Review Article

Biosynthesis of lanthionine-constrained agonists of G protein-coupled receptors

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The conformation with which natural agonistic peptides interact with G protein-coupled receptor(s) (GPCR(s)) partly results from intramolecular interactions such as hydrogen bridges or is induced by ligand–receptor interactions. The conformational freedom of a peptide can be constrained by intramolecular cross-links. Conformational constraints enhance the receptor specificity, may lead to biased activity and confer proteolytic resistance to peptidic GPCR agonists. Chemical synthesis allows to introduce a variety of cross-links into a peptide and is suitable for bulk production of relatively simple lead peptides. Lanthionines are thioether bridged alanines of which the two alanines can be introduced at different distances in chosen positions in a peptide. Thioether bridges are much more stable than disulfide bridges. Biosynthesis of lanthionine-constrained peptides exploiting engineered Gram-positive or Gram-negative bacteria that contain lanthionine-introducing enzymes constitutes a convenient method for discovery of lanthionine-stabilized GPCR agonists. The presence of an N-terminal leader peptide enables dehydratases to dehydrate serines and threonines in the peptide of interest after which a cyclase can couple the formed dehydroamino acids to cysteines forming (methyl)lanthionines. The leader peptide also guides the export of the formed lanthionine-containing precursor peptide out of Gram-positive bacteria via a lanthipeptide transporter. An engineered cleavage site in the C-terminus of the leader peptide allows to cleave off the leader peptide yielding the modified peptide of interest. Lanthipeptide GPCR agonists are an emerging class of therapeutics of which a few examples have demonstrated high efficacy in animal models of a variety of diseases. One lanthipeptide GPCR agonist has successfully passed clinical Phase Ia.

Introduction

Today nearly 50 G protein-coupled receptor (GPCR) peptide drugs have been approved, and the pipeline is filled with an increasing number [1]. Many therapeutic peptides on the market are agonists of GPCRs [1,2]. GPCRs contain seven transmembrane segments and extracellular and intracellular loops. GPCR activity can be modulated by ligands that bind to their extracellular or transmembrane parts [3,4]. GPCRs can be activated by a variety of extracellular stimuli, among which interaction with peptides. GPCRs and their selectivity are very susceptible to allosteric modulation [5,6]. Extracellular GPCR stimulation activates G proteins that initiate intracellular signal transduction cascades. In addition, other GPCR-linked pathways, independent of G proteins, can be activated as well [7].

Structural stability of peptides can be imposed by hydrophobic or electrostatic intramolecular interactions and/or cross-links and may lead to increased GPCR-binding affinity by decreasing the entropic cost to adopt the GPCR-binding conformation. In the case of GPCR agonists, a conformational constraint imposed on a peptide, may induce a structure which is suitable or required for functional binding to a GPCR.
Importantly, lanthionine-induced cyclization of a peptide may enhance the receptor specificity [8] or even the receptor subtype specificity [9]. A lanthionine somatostatin showed a similar high affinity for rat somatostatin receptor 5 (rsSSTR5) compared with somatostatin [1–14] and sandostatin. However, it exhibited about 50 times weaker binding affinity for mouse somatostatin receptor 2b (mSSTR2b) than sandostatin [8]. Lanthionine enkephalin analogs with D-Ala in position 2 bind to both μ- and δ-opioid receptors and showed picomolar potencies [9]. The receptor specificity of a cyclized ligand has an additional impact, when the ligand up-regulates the GPCR level as reported for the angiotensin II type 2 receptor [10]. Furthermore, in specific cases, cyclized peptides can exert biased receptor agonism. Biased agonism by lanthi-apelins via the APJ receptor has been observed as witnessed by relatively stronger stimulation of the G protein pathway compared with the arrestin pathway [11]. This can translate to improved efficacy in the clinic by selectively stimulating therapeutic actions but avoiding activating detrimental β-arrestin-dependent pathways [12]. Selected lanthi-apelins, which more strongly stimulated the G protein pathway than the β-arrestin pathway did neither cause vasodilation nor increase heart rate in rats (Lanthio Pharma unpublished data, Figure 1).

In addition to the primary importance of conformational constraints of receptor agonists for their interaction with the receptor, several secondary advantages may occur such as the enhanced capacity to pass membranes and resistance to breakdown by peptidases and increased physical stability. Resistance to breakdown by peptidases in organ homogenates has been demonstrated amongst others for lanthionine-stabilized angiotensin-(1-7) [13,14] and for lanthionine-stabilized analogs of gonadotropin-releasing hormone I [15]. The capacity to pass membranes and the property of proteolytic resistance may lead to increased bioavailability after oral and

![Figure 1. Lanthipeptide biased agonism via APJ receptor.](image)

Cartoon of hypothetical stimulation of the APJ receptor by a strictly biased agonist that stimulates G protein-dependent pathways but not β-arrestin dependent pathways. APJ apelin receptor; AT1R angiotensin II type 1 receptor; HNE1 sodium/hydrogen exchanger 1; NCX sodium–calcium exchanger; AC adenylyl cyclase; PKA protein kinase A; MEK 1/2 dual specificity mitogen-activated protein kinase kinase 1/2; PKCε protein kinase C epsilon type; MLCK myosin light-chain kinase; MLC myosin light-chain; PLC phospholipase C; IP3 inositol trisphosphate; SR sarcoplasmic reticulum; AQP2 aquaporin 2; BP blood pressure.
pulmonary delivery as observed for lanthionine-stabilized angiotensin-(1-7) [14], less frequent administration, lower doses and prolonged shelf life. These features apply also to conformationally constrained antagonists/blockers [16], which are outside the scope of this mini-review which focuses on agonistic peptides. Some naturally occurring peptide GPCR agonists are constrained by disulfide bridges, e.g. vasopressin, oxytocin, cortistatin, urotensin II, endothelin I, somatostatin, adrenomedullin and calcitonin.

GPCR peptide agonists have been cyclized synthetically by a variety of cross-links, such as beta lactam [17], lysinoalanine [18], disulfide [12], artificial lipidated crosslinkers [19] and lanthionines [9]. Known lanthionine rings in lanthipeptide GPCR agonists range from i, i+3 (two amino acids between the lanthionine) to i, i+6 (five amino acids between the lanthionine) [8,9,11,13,20,21]. A thioether bridge is shorter than a disulfide bridge and a lysinoalanine. The exact structures resulting from the introduction of a lanthionine depend on the amino acid sequence and the position of the lanthionine and the resulting ring-size. Bacterial production of lanthionine-constrained agonistic peptides is a convenient method in the discovery of drug candidates. Obviously, the introduction of a lanthionine, either by insertion into the existing amino acid sequence or by replacing existing amino acids with (half of) a lanthionine, may strongly modulate the agonistic activity of the peptide, ranging from completely loss of activity to enhanced activity. Screening of lanthionine-containing variants will be required to select pharmacologically valuable peptides.

In many therapeutic peptides, the C-terminus is amidated which precludes carboxypeptidase action and may contribute to optimal receptor interaction. Peptides can be enzymatically amidated by peptidyl-glycine alpha-amidating monooxygenase or carboxypeptidase Y, which is likely compatible with bacterial production of lanthionine-containing peptides. Following bacterial production chemical and enzymatic amidation has been demonstrated for gonadotropin-releasing hormone I [15].

As broadly applicable, highly convenient methods have been developed for the bacterial synthesis of lanthionine-stabilized peptides, we here provide an overview on their emerging application in the discovery of lanthionine-constrained GPCR agonists.

Biosynthesis of lanthipeptides

The word lanthionine stems from the latin word lana which means wool, in which lanthionines were first observed, and thiol (sulfhydryl). Lanthionines are thioether bridged amino acids such as alanine-S-alanine, aminobutyryc acid-S-alanine or alanine-S-aminobutyric acid. Depending on the distances between the thioether bridged residues, smaller or larger ring structures are formed in the peptide. Lanthionine-containing peptides, so-called lanthipeptides [22] are naturally produced for instance by several Gram-positive bacteria [23].

Lanthipeptides are synthesized as precursor peptides that contain an N-terminal leader peptide and a modifiable core peptide. This leader peptide enables the interaction with modification enzymes that can dehydrate serines and threonines in the core peptide and couple the formed dehydroamino acids to cysteines thus forming lanthionines and methyllanthionines. The cyclization is stereospecific: either D,L (methyl)lanthionines or L,D (methyl)lanthionines are formed. The enzymatic cyclization is also regiospecific. This means that a dehydroamino acid in a certain position is coupled to only one cysteine position yielding only one ring position structure and, in case of more than one lanthionine in one peptide, only one ring pattern. In Lactococcus lactis, the leader peptide allows the export of the modified precursor peptide out of the cell. These leader peptides and the corresponding modification and transporter enzymes have been subject of a detailed review [24]. For some of these enzyme systems, a membrane-bound enzyme complex has been described which is composed of the modification enzymes and a dedicated transporter. The leader peptide is cleaved off either intracellularly by a bifunctional transporter/leaderpeptidase or extracellularly by a leader peptidase or non-dedicated peptidases.

The best-known lanthipeptide is the pentacyclic lantibiotic nisin [25] which is produced by L. lactis. The nisin precursor peptide is dehydrated by the dehydratase NisB [26,27], after which a cyclase NisC [28] couples dehydroamino acids to cysteines followed by export by the transporter NisT and removal of the leader peptide by the extracellular leader peptidase NisP [29].

Exploitation of bacteria for the biosynthesis of engineered lanthipeptides

Following the elucidation of the precursor sequence of the lanthipeptide gallidermin, as early as 1988, it was recognized that the introduction of (methyl)lanthionines into therapeutic peptides, such as agonistic peptide hormones might constitute a valuable opportunity to discover specific and stable lanthionine-containing therapeutics [30].
However, this concept was entirely dependent on the yet unknown substrate specificity of the complex of lanthipeptide modification and transport enzymes. In 2004, the first GPCR agonist peptide, an angiotensin-(1-7) variant fused to the nisin leader, which was totally unrelated to nisin, was modified by the nisin dehydratase and exported out of *L. lactis* by the nisin transporter NisT [31]. Subsequent studies demonstrated that the dehydratase NisB and the nisin cyclase NisC could modify a broad range of peptides unrelated to nisin, and that also the transporter could accommodate the export of a variety of fusion peptides of the nisin leader peptide and a peptide of interest [32–35]. In the C-terminal part of the nisin leader peptide, general cleavage sites could be engineered to allow convenient clean removal of the leader peptide from the produced peptide of interest [29]. By engineering a signal sequence at the N-terminus of the leader peptide constructs peptides could also be exported out of *L. lactis* via the SEC secretion system provided they had limited bulkiness [36,37]. Excellent *in vitro* studies by Wilfred van der Donk and co-workers furthermore demonstrated that a bifunctional lanthionine-introducing LanM enzyme, which performs both the dehydration as well as the cyclization step, also has a broad substrate specificity [38].

Several types of lanthionine-introducing enzymes were demonstrated to be functional in *Escherichia coli* [39,40] allowing intracellular modification and subsequent harvesting after disruption of the cells. The use of either Gram-positive or Gram-negative bacteria would thus constitute a cheap and effective method for producing a variety of rationally designed lanthipeptides to subsequently pursue the discovery of GPCR agonists with interesting pharmacokinetics and -dynamics.

### Substrate specificity of lanthionine-introducing enzymes

An excellent, comprehensive and thorough review focuses on lanthionine-introducing enzymes, their structure and mechanism of action [23]. Here, we summarize some of the progress made with respect to engineering novel lanthipeptides mainly by using bacteria containing the nisin modification enzymes. While the attainment of strict rules with respect to the substrate specificities of lanthionine-introducing enzymes has been challenging, some practical guidelines for the design of modifiable peptides have been reached for some lanthipeptide biosynthesis systems [32]. The substrate specificity of lanthionine-introducing enzymes was first investigated via an *in silico* study on all available lanthipeptide structures and subsequently experimentally validated with the nisin modification enzymes [32,34]. Directly flanking residues of serines and threonines clearly had an impact on the extent of dehydration by NisB (Figure 2). Hydrophobic amino acids, especially when present on both

![Figure 2. Biosynthesis of a lanthipeptide.](https://doi.org/10.1042/BST20200427)

A threonine in the core peptide of interest preceded by a cleavable leader peptide (Lp) is dehydrated by a LanB dehydratase forming a dehydrobutyrine (Dhb) after which a LanC cyclase couples the formed dehydrobutyrine to a cysteine forming a *d,L* methyllanthionine (dAbu-S-Ala). Directly flanking amino acids (light green) of the Thr and of the Cys (dark green) may affect the extent of dehydration and cyclization [32,34,35]. The enzymes LanB and LanC can also introduce a lanthionine via LanB-catalyzed dehydration of a serine yielding dehydroalanine and subsequent LanC-catalyzed coupling of the dehydroalanine to a cysteine. However, dehydroalanines are much more reactive than dehydrobutyrines and can spontaneously react with cysteines without stereospecificity.
sides of the Ser/Thr favored dehydration whereas hydrophilic amino acids did not. Hydrophilic amino acids, especially Arg, Asp, Glu and Gly, simultaneously present at both sides of the Ser/Thr, prevented dehydration. The requirement of hydrophobic amino acids directly flanking dehydratable serines/threonines might be caused by the negative charge of glutamyl tRNA which acts as cofactor in the dehydration process [23]. The influence on dehydration of the combination of one hydrophobic and one hydrophilic amino acid as directly flanking residues of Ser/Thr differed from case to case, and often led to partial dehydration. Thr was generally dehydrated to a larger extent than Ser. Ser/Thr at the very C-terminus escaped dehydration possibly as a result of the hydrophilicity of the carboxyl group. The presence of an already present lanthionine ring might in specific sequence contexts hamper the dehydration of Ser/Thr by a LanM enzyme [41]. Dehydration at a position as remote from the leader as 46 positions could still be dehydrated [34], but it is not known yet where the upper limit is. Interestingly, an elegant high-throughput screening system allowed to obtain mutant NisB enzymes with adapted substrate specificity with respect to the amino acids that directly flank Ser/Thr [42].

The substrate specificity of NisC was first investigated with model peptides. Dehydrolanine appeared to be very reactive leading to spontaneous lanthionine formation in bacterially produced peptides even in the absence of NisC as evidenced by comparing cells expressing NisBTC with cells with only NisBT [35]. In the case of spontaneous cyclization in which highly reactive dehydrolanines readily couple to cysteines, more than one isomer can be formed. To be complete it should also be mentioned that in specific sequences spontaneous cyclization between dehydrolanines and cysteines can be stereospecific as well. In these cases, the sequence imposes the stereochemistry. For NisC-catalyzed cyclization, it appears complex to generate guidelines for the design of peptides that are well cyclized or escape cyclization. Bulky residues in small ring structures appear to impose the stereochemistry. For NisC-catalyzed cyclization, it appears complex to generate guidelines for the design of peptides that are well cyclized or escape cyclization. Bulky residues in small ring structures appear to be unfavorable [35]. Glycines appear to be favorable and the impact of prolines is hard to predict. For cyclization, negatively charged amino acids as N-terminally directly flanking residues of a cysteine seem to be favored over positively charged amino acids.

The stereospecificity of NisC-cyclized GPCR agonist, 4,7 lanthionine-angiotensin-(1-7) was investigated using the Ni2B method, which opens the ring structure while retaining the D or L conformation [43]. Using reference peptide with combinations of D-Ala/L-Ala in positions 4 and 7, the NisC-cyclized cAng-(1-7) isomer turned out to be D,L [R. Rink unpublished data]. Using the same method it was demonstrated that also 4,7 NisC-cyclized LHRH had the D,L conformation [15]. This is in accordance with the D,L stereospecificity of all the lanthionine and methyllanthionine rings in nisin. Designed, NisC-cyclized intertwined lanthionine structures of different sizes have been synthesized [35].

The substrate specificity of the nisin transporter is not precisely known yet. Generally, positively charged peptides [44] seem to be better exported than negatively charged peptides in correspondence with the cationicity of the natural substrate, nisin. The mechanism of export and the involvement of NisT in the observed export of a large cell-wall spanning protein out of L. lactis may require detailed mechanistic analyses [45]. Generally, the efficiency of NisT-mediated export of peptides that are longer than nisin decreases significantly with increasing peptide length.

The membrane-bound leader peptidase NisP appears to be highly specific for lanthionine-containing precursor nisin [31]. In contrast, an engineered water-soluble truncated NisP was able to cleave off the leader peptide from a variety of substrate peptides [46]. Apart from the nisin biosynthetic machinery, many other lanthipeptide biosynthesis systems exist. Interestingly, the single bifunctional enzyme ProcM naturally introduces lanthionine rings in 29 different substrate peptides [47]. Clearly, this enzyme is a valuable tool in the design and synthesis of lanthionine-containing GPCR agonists. Recently, a powerful high-throughput screening method of NisB mutants with adapted substrate specificity has been demonstrated, thus opening the way to analogously obtain many tailor-made modification enzymes [42].

### Lanthipeptide GPCR agonists

Lanthipeptide GPCR agonists are an emerging class of peptide therapeutics. A number of them is listed in Table 1. Most data are available on 4,7 lanthionine-constrained angiotensin-(1-7) which has shown high efficacy in many animal disease models such as acute respiratory distress syndrome [49,50], acute lung injury [20], pulmonary arterial hypertension [51], myocardial infarct [52], diabetic nephropathy [54], type 1 and 2 diabetes [55] and cerebral stroke [56]. It is completely resistant to breakdown by ACE [13] and pulmonary delivery is feasible [14]. Its ex vivo vasodilatory action was inhibited by two peptides, D-Pro7-Ang-(1-7) and D-Ala7-Ang-(1-7), that are considered antagonists of the MAS1 receptor [13].
Lanthionine naturally occurs in lanthionine-ketimine present in the human brain [58]; thioethers are ubiquitously present in the human body in methionines. The lanthipeptide AT2R agonist LP2 [20,57] has been tested in clinical Phase I where it showed at the tested doses safety and favorable pharmacokinetics. Also complex lanthipeptides, other than receptor agonists, like the lantibiotics NBV302 ([59], BioCentury|August 13, 2012) and duramycin [60], have been successfully tested in clinical safety trials. Duramycin interacts with phosphatidyethanolamine and it can uncouple mitochondrial respiration in isolated mitochondria [61]. Duramycin activates an alternative chloride channel in cystic fibrosis nasal and airway epithelia. By this, duramycin bypasses the transmembrane conductance regulator, a chloride channel, which is dysfunctional in cystic fibrosis. Under therapeutic conditions after administration via inhalation, duramycin plasma concentration stays below 0.5 nM [62]. Until now no data directly point at any intrinsic toxicity of lanthionines.

Next to potentially high value as therapeutics lanthipeptide, GPCR agonists may have value as stable research tools with higher GPCR specificity than the corresponding natural linear peptides. Natural peptides such as those derived from angiotensin I are in vivo rapidly degraded, making it difficult to assign their precise effects. In this respect, stable analogs may allow more reliable conclusions as to which peptide acts via which receptor leading to which effects. High GPCR specificity of lanthionine-constrained agonists may also prove useful in the elucidation of GPCR heterodimerization which occurs for instance for MAS1 and AT2 receptors [63]. While biosynthesis methods are very convenient in the discovery process of therapeutic candidates, upscaled chemical production methods [64–67] are in development and base-assisted sulfur extrusion has already successfully been applied for bulk production of GMP material of the clinically tested LP2.

**Perspectives**

- The target specificity, stability and safety of lanthipeptide GPCR agonists make them an important class of candidate therapeutics.

- Currently, it is clear that several biosynthetic systems have relaxed substrate specificity which allows their exploitation in lanthipeptide drug discovery. In addition, the feasibility of high-throughput screening for tailor-made lanthionine-introducing enzymes with adapted substrate specificity for rational design and biosynthesis of lanthipeptide GPCR agonists has recently been demonstrated.

- Future developments comprise the discovery and clinical development of multiple lanthipeptide GPCR agonists as therapeutics for treating human diseases.

**Competing Interest**

All authors are employees of LanthioPep B.V., which organization is the owner of patents on the biosynthesis of lanthipeptides and specific lanthipeptide GPCR agonists. G.N.M. is the director of LanthioPep B.V.
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Author Contributions
G.N.M. contributed to the overall outline of the manuscript; R.R. with unpublished data, A.K., R.R. and T.B. to the paragraphs on biosynthesis, R.R. to the chemical synthesis, A.K., L.d.V. and P.N. to GPCRs; P.N. made Figure 1.

Abbreviations
GPCRs, G protein-coupled receptors; mSSTR2b, mouse somatostatin receptor 2b; rSSTR5, rat somatostatin receptor 5.

References
1 Davenport, A.P., Scully, C.C.G., de Graaf, C., Brown, A.J.H. and Maguire, J.J. (2020) Advances in therapeutic peptides targeting G protein-coupled receptors. Nat. Rev. Drug Discov. 19, 389–413 https://doi.org/10.1038/s41573-020-0062-z
2 Reichert, J., Pechon, P., Tartar, A., and Dunn, M.K. (2019) Development Trends for Peptide Therapeutics. Peptide Therapeutics Foundation. San Diego, California 92121
3 Wang, W., Oiao, Y. and Li, Z. (2018) New insights into modes of GPCR activation. Trends Pharmacol. Sci. 39, 367–386 https://doi.org/10.1016/j.tips.2018.01.001
4 Weis, W.I. and Kobilka, B.K. (2018) The molecular basis of G protein-coupled receptor activation. Annu. Rev. Biochem. 87, 897–919 https://doi.org/10.1146/annurev-biochem-060614-033910
5 van der Westhuizen, E.T., Valant, C., Sexton, P.M. and Christopoulos, A. (2015) Endogenous allosteric modulators of G protein-coupled receptors. J. Pharmacol. Exp. Ther. 353, 246–260 https://doi.org/10.1124/jpet.114.221606
6 Thal, D.M., Guikova, A., Sexton, P.M. and Christopoulos, A. (2018) Structural insights into G-protein-coupled receptor allostery. Nature 559, 45–53 https://doi.org/10.1038/s41586-018-0259-z
7 Walther, C. and Ferguson, S.S. (2015) Minireview: role of intracellular scaffolding proteins in the regulation of endocrine G protein-coupled receptor signaling. Mol. Endocrinol. 29, 814–830 https://doi.org/10.1210/me.2015-091
8 Ösapay, G., Prokai, L., Kim, H.S., Medzihradszky, K.F., Coy, D.H., Liapakis, G. et al. (1997) Lanthionine-somatostatin analogs: synthesis, characterization, biological activity, and enzymatic stability studies. J. Med. Chem. 40, 2241–2251 https://doi.org/10.1021/jm960850i
9 Rev, Y., Malkmus, S., Svensson, C., Yaksi, T.L., Chung, N.N., Schiller, P.W. et al. (2002) Synthesis and biological activities of cyclic lanthionine enkephalin analogues: delta-opioid receptor selective ligands. J. Med. Chem. 45, 3746–3754 https://doi.org/10.1021/jm020108k
10 Rink, R. (2019) Cyclic apelin analogs. Patent EP3122764 (B1)
11 Rink, R. (2019) Cyclic apelin analogs. Patent US 10501514, B2
12 Rink, R. (2019) Cyclic apelin analogs. Patent EP3122764 (B1)
13 Rink, R. (2019) Cyclic apelin analogs. Patent US 10501514, B2
14 Rink, R. (2019) Cyclic apelin analogs. Patent EP3122764 (B1)
15 Rink, R. (2019) Cyclic apelin analogs. Patent US 10501514, B2
16 Rink, R. (2019) Cyclic apelin analogs. Patent EP3122764 (B1)
17 Rink, R. (2019) Cyclic apelin analogs. Patent US 10501514, B2
18 Rink, R. (2019) Cyclic apelin analogs. Patent EP3122764 (B1)
19 Rink, R. (2019) Cyclic apelin analogs. Patent US 10501514, B2
20 Rink, R. (2019) Cyclic apelin analogs. Patent EP3122764 (B1)
21 Rink, R. (2019) Cyclic apelin analogs. Patent US 10501514, B2
22 Rink, R. (2019) Cyclic apelin analogs. Patent EP3122764 (B1)
53 Pessôa, S., Becher, B., Van Veghel, P.M., De Vries, R., Tempel, R., Sneep, D. (2015) Effect of a stable angiotensin-(1-7) analogue on progenitor cell recruitment and cardiovascular function post myocardial infarction. J. Am. Heart Assoc. 4, e001510 https://doi.org/10.1161/JAHA.114.001510

54 Cassis, P., Locatelli, M., Corna, D., Villa, S., Rottoli, D., Cerullo, D. et al. (2019) Addition of cyclic angiotensin-(1-7) to angiotensin-converting enzyme inhibitor therapy has a positive add-on effect in experimental diabetic nephropathy. Kidney Int. 96, 906–917 https://doi.org/10.1016/j.kint.2019.04.024

55 Kuipers, A., Moll, G.N., Wagner, E. and Franklin, R. (2019) Efficacy of lanthionine-stabilized angiotensin-(1-7) in type 1 and type 2 diabetes mouse models. Peptides 112, 78–84 https://doi.org/10.1016/j.peptides.2018.10.015

56 Kuipers, A., Moll, G.N., Levy, A., Krakovsky, M. and Franklin, R. (2020) Cyclic angiotensin-(1-7) contributes to rehabilitation of animal performance in a rat model of cerebral stroke. Peptides 123, 170193 https://doi.org/10.1016/j.peptides.2019.170193

57 Wagenaar, G.T., Sengers, R.M., El Laghmani, H., Chen, X., Lindeboom, M.P., Roks, A.J. et al. (2014) Angiotensin II type 2 receptor ligand PD123319 attenuates hyperoxia-induced lung and heart injury at a low dose in newborn rats. Am. J. Physiol. Lung Cell. Mol. Physiol. 307, L261–L272 https://doi.org/10.1152/ajplung.00345.2013

58 Fontana, M., Brunori, A., Costa, M. and Antonucci, A. (1997) Detection of cystathione ketimine and lanthionine ketimine in human brain. Neurochem. Res. 22, 821–824 https://doi.org/10.1023/A:1022083809994

59 Sandford, S.K. (2019) Current developments in lantibiotic discovery for treating Clostridium difficile infection. Expert Opin. Drug Discov. 14, 71–79 https://doi.org/10.1080/17460441.2019.1549032

60 Zeitlin, P.L., Boyle, M.P., Guggino, W.B. and Molina, L. (2004) A phase I trial of intranasal Moli1901 for cystic fibrosis. Chest 125, 143–149 https://doi.org/10.1378/chest.125.1.14

61 Sokolove, P.M., Kester, M.B. and Westphal, P.A. (1991) Duramycin effects on the structure and function of heart mitochondria. II. Energy conversion reactions. Arch. Biochem. Biophys. 287, 180–185 https://doi.org/10.1016/0003-9861(91)90404-7

62 Zebedin, E., Koenig, X., Radenkovic, M., Pankeyvich, H., Todt, H., Freissmuth, M. et al. (2008) Effects of duramycin on cardiac voltage-gated ion channels. Naunyn Schmiedebergs Arch. Pharmacol. 377, 87–100 https://doi.org/10.1007/s00210-007-0248-5

63 Leonhardt, J., Villela, D.C., Teichmann, A., Münter, L.M., Mayer, M.C. and Maridis, M. (2017) Evidence for heterodimerization and functional interaction of the angiotensin type 2 receptor and the receptor MAS. Hypertension 69, 1128–1135 https://doi.org/10.1161/HYPERTENSIONAHA.116.08814

64 Glande, A.K., Trent, J.O. and Spatola, A.F. (2003) Understanding base-assisted desulfurization using a variety of disulfide-bridged peptides. Biopolymers 71, 534–551 https://doi.org/10.1002/bip.10532

65 Knerr, P.J. and van der Donk, W.A. (2012) Chemical synthesis and biological activity of analogues of the lantibiotic epilancin 15X. J. Am. Chem. Soc. 134, 7648–7651 https://doi.org/10.1021/ja302435y

66 Gieselman, M.D., Zhu, Y., Zhou, H., Galonic, D. and van der Donk, W.A. (2002) Selenocysteine derivatives for chemoselective ligations. Chembiochem 3, 709–716 https://doi.org/10.1002/1439-7633(20020620)3:8<709::AID-CBIC709>3.0.CO;2-8

67 Ongey, E.L. and Neubauer, P. (2016) Lanthipeptides: chemical synthesis versus in vivo bioconversion as tools for pharmaceutical production. Microb. Cell. Fact. 15, 97 https://doi.org/10.1186/s12934-016-0502-y