Can peptides be mimicked?

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The biological activity of peptides is of enormous interest to the pharmaceutical industry, but endogenous peptides themselves typically have some limitations regarding bioavailability and oral activity. Peptide mimicry by design used to be touted as a solution to these problems and was focused on impersonating secondary structural motifs, particularly β-turns, but this approach has yielded few pharmaceutical products. Today, the process of identifying and optimizing peptide mimics is driven mainly by screening to obtain hits, followed by optimization, which might include design based on arranging pharmacophores appropriately in three dimensions. A consequence of this is that one of the more difficult problems in drug discovery, namely the identification of non-peptide agonists at peptide receptors, is beginning to be solved.

It is many years since chemists first attempted to devise non-peptide molecules that resembled peptides in some way. Early and continuing attempts[1] have focused on the geometry of the peptide bond and secondary structural features of proteins such as α-helices and β-turns, the introduction of unnatural amino acids into the sequence of endogenous peptides, and the cyclization of peptide functional groups that were predicted or known to be in close proximity to each other. The process was heavily influenced by the peptide side of chemistry and by structural analysis compared with the peptide being mimicked. However, few of these classical peptidomimetics have found their way to the pharmaceutical market.

Although medicinal chemists were familiar with the connection between often-complex natural products (such as alkaloids) and biological activity, not many had realized that there might be cases where some structural connection between these and endogenous peptides could exist. The alkaloid component of the prototypic example of this had been around since the beginnings of civilization, yet it was not until 1975 that endogenous peptides, such as the enkephalins[2] and β-endorphin[3,4], were shown to have similar agonist properties to the classical opiates and to bind to the same family of receptors. Chemists scrambled to embrace this realization that two structurally diverse species might have a common mode of agonist action and, hence, a possible common interaction with a receptor. In those days, many medicinal and peptide chemists had become so entrenched in the use of the three-letter code for peptide sequences, which conveys nothing of the three-dimensional structure of a peptide, that such connections were initially dismissed.

Today, large numbers of strange and wonderful organic molecules are known to act as antagonists at peptide receptors or inhibitors at the active sites of enzymes that accept peptide substrates. By contrast, there are still relatively few examples of peptide mimicry by non-peptides that have functional similarity to the corresponding peptide or protein in a biological system. The purpose of this article is to provide a brief historical perspective and to summarize the current state-of-the-art concerning this aspect of peptide mimicry (i.e. non-peptide agonists at peptide receptors).

The opiates

The recognition that the classical opiates, such as morphine, interact at a receptor that accepts peptides occurred over several years. Receptor binding sites were first mapped out in mouse brain[5] and nervous tissue[6] using radiolabeled ligands. Several research groups then searched for endogenous ligands, resulting in the isolation, characterization and synthesis of the enkephalins[2] and

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β-endorphin[3,4]. What was interesting was that many of the opiate alkaloids produced a similar functional response compared with the endogenous ligands (i.e. they acted as agonists)[7]. The opiates also served as an important milestone in the analysis of structure–activity relationships (SARs), particularly because this was one of the first areas of medicinal chemistry to be widely subjected to structure–activity rationale. First published in 1976, the possible structural connection between the morphine skeleton and the enkephalins has remained a subject of controversy to this date, because of the conflicting nature of structural data that has emerged[8–10].

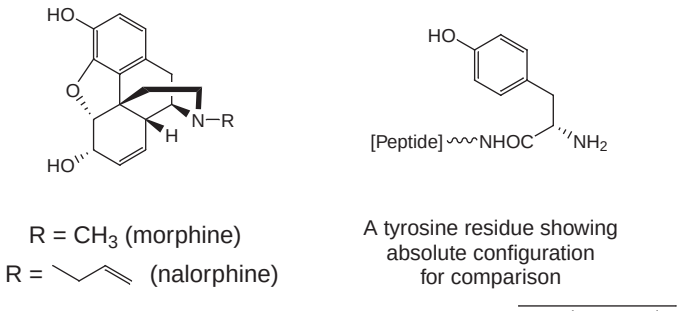


Figure 1. *Morphine (opiate agonist) and nalorphine (opiate antagonist) compared with the N-terminal residue of enkephalins and β-endorphin, a tyrosine, showing the absolute configurations.*

At first sight, the absolute configuration of the N-terminal amino group, a tyrosine residue, did not seem to fit the configuration of the N-methyl group of morphine (Fig. 1). However, such simplistic analyses of structural connections are easily changed with the flip of a bond or the rotation of an angle and certainly look different after 25 years of medicinal chemistry research. Of particular interest in the context of the future discovery of agonists is the fact that a plethora of opiate antagonists have been described that, in general, are all related to their corresponding agonists via simple structural changes. It is tempting to speculate that this is a general phenomena (i.e. within classes of non-peptide antagonists for peptide receptors, occasional agonists can consistently be found). By contrast, as will be seen later, the advent of functional HTS has meant that non-peptide agonists are sometimes discovered using paradigms that would not identify antagonists.

Has molecular biology had an impact?

Although the opiate story emerged at the beginning of the era of molecular biology, it remained the only example of a non-peptide agonist at a peptide receptor for approximately 15 years. This was despite the increasing availability of both wild-type and recombinant receptors, novel receptor subtypes, peptide endogenous ligands and their radiolabeled equivalents, newly discovered ligand receptor pairs, novel orphan receptors in the peptide class, libraries of peptides and non-peptides for screening, and continuing increases in screening throughput. It is only in the past few years that other examples of non-peptide agonists at peptide receptors have been reported and, although molecular biology has played a role in that process, there is a much closer temporal correlation between the advances in HTS and the discovery of more non-peptide agonists. It could be argued that combinatorial chemistry has also played a role, but the evidence from the literature is that the more traditional pharmaceutical company activities have had a greater impact, particularly in the ability to screen historical collections of compounds and the Merck (Rahway, NJ,

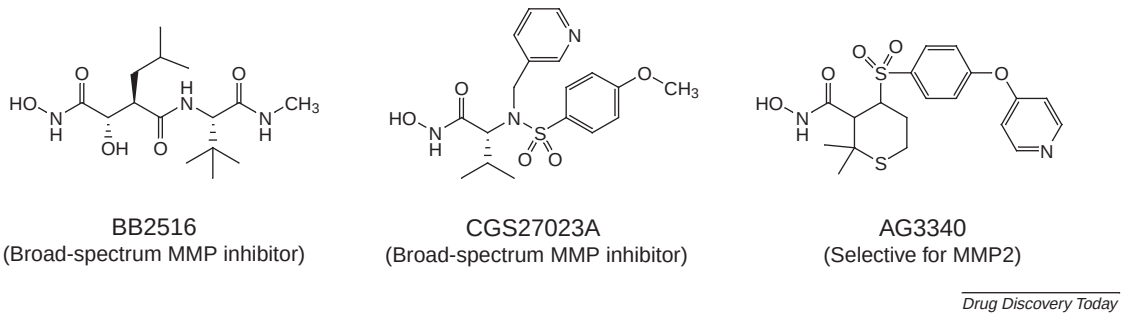


Figure 2. *A comparison of recent inhibitors of matrix metalloproteinases (MMPs), CGS27023A and AG3340 with the earlier 'peptoid' inhibitor BB2516.*

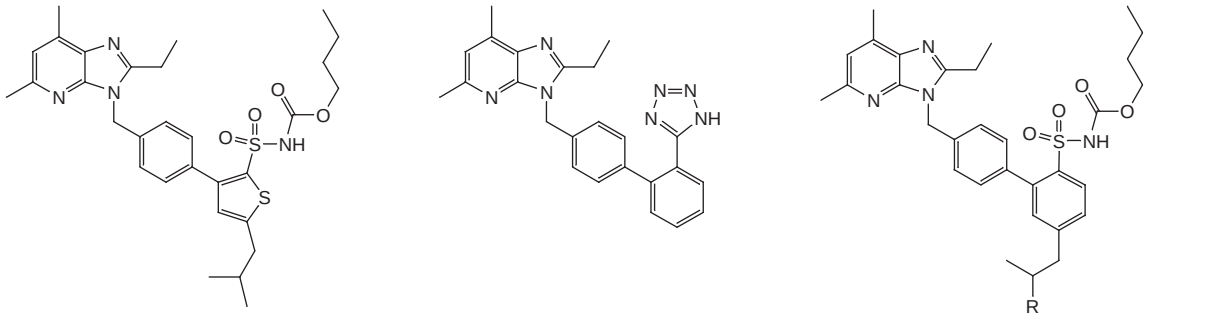


Figure 3. *A comparison of two angiotensin II partial agonists with closely related antagonists.*

USA) 'privileged structure' concept[11], which is now widely practised.

Enzyme active sites

It could be argued that peptide mimicry exists in the area of enzyme inhibition. The now classical captopril/enalapril ACE inhibitor story began with a decapeptide first isolated from the venom of a snake (*Bothrops jararaca*)[12,13]. A family of enzymes where two forms of endogenous inhibition are known are the matrix metalloproteinases (MMPs). The first type of inhibition concerns the so-called tissue inhibitors of MMPs (TIMPs)[14–16]. The second form is the process of self-inhibition shown by the latent forms of these enzymes. This is based on a key cysteine residue providing a fourth ligand to the active-site zinc atom, along with binding of adjacent peptide residues into the active site[17] (the cysteine switch) and has proved to be useful in the design of selective inhibitors[18]. Some early 'peptoid' hydroxamic acid inhibitors are well known, and more recent work has reported non-peptidic inhibitors (Fig. 2). CGS27023A (Ref. 19) is a broad-spectrum MMP inhibitor with low-nanomolar IC_{50}s, whereas AG3340 (Ref. 20) is potent and selective for MMP_2, which has an IC_{50} of 0.08 nM. Both have benefited from the hypothesis established with earlier inhibitors such as BB2516 (Ref. 21) that a sterically demanding group in the P_2' position assists with oral activity by preventing hydrolysis at adjacent hydrolyzable centers. However, none of these examples provides insight into surmounting the hurdle of achieving a direct functional response that has to be overcome with mimicry at peptide receptors.

Non-peptide agonists at G protein-coupled receptors

G protein-coupled receptors (GPCRs) have proven to be the most important receptors in the search for today's pharmaceuticals, with >60% of the worldwide market having some such lineage. Many of the peptide hormones identified in recent years exert their biological function via a subfamily of GPCRs. The prototypical morphine/enkephalin story already described is such an example of receptor mediation. Although the identification of non-peptide, small-molecule antagonists at such receptors has become a fairly straightforward screening exercise, the identification of the corresponding non-peptide agonists has thus far been one of the more difficult problems facing pharmaceutical researchers today.

Angiotensin II agonists

The potent vasoconstrictor, angiotensin II, is an endogenous octapeptide (DRVYIHPF-*OH*) formed from the removal of two amino acid residues from the C-terminus of the decapeptide angiotensin I (DRVYIHPFHL-*OH*) by angiotensin converting enzyme (ACE). The widespread success of ACE inhibitors encouraged researchers to examine alternative mechanisms for regulating the renin–angiotensin system and several angiotensin II inhibitors have progressed through research to the cardiovascular market[22]. It is currently thought that angiotensin II exerts its action through two closely related GPCRs, namely the AT_1 and AT_2 receptors. Careful examination in functional assays of molecules previously identified as potential antagonists in binding studies revealed that L162313 (Fig. 3) was a partial

AT_1-receptor agonist *in vitro* with EC_{50}s of 33 nM and 13 nM, respectively versus inositol phosphate accumulation in either CHO (Chinese hamster ovary) or COS-7 (African green monkey kidney) cells transfected with the rat AT_1 receptor. When rats were evaluated for increases in blood pressure *in vivo*, L162313 behaved as a full agonist and was the first example since the opiates of a non-peptide agonist at a peptide receptor[23]. Some interesting observations concerning this molecule include that in point mutation studies on the ligand-binding domain of the AT_1 receptor, L162313 does not bind to the same site as angiotensin II. In addition, as is the case with the opiates, a change of profile from antagonist to agonist is structurally related. An even more striking example was published later[24], where the structural change relating another partial agonist L162782 to an antagonist L162389 was shown to be as simple as the removal of a methyl group (Fig. 3).

Bradykinin-receptor agonists and antagonists

The endogenous peptide bradykinin is a pro-inflammatory nonapeptide having the sequence RPPGFSPFR-*OH*. Two GPCRs for this peptide, designated B_1 and B_2, have been identified by both molecular cloning and pharmacological methods. Agonism at the B_2 receptor appears to play a role in mediating pain, inflammation, asthma and hypotension. Considerable efforts have established an understanding of the SAR of bradykinin, resulting in many peptide agonists. Others have discovered novel hybrid and non-peptide antagonists. One of the more interesting examples that has emerged recently concerns the two structurally related analogs, FR165649 and FR190997 (Fig. 4)[25–27]. Both displace tritiated bradykinin from guinea pig ileum membranes with IC_{50}s of 0.47 nM and 1.5 nM, respectively. However, in the isolated guinea pig ileum, FR165649 behaves as an antagonist, having no effects on contraction and causing a concentration-dependent, parallel shift to the right of the dose–response curve for contractions induced by bradykinin. By contrast, FR190997 behaves as an agonist, inducing contractions in a dose-dependent manner with a pD_2 (the negative logarithm of EC_{50}) of 7.9. This particular molecule emerged from an extensive series of bradykinin-receptor antagonists[28–31]. Apparently, the introduction of a 2-pyridylmethoxy group on the quinoline nucleus results in a remarkable switch from potent antagonist to potent agonist.

Cholecystokinin agonists

Several cholecystokinin sequences are known to be endogenous hormones, including CCK-58, CCK-39, CCK-33, CCK-8 and CCK-4. They are found in the CNS and the gastrointestinal tract, although CCK-8, having the sequence DY(SO_3H)MGWMDF-*NH_2*, is considered to be the prototypical cholecystokinin. Responses are mediated through CCK_1 and CCK_2 GPCRs, of which CCK_1 has attracted particular attention in the context of obesity as it appears to be an intermediary in the regulation of satiety. Several groups have worked on both peptide and non-peptide antagonists and a 'peptoid' approach to CCK_1-receptor agonists has been reported, an example of which is shown in Fig. 5 (Ref. 32).

One of the more interesting efforts in this area has come from the GlaxoWellcome (Research Triangle Park, NC, USA) group who screened a series of carefully chosen analogs from their registry files for contractile activity on the isolated guinea pig gall bladder, a CCK_1-mediated effect. The NH and N-methyl derivatives (Fig. 6) were both

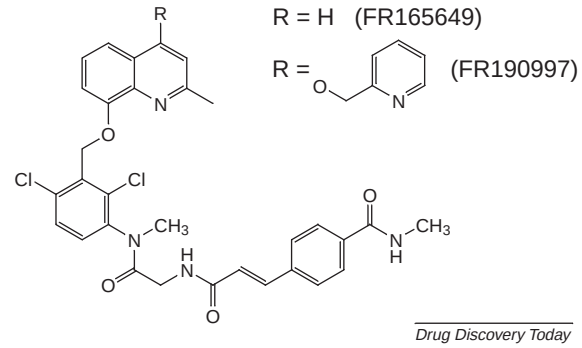


Figure 4. *A bradykinin-receptor agonist/antagonist pair: FR165649, a B_2-receptor antagonist and FR190997, a B_2-receptor agonist.*

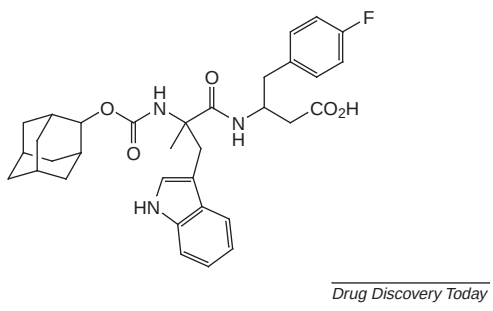


Figure 5. *A 'peptoid' CCK_1-receptor agonist/antagonist pair from Parke Davis (Cambridge, UK): the* RS*-diastereomer is a CCK_1-receptor agonist and the* SR*-diastereomer is a CCK_1-receptor antagonist.*

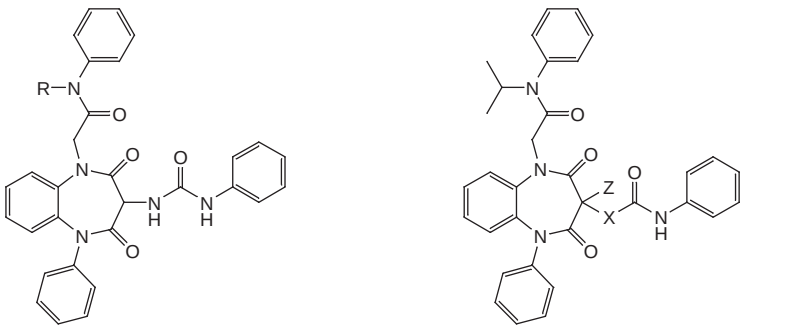


Figure 6. *Two series of GlaxoWellcome (Research Triangle Park, NC, USA) CCK_1-receptor agonists and antagonists.*

antagonists whereas the N-ethyl, N-propyl and N-isobutyl derivatives were all full CCK_1-receptor agonists[33]. Further optimization resulted in the discovery of orally active agonists such as GW5823 (Ref. 34; not shown) and GW7854 (Ref. 35) with potential for treating obesity. Subsequent work reported optimization at the C-3 position as illustrated by the analog where X = CH_2 and Z = CH_3 (Ref. 36) as well as unsuccessful attempts to optimize GW5823 (Fig. 6)[37].

Growth hormone secretagogues

The release of growth hormone (GH), a 191-amino acid regulator of body growth synthesized in the anterior pituitary gland, is positively regulated by growth hormone releasing hormone (GHRH) and negatively regulated by somatostatin. Some years ago, a set of synthetic hexapeptides was reported to stimulate GH secretion from pituitary cell cultures, apparently via a novel mechanism[38,39]. The intermediary of a receptor was postulated. These peptides became the prototypic GH secretagogues and were the subject of conventional SAR studies; for example, a series of cyclic tetrapeptides from Genentech (San Francisco, CA, USA)[40]. In recent years, a set of non-peptides has emerged as being potent GH secretagogues with a mechanism of action that shows that these molecules act as agonists at a seven-transmembrane (7-TM) GPCR (Refs 41,42) for which the endogenous ligand is currently not known. These small-molecule agonists were initially identified from HTS but more recently from the usual rationale, screening and serendipity. The benzolactam L692429 (Fig. 7) was one of the first to be discovered, has an EC_{50} of 60 nM for GH release from cultured pituitary cells, and at one time, was examined in the clinic but discontinued after Phase I studies because of poor oral bioavailability[43]. A later compound was the unusual dipeptide-like compound MK0677 (Fig. 7), with an EC_{50} of 1.3 nM (Ref. 44). This analog had 60% oral bioavailability in beagles and was long-acting but showed little efficacy in Phase II clinical trials in short-stature GH-deficient children and in a frailty prevention study in the elderly.

Other researchers have developed their own versions of dipeptide-like GH secretagogues that are being evaluated clinically. Two examples, NN703 and CP424391, are shown in Fig. 8. NN703 (NNC260703) has an 18 nM EC_{50}, a long half-life in dogs (4 h) after intravenous administration, and 30% oral bioavailability[45,46]. CP424391 has an EC_{50} of 3 nM, a short half-life in dogs of 1.5 h and high oral bioavailability in dogs and rats of 44% and 65%, respectively[47]. A recent report describes the quinazolinone derivative (Fig. 8) that has an IC_{50} of 16 nM versus the human GH secretagogue receptor and an ED_{50} of 0.63 nM for the secretion of GH from cultured rat pituitary cells[48].

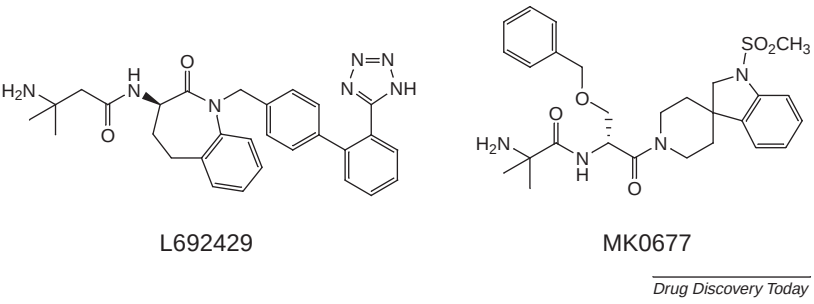


Figure 7. *Non-peptide growth hormone secretagogues L692429 and MK0677.*

Agonists at somatostatin receptors

Somatostatins are endogenous hormones that inhibit GH secretion, among other effects. Somatostatin-28 (SRIF-28) and SRIF-14 are the principal natural forms having sequences characterized by a single disulphide bridge (Fig. 9).

It is now known that they exert their actions via a set of 7TM GPCRs of which five subtypes have been identified, namely sst_1, sst_2, sst_3, sst_4, and sst_5. In recent years, this area has yielded almost as many agonists as the opiates. Initial progress towards small molecules was heavily influenced by a drug design approach making use of conformational knowledge of several cyclic peptide derivatives synthesized in the era prior to detailed knowledge of somatostatin receptors, such as octreotide[49] and MK678 (Ref. 50), which enabled key pharmacophores to adopt a similar spatial orientation. The discovery of a series of sugar derivatives (Fig. 10) that showed weak somatostatin mimicry with little selectivity for the receptor subtypes[51] was later complemented by a report that the related imidazole indole was somewhat selective for the sst_4 receptor, with an EC_{50} potency of 100 nM (Fig. 10)[52].

The Merck group has recently reported three related series of molecules, all of which are potent and selective sst_2-receptor agonists (Fig. 11). A representative of the first series, which emerged from a design exercise using the side-chain atomic coordinates of the cyclic hexapeptide L363377, is L054264, a selective sst_2-receptor agonist with a binding affinity of 1.6 nM and a selectivity ratio of >1000 versus other sst receptors[53]. The related compound L779976 emerged from deconvolution of a directed combinatorial library and shows remarkable potency (0.05 nM) and selectivity (6000–80,000) for the sst_2 receptor[54]. Interestingly, the cyclic ureas, although less potent (8.5 nM) versus sst_2, are orally active agonists *in vivo* (Fig. 11)[55].

A further set of agonists that are selective for sst_4 receptors are exemplified by two structures (Fig. 12). NNC2691100 has a binding affinity of 6 nM versus sst_4 receptors and is a full agonist having an EC_{50} of 2 nM in an sst_4-expressing cell-based cAMP assay[56]. L803087, which was produced using combinatorial chemistry, is also a full sst_4-receptor agonist in the pituitary cell functional assay and has a binding affinity of 0.7 nM for sst_4 receptors[57].

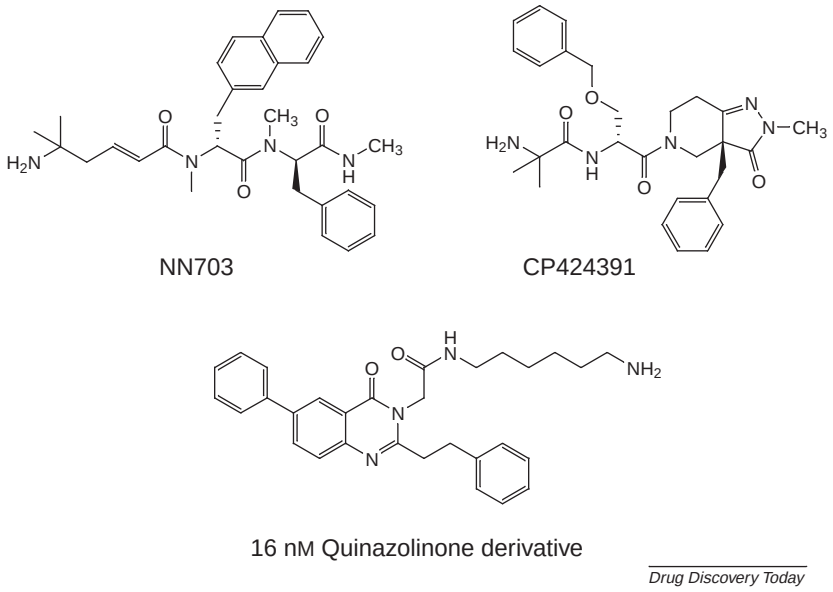


Figure 8. *Some recent growth hormone secretagogues.*

Tyrosine kinase receptors

There has been a quest for an insulin mimetic ever since the discovery of insulin in the 1920s. Other than variants of the protein itself, progress has been slow until recently. One of the most exciting reports is from the Merck group who have identified a constituent of a fungal (*Pseudomassaria* sp.) extract, L783281 (Fig. 13), as being an insulin mimetic in a variety of *in vitro* and *in vivo* assays[58]. Approximately 50,000 natural product extracts were screened in a cell-based assay using an over-expressed human insulin receptor in CHO cells from which insulin receptor tyrosine kinase activity could be measured.

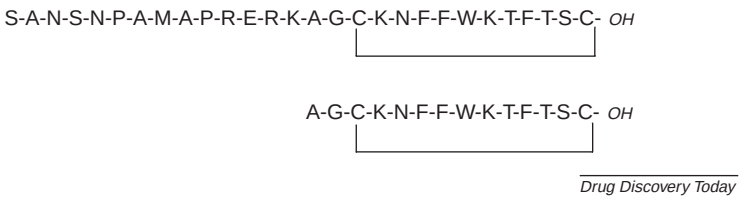
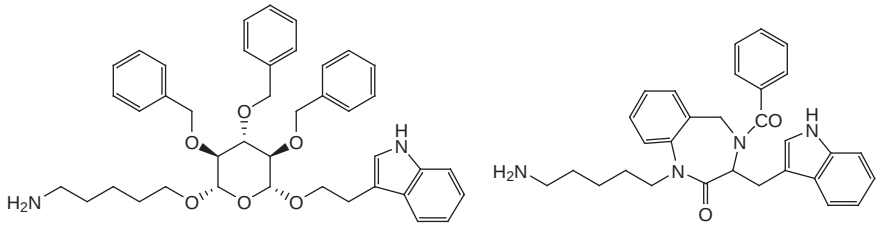
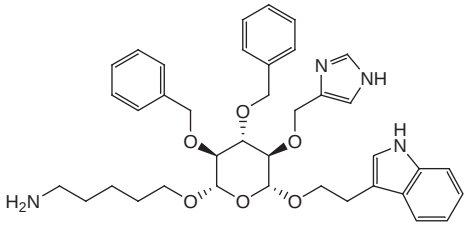


Figure 9. *A comparison of somatostatin-28 with somatostatin-14.*



Two non-selective, weak sst-receptor partial agonists



A 100 nM agonist selective for sst_4 receptors

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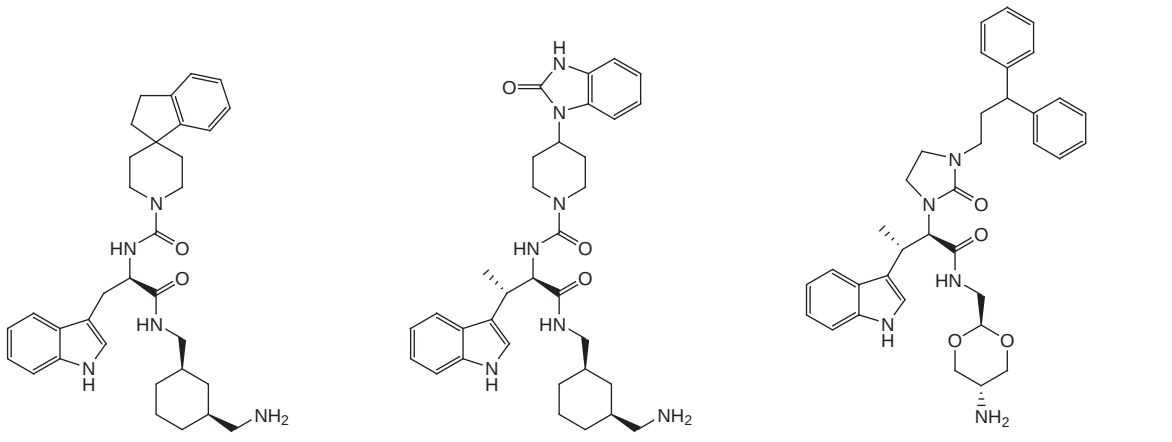
Figure 10. *A comparison between Hirschmann's sugar-based somatostatin agonists with an early Merck (Rahway, NJ, USA) benzodiazepinone agonist.*

L783281 emerged with a micromolar dose–response curve, whereas the closely related analogue L767827 (Fig. 13) was 100-times less active. L783281 showed antidiabetic activity on acute and chronic oral administration in *db/db* mice (a mouse model of diabetes and obesity driven by a defect in the *db* gene resulting in production of defective leptin receptors) at doses of 5–25 mg kg^{-1}, as measured by glucose levels and in *ob/ob* mice (a mouse model of diabetes and obesity driven by a defect in the *ob* gene resulting in production of truncated leptin) at doses of 5–20 mg kg^{-1}, as measured by glucose and insulin levels. It is particularly interesting to note that the symmetrical molecule is less active because receptor dimerization (often a feature of tyrosine kinase-based receptors) is somewhat different for the insulin receptor, which is a permanently dimerized crosslinked structure containing two A chains that constitute the receptor binding site and two B chains that are tyrosine kinases. There is evidence to suggest that the mechanism of action of L783281 differs from insulin in that direct activation of the tyrosine kinase domain of the insulin receptor (the intracellular β subunit) occurs.



L054264
(sst_2-receptor agonist)

L779976
(0.05 nM sst_2-receptor agonist)

Cyclic urea
(Orally active sst_2-receptor agonist)

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Figure 11. *Some recent somatostatin-receptor agonists selective for the somatostatin sst_2 receptor.*

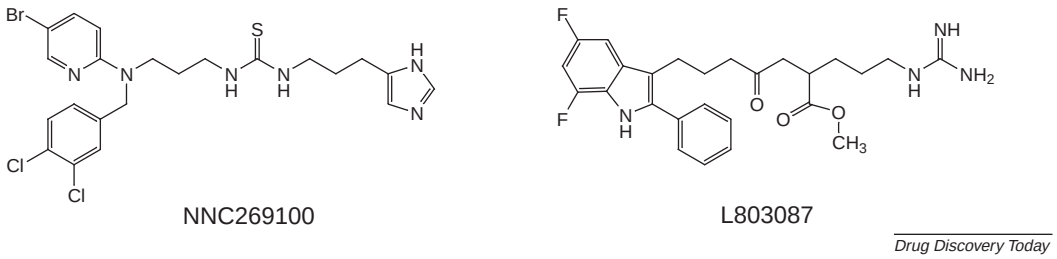


Figure 12. *Two potent and selective somatostatin sst_4-receptor agonists, NNC269100 and L803087.*

Growth factor and cytokine receptors

The GCSF (granulocyte colony stimulating factor) receptor belongs to a family of growth factor and cytokine receptors that are activated by ligand-stimulated oligomerization. The ligand GCSF induces homodimerization of the receptor that activates the intracellular tyrosine kinases, janus kinase 1 (JAK1) and JAK2, which can then associate with the receptor and phosphorylate tyrosine residues on the cytoplasmic face of the receptor. These serve as binding sites for various signaling proteins, which include the STATs (signal transducers and activators of transcription). The JAKs phosphorylate the STATs on tyrosine residues, the phosphorylated STATs then dimerize, translocate to the nucleus and bind to DNA sequences in the promoter region of responsive genes, thus regulating transcription. The Ligand (San Diego, CA, USA) and SmithKline Beecham (Philadelphia, PA, USA) groups recently reported the identification of a small-molecule GCSF agonist, the structure of which is shown in Fig. 14 (Ref. 59). This compound's symmetrical appearance strongly suggests a role in receptor dimerization. SB247464 was originally identified from a cell-based (4B6 clone of a murine myeloid cell line NFS60 containing a GCSF reporter construct using luciferase to generate a readout) screen of a large number of compounds. It was shown to be active in several *in vitro* and *ex vivo* models, as well as demonstrating granulopoietic activity *in vivo* on subcutaneous administration in female BDF-1 mice at doses between 20 and 50 mg kg^{-1}. Interestingly, this particular molecule would not be expected to work in humans, as careful examination of two chimeric murine/human receptors versus the murine receptor showed that SB247464 exerts its action exclusively at a murine extracellular domain adjacent to the transmembrane-spanning region of the receptor. This also demonstrates that the mode of receptor binding of SB247464 is probably different from that of GCSF and provides an intriguing counter to the classical methods of drug discovery, as this molecule could not have been identified from GCSF receptor-binding studies.

Conclusions

Out of eight different examples of non-peptide agonists at peptide receptors, four cases show a close structural relationship to corresponding antagonists. In four other cases, the screening used would not have detected antagonists and so the general hypothesis that, for a given receptor, agonist and antagonist are structurally related, might still be valid. The bottom line is that screening in functional assays leads to identification of agonists. Although all of the cases demonstrate functional mimicry between the peptide and the non-peptide, those that have been subjected to more detailed biochemical analysis do not appear to interact with their receptor in the same way that the natural ligand

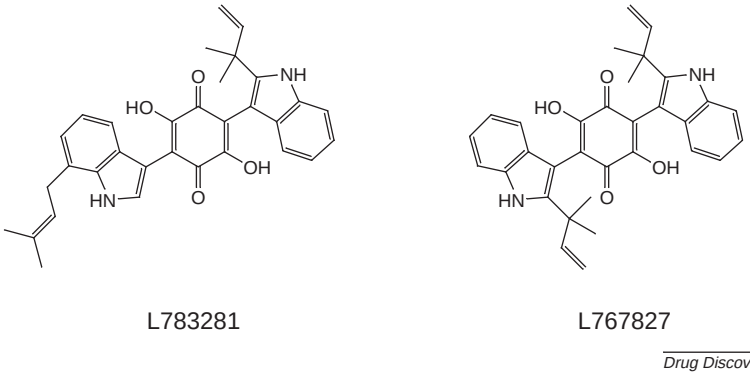
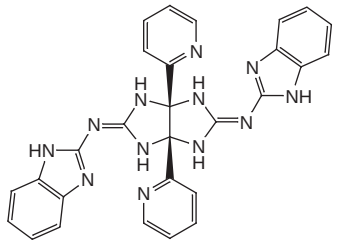


Figure 13. *Two insulin mimetics, L783281 and L767827 (100-times less active).*



SB247464

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Figure 14. *SB247464, which mimics the agonist activity of granulocyte colony stimulating factor (GCSF), but only in mice.*

does. The advent of the consequences of the Human Genome Project, together with ingenious screening techniques such as the use of constitutively active known or orphan receptors where neither the presence or knowledge of endogenous ligand is necessary[60], will result in a plethora of non-peptide agonists for peptide receptors in the near future.

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