Discovery and Validation of Novel Peptide Agonists for G-protein-coupled Receptors*

Ronen Shemesh 1, Amir Toporik, Zurit Levine, Iris Hecht, Galit Rotman, Assaf Wool, Dvir Dahary, Eyal Gofer, Yossef Kliger, Michal Ayalon Soffer, Avi Rosenberg, Dani Eshel, and Yossi Cohen

From Compugen Limited, 72 Pinchas Rosen St., Tel Aviv 69512, Israel

G-protein-coupled receptors (GPCRs) 2 represent a large group of receptors that are directly involved in cellular signaling networks and are considered to be an important family of targets for pharmacological intervention (reviewed in Ref. 1). It is estimated that approximately one-third of therapeutic development in the pharmaceutical industry is targeted toward drug discovery in the area of GPCRs (2). Recent analysis of the human genome identified over 800 putative members of the GPCR superfamily, over half of which are thought to be involved in sensory mechanisms and are not considered to be drug targets. Among the known GPCRs, ~300 are considered potential pharmaceutical targets (3). Out of this group around 150 GPCRs have no designated natural/endogenous ligand or activator. These receptors are termed “orphan GPCRs” and have recently become very attractive to many industrial and academic researchers as they hold great potential as targets for novel drug discovery (4).

Lately, a major effort was made by many academic institutes as well as biotech and pharmaceutical companies to identify both natural and surrogate ligands for both known and orphan GPCRs (5, 6). The most common method to screen for novel GPCR activators is through large scale screening of hundreds and thousands of compounds on many GPCRs, testing for in vitro activation of any given receptor by assessing its signaling response of an expressing cell to binding of a specific compound or tissue extract. This method, called “reverse pharmacology” (7, 8), gave rise in the past decade to over 30 new receptor/ligand pairs and many previously known or novel ligands, most of them peptides (5). However, this method is very expensive and time-consuming and might lead to many potential ligands either being missed or falsely annotated (6, 9). To increase the feasibility of this type of testing, it is necessary to pre-screen any potential group of ligands and focus on high chance, low risk candidates. This might allow more thorough screening and validation of true novel activators with better chances of becoming therapies.

Computational screening can supplement experimental efforts as a preliminary screening tool for efficient identification of natural ligands, especially peptides (10). Bioinformatics can serve as a powerful tool that provides reliable predictive measures to select for the high potential candidates and provide a spotlight pointed at potential new candidates for experimental discovery, thereby enabling higher success rates in identification of novel GPCR ligands (11).

In this study, we describe the experimental validation of computationally discovered novel GPCR-activating peptides. A subgroup of 33 potential candidate peptides was experimentally screened. Here we report the in vitro validation of eight novel GPCR peptide activators (hit rate of ~25%) as potential candidates for related clinical utilities. One of the examined peptides was tested in vivo and was found effective in a mouse inflammation model.

EXPERIMENTAL PROCEDURES

Data Set Preparation for the Proteolytic Site Predictor—All mammalian proteins (28,780) were downloaded from the Swiss Protein Database (release 43) (12). Of these, 11,705 proteins were classified as secreted or membrane proteins according to Swiss-Prot annotation. From these 11,705 proteins, 553 experimentally validated 18-mer peptide convertase proteolytic sites were extracted based on Swiss-Prot FT annotation lines. This subset of sequences was used as a learning set for Breiman’s random forest classifier algorithm (13), whereas the negative set (non proteolytic sites) consisted of all extracellular 18-mers.

1 To whom correspondence should be addressed: Compugen Ltd., 72 Pinchas Rosen St., Tel Aviv 69512, Israel. Tel.: 972-3-7658138; Fax: 972-3-7658555; E-mail: ronens@compugen.co.il.

The abbreviation used are: GPCR, G-protein-coupled receptor; PBS, phosphate-buffered saline; PMN, polymorphonuclear leukocyte; Ang, angiotensin.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
The predicted human secretome was generated by analyzing human proteins from the following two sources: (i) the Uniprot/Swiss Protein Database (version 47) and (ii) Refseq sequences from the NCBI nr protein data base (GenBank\textsuperscript{TM} version 149). There were 29,530 proteins from NCBI nr, including proteins with various validation annotations, reviewed, predicted, and modeled. The annotation for Uniprot proteins was used to filter out proteins that do not have a signal peptide, validated or predicted. For NCBI nr proteins, the SignalP 3.0 software (14) was used to predict the existence and location of a signal peptide.

For each protein precursor sequence, the following procedure was used to create the potential peptide products of the precursor. (The full cleavage prediction concept is detailed in Kliger et al. (15).) The predicted signal peptide was removed, and potential cleavage sites were scored based on the outcome of the machine learning algorithm used for cleavage site prediction. Based on precision/recall analysis, each putative cleavage site was assigned a score value between 0 and 10, where 0 represents a known cleavage site (as annotated on Uniprot) and 10 corresponds to unlikely cleavage sites. Sites with a score of 4 or less, and sites that contained two consecutive basic amino acids (e.g., Arg or Lys), regardless of their score, were selected. Peptides whose end points were either a selected cleavage site or an end point of the precursor (signal peptide cleavage site at the N terminus or the C terminus of the precursor) were created. Peptides longer than 100 amino acids were discarded. It should be noted that different peptides from the same precursor may have an overlapping sequence, and one can be a sub-sequence of another. The peptide set was extended by sequentially removing C-terminal basic amino acids and removing an additional C-terminal glycine, leaving an amide attached to the C terminus of the peptide (this mimics a known natural peptide maturation process called “glycine-directed peptide amida-tion”). All the intermediate peptides resulting from this extension were considered as separate peptide products. The complete list of predicted peptides (the “peptidome”) contained hundreds of thousands of peptides, which were then analyzed as a source data by the GPCR-activating ligand predictor.

**GPCR Ligand Predictor**—The set of known GPCR peptide ligands was extracted from Uniprot, using the Uniprot annotation (SRS from Swiss-Prot and by relevant keywords from GPCRDB (16)) and was manually curated based on literature search. The sequence lengths and positions of the peptides were extracted from the Swiss-Prot feature table. The learning data set contained 64 precursors and 123 peptides. Of these, 87 did not overlap with another peptide of the same precursor. Several known GPCR ligands were removed from the learning set (for example, the chemokine family and the hormone proteins that are larger than 100 amino acids) giving an average peptide size of 26 amino acids. The learning process also required a negative set of peptides that are not known as GPCR peptide ligands. Two negative sets of peptides were constructed. The first set was extracted based on Uniprot using annotations to filter peptides that are not GPCR peptide ligands. The second set was a collection of 3000 randomly chosen peptides from the novel peptide data base (peptidome) described above.

Using the generated learning set, a classifier based on the “Random Forest” algorithm (13) was implemented to select peptides that are likely to be GPCR ligands. Following a thorough optimization process, the classifier was programmed to analyze the following index parameters. The length of the peptide (in amino acids). The frequency of each amino acid in the peptide relative to the peptide length (21 parameters, one for each amino acid and an extra parameter for those that are not conventional amino acids). The amino acids in proximity to the N-terminal cleavage site from both sides as follows: the first four amino acids in the N terminus of the peptide and the last four amino acids of the precursor preceding the cleavage site (eight parameters, and each can be one of 21 values, one for each amino acid and an extra value for other letters or for amino acids beyond the signal peptide cleavage site). This parameter (four amino acids) was found most effective in the optimization process. The amino acids in proximity to the C-terminal cleavage site from both sides are as follows: the last three amino acids in the C terminus of the peptide and the first three amino acids of the precursor following the cleavage site (six parameters, each can be one of 21 values, one for each amino acid and an extra value for other letters or for amino acids beyond the precursor end). This parameter (3 amino acids) was found most effective in the optimization process. The last entry is the number of coding exons in the gene coding for of the precursor.

Based on these numeric parameters the classifying program was trained to distinguish between the learning set of known ligands (true set) and the negative (false) set. This program was then used to give each predicted peptide a score signifying the likelihood of being a GPCR-activating ligand. At the end of the process, a dataset of 100 peptides with the highest classifier scores was created. These peptides underwent a manual expert analysis process and literature review by a few different biologists. The selection of GPCR-activating peptides was based on the following criteria: expression profile and tissue specificity of the precursor, with relation to the receptor; comparison of the cleavage and GPCR classifier scores of the candidate peptides and their mouse orthologs; conservation of the sequence and cleavage sites of the peptide in the precursor proteins of all orthologs; position of the peptide within the precursor with respect to known domains and features (as a negative rule); and the number of cysteine residues and disulfide bridge annotations.

The peptides were also checked for their novelty status based upon the comparison with sequences appearing in patent data bases with very strict exclusion parameters. This process resulted in a subset of 35 confirmed novel peptides most likely to be GPCR ligands.

**Peptide Synthesis**—For each peptide, from the subset of 35 peptides, a number of modifications were made to enhance stability and functionality. In peptides containing a single cysteine residue, the cysteine was replaced by either a serine or a valine depending on the hydrophobicity and amino acid content of the peptide. For peptides containing a glycine residue at the C terminus, the glycine was replaced by an amide. Peptides were synthesized (10 mg) by Pepscan Inc. (The Netherlands)
and purified by high pressure liquid chromatography to >90\% (confirmed by mass spectrometry). Apart from one peptide that failed synthesis, and one insoluble peptide, which was discarded, all remaining 33 peptides were soluble in PBS or in Lab-grade purified water.

**G-protein-coupled Receptor Selection**—The list of receptors was generated by ranking all human (GPCRDB (16)) receptors according to their clinical relevance. All known odorant and taste receptors were omitted. GPCRs known to be activated by non-peptide ligands and small molecules (such as dopamine, serotonin, purines, etc.) were removed from the list together with receptors for proteins (size over 50 amino acids) such as chemokines. The final list of receptors contained mainly peptide-activated GPCRs together with receptors annotated as orphan, with a predicted high probability of being activated by a peptide (based upon sequence similarity and evolutionary relation to GPCRs activated by peptides and literature searches). Each receptor was also assessed for its most potent known ligand as a positive control for the screening experiments.

**GPCR Screening for Ca\(^{2+}\) Activation by the Predicted Peptides**—The experimental screening was done by Applied Cell Sciences, Inc., on all the selected GPCRs by utilizing the promiscuous Go\(_{16}\) to divert signaling to the G\(_q\) pathway, thus enabling readout of GPCR activation by testing for Ca\(^{2+}\) flux as described (17). Peptides were diluted in PBS containing 0.1\% bovine serum albumin. All plates were stored at \(-80^\circ\)C until used. cDNA clones of the GPCRs were commercially obtained in one of the following expression vectors: pcDNA3.1, pCMV6, or MO2.

Transient transfections were performed using CHO-K1 cells as host cells. Cells (12 millions) were plated into T75 flasks on the day preceding transfection. Cells were transfected with a GPCR DNA and Go\(_{16}\) using a lipid technique according to the manufacturer’s recommendation. Cells were transfected for 5 h, then re-plated into 96-well dishes (60,000 cells per well), and grown overnight.

On the day of the experiment, cells were loaded with Fluo4-NW (Invitrogen) according to the manufacturer’s recommendation. Plates were loaded into a FlexStation\(^TM\) (Molecular Devices) plate reader, and fluorescence was monitored. Seventeen seconds following initiation of reading, cells were stimulated with the indicated agonist/compound at final concentration of 1 \(\mu\)M. Each 96-well plate contained each of the examined peptides in triplicate. We defined a hit as a peptide that elicited a clear and distinct increase in intracellular calcium that is clearly visible and statistically significant (using a \(t\) test comparing the levels of calcium before and after peptide addition with \(p\) value lower than 0.001) upon examination of the calcium trace for at least two repeats.

**GPCR Screening for Cyclic AMP Accumulation with Candidate Peptides**—Applied Cell Sciences transiently transfected 13 different GPCRs included within the list of screened receptors into CHO-K1 cells as described previously. Transfected cells were plated into 24 wells of a 96-well plate. Cells were pretreated with 0.5 \(\mu\)M isobutylmethylxanthine (stimulation buffer) for 10 min at 37 °C, then stimulated with either a positive control or a candidate peptide for G\(_i\) functional examination, followed by stimulation or a preincubation with 10 \(\mu\)M forskolin (for G\(_i\) functional examination). Following a 20-min stimulation with 1 \(\mu\)M of either positive control or the tested peptides, either with or without forskolin, intracellular cAMP was assayed using the Hit Hunter cAMP kit (DiscoveRx Corp.), according to the manufacturer’s recommended protocol. Data were converted to nanomoles of cAMP by running a standard cAMP curve.

**Dose Response of GPCR Activation and Affinity (EC\(_{50}\)) Measurements**—Cells were transfected as described above. Each peptide was added, in triplicate, according to functional assay selected (either Ca\(^{2+}\) or cAMP) in final concentrations of 1, 3, 10, 30, 100, and 300 \(\mu\)M and 1 and 3 \(\mu\)M. All peptides were examined and compared with similar concentrations of a selected positive control (as described in Table 1). EC\(_{50}\) best fit values (representing affinity) were calculated by nonlinear regression of sigmoidal dose-response curves, using Prism version 4 (GraphPad Software Inc., San Diego). The formula for the sigmoidal dose-response curves was defined as \(Y = bottom + (top - bottom)/(1 + 10^{(logEC_{50} - X)} \times Hill slope)\).

**In Vivo Activity of P58 and Its Derivates**—Male out-bred Swiss albino mice were purchased from Harlan, UK (T.O. strain). All animals were housed for 7 days prior to experimentation to allow body weight to reach \(\sim 25\) g on the day of the experiment. Air pouches were formed by injecting 2.5 ml of sterile air subcutaneously to the back of the mice 3 and 6 days before the experiment. Before use, peptides were dissolved with pyrogen-free PBS (Invitrogen, catalog number 14190-094). Peptides or vehicle were administered intravenously at a final volume of 200 \(\mu\)l at the indicated doses immediately before intra-pouch injection of 1 mg of zymosan A (Sigma). Four hours post-treatment, air pouches were washed with 2 ml of ice-cold PBS containing 3 \(\mu\)M EDTA and 25 units/ml of heparin and kept on ice. Polymorphonuclear neutrophil neutrophil content was determined by staining an aliquot of the lavage with phycoerythrin-conjugated anti-GR-1 monoclonal antibody (eBiosciences). An irrelevant phycoerythrin-conjugated rat IgG2b antibody served as isotype control. Samples were analyzed by FACScan (BD Biosciences). For determination of total leukocytes, lavages diluted 1:10 in Turk’s solution (0.01% crystal violet in 3% acetic acid) were analyzed using Neubauer hemocytometer and light microscope (Olympus B061). Statistical differences were determined by analysis of variance, plus Student Newman Keuls test. A \(p\) value <0.05 was taken as significant.

**RESULTS**

**Generation of in Silico Predicted Human GPCR-activating Peptides**—A list of the 100 highest scoring peptides as potential GPCR ligands were manually examined and ranked according to additional peptide characteristics (see “Experimental Procedures”). Finally, 33 peptides were synthesized and screened against 152 GPCRs.

**Screening Results, Ca\(^{2+}\) Flux Assay**—Screening was carried out on all types of GPCRs by utilizing the promiscuous Go\(_{16}\) to divert signaling to the G\(_q\) pathway, thus enabling readouts of G\(_q\), G\(_i\), and G\(_s\) for the GPCR activation by testing for Ca\(^{2+}\) flux (17). The efficiency of this method in diverting either cAMP
TABLE 1
Novel peptides that were found to activate GPCRs
Description of the precursor proteins and peptides that were found to functionally activate GPCRs in the screening process (at 1 μM) and in dose-response activation assays.

| Peptide name | Protein name of precursor | Swiss-Prot ID | Sequence |
|--------------|--------------------------|---------------|----------|
| P35          | HWKM1940                 | Q6UWF9_HUMAN  | SMCHRWSRAVLFPAAHRP \(^a\) |
| P58          | B9                       | Q9UPM9_HUMAN  | TLIPVYESTSESQKPTSWFMC \(^b\) |
| P58-5        |                          |               | LQKFTSWFMC \(^b\) |
| P58-4        |                          |               | FTSNFMG \(^b\) |
| P59          | Complement C1q tumor necrosis factor-related protein 8 (Precursor) | CIQT8_HUMAN | GQKQQVGGPAAQRRAAYASFVG\(^+\) |
| P74          | Complement C1q tumor necrosis factor-related protein 8 (Precursor) | CIQT8_HUMAN | GQKQQVGGPAAQRRAAYASFVG\(^+\) |
| P60          | Uncharacterized protein C5orf29 | CE029_HUMAN | GIGCWWRKHRVATRTLPRPLQ\(^a\) |
| P61          | TMEM9                    | Q6UX7_HUMAN   | FLGTYLPE5REERGDPAPKRELGD\(^b\) |
| P63          | Bone morphogenetic protein 3b (Precursor) | BMP3B_HUMAN | AHAQSEKHIQLPSFPRALKPRPG\(^b\) |
| P94          | Bone morphogenetic protein 3b (Precursor) | BMP3B_HUMAN | AQAQTLPQDNLGLDERPRRAHQKFHKQLPSFPRALKPRPG\(^b\) |

\(^a\) Underlined Cys was replaced by Val for the validation experiment.
\(^b\) C-terminal glycine was replaced by an amide.
\(^c\) Underlined Cys was replaced by Ser for the validation experiment.

TABLE 2
GPCRs activation and specificity by screened peptides
Positive hits and calculated EC\(_{50}\) values for Compugen’s peptides and receptor-specific positive controls. Each hit is indicated by a “+” mark as well as a calculated EC\(_{50}\) value (μM). The observed affinity (EC\(_{50}\)) value is also indicated for the positive control peptides (with the exception of Ang 1–7, where no activation of the MAS1 receptor was observed, NA). ND means no data.

| Receptor | Positive controls (EC\(_{50}\) μM) | P60 | P61 | P94 | P63 | P58 | P33 | P59 | P74 |
|----------|-----------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|
| MRGX1    | BAM22 (0.08)                      | (0.3)| (0.9)| (0.6)| (0.5)|     |     |     |     |
| MRGX2    | Cort14 (0.61)                     | (1.0)| (0.57)| (0.6)| (0.5)|     |     |     |     |
| MAS1     | Ang1–7 (NA)                       | (1.5)| (1.3)| (0.55)| (2.0)|     |     |     |     |
| FPRL1    | FMLP (0.85)                       |     |     |     |     |     |     |     |     |
| LGR7 (RXFP1) \(^a\) | Relaxin (ND)                    |     |     |     |     |     |     |     |     |
| LGR8 (RXFP2) \(^b\) | Relaxin (ND)                     |     |     |     |     |     |     |     |     |

\(^a\) Receptors were included in the screening. No activation by Compugen peptides was observed. Activation by positive control is indicated from the literature.
\(^b\) Data were examined by cAMP inhibition assay. No dose response was performed (ND).

3. I. Hecht, J. Rong, A. L. F. Samapio, C. Herzem, C. Rutledge, R. Shemesh, A. Toporkir, M. Beiman, L. Dassa, H. Niv, G. Gojocaru, A. Zuberlen, G. Rotman, M. Perrett, J. Vinten-Johansen, and Y. Cohen, manuscript in preparation.
control (Ang 1–7), a known ligand for MAS1 (23). However, our peptides did activate the receptor at concentrations similar to that found for dynorphin A (an established surrogate, lower affinity activator of MAS1). Our peptides were specific to MAS1 and did not activate AT1 or AT2, angiotensin II receptors which are known to be weakly activated by Ang 1–7 (Table 2).

Cyclic AMP Screening Assay Results—Because the promiscuous Gα16 does not always divert a cAMP-related signal to Ca2+ accumulation, a subset of 13 GPCRs with very low activation by either the positive controls or any of the novel examined peptides was selected to be tested for Gi/Gs activation by 2 of the 33 examined peptides. These receptors were screened for cAMP accumulation (Gs) or inhibition (Gi) as described below.

Two of the peptides (P59 and P74, see Table 1) showed distinct Gs-dependent activation of two related GPCRs (LGR7 (RXFP1) and LGR8 (RXFP2), see Table 2 and Fig. 2). Both of these receptors were recently identified as being activated by Relaxin and INSL3, respectively (24). A much weaker hit on LGR4, an orphan family member (data not shown), was also observed. Specificity was examined by GPR135 (RXFP3), a receptor for Relaxin 3, which was not activated by peptide P59 (Table 2).
In Vivo Activation of the FPRL1 Receptor by P58—FPRL1 activation promotes resolution of inflammation, an active and tightly synchronized process, involving counter-regulation of leukocytes, which leads to a prominent anti-inflammatory effect (21). To study the in vivo activity of our novel FPRL1 peptide agonist, P58, and two of its derivatives (P58-4 and P58-5), we used a model of acute inflammation, involving zymosan-induced leukocyte recruitment into the murine dorsal air pouch (see “Experimental Procedures”). The novel peptide P58 as well as P58-4 and P58-5 were tested at doses of 20 and 80 nmol per mouse and were given intravenously at the time of zymosan A injection (time 0). These doses correspond to 50 and 200 μg of P58 per mouse, respectively; and equivalent doses were used for the shorter peptides. Cell recruitment into the air pouches was determined at the 4-h time point, using both light microscopy (for total leukocyte count) and fluorescence-activated cell sorter analysis for Gr1-stained cells (for assessment of PMNs). A significant inhibition of about 50% was observed in the infiltration of PMNs into the air pouch with P58 at 20 nmol/mouse (Fig. 3). Out of the two derivatives tested, P58-4 displayed a 47% inhibition of Gr1+ cell migration into air pouches at the highest dose tested (80 nmol/mouse). P58-5 produced a discrete effect at the highest dose that was not statistically significant. Similar effects were observed on total leukocyte counts (data not shown).

**DISCUSSION**

In this work we describe the results of a screen for novel peptide GPCR agonists. A subset of the predicted candidate peptides was screened on a large group of GPCRs, and eight peptide agonists were identified for six different GPCRs. The peptides activity was further validated experimentally using established biochemical assays such as dose-dependent induction of calcium flux and by assessing the efficacy in vivo in one case.

By utilizing bioinformatic capabilities and machine learning, tools both related to identifying all potential secreted proteins (the secretome), and predicting all potential Arg/Lys cleavage sites, we were able to create a comprehensive peptidome that includes hundreds of thousands of potential peptides, ranked and prioritized by computational scores. Sequences of the predicted peptidome were further analyzed for features and assigned a probability score of being GPCR ligands by a machine learning algorithm. This collection contained hundreds of candidate peptides that were scored and ranked by distinct parameters and expert examination. The highest scoring candidates were checked for novelty and synthesized for further screening for activation on a group of target GPCRs. The screening included 33 peptides that were screened for activation of 152 GPCRs selected according to relevance and availability.

Out of the 33 peptides screened, 8 (~25%) showed a distinct activation of at least one receptor. The ratio of successful candidates is remarkable when compared with the average ratio of novel GPCR peptide ligand discovery rate, which is on average around two to three new candidates per year in the past decade (25). All of these peptides are novel, and most of them are derived from hypothetical protein precursors. Most of the peptides that showed initial hits also displayed dose-response activation. Furthermore, one of the peptides, designated peptide P58 (now named CGEN-855A), which showed a dose-dependent activation of the FPRL1 receptor, was further examined for in vivo activation of the FPRL1 receptor using a murine model of acute inflammation. The activation of FPRL1, as determined by inhibition of PMNs infiltration, was found to be significant. Moreover, out of two subsequent peptides derived from P58, P58-4 (now CGEN-855B) and P58-5 (now CGEN-855C), at least one (P58-4) was found to invoke an in vivo response mediated by the FPRL1 receptor, similar to the original P58 peptide. An examination of the activation of the receptors by the novel peptides shows cross-hits of the same peptides on several different GPCRs. This does not seem surprising because some of the receptors, such as the two relaxin-related GPCRs, namely RXFP1 (LGR7) and RXFP2 (LGR8), belong to the same family, whereas the others (MAS1, MRGX1, MRGX2, and FPRL1) belong to the same evolutionary branch (according to Ref. 26).

Even though no sequence homology was found between the newly discovered peptides and the known GPCR ligands, we predict that other structural properties (such as secondary structure) might show a mechanistic resemblance between the novel and known peptides. A preliminary computational structural prediction of the eight novel peptides by using the PSIPRED (27) secondary structure predictor revealed that all of them contain at least one helical stretch, a feature common in many known peptide GPCR ligands (data not shown).

We conclude that we were able to show efficacy and a proof of concept for our discovery platform of GPCR peptide agonists. We believe that we will be able to broaden our analysis and discover additional novel peptide GPCR ligands by this method. We further predict that more of the newly discovered and validated activators found for these GPCRs will be further investigated for their potential as therapeutic compounds targeted at receptors involved in distinct diseases and conditions.

**REFERENCES**

1. Imming, P., Sinning, C., and Meyer, A. (2006) Nat. Rev. Drug Discov. 5, 821–834
Novel GPCR Peptide Agonists

2. Hill, S. J. (2006) *Br. J. Pharmacol.* **147**, Suppl. 1, 27–37
3. Lin, S. H., and Civelli, O. (2004) *Ann. Med.* **36**, 204–214
4. Leifert, W. R., Aloia, A. L., Bucco, O., Glatz, R. V., and McMurchie, E. J. (2005) *J. Biol. Chem. Screen.* **10**, 765–779
5. Kutzleb, C., Busmann, A., Wendland, M., and Maronde, E. (2005) *Curr. Protein Pept. Sci.* **6**, 265–278
6. Jiang, Z., and Zhou, Y. (2006) *Curr. Protein Pept. Sci.* **7**, 459–464
7. Howard, A. D., McAllister, G., Feighner, S. D., Liu, Q., Nargund, R. P., Van der Ploeg, L. H., and Patchett, A. A. (2001) *Trends Pharmacol. Sci.* **22**, 132–140
8. Civelli, O. (2005) *Trends Pharmacol. Sci.* **26**, 15–19
9. Eglen, R. M. (2005) *Comb. Chem. High Throughput Screen.* **8**, 311–318
10. Bock, J. R., and Gough, D. A. (2004) *J. Chem. Inf. Model.* **45**, 1402–1414
11. Huang, E. S. (2005) *Drug Discov. Today* **10**, 69–73
12. Bairoch, A., Apweiler, R., Wu, C. H., Barker, W. C., Boeckmann, B., Ferro, S., Gasteiger, E., Huang, H., Lopez, R., Magrane, M., Martin, M. J., Natale, D. A., O’Donovan, C., Redaschi, N., and Yeh, L. S. (2005) *Nucleic Acids Res.* **33**, D154–D159
13. Breiman, L. (2001) *Machine Learning* **45**, 5–32
14. Bendtsen, J. D., Nielsen, H., von Heijne, G., and Brunck, S. (2004) *J. Mol. Biol.* **340**, 783–795
15. Kliger, Y., Gofer, E., Wool, A., Toporik, A., Apatoff, A., and Olshansky, M. (2008) *Bioinformatics (Oxf)* **24**, 1049–1055
16. Horn, F., Bettler, E., Oliveira, L., Campagne, F., Cohn, F. E., and Vriend, G. (2003) *Nucleic Acids Res.* **31**, 294–297
17. Liu, A. M., Ho, M. K., Wong, C. S., Chan, J. H., Pau, A. H., and Wong, Y. H. (2003) *J. Biol. Mol. Screen.* **8**, 39–49
18. Burstein, E. S., Ott, T. R., Feddock, M., Ma, J. N., Fuhs, S., Wong, S., Schiffer, H. H., Brann, M. R., and Nash, N. R. (2006) *Br. J. Pharmacol.* **147**, 73–82
19. Wollemann, M., and Benyhe, S. (2004) *Life Sci.* **75**, 257–270
20. Iribarren, P., Zhou, Y., Hu, J., Le, and Wang, J. M. (2005) *Immunol. Res.* **31**, 165–176
21. Perretti, M., and D’Acquisto, F. (2006) *Inflamm. Allergy Drug Targets* **5**, 107–114
22. VanCompernolle, S. E., Claek, K. L., Rummel, K. A., and Todd, S. C. (2003) *J. Immunol.* **171**, 2050–2056
23. Santos, R. A., Simoes e Silva, A. C., Maric, C., Silva, D. M., Machado, R. P., de Buhr, I., Heringer-Walther, S., Pinheiro, S. V., Lopes, M. T., Bader, M., Mendes, E. P., Lemos, V. S., Campagnole-Santos, M. J., Schulteiss, H.-P., Speth, R., and Walther, T. (2003) *Proc. Natl. Acad. Sci. U. S. A.* **100**, 8258–8263
24. Halls, M. L., Bathgate, R. A., and Summers, R. J. (2006) *Mol. Pharmacol.* **70**, 214–226
25. Katugampola, S., and Davenport, A. (2003) *Trends Pharmacol. Sci.* **24**, 30–35
26. Joost, P., and Methner, A. (2002) *Genome Biol.* **3**, RESEARCH0063
27. McGuffin, L. J., Bryson, K., and Jones, D. T. (2000) *Bioinformatics (Oxf)* **16**, 404–405