuya, Y.; Mochizuki, N.; Fukamizu, A. *J. Biol. Chem.* **2004**.
(56) Fan, X.; Zhou, N.; Zhang, X.; Mukhtar, M.; Lu, Z.; Fang, J.; DuBois, G. C.; Pomerantz, R. J. *Biochemistry* **2003**, *42*, 10163.
(57) Medhurst, A. D.; Jennings, C. A.; Robbins, M. J.; Davis, R. P.; Ellis, C.; Winborn, K. Y.; Lawrie, K. W.; Hervieu, G.; Riley, G.; Bolaky, J. E.; Herrity, N. C.; Murdock, P.; Darker, J. G. *J. Neurochem.* **2003**, *84*, 1162.
(58) Wright, J. W.; Reichert, J. R.; Davis, C. J.; Harding, J. W. *Neurosci. Biobehav. Rev.* **2002**, *26*, 529.
(59) de Gasparo, M.; Catt, K. J.; Inagami, T.; Wright, J. W.; Unger, T. *Pharmacol. Rev.* **2000**, *52*, 415.
(60) Spyroulias, G. A.; Nikolakopoulou, P.; Tzakos, A.; Gerothanassis, I. P.; Magafa, V.; Manessi-Zoupa, E.; Cordopatis, P. *Eur. J. Biochem.* **2003**, *270*, 2163.
(61) Nikiforovich, G. V.; Kao, J. L.; Plucinska, K.; Zhang, W. J.; Marshall, G. R. *Biochemistry* **1994**, *33*, 3591.
(62) Garcia, K. C.; Ronco, P. M.; Verroust, P. J.; Brunger, A. T.; Amzel, L. M. *Science* **1992**, *257*, 502.
(63) Carpenter, K. A.; Wilkes, B. C.; Schiller, P. W. *Eur. J. Biochem.* **1998**, *251*, 448.
(64) Cho, N.; Asher, S. A. *Biospectroscopy* **1996**, *2*, 71.
(65) Nikiforovich, G. V.; Marshall, G. R. *Biochem. Biophys. Res. Commun.* **1993**, *195*, 222.
(66) Spear, K. L.; Brown, M. S.; Reinhard, E. J.; McMahon, E. G.; Olins, G. M.; Palomo, M. A.; Patton, D. R. *J. Med. Chem.* **1990**, *33*, 1935.
(67) Schmidt, B.; Lindman, S.; Tong, W.; Lindeberg, G.; Gogoll, A.; Lai, Z.; Thornwall, M.; Synnergren, B.; Nilsson, A.; Welch, C. J.; Sohtell, M.; Westerlund, C.; Nyberg, F.; Karlen, A.; Hallberg, A. *J. Med. Chem.* **1997**, *40*, 903.
(68) Rosenstrom, U.; Skold, C.; Lindeberg, G.; Botros, M.; Nyberg, F.; Karlen, A.; Hallberg, A. *J. Med. Chem.* **2004**, *47*, 859.
(69) Yamada, K.; Wada, E.; Wada, K. *Ann. Med.* **2000**, *32*, 519.
(70) Horwell, D. C.; Howson, W.; Naylor, D.; Osborne, S.; Pinnock, R. D.; Ratcliffe, G. S.; Suman-Chauhan, N. *Int. J. Pept. Protein Res.* **1996**, *48*, 522.
(71) Carver, J. A. *Eur. J. Biochem.* **1987**, *168*, 193.
(72) Carver, J. A.; Collins, J. G. *Eur. J. Biochem.* **1990**, *187*, 645.
(73) Erne, D.; Schwyzer, R. *Biochemistry* **1987**, *26*, 6316.
(74) Polverini, E.; Casadio, R.; Neyroz, P.; Masotti, L. *Arch. Biochem. Biophys.* **1998**, *349*, 225.
(75) Lee, S.; Kim, Y. *FEBS Lett.* **1999**, *460*, 263.
(76) Cristau, M.; Devin, C.; Oiry, C.; Chaloin, O.; Amblard, M.; Bernad, N.; Heitz, A.; Fehrentz, J. A.; Martinez, J. *J. Med. Chem.* **2000**, *43*, 2356.
(77) Eden, J. M.; Hall, M. D.; Higginbottom, M.; Horwell, D. C.; Howson, W.; Hughes, J.; Jordan, R. E.; Lewthwaite, R. A.; Martin, K.; McKnight, A. T.; OToole, J. C.; Pinnock, R. D.; Pritchard, M. C.; SumanChauhan, N.; Williams, S. C. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2617.
(78) Knight, M.; Takahashi, K.; Chandrasekhar, B.; Geblaoui, A. Z.; Jensen, R. T.; Strader, D.; Moody, T. W. *Peptides* **1995**, *16*, 1109.
(79) Ellis, K. M.; Fozard, J. R. *Auton. Autacoid Pharmacol.* **2002**, *22*, 3.
(80) Lee, S. C.; Russell, A. F.; Laidig, W. D. *Int. J. Pept. Protein Res.* **1990**, *35*, 367.
(81) Miskolzie, M.; Gera, L.; Stewart, J. M.; Kotovych, G. *J. Biomol. Struct. Dyn.* **2000**, *18*, 249.
(82) Sejbal, J.; Cann, J. R.; Stewart, J. M.; Gera, L.; Kotovych, G. *J. Med. Chem.* **1996**, *39*, 1281.
(83) Kotovych, G.; Cann, J. R.; Stewart, J. M.; Yamamoto, H. *Biochem. Cell Biol.* **1998**, *76*, 257.
(84) Sawada, Y.; Kayakiri, H.; Abe, Y.; Mizutani, T.; Inamura, N.; Asano, M.; Hatori, C.; Aramori, I.; Oku, T.; Tanaka, H. *J. Med. Chem.* **2004**, *47*, 2853.
(85) Thurieau, C.; Feletou, M.; Hennig, P.; Raimbaud, E.; Canet, E.; Fauchere, J. L. *J. Med. Chem.* **1996**, *39*, 2095.
(86) Pineda, L. F.; Liebmann, C.; Hensellek, S.; Paegelow, I.; Steinmetzer, T.; Schweinitz, A.; Stürzebecher, J.; Reissmann, S. *Lett. Pept. Sci.* **2000**, *7*, 69.
(87) Breimer, L. H.; MacIntyre, I.; Zaidi, M. *Biochem. J.* **1988**, *255*, 377.
(88) Inzerillo, A. M.; Zaidi, M.; Huang, C. L. *Thyroid* **2002**, *12*, 791.
(89) Motta, A.; Pastore, A.; Goud, N. A.; Castiglione Morelli, M. A. *Biochemistry* **1991**, *30*, 10444.
(90) Motta, A.; Andreotti, G.; Amodeo, P.; Strazzullo, G.; Castiglione Morelli, M. A. *Proteins* **1998**, *32*, 314.
(91) Hashimoto, Y.; Toma, K.; Nishikido, J.; Yamamoto, K.; Haneda, K.; Inazu, T.; Valentine, K. G.; Opella, S. J. *Biochemistry* **1999**, *38*, 8377.
(92) Kapurniotu, A.; Kayed, R.; Taylor, J. W.; Voelter, W. *Eur. J. Biochem.* **1999**, *265*, 606.

(93) Edvinsson, L.; Alm, R.; Shaw, D.; Rutledge, R. Z.; Koblan, K. S.; Longmore, J.; Kane, S. A. *Eur. J. Pharmacol.* **2002**, *434*, 49.
(94) Carpenter, K. A.; Schmidt, R.; von Mentzer, B.; Haglund, U.; Roberts, E.; Walpole, C. *Biochemistry* **2001**, *40*, 8317.
(95) Teschemacher, H.; Koch, G.; Brantl, V. *Biopolymers* **1997**, *43*, 99.
(96) Meisel, H. *Biopolymers* **1997**, *43*, 119.
(97) Clare, D. A.; Swaisgood, H. E. *J. Dairy Sci.* **2000**, *83*, 1187.
(98) Carpenter, K. A.; Schiller, P. W.; Schmidt, R.; Wilkes, B. C. *Int. J. Pept. Protein Res.* **1996**, *48*, 102.
(99) Kazmierski, W. M.; Yamamura, H. I.; Hruby, V. J. *J. Am. Chem. Soc.* **1991**, *113*, 2275.
(100) Handel, T. M.; Lau, E. K. *Ernst Schering Res. Found Workshop* **2004**, 101.
(101) In *Guide to Receptors and Channels, Br. J. Pharmacol.*; Alexander, S. P. H., Mathie, A., Peters, J. A., Eds.; 2004; Vol. 141.
(102) Gonzalez-Barcena, D.; Vadillo-Buenfil, M.; Cortez-Morales, A.; Fuentes-Garcia, M.; Cardenas-Cornejo, I.; Comaru-Schally, A. M.; Schally, A. V. *Urology* **1995**, *45*, 275.
(103) Bacon, K. B.; Oppenheim, J. J. *Cytokine Growth Factor Rev.* **1998**, *9*, 167.
(104) Cascieri, M. A.; Springer, M. S. *Curr. Opin. Chem. Biol.* **2000**, *4*, 420.
(105) Houshmand, P.; Zlotnik, A. *Curr. Opin. Chem. Biol.* **2003**, *7*, 457.
(106) Fujii, N.; Oishi, S.; Hiramatsu, K.; Araki, T.; Ueda, S.; Tamamura, H.; Otaka, A.; Kusano, S.; Terakubo, S.; Nakashima, H.; Broach, J. A.; Trent, J. O.; Wang, Z. X.; Peiper, S. C. *Angew. Chem., Int. Ed. Engl.* **2003**, *42*, 3251.
(107) Noble, F.; Wank, S. A.; Crawley, J. N.; Bradwejn, J.; Seroogy, K. B.; Hamon, M.; Roques, B. P. *Pharmacol. Rev.* **1999**, *51*, 745.
(108) Fournie-Zaluski, M. C.; Belleney, J.; Lux, B.; Durieux, C.; Gerard, D.; Gacel, G.; Maigret, B.; Roques, B. P. *Biochemistry* **1986**, *25*, 3778.
(109) Roy, P.; Charpentier, B.; Durieux, C.; Dor, A.; Roques, B. P. *Biopolymers* **1989**, *28*, 69.
(110) Pattou, D.; Maigret, B.; Fournie-Zaluski, M. C.; Roques, B. P. *Int. J. Pept. Protein Res.* **1991**, *37*, 440.
(111) Pellegrini, M.; Mierke, D. F. *Biochemistry* **1999**, *38*, 14775.
(112) Keire, D. A.; Solomon, T. E.; Reeve, J. R., Jr. *Biochem. Biophys. Res. Commun.* **2002**, *293*, 1014.
(113) Albrizio, S.; Carotenuto, A.; Fattorusso, C.; Moroder, L.; Picone, D.; Temussi, P. A.; D'Ursi, A. *J. Med. Chem.* **2002**, *45*, 762.
(114) de Tullio, P.; Delarge, J.; Pirotte, B. *Curr. Med. Chem.* **1999**, *6*, 433.
(115) Martin-Martinez, M.; De La Figuera, N.; LaTorre, M.; Herranz, R.; Garcia-Lopez, M. T.; Cenarruzabeitia, E.; Del Rio, J.; Gonzalez-Muniz, R. *J. Med. Chem.* **2000**, *43*, 3770.
(116) Horwell, D. C.; Hughes, J.; Hunter, J. C.; Pritchard, M. C.; Richardson, R. S.; Roberts, E.; Woodruff, G. N. *J. Med. Chem.* **1991**, *34*, 404.
(117) Smits, G.; Campillo, M.; Govaerts, C.; Janssens, V.; Richter, C.; Vassart, G.; Pardo, L.; Costagliola, S. *EMBO J.* **2003**, *22*, 2692.
(118) McClamrock, H. D. *Clin. Obstet. Gynecol.* **2003**, *46*, 298.
(119) Wu, H.; Lustbader, J. W.; Liu, Y.; Canfield, R. E.; Hendrickson, W. A. *Structure* **1994**, *2*, 545.
(120) Tegoni, M.; Spinelli, S.; Verhoeyen, M.; Davis, P.; Cambillau, C. *J. Mol. Biol.* **1999**, *289*, 1375.
(121) Jiang, X.; Dreano, M.; Buckler, D. R.; Cheng, S.; Ythier, A.; Wu, H.; Hendrickson, W. A.; el Tayar, N. *Structure* **1995**, *3*, 1341.
(122) Zuiderweg, E. R.; Nettesheim, D. G.; Mollison, K. W.; Carter, G. W. *Biochemistry* **1989**, *28*, 172.
(123) March, D. R.; Proctor, L. M.; Stoermer, M. J.; Sbaglia, R.; Abbenante, G.; Reid, R. C.; Woodruff, T. M.; Wadi, K.; Paczkowski, N.; Tyndall, J. D.; Taylor, S. M.; Fairlie, D. P. *Mol. Pharmacol.* **2004**, *65*, 868.
(124) Wong, A. K.; Finch, A. M.; Pierens, G. K.; Craik, D. J.; Taylor, S. M.; Fairlie, D. P. *J. Med. Chem.* **1998**, *41*, 3417.
(125) Finch, A. M.; Wong, A. K.; Paczkowski, N. J.; Wadi, S. K.; Craik, D. J.; Fairlie, D. P.; Taylor, S. M. *J. Med. Chem.* **1999**, *42*, 1965.
(126) Strachan, A. J.; Shiels, I. A.; Reid, R. C.; Fairlie, D. P.; Taylor, S. M. *Br. J. Pharmacol.* **2001**, *134*, 1778.
(127) Woodruff, T. M.; Strachan, A. J.; Dryburgh, N.; Shiels, I. A.; Reid, R. C.; Fairlie, D. P.; Taylor, S. M. *Arthritis Rheum.* **2002**, *46*, 2476.
(128) Woodruff, T. M.; Arumugam, T. V.; Shiels, I. A.; Reid, R. C.; Fairlie, D. P.; Taylor, S. M. *J. Immunol.* **2003**, *171*, 5514.
(129) Sumichika, H.; Sakata, K.; Sato, N.; Takeshita, S.; Ishibuchi, S.; Nakamura, M.; Kamahori, T.; Ehara, S.; Itoh, K.; Ohtsuka, T.; Ohbora, T.; Mishina, T.; Komatsu, H.; Naka, Y. *J. Biol. Chem.* **2002**, *277*, 49403.
(130) Takabayashi, T.; Vannier, E.; Clark, B. D.; Margolis, N. H.; Dinarello, C. A.; Burke, J. F.; Gelfand, J. A. *J. Immunol.* **1996**, *156*, 3455.
(131) Fischer, W. H.; Jagels, M. A.; Hugli, T. E. *J. Immunol.* **1999**, *162*, 453.
(132) Huber, R.; Scholze, H.; Paques, E. P.; Deisenhofer, J. *Hoppe Seylers Z. Physiol. Chem.* **1980**, *361*, 1389.
(133) Morgan, E. L.; Weigle, W. O.; Hugli, T. E. *Fed. Proc.* **1984**, *43*, 2543.
(134) Morgan, E. L. *Complement* **1986**, *3*, 128.
(135) Humbles, A. A.; Lu, B.; Nilsson, C. A.; Lilly, C.; Israel, E.; Fujiwara, Y.; Gerard, N. P.; Gerard, C. *Nature* **2000**, *406*, 998.
(136) Bautsch, W.; Hoymann, H. G.; Zhang, Q.; Meier-Wiedenbach, I.; Raschke, U.; Ames, R. S.; Sohns, B.; Flemme, N.; Meyer zu Vilsendorf, A.; Grove, M.; Klos, A.; Kohl, J. *J. Immunol.* **2000**, *165*, 5401.
(137) Ember, J. A.; Johansen, N. L.; Hugli, T. E. *Biochemistry* **1991**, *30*, 3603.
(138) Reul, J. M.; Holsboer, F. *Curr. Opin. Pharmacol.* **2002**, *2*, 23.
(139) Keller, P. A.; Elfick, L.; Garner, J.; Morgan, J.; McCluskey, A. *Bioorg. Med. Chem.* **2000**, *8*, 1213.
(140) Romier, C.; Bernassau, J. M.; Cambillau, C.; Darbon, H. *Protein Eng.* **1993**, *6*, 149.
(141) Lau, S. H.; Rivier, J.; Vale, W.; Kaiser, E. T.; Kezdy, F. J. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 7070.
(142) Spyroulias, G. A.; Papazacharias, S.; Pairas, G.; Cordopatis, P. *Eur. J. Biochem.* **2002**, *269*, 6009.
(143) Miranda, A.; Lahrichi, S. L.; Gulyas, J.; Koerber, S. C.; Craig, A. G.; Corrigan, A.; Rivier, C.; Vale, W.; Rivier, J. *J. Med. Chem.* **1997**, *40*, 3651.
(144) Yamada, Y.; Mizutani, K.; Mizusawa, Y.; Hantani, Y.; Tanaka, M.; Tanaka, Y.; Tomimoto, M.; Sugawara, M.; Imai, N.; Yamada, H.; Okajima, N.; Haruta, J. *J. Med. Chem.* **2004**, *47*, 1075.
(145) Przewlocki, R.; Przewlocka, B. *Eur. J. Pharmacol.* **2001**, *429*, 79.
(146) Tessmer, M. R.; Kallick, D. A. *Biochemistry* **1997**, *36*, 1971.
(147) Tessmer, M. R.; Meyer, J. P.; Hruby, V. J.; Kallick, D. A. *J. Med. Chem.* **1997**, *40*, 2148.
(148) Spadaccini, R.; Crescenzi, O.; Picone, D.; Tancredi, T.; Temussi, P. A. *J. Pept. Sci.* **1999**, *5*, 306.
(149) Hruby, V. J.; Agnes, R. S. *Biopolymers* **1999**, *51*, 391.
(150) Lung, F. D.; Collins, N.; Stropova, D.; Davis, P.; Yamamura, H. I.; Porreca, F.; Hruby, V. J. *J. Med. Chem.* **1996**, *39*, 1136.
(151) Becker, J. A.; Wallace, A.; Garzon, A.; Ingallinella, P.; Bianchi, E.; Cortese, R.; Simonin, F.; Kieffer, B. L.; Pessi, A. *J. Biol. Chem.* **1999**, *274*, 27513.
(152) Okada, Y.; Tsuda, Y.; Bryant, S. D.; Lazarus, L. H. *Vitam. Horm.* **2002**, *65*, 257.
(153) Mizoguchi, H.; Tseng, L. F.; Suzuki, T.; Sora, I.; Narita, M. *Jpn. J. Pharmacol.* **2002**, *89*, 229.
(154) Zadina, J. E.; Hackler, L.; Ge, L. J.; Kastin, A. J. *Nature* **1997**, *386*, 499.
(155) Doi, M.; Asano, A.; Komura, E.; Ueda, Y. *Biochem. Biophys. Res. Commun.* **2002**, *297*, 138.
(156) In, Y.; Minoura, K.; Ohishi, H.; Minakata, H.; Kamigauchi, M.; Sugiura, M.; Ishida, T. *J. Pept. Res.* **2001**, *58*, 399.
(157) Podlogar, B. L.; Paterlini, M. G.; Ferguson, D. M.; Leo, G. C.; Demeter, D. A.; Brown, F. K.; Reitz, A. B. *FEBS Lett.* **1998**, *439*, 13.
(158) Okada, Y.; Fukumizu, A.; Takahashi, M.; Shimizu, Y.; Tsuda, Y.; Yokoi, T.; Bryant, S. D.; Lazarus, L. H. *Biochem. Biophys. Res. Commun.* **2000**, *276*, 7.
(159) Yamazaki, T.; Ro, S.; Goodman, M.; Chung, N. N.; Schiller, P. W. *J. Med. Chem.* **1993**, *36*, 708.
(160) Keller, M.; Boissard, C.; Patiny, L.; Chung, N. N.; Lemieux, C.; Mutter, M.; Schiller, P. W. *J. Med. Chem.* **2001**, *44*, 3896.
(161) Saviano, G.; Crescenzi, O.; Picone, D.; Temussi, P.; Tancredi, T. *J. Pept. Sci.* **1999**, *5*, 410.
(162) Bolli, M. H.; Marfurt, J.; Grisostomi, C.; Boss, C.; Binkert, C.; Hess, P.; Treiber, A.; Thorin, E.; Morrison, K.; Buchmann, S.; Bur, D.; Ramuz, H.; Clozel, M.; Fischli, W.; Weller, T. *J. Med. Chem.* **2004**, *47*, 2776.
(163) Davenport, A. P. *Pharmacol. Rev.* **2002**, *54*, 219.
(164) Janes, R. W.; Peapus, D. H.; Wallace, B. A. *Nat. Struct. Biol.* **1994**, *1*, 311.
(165) Andersen, N. H.; Chen, C. P.; Marschner, T. M.; Krystek, S. R., Jr.; Bassolino, D. A. *Biochemistry* **1992**, *31*, 1280.
(166) Hewage, C. M.; Jiang, L.; Parkinson, J. A.; Ramage, R.; Sadler, I. H. *J. Pept. Sci.* **1997**, *3*, 415.
(167) Huggins, J. P.; Pelton, J. T.; Miller, R. C. *Pharmacol. Ther.* **1993**, *59*, 55.
(168) Wallace, B. A.; Janes, R. W.; Bassolino, D. A.; Krystek, S. R., Jr. *Protein Sci.* **1995**, *4*, 75.
(169) Orry, A. J.; Wallace, B. A. *Biophys. J.* **2000**, *79*, 3083.
(170) Arvidsson, K.; Nemoto, T.; Mitsui, Y.; Ohashi, S.; Nakanishi, H. *Eur. J. Biochem.* **1998**, *257*, 380.
(171) Mills, R. G.; O'Donoghue, S. I.; Smith, R.; King, G. F. *Biochemistry* **1992**, *31*, 5640.
(172) Atkins, A. R.; Ralston, G. B.; Smith, R. *Int. J. Pept. Protein Res.* **1994**, *44*, 372.
(173) Coles, M.; Sowemimo, V.; Scanlon, D.; Munro, S. L.; Craik, D. J. *J. Med. Chem.* **1993**, *36*, 2658.
(174) Camerman, A.; Mastropaolo, D.; Karle, I.; Karle, J.; Camerman, N. *Nature* **1983**, *306*, 447.
(175) Griffin, J. F.; Langs, D. A.; Smith, G. D.; Blundell, T. L.; Tickle, I. J.; Bedarkar, S. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 3272.
(176) Blundell, T. L.; Hearn, L.; Tickle, I. J.; Palmer, R. A.; Morgan, B. A.; Smith, G. D.; Griffin, J. F. *Science* **1979**, *205*, 220.

(177) Aubry, A.; Birlirakis, N.; Sakarellos-Daitsiotis, M.; Sakarellos, C.; Marraud, M. *J. Chem. Soc., Chem. Commun.* **1988**, 963.
(178) Garbay-Jaureguiberry, C.; Roques, B. P.; Oberlin, R. *FEBS Lett.* **1977**, *76*, 93.
(179) Roques, B. P.; Garbay-Jaureguiberry, C.; Oberlin, R.; Anteunis, M.; Lala, A. K. *Nature* **1976**, *262*, 778.
(180) Milon, A.; Miyazawa, T.; Higashijima, T. *Biochemistry* **1990**, *29*, 65.
(181) Picone, D.; D'Ursi, A.; Motta, A.; Tancredi, T.; Temussi, P. A. *Eur. J. Biochem.* **1990**, *192*, 433.
(182) RudolphBohner, S.; Quarzago, D.; Czisch, M.; Ragnarsson, U.; Moroder, L. *Biopolymers* **1997**, *41*, 591.
(183) Malicka, J.; Groth, M.; Czaplewski, C.; Wiczk, W.; Liwo, A. *Biopolymers* **2002**, *63*, 217.
(184) Goodman, M. *Biopolymers* **2001**, *60*, 229.
(185) Rew, Y.; Malkmus, S.; Svensson, C.; Yaksh, T. L.; Chung, N. N.; Schiller, P. W.; Cassel, J. A.; DeHaven, R. N.; Taulane, J. P.; Goodman, M. *J. Med. Chem.* **2002**, *45*, 3746.
(186) Fox, K. M.; Dias, J. A.; Van Roey, P. *Mol. Endocrinol.* **2001**, *15*, 378.
(187) Ludwig, M.; Westergaard, L. G.; Diedrich, K.; Andersen, C. Y. *Best Pract. Res. Clin. Obstet. Gynecol.* **2003**, *17*, 231.
(188) Le, Y.; Murphy, P. M.; Wang, J. M. *Trends Immunol.* **2002**, *23*, 541.
(189) Edmundson, A. B.; Ely, K. R. *Mol. Immunol.* **1985**, *22*, 463.
(190) Gavuzzo, E.; Mazza, F.; Pochetti, G.; Scatturin, A. *Int. J. Pept. Protein Res.* **1989**, *34*, 409.
(191) Dentino, A. R.; Raj, P. A.; De Nardin, E. *Arch. Biochem. Biophys.* **1997**, *337*, 267.
(192) Dalpiaz, A.; Ferretti, M. E.; Pecoraro, R.; Fabbri, E.; Traniello, S.; Scatturin, A.; Spisani, S. *Biochim. Biophys. Acta* **1999**, *1432*, 27.
(193) Dalpiaz, A.; Vertuani, G.; Scatturin, A.; Vitali, F.; Varani, K.; Spisani, S. *Arzneimittelforschung* **2003**, *53*, 793.
(194) Le, Y.; Oppenheim, J. J.; Wang, J. M. *Cytokine Growth Factor Rev.* **2001**, *12*, 91.
(195) Joyce, J. G.; Hurni, W. M.; Bogusky, M. J.; Garsky, V. M.; Liang, X.; Citron, M. P.; Danzeisen, R. C.; Miller, M. D.; Shiver, J. W.; Keller, P. M. *J. Biol. Chem.* **2002**, *277*, 45811.
(196) Bedecs, K.; Berthold, M.; Bartfai, T. *Int. J. Biochem. Cell Biol.* **1995**, *27*, 337.
(197) Branchek, T.; Smith, K. E.; Walker, M. W. *Ann. N. Y. Acad. Sci.* **1998**, *863*, 94.
(198) Ohtaki, T.; Kumano, S.; Ishibashi, Y.; Ogi, K.; Matsui, H.; Harada, M.; Kitada, C.; Kurokawa, T.; Onda, H.; Fujino, M. *J. Biol. Chem.* **1999**, *274*, 37041.
(199) Ohman, A.; Lycksell, P. O.; Jureus, A.; Langel, U.; Bartfai, T.; Graslund, A. *Biochemistry* **1998**, *37*, 9169.
(200) Wennerberg, A. B.; Cooke, R. M.; Carlquist, M.; Rigler, R.; Campbell, I. D. *Biochem. Biophys. Res. Commun.* **1990**, *166*, 1102.
(201) Morris, M. B.; Ralston, G. B.; Biden, T. J.; Browne, C. L.; King, G. F.; Iismaa, T. P. *Biochemistry* **1995**, *34*, 4538.
(202) Kojima, M.; Hosoda, H.; Date, Y.; Nakazato, M.; Matsuo, H.; Kangawa, K. *Nature* **1999**, *402*, 656.
(203) Rosicka, M.; Krsek, M.; Jarkovska, Z.; Marek, J.; Schreiber, V. *Physiol. Res.* **2002**, *51*, 435.
(204) Gualillo, O.; Lago, F.; Gomez-Reino, J.; Casanueva, F. F.; Dieguez, C. *FEBS Lett.* **2003**, *552*, 105.
(205) Nagaya, N.; Kangawa, K. *Regul. Pept.* **2003**, *114*, 71.
(206) Bednarek, M. A.; Feighner, S. D.; Pong, S. S.; McKee, K. K.; Hreniuk, D. L.; Silva, M. V.; Warren, V. A.; Howard, A. D.; Van Der Ploeg, L. H.; Heck, J. V. *J. Med. Chem.***2000**, *43*, 4370.
(207) McDowell, R. S.; Elias, K. A.; Stanley, M. S.; Burdick, D. J.; Burnier, J. P.; Chan, K. S.; Fairbrother, W. J.; Hammonds, R. G.; Ingle, G. S.; Jacobsen, N. E.; et al. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 11165.
(208) Momany, F. A. *Growth Hormone Secratogues*; Springer-Verlag: New York, 1996.
(209) Schoen, W. R.; Pisano, J. M.; Prendergast, K.; Wyvratt, M. J., Jr.; Fisher, M. H.; Cheng, K.; Chan, W. W.; Butler, B.; Smith, R. G.; Ball, R. G. *J. Med. Chem.* **1994**, *37*, 897.
(210) DeVita, R. J.; Frontier, A. J.; Schoen, W. R.; Wyvratt, M. J.; Fisher, M. H.; Cheng, K.; Chan, W. W. S.; Butler, B. S.; Smith, R. G. *Helv. Chim. Acta* **1997**, *80*, 1244.
(211) Mayo, K. E.; Miller, L. J.; Bataille, D.; Dalle, S.; Goke, B.; Thorens, B.; Drucker, D. J. *Pharmacol. Rev.* **2003**, *55*, 167.
(212) Sasaki, K.; Dockerill, S.; Adamiak, D. A.; Tickle, I. J.; Blundell, T. *Nature* **1975**, *257*, 751.
(213) Braun, W.; Wider, G.; Lee, K. H.; Wuthrich, K. *J. Mol. Biol.* **1983**, *169*, 921.
(214) Sturm, N. S.; Lin, Y.; Burley, S. K.; Krstenansky, J. L.; Ahn, J. M.; Azizeh, B. Y.; Trivedi, D.; Hruby, V. J. *J. Med. Chem.* **1998**, *41*, 2693.
(215) Ahn, J. M.; Gitu, P. M.; Medeiros, M.; Swift, J. R.; Trivedi, D.; Hruby, V. J. *J. Med. Chem.* **2001**, *44*, 3109.
(216) Holz, G. G.; Chepurny, O. G. *Curr. Med. Chem.* **2003**, *10*, 2471.
(217) Drucker, D. J. *Mol. Endocrinol.* **2003**, *17*, 161.
(218) Drucker, D. J. *Diabetes* **1998**, *47*, 159.
(219) Chang, X. Q.; Keller, D.; Bjorn, S.; Led, J. J. *Magn. Res. Chem.* **2001**, *39*, 477.
(220) Giannoukakis, N. *Curr. Opin. Invest. Drugs* **2003**, *4*, 459.
(221) Neidigh, J. W.; Fesinmeyer, R. M.; Prickett, K. S.; Andersen, N. H. *Biochemistry* **2001**, *40*, 13188.
(222) Hinke, S. A.; Manhart, S.; Pamir, N.; Demuth, H.; R, W. G.; Pederson, R. A.; McIntosh, C. H. *Biochim. Biophys. Acta* **2001**, *1547*, 143.
(223) Vahl, T.; D'Alessio, D. *Curr. Opin. Clin. Nutr. Metab. Care* **2003**, *6*, 461.
(224) Miyawaki, K.; Yamada, Y.; Ban, N.; Ihara, Y.; Tsukiyama, K.; Zhou, H.; Fujimoto, S.; Oku, A.; Tsuda, K.; Toyokuni, S.; Hiai, H.; Mizunoya, W.; Fushiki, T.; Holst, J. J.; Makino, M.; Tashita, A.; Kobara, Y.; Tsubamoto, Y.; Jinnouchi, T.; Jomori, T.; Seino, Y. *Nat. Med.* **2002**, *8*, 738.
(225) Morrow, G. W.; Kieffer, T. J.; McIntosh, C. H.; MacGillivray, R. T.; Brown, J. C.; St Pierre, S.; Pederson, R. A. *Can. J. Physiol. Pharmacol.* **1996**, *74*, 65.
(226) Manhart, S.; Hinke, S. A.; McIntosh, C. H.; Pederson, R. A.; Demuth, H. U. *Biochemistry* **2003**, *42*, 3081.
(227) Millar, R.; Lowe, S.; Conklin, D.; Pawson, A.; Maudsley, S.; Troskie, B.; Ott, T.; Millar, M.; Lincoln, G.; Sellar, R.; Faurholm, B.; Scobie, G.; Kuestner, R.; Terasawa, E.; Katz, A. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 9636.
(228) Bolla, M.; Gonzalez, D.; Warde, P.; Dubois, J. B.; Mirimanoff, R. O.; Storme, G.; Bernier, J.; Kuten, A.; Sternberg, C.; Gil, T.; Collette, L.; Pierart, M. *N. Engl. J. Med.* **1997**, *337*, 295.
(229) Wheeler, J. M.; Knittle, J. D.; Miller, J. D. *Am. J. Obstet. Gynecol.* **1992**, *167*, 1367.
(230) Garnick, M. B. *N. Engl. J. Med.* **1984**, *311*, 1281.
(231) Brogden, R. N.; Buckley, M. M.; Ward, A. *Drugs* **1990**, *39*, 399.
(232) Parmar, H.; Phillips, R. H.; Lightman, S. L.; Edwards, L.; Allen, L.; Schally, A. V. *Lancet* **1985**, *2*, 1201.
(233) Albano, C.; Smitz, J.; Camus, M.; Riethmuller-Winzen, H.; Van Steirteghem, A.; Devroey, P. *Fertil. Steril.* **1997**, *67*, 917.
(234) Devroey, P. *Fertil. Steril.* **2000**, *73*, 15.
(235) Huirne, J. A.; Lambalk, C. B. *Lancet* **2001**, *358*, 1793.
(236) Digilio, G.; Bracco, C.; Barbero, L.; Chicco, D.; Del Curto, M. D.; Esposito, P.; Traversa, S.; Aime, S. *J. Am. Chem. Soc.* **2002**, *124*, 3431.
(237) Sealfon, S. C.; Weinstein, H.; Millar, R. P. *Endocr. Rev.* **1997**, *18*, 180.
(238) Rizo, J.; Sutton, R. B.; Breslau, J.; Koerber, S. C.; Porter, J.; Hagler, A. T.; Rivier, J. E.; Gierasch, L. M. *J. Am. Chem. Soc.* **1996**, *118*, 970.
(239) Karten, M. J.; Rivier, J. E. *Endocr. Rev.* **1986**, *7*, 44.
(240) Dutta, A. S. *Drugs Future* **1988**, *13*, 43.
(241) Cho, N.; Harada, M.; Imaeda, T.; Imada, T.; Matsumoto, H.; Hayase, Y.; Sasaki, S.; Furuya, S.; Suzuki, N.; Okubo, S.; Ogi, K.; Endo, S.; Onda, H.; Fujino, M. *J. Med. Chem.* **1998**, *41*, 4190.
(242) Kakar, S. S.; Musgrove, L. C.; Devor, D. C.; Sellers, J. C.; Neill, J. D. *Biochem. Biophys. Res. Commun.* **1992**, *189*, 289.
(243) Gaylinn, B. D. *Receptors Channels* **2002**, *8*, 155.
(244) Lin-Su, K.; Wajnrajch, M. P. *Rev. Endocr. Metab. Disord.* **2002**, *3*, 313.
(245) Cervini, L. A.; Donaldson, C. J.; Koerber, S. C.; Vale, W. W.; Rivier, J. E. *J. Med. Chem.* **1998**, *41*, 717.
(246) Digilio, G.; Barbero, L.; Bracco, C.; Corpillo, D.; Esposito, P.; Piquet, G.; Traversa, S.; Aime, S. *J. Am. Chem. Soc.* **2003**, *125*, 3458.
(247) Clore, G. M.; Martin, S. R.; Gronenborn, A. M. *J. Mol. Biol.* **1986**, *191*, 553.
(248) Brunger, A. T.; Clore, G. M.; Gronenborn, A. M.; Karplus, M. *Protein Eng.* **1987**, *1*, 399.
(249) Rao, C. V. *Fertil. Steril.* **2001**, *76*, 1097.
(250) Evans, J. J. *Arch. Physiol. Biochem.* **2002**, *110*, 154.
(251) Vitale, R. M.; Zaccaro, L.; Di Blasio, B.; Fattorusso, R.; Isernia, C.; Amodeo, P.; Pedone, C.; Saviano, M. *ChemBioChem* **2003**, *4*, 73.
(252) Mori, M.; Harada, M.; Terao, Y.; Sugo, T.; Watanabe, T.; Shimomura, Y.; Abe, M.; Shintani, Y.; Onda, H.; Nishimura, O.; Fujino, M. *Biochem. Biophys. Res. Commun.* **2001**, *283*, 1013.
(253) Bednarek, M. A.; Tan, C.; Hreniuk, D. L.; Palyha, O. C.; MacNeil, D. J.; Van Der Ploeg, L. H.; Howard, A. D.; Feighner, S. D. *J. Biol. Chem.* **2002**, *277*, 13821.
(254) Gantz, I.; Fong, T. M. *Am. J. Physiol. Endocrinol. Metab.* **2003**, *284*, E468.
(255) Lee, J. H.; Lim, S. K.; Huh, S. H.; Lee, D.; Lee, W. *Eur. J. Biochem.* **1998**, *257*, 31.
(256) Li, S. Z.; Lee, J. H.; Lee, W.; Yoon, C. J.; Baik, J. H.; Lim, S. K. *Eur. J. Biochem.* **1999**, *265*, 430.
(257) Grieco, P.; Lavecchia, A.; Cai, M.; Trivedi, D.; Weinberg, D.; MacNeil, T.; Van der Ploeg, L. H.; Hruby, V. J. *J. Med. Chem.* **2002**, *45*, 5287.
(258) Fotsch, C.; Smith, D. M.; Adams, J. A.; Cheetham, J.; Croghan, M.; Doherty, E. M.; Hale, C.; Jarosinski, M. A.; Kelly, M. G.; Norman, M. H.; Tamayo, N. A.; Xi, N.; Baumgartner, J. W. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2337.

(259) Bednarek, M. A.; Macneil, T.; Kalyani, R. N.; Tang, R.; Van der Ploeg, L. H.; Weinberg, D. H. *Biochem. Biophys. Res. Commun.* **1999**, *261*, 209.
(260) Victoria Silva Elipe, M.; Mosley, R. T.; Bednarek, M. A.; Arison, B. H. *Biopolymers* **2003**, *68*, 512.
(261) Bednarek, M. A.; MacNeil, T.; Kalyani, R. N.; Tang, R.; Van der Ploeg, L. H.; Weinberg, D. H. *J. Med. Chem.* **2001**, *44*, 3665.
(262) Haskell-Luevano, C.; Rosenquist, A.; Souers, A.; Khong, K. C.; Ellman, J. A.; Cone, R. D. *J. Med. Chem.* **1999**, *42*, 4380.
(263) Brown, J. C.; Cook, M. A.; Dryburgh, J. R. *Can. J. Biochem.* **1973**, *51*, 533.
(264) Itoh, Z. *Peptides* **1997**, *18*, 593.
(265) Feighner, S. D.; Tan, C. P.; McKee, K. K.; Palyha, O. C.; Hreniuk, D. L.; Pong, S. S.; Austin, C. P.; Figueroa, D.; MacNeil, D.; Cascieri, M. A.; Nargund, R.; Bakshi, R.; Abramovitz, M.; Stocco, R.; Kargman, S.; O'Neill, G.; Van Der Ploeg, L. H.; Evans, J.; Patchett, A. A.; Smith, R. G.; Howard, A. D. *Science* **1999**, *284*, 2184.
(266) Edmondson, S.; Khan, N.; Shriver, J.; Zdunek, J.; Graslund, A. *Biochemistry* **1991**, *30*, 11271.
(267) Jarvet, J.; Zdunek, J.; Damberg, P.; Graslund, A. *Biochemistry* **1997**, *36*, 8153.
(268) Andersson, A.; Maler, L. *J. Biomol. NMR* **2002**, *24*, 103.
(269) Haramura, M.; Okamachi, A.; Tsuzuki, K.; Yogo, K.; Ikuta, M.; Kozono, T.; Takanashi, H.; Murayama, E. *Chem. Pharm. Bull. (Tokyo)* **2001**, *49*, 40.
(270) Roumy, M.; Zajac, J. M. *Eur. J. Pharmacol.* **1998**, *345*, 1.
(271) Miskolzie, M.; Kotovych, G. *Biopolymers* **2003**, *69*, 201.
(272) Elshourbagy, N. A.; Ames, R. S.; Fitzgerald, L. R.; Foley, J. J.; Chambers, J. K.; Szekeres, P. G.; Evans, N. A.; Schmidt, D. B.; Buckley, P. T.; Dytko, G. M.; Murdock, P. R.; Milligan, G.; Groarke, D. A.; Tan, K. B.; Shabon, U.; Nuthulaganti, P.; Wang, D. Y.; Wilson, S.; Bergsma, D. J.; Sarau, H. M. *J. Biol. Chem.* **2000**, *275*, 25965.
(273) Bonini, J. A.; Jones, K. A.; Adham, N.; Forray, C.; Artymyshyn, R.; Durkin, M. M.; Smith, K. E.; Tamm, J. A.; Boteju, L. W.; Lakhlani, P. P.; Raddatz, R.; Yao, W. J.; Ogozalek, K. L.; Boyle, N.; Kouranova, E. V.; Quan, Y.; Vaysse, P. J.; Wetzel, J. M.; Branchek, T. A.; Gerald, C.; Borowsky, B. *J. Biol. Chem.* **2000**, *275*, 39324.
(274) In *Peptides*; Kalra, S. P., Ed.; Elsevier: 2001; Vol. 22.
(275) Michel, M. C.; Beck-Sickinger, A.; Cox, H.; Doods, H. N.; Herzog, H.; Larhammar, D.; Quirion, R.; Schwartz, T.; Westfall, T. *Pharmacol. Rev.* **1998**, *50*, 143.
(276) Monks, S. A.; Karagianis, G.; Howlett, G. J.; Norton, R. S. *J. Biomol. NMR* **1996**, *8*, 379.
(277) Bader, R.; Bettio, A.; Beck-Sickinger, A. G.; Zerbe, O. *J. Mol. Biol.* **2001**, *305*, 307.
(278) Keire, D. A.; Kobayashi, M.; Solomon, T. E.; Reeve, J. R., Jr. *Biochemistry* **2000**, *39*, 9935.
(279) Li, X. A.; Sutcliffe, M. J.; Schwartz, T. W.; Dobson, C. M. *Biochemistry* **1992**, *31*, 1245.
(280) Lerch, M.; Gafner, V.; Bader, R.; Christen, B.; Folkers, G.; Zerbe, O. *J. Mol. Biol.* **2002**, *322*, 1117.
(281) Ekblad, E.; Edvinsson, L.; Wahlestedt, C.; Uddman, R.; Hakanson, R.; Sundler, F. *Regul. Pept.* **1984**, *8*, 225.
(282) Doughty, M. B.; Hu, L. *Biopolymers* **1993**, *33*, 1195.
(283) Yao, S.; Smith-White, M. A.; Potter, E. K.; Norton, R. S. *J. Med. Chem.* **2002**, *45*, 2310.
(284) Jois, S. D.; Balasubramaniam, A. *Peptides* **2003**, *24*, 1035.
(285) Balasubramaniam, A.; Dhawan, V. C.; Mullins, D. E.; Chance, W. T.; Sheriff, S.; Guzzi, M.; Prabhakaran, M.; Parker, E. M. *J. Med. Chem.* **2001**, *44*, 1479.
(286) Ferris, C. F. In *Handbook of Physiology*; Makhlouf, G. M., Ed.; American Physiological Society: Bethesda, 1989; Vol. 11.
(287) Reches, A.; Burke, R. E.; Jiang, D.; Wagner, H. R.; Fahn, S. *Peptides* **1983**, *4*, 43.
(288) Binder, E. B.; Kinkead, B.; Owens, M. J.; Nemeroff, C. B. *Pharmacol. Rev.* **2001**, *53*, 453.
(289) Nieto, J. L.; Rico, M.; Santoro, J.; Herranz, J.; Bermejo, F. J. *Int. J. Pept. Protein Res.* **1986**, *28*, 315.
(290) Xu, G. Y.; Deber, C. M. *Int. J. Pept. Protein Res.* **1991**, *37*, 528.
(291) St-Pierre, S.; Lalonde, J. M.; Gendreau, M.; Quirion, R.; Regoli, D.; Rioux, F. *J. Med. Chem.* **1981**, *24*, 370.
(292) Sefler, A. M.; He, J. X.; Sawyer, T. K.; Holub, K. E.; Omecinsky, D. O.; Reily, M. D.; Thanabal, V.; Akunne, H. C.; Cody, W. L. *J. Med. Chem.* **1995**, *38*, 249.
(293) Pang, Y. P.; Cusack, B.; Groshan, K.; Richelson, E. *J. Biol. Chem.* **1996**, *271*, 15060.
(294) Luca, S.; White, J. F.; Sohal, A. K.; Filippov, D. V.; van Boom, J. H.; Grisshammer, R.; Baldus, M. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 10706.
(295) Amodeo, P.; Lopez Mendez, B.; Guerrini, R.; Salvadori, S.; Temussi, P. A.; Tancredi, T. *FEBS Lett.* **2000**, *473*, 157.
(296) Salvadori, S.; Picone, D.; Tancredi, T.; Guerrini, R.; Spadaccini, R.; Lazarus, L. H.; Regoli, D.; Temussi, P. A. *Biochem. Biophys. Res. Commun.* **1997**, *233*, 640.
(297) Zhang, C.; Miller, W.; Valenzano, K. J.; Kyle, D. J. *J. Med. Chem.* **2002**, *45*, 5280.
(298) Van Cauwenberghe, S.; Simonin, F.; Cluzeau, J.; Becker, J. A.; Lubell, W. D.; Tourwe, D. *J. Med. Chem.* **2004**, *47*, 1864.
(299) Lee, J. H.; Bang, E.; Chae, K. J.; Kim, J. Y.; Lee, D. W.; Lee, W. *Eur. J. Biochem.* **1999**, *266*, 831.
(300) Gerashchenko, D.; Shiromani, P. J. *Mol. Neurobiol.* **2004**, *29*, 41.
(301) Darker, J. G.; Porter, R. A.; Eggleston, D. S.; Smart, D.; Brough, S. J.; Sabido-David, C.; Jerman, J. C. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 737.
(302) Miskolzie, M.; Lucyk, S.; Kotovych, G. *J. Biomol. Struct. Dyn.* **2003**, *21*, 341.
(303) Asahi, S.; Egashira, S. I.; Matsuda, M.; Iwaasa, H.; Kanatani, A.; Ohkubo, M.; Ihara, M.; Morishima, H. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 111.
(304) Rose, J. P.; Wu, C. K.; Hsiao, C. D.; Breslow, E.; Wang, B. C. *Nat. Struct. Biol.* **1996**, *3*, 163.
(305) Wood, S. P.; Tickle, I. J.; Treharne, A. M.; Pitts, J. E.; Mascarenhas, Y.; Li, J. Y.; Husain, J.; Cooper, S.; Blundell, T. L.; Hruby, V. J.; et al. *Science* **1986**, *232*, 633.
(306) *Pharm. J.* **2000**, *264*, 871.
(307) Hock, D.; Magerlein, M.; Heine, G.; Ochlich, P. P.; Forssmann, W. G. *FEBS Lett.* **1997**, *400*, 221.
(308) Chorev, M. *Receptors Channels* **2002**, *8*, 219.
(309) Usdin, T. B. *Endocrinology* **1997**, *138*, 831.
(310) Usdin, T. B.; Bonner, T. I.; Hoare, S. R. *Receptors Channels* **2002**, *8*, 211.
(311) Fox, J. *Curr. Opin. Pharmacol.* **2002**, *2*, 338.
(312) Jin, L.; Briggs, S. L.; Chandrasekhar, S.; Chirgadze, N. Y.; Clawson, D. K.; Schevitz, R. W.; Smiley, D. L.; Tashjian, A. H.; Zhang, F. *J. Biol. Chem.* **2000**, *275*, 27238.
(313) Marx, U. C.; Austermann, S.; Bayer, P.; Adermann, K.; Ejchart, A.; Sticht, H.; Walter, S.; Schmid, F. X.; Jaenicke, R.; Forssmann, W. G.; et al. *J. Biol. Chem.* **1995**, *270*, 15194.
(314) Marx, U. C.; Adermann, K.; Bayer, P.; Forssmann, W. G.; Rosch, P. *Biochem. Biophys. Res. Commun.* **2000**, *267*, 213.
(315) Condon, S. M.; Morize, I.; Darnbrough, S.; Burns, C. J.; Miller, B. E.; Uhl, J.; Burke, K.; Jariwala, N.; Locke, K.; Krolikowski, P. H.; Kumar, N. V.; Labaudiniere, R. F. *J. Am. Chem. Soc.* **2000**, *122*, 3007.
(316) Shimizu, M.; Potts, J. T., Jr.; Gardella, T. J. *J. Biol. Chem.* **2000**, *275*, 21836.
(317) Tsomaia, N.; Pellegrini, M.; Hyde, K.; Gardella, T. J.; Mierke, D. F. *Biochemistry* **2004**, *43*, 690.
(318) Vaudry, D.; Gonzalez, B. J.; Basille, M.; Yon, L.; Fournier, A.; Vaudry, H. *Pharmacol. Rev.* **2000**, *52*, 269.
(319) Laburthe, M.; Couvineau, A.; Marie, J. C. *Receptors Channels* **2002**, *8*, 137.
(320) Inooka, H.; Endo, S.; Kitada, C.; Mizuta, E.; Fujino, M. *Int. J. Pept. Protein Res.* **1992**, *40*, 456.
(321) Wray, V.; Kakoschke, C.; Nokihara, K.; Naruse, S. *Biochemistry* **1993**, *32*, 5832.
(322) Inooka, H.; Ohtaki, T.; Kitahara, O.; Ikegami, T.; Endo, S.; Kitada, C.; Ogi, K.; Onda, H.; Fujino, M.; Shirakawa, M. *Nat. Struct. Biol.* **2001**, *8*, 161.
(323) Robberecht, P.; Gourlet, P.; De Neef, P.; Woussen-Colle, M. C.; Vandermeers-Piret, M. C.; Vandermeers, A.; Christophe, J. *Eur. J. Biochem.* **1992**, *207*, 239.
(324) Hinuma, S.; Habata, Y.; Fujii, R.; Kawamata, Y.; Hosoya, M.; Fukusumi, S.; Kitada, C.; Masuo, Y.; Asano, T.; Matsumoto, H.; Sekiguchi, M.; Kurokawa, T.; Nishimura, O.; Onda, H.; Fujino, M. *Nature* **1998**, *393*, 272.
(325) Roland, B. L.; Sutton, S. W.; Wilson, S. J.; Luo, L.; Pyati, J.; Huvar, R.; Erlander, M. G.; Lovenberg, T. W. *Endocrinology* **1999**, *140*, 5736.
(326) Ibata, Y.; Iijima, N.; Kataoka, Y.; Kakihara, K.; Tanaka, M.; Hosoya, M.; Hinuma, S. *Neurosci. Res.* **2000**, *38*, 223.
(327) D'Ursi, A. M.; Albrizio, S.; Di Fenza, A.; Crescenzi, O.; Carotenuto, A.; Picone, D.; Novellino, E.; Rovero, P. *J. Med. Chem.* **2002**, *45*, 5483.
(328) Macfarlane, S. R.; Seatter, M. J.; Kanke, T.; Hunter, G. D.; Plevin, R. *Pharmacol. Rev.* **2001**, *53*, 245.
(329) Matsoukas, J. M.; Panagiotopoulos, D.; Keramida, M.; Mavromoustakos, T.; Yamdagni, R.; Wu, Q.; Moore, G. J.; Saifeddine, M.; Hollenberg, M. D. *J. Med. Chem.* **1996**, *39*, 3585.
(330) Alexopoulos, K.; Panagiotopoulos, D.; Mavromoustakos, T.; Fatseas, P.; Paredes-Carbajal, M. C.; Mascher, D.; Mihailescu, S.; Matsoukas, J. *J. Med. Chem.* **2001**, *44*, 328.
(331) Ceruso, M. A.; McComsey, D. F.; Leo, G. C.; Andrade-Gordon, P.; Addo, M. F.; Scarborough, R. M.; Oksenberg, D.; Maryanoff, B. E. *Bioorg. Med. Chem.* **1999**, *7*, 2353.
(332) Hsu, S. Y.; Nakabayashi, K.; Nishi, S.; Kumagai, J.; Kudo, M.; Sherwood, O. D.; Hsueh, A. J. *Science* **2002**, *295*, 671.
(333) Samuel, C. S.; Parry, L. J.; Summers, R. J. *Curr. Opin. Pharmacol.* **2003**, *3*, 152.
(334) Dschietzig, T.; Stangl, K. *Cell. Mol. Life Sci.* **2003**, *60*, 688.
(335) Eigenbrot, C.; Randal, M.; Quan, C.; Burnier, J.; O'Connell, L.; Rinderknecht, E.; Kossiakoff, A. A. *J. Mol. Biol.* **1991**, *221*, 15.
(336) Bullesbach, E. E.; Yang, S.; Schwabe, C. *J. Biol. Chem.* **1992**, *267*, 22957.

(337) Dong, M.; Miller, L. J. *Receptors Channels* **2002**, *8*, 189.
(338) Bayliss, W. M.; Starling, E. H. *J. Physiol. (London)* **1902**, *28*, 325.
(339) Ishihara, T.; Nakamura, S.; Kaziro, Y.; Takahashi, T.; Takahashi, K.; Nagata, S. *EMBO J.* **1991**, *10*, 1635.
(340) Gronenborn, A. M.; Bovermann, G.; Clore, G. M. *FEBS Lett.* **1987**, *215*, 88.
(341) Hofland, L. J.; Lamberts, S. W. *Endocr. Rev.* **2003**, *24*, 28.
(342) Veber, D. F.; Freidlinger, R. M.; Perlow, D. S.; Paleveda, W. J., Jr.; Holly, F. W.; Strachan, R. G.; Nutt, R. F.; Arison, B. H.; Homnick, C.; Randall, W. C.; Glitzer, M. S.; Saperstein, R.; Hirschmann, R. *Nature* **1981**, *292*, 55.
(343) Melacini, G.; Zhu, Q.; Goodman, M. *Biochemistry* **1997**, *36*, 1233.
(344) Pohl, E. *Acta Crystallogr., D: Biol. Crystallogr.* **1995**, *51*, 48.
(345) Falb, E.; Salitra, Y.; Yechezkel, T.; Bracha, M.; Litman, P.; Olender, R.; Rosenfeld, R.; Senderowitz, H.; Jiang, S.; Goodman, M. *Bioorg. Med. Chem.* **2001**, *9*, 3255.
(346) Jiang, S.; Gazal, S.; Gelerman, G.; Ziv, O.; Karpov, O.; Litman, P.; Bracha, M.; Afargan, M.; Gilon, C.; Goodman, M. *J. Pept. Sci.* **2001**, *7*, 521.
(347) vonRoedern, E. G.; Lohof, E.; Hessler, G.; Hoffmann, M.; Kessler, H. *J. Am. Chem. Soc.* **1996**, *118*, 10156.
(348) Hirschmann, R. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1278.
(349) Severini, C.; Improta, G.; Falconieri-Erspamer, G.; Salvadori, S.; Erspamer, V. *Pharmacol. Rev.* **2002**, *54*, 285.
(350) Young, J. K.; Anklin, C.; Hicks, R. P. *Biopolymers* **1994**, *34*, 1449.
(351) Keire, D. A.; Fletcher, T. G. *Biophys. J.* **1996**, *70*, 1716.
(352) Chandrashekar, I. R.; Cowsik, S. M. *Biophys. J.* **2003**, *85*, 4002.
(353) Whitehead, T. L.; McNair, S. D.; Hadden, C. E.; Young, J. K.; Hicks, R. P. *J. Med. Chem.* **1998**, *41*, 1497.
(354) Grace, R. C.; Chandrashekar, I. R.; Cowsik, S. M. *Biophys. J.* **2003**, *84*, 655.
(355) Grace, R. C.; Lynn, A. M.; Cowsik, S. M. *J. Biomol. Struct. Dyn.* **2001**, *18*, 611.
(356) Bitan, G.; Zeltser, I.; Byk, G.; Halle, D.; Mashriki, Y.; Gluhov, E. V.; Sukhotinsky, I.; Hanani, M.; Selinger, Z.; Gilon, C. *J. Pept. Sci.* **1996**, *2*, 261.
(357) Weisshoff, H.; Nagel, T.; Hansicke, A.; Zschunke, A.; Mugge, C. *FEBS Lett.* **2001**, *491*, 299.
(358) Catalioto, R. M.; Criscuoli, M.; Cucchi, P.; Giachetti, A.; Gianotti, D.; Giuliani, S.; Lecci, A.; Lippi, A.; Patacchini, R.; Quartara, L.; Renzetti, A. R.; Tramontana, M.; Arcamone, F.; Maggi, C. A. *Br. J. Pharmacol.* **1998**, *123*, 81.
(359) Horwell, D. C.; Naylor, D.; Willems, H. M. G. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 31.
(360) Hirschmann, R.; Nicolaou, K. C.; Pietranico, S.; Salvino, J.; Leahy, E. M.; Sprengeler, P. A.; Furst, G.; Smith, A. B.; Strader, C. D.; Cascieri, M. A.; Candelore, M. R.; Donaldson, C.; Vale, W.; Maechler, L. *J. Am. Chem. Soc.* **1992**, *114*, 9217.
(361) Hirschmann, R.; Yao, W. Q.; Cascieri, M. A.; Strader, C. D.; Maechler, L.; CichyKnight, M. A.; Hynes, J.; vanRijn, R. D.; Sprengeler, P. A.; Smith, A. B. *J. Med. Chem.* **1996**, *39*, 2441.
(362) Emerson, C. H.; Torres, M. S. *BioDrugs* **2003**, *17*, 19.
(363) Cooper, D. S. *Lancet* **2003**, *362*, 459.
(364) Rapoport, B.; Chazenbalk, G. D.; Jaume, J. C.; McLachlan, S. M. *Endocr. Rev.* **1998**, *19*, 673.
(365) Sun, Y.; Lu, X.; Gershengorn, M. C. *J. Mol. Endocrinol.* **2003**, *30*, 87.
(366) Nillni, E. A.; Sevarino, K. A. *Endocr. Rev.* **1999**, *20*, 599.
(367) Prokai, L. *Prog. Drug Res.* **2002**, *59*, 133.
(368) Zhang, W. J.; Berglund, A.; Kao, J. L.; Couty, J. P.; Gershengorn, M. C.; Marshall, G. R. *J. Am. Chem. Soc.* **2003**, *125*, 1221.
(369) Howell, P. L.; Pangborn, W. A.; Marshall, G. R.; Zabrocki, J.; Smith, G. D. *Acta Crystallogr., C* **1995**, *51* (Pt 12), 2575.
(370) Chu, W.; Perlman, J. H.; Gershengorn, M. C.; Moeller, K. D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3093.
(371) Li, W. H.; Moeller, K. D. *J. Am. Chem. Soc.* **1996**, *118*, 10106.
(372) Kamiya, K.; Takamoto, M.; Wada, Y.; Fujino, M.; Nishikawa, M. *J. Chem. Soc., Chem. Commun.* **1980**, 438.
(373) Vicar, J.; Abillon, E.; Toma, F.; Piriou, F.; Lintner, K.; Blaha, K.; Fromageot, P.; Fermandjian, S. *FEBS Lett.* **1979**, *97*, 275.
(374) Moore, M. L., Ph.D., Washington, 1978.
(375) Usdin, T. B.; Gruber, C.; Bonner, T. I. *J. Biol. Chem.* **1995**, *270*, 15455.
(376) Usdin, T. B.; Dobolyi, A.; Ueda, H.; Palkovits, M. *Trends Endocrinol. Metab.* **2003**, *14*, 14.
(377) Piserchio, A.; Usdin, T.; Mierke, D. F. *J. Biol. Chem.* **2000**, *275*, 27284.
(378) Coste, S. C.; Quintos, R. F.; Stenzel-Poore, M. P. *Trends Cardiovasc. Med.* **2002**, *12*, 176.
(379) Dautzenberg, F. M.; Hauger, R. L. *Trends Pharmacol. Sci.* **2002**, *23*, 71.
(380) Rivier, J.; Gulyas, J.; Kirby, D.; Low, W.; Perrin, M. H.; Kunitake, K.; DiGruccio, M.; Vaughan, J.; Reubi, J. C.; Waser, B.; Koerber, S. C.; Martinez, V.; Wang, L.; Tache, Y.; Vale, W. *J. Med. Chem.* **2002**, *45*, 4737.
(381) Ames, R. S.; Sarau, H. M.; Chambers, J. K.; Willette, R. N.; Aiyar, N. V.; Romanic, A. M.; Louden, C. S.; Foley, J. J.; Sauermelch, C. F.; Coatney, R. W.; Ao, Z.; Disa, J.; Holmes, S. D.; Stadel, J. M.; Martin, J. D.; Liu, W. S.; Glover, G. I.; Wilson, S.; McNulty, D. E.; Ellis, C. E.; Elshourbagy, N. A.; Shabon, U.; Trill, J. J.; Hay, D. W.; Douglas, S. A.; et al. *Nature* **1999**, *401*, 282.
(382) Grieco, P.; Carotenuto, A.; Campiglia, P.; Zampelli, E.; Patacchini, R.; Maggi, C. A.; Novellino, E.; Rovero, P. *J. Med. Chem.* **2002**, *45*, 4391.
(383) Douglas, S. A.; Ohlstein, E. H. *Trends Cardiovasc. Med.* **2000**, *10*, 229.
(384) Flohr, S.; Kurz, M.; Kostenis, E.; Brkovich, A.; Fournier, A.; Klabunde, T. *J. Med. Chem.* **2002**, *45*, 1799.
(385) Grieco, P.; Carotenuto, A.; Patacchini, R.; Maggi, C. A.; Novellino, E.; Rovero, P. *Bioorg. Med. Chem.* **2002**, *10*, 3731.
(386) Carotenuto, A.; Grieco, P.; Campiglia, P.; Novellino, E.; Rovero, P. *J. Med. Chem.* **2004**, *47*, 1652.
(387) Croston, G. E.; Olsson, R.; Currier, E. A.; Burstein, E. S.; Weiner, D.; Nash, N.; Severance, D.; Allenmark, S. G.; Thunberg, L.; Ma, J. N.; Mohell, N.; O'Dowd, B.; Brann, M. R.; Hacksell, U. *J. Med. Chem.* **2002**, *45*, 4950.
(388) Nicole, P.; Lins, L.; Rouyer-Fessard, C.; Drouot, C.; Fulcrand, P.; Thomas, A.; Couvineau, A.; Martinez, J.; Brasseur, R.; Laburthe, M. *J. Biol. Chem.* **2000**, *275*, 24003.
(389) Theriault, Y.; Boulanger, Y.; St-Pierre, S. *Biopolymers* **1991**, *31*, 459.
(390) Blankenfeldt, W.; Nokihara, K.; Naruse, S.; Lessel, U.; Schomburg, D.; Wray, V. *Biochemistry* **1996**, *35*, 5955.
(391) Langs, D. A.; Smith, G. D.; Stezowski, J. J.; Hughes, R. E. *Science* **1986**, *232*, 1240.
(392) Wu, C. K.; Hu, B.; Rose, J. P.; Liu, Z. J.; Nguyen, T. L.; Zheng, C.; Breslow, E.; Wang, B. C. *Protein Sci.* **2001**, *10*, 1869.
(393) Walse, B.; Kihlberg, J.; Drakenberg, T. *Eur. J. Biochem.* **1998**, *252*, 428.
(394) Antohi, O.; Marepalli, H. R.; Yang, W.; Becker, J. M.; Naider, F. *Biopolymers* **1998**, *45*, 21.
(395) Zhang, Y. L.; Marepalli, H. R.; Lu, H. F.; Becker, J. M.; Naider, F. *Biochemistry* **1998**, *37*, 12465.
(396) Lee, B. K.; Lee, Y. H.; Hauser, M.; Son, C. D.; Khare, S.; Naider, F.; Becker, J. M. *Biochemistry* **2002**, *41*, 13681.
(397) Day, T. A.; Maule, A. G. *Peptides* **1999**, *20*, 999.
(398) Miskolzie, M.; Kotovych, G. *J. Biomol. Struct. Dyn.* **2002**, *19*, 991.
(399) Nachman, R. J.; Moyna, G.; Williams, H. J.; Tobe, S. S.; Scott, A. I. *Bioorg. Med. Chem.* **1998**, *6*, 1379.
(400) Konopinska, D.; Rosinski, G. *J. Pept. Sci.* **1999**, *5*, 533.
(401) Odell, B.; Hammond, S. J.; Osborne, R.; Goosey, M. W. *J. Comput.-Aided Mol. Des.* **1996**, *10*, 89.
(402) Zubrzycki, I. Z.; Gade, G. *Biochem. Biophys. Res. Commun.* **1994**, *198*, 228.
(403) Horne, T. J.; Doak, D. G.; Rayne, R. C.; Balacco, G.; O'Shea, M.; Campbell, I. D. *Proteins* **1994**, *20*, 356.
(404) Nittoli, T.; Coast, G. M.; Sieburth, S. M. *J. Pept. Res.* **1999**, *53*, 99.
(405) Roberts, V. A.; Nachman, R. J.; Coast, G. M.; Hariharan, M.; Chung, J. S.; Holman, G. M.; Williams, H.; Tainer, J. A. *Chem. Biol.* **1997**, *4*, 105.
(406) Shalev, D. E.; Yerushalmi, Y.; Pener, M. P.; Kustanovich, I. *Insect Biochem. Mol. Biol.* **2003**, *33*, 489.
(407) Chiang, H. *Gen. Eng. News* **2004**, *24* (9), 27−28.
(408) Harmar, A. J. *Genome Biol.* **2001**, *2*, REVIEWS3013.
(409) Bittermann, H.; Einsiedel, J.; Hubner, H.; Gmeiner, P. *J. Med. Chem.* **2004**, *47*, 5587.
(410) Tyndall, J. D. A.; Fairlie, D. P. *J. Mol. Recognit.* **1999**, *12*, 363.
(411) Fairlie, D. P.; Tyndall, J. D. A.; Reid, R. C.; Wong, A. K.; Abbenante, G.; Scanlon, M. J.; March, D. R.; Bergman, D. A.; Chai, C. L.; Burkett, B. A. *J. Med. Chem.* **2000**, *43*, 1271.
(412) Tyndall, J. D. A.; Nall, T.; Fairlie, D. P. *Chem. Rev.* **2005**, *105*, 973.
(413) Fan, Q. R.; Hendrickson, W. A. *Nature* **2005**, *433*, 269.

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