Patent Highlights August–September 2018

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A snapshot of noteworthy recent developments in the patent literature of relevance to pharmaceutical and medical research and development.

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WO/2018/140299

N-[4-Fluoro-5-[[[2S,4S]-2-methyl-4-[(5-methyl-1-2-4-oxadiazol-3-yl)methoxy]-1-piperidyl]methyl]thiazol-2-yl]acetamide as OGA inhibitor

Inventors: Dreyfus NJF, Lindsay-Scott PJ

Assignee: Eli Lilly & Co, IN (USA)

The tau protein has proven to be a quite difficult target for the pharmacotherapy of neurodegenerative diseases. While reduction or prevention of its hyperphosphorylation has been pursued for many years, inhibiting O-glycoprotein-2-acetamido-2-deoxy-β-D-glucopyranosidase (O-GlcNAcase, OGA) is a new route. Chronic inhibition of OGA reduces pathological tau in the brain of rTg4510 transgenic mice, most likely by directly increasing O-GlcNAcylation of tau, reducing its aggregation and the subsequent deleterious post-translational modifications [1,2]. Eli Lilly, IN, USA is highly active in this field; they acquired the tau PET tracer program developed by Siemens Medical Solutions USA, Inc., in 2013, and its antibody LY-3303560 (directed against aggregated tau) is in clinical trials. Now the company has filed a patent application claiming a single compound (a quite unusual move nowadays) as an OGA inhibitor, for the treatment of Alzheimer's disease, the mild cognitive impairment preceding it and for progressive supranuclear palsy. For the last-mentioned condition the Swiss company Asceneuron (Lausanne, Switzerland) SA (a spinout of Merck Serono's [Darmstadt, Germany] Alzheimer portfolio) is planning a Phase II trial for its lead OGA inhibitor, ASN120290. For more OGA inhibitors, see Janssen Pharmaceutica's (Beerse, Belgium) WO/2018/109198 (reviewed in PPA 7[6]). One problem with such compounds is that they are potentially diabetogenic: streptozotocin and alloxan both are OGA inhibitors [3].

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MeSH keywords: beta-N-Acetylhexosaminidases/hexosaminidase C/tau proteins

WO/2018/140563

Use of apolipoprotein E4 (ApoE) mimetic peptides for reducing the likelihood of epileptogenesis

Inventors: Laskowitz DT, Mace BE, Kolls B

Assignee: Duke University, NC (USA)

Epileptogenesis – the onset of seizures weeks or months after a traumatic brain injury or hemorrhagic stroke, caused by a developing 'seizure focus' – is generally not prevented by conventional anticonvulsants; disruption of the blood–brain barrier and neuroinflammation seem to play a role [4,5]. The inventors have made the truly astonishing discovery that apolipoprotein E4 (ApoE)-derived peptides, such as, acetyl-Val-Ser-Arg-Arg-Arg-NH₂ (CN-105) can reduce the likelihood of epileptogenesis in a validated rat model of status epilepticus. The administration of CN-105 (two 0.1–1.0 mg/kg doses) in the first few hours after onset of status had no impact on the ongoing seizures with the first injection, but there were no ongoing seizures at the 6-h second injection. CN-105 reduced the likelihood of developing the subsequent, late seizures at 10–14 days. Nothing is said concerning the mechanistic background, which cannot be readily deduced from the literature. As part of their normal contributions to brain homeostasis, astrocytes release ApoE which transports cholesterol to neurons via a specific receptor and supports

neurotransmission, growth factor release and anti-inflammatory immune responses [6]. On the other hand, such protection is not universal for all ApoE isoforms: post-traumatic seizures seem to be associated with the *APOE4* allele [7], which is also a well-known genetic-risk factor for Alzheimer's disease. In traumatic brain injury, ApoE is associated with increased amyloid deposition, amyloid angiopathy and larger intracranial hematomas [8]. All taken together, it is not obvious from the prior art how small ApoE peptide fragments (which would not be able to transport cholesterol) could prevent epileptogenesis, especially not after contusional head injury.

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MeSH keywords: Apolipoproteins E/Epilepsy, Post-Traumatic/Head Injuries, Closed/Intracranial Hemorrhages

WO/2018/141854

Piperazine derivatives for influenza virus inhibition

Inventors: Van Donger M, Juraszek J, Schepens WBG, et al.

Assignee: Janssen Vaccines & Prevention BV, Leiden (The Netherlands)

The discovery of novel influenza antivirals acting on the surface protein hemagglutinin as an alternative strategy to prevent and/or treat infection is hampered by the large sequence variability of this protein. Antibodies capable of interacting with a large variety of hemagglutinin proteins and capable of neutralizing a broad spectrum of influenza A and/or B viruses have recently been described. Their binding to an 'influenza-universal' epitope in the stem region of hemagglutinin causes them to interfere with large structural rearrangements which are critical for membrane fusion [9]. Examples are CR9114 and CR6261 (disclosed in WO/2013/007770 and WO/2008/028946, resp.; both by Crucell, another Johnson & Johnson company) [10]. The small molecules disclosed in the present patent application employ the same mechanism. They show virus neutralization and competitive binding activity towards hemagglutinin of influenza H1N1 strains A/California/07/2009 and A/New Caledonia/20/1999, and also towards the H5N1 strain A/Vietnam/1203/2004. One representative compound is methyl 2-(4-(3-(5-cyclopropoxybenzo[d]oxazol-2-yl)benzoyl)piperazin-1-yl)-2-phenylacetate; pIC₅₀ for binding 7.1–8.9, pEC₅₀ for neutralization of viral infectivity on the MDCK canine kidney cell line, 6.7–8.6. Note that with the above-mentioned antibodies, such *in vitro* neutralization was not predictive of prophylactic *in vivo* activity [11]. Also see Bristol-Myers Squibb Co.'s WO/2012/033736 for other piperazine-based compounds with anti-influenza activity.

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MeSH keywords: Hemagglutinin Glycoproteins, Influenza Virus/Influenza, Human/Influenza A Virus, H1N1 Subtype/Influenza A Virus, H5N1 Subtype

WO/2018/143638

4'-Phosphopantetheinyl transferase enzyme inhibitor derived from *Mycobacterium tuberculosis* & pharmaceutical composition for preventing or treating tuberculosis, containing same as active ingredient

Inventors: Lee B-J, Lee K-Y, Kim D-G, Lee S-J

Assignee: Seoul National University R&DB Foundation, Seoul (Korea)

4'-Phosphopantetheinyl transferases (PPTases) post-translationally modify carrier proteins with phosphopantetheine. In mycobacteria, the Sfp-type PPTase activates pathways necessary for the biosynthesis of cell wall components and small molecule virulence factors. The recent characterization of *M. tuberculosis* PPTase PptT [12], *in silico* docking experiments [13] and the availability of its x-ray crystal structure [14,15] have made this enzyme a promising new target for tuberculosis therapy. *N*-(1-(6-(4H-1,2,4-triazol-4-yl)pyridin-2-yl)vinyl)-5-(benzo[d]thiazol-2-yl)-2-methyl-benzeneamine (IC₅₀ of 635 nM for recombinant PptT PPTase) and the three related compounds, obtained by structure-based design, that are presented here add to the few small molecule inhibitors (such as ML267) that have been reported for this enzyme [16]. Some of the work appears to have been done using PptT PPTase from *M. smegmatis*, which is very similar to that of *M. tuberculosis*. No *in vivo* data are disclosed.

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MeSH keywords: phosphopantetheinyl transferase/Tuberculosis

WO/2018/148666**Focal adhesion kinase inhibitor as a therapeutic agent in diabetes***Inventors: Esni F, Hu J*

Assignee: University of Pittsburgh – Of the Commonwealth Systems of Higher Education, PA (USA)

FAK is a cytoplasmic nonreceptor protein tyrosine kinase that is crucial for cellular adhesion, with essential roles in cell motility. It is overexpressed and activated in many solid tumors, being responsible for both tumorigenesis and invasiveness [17], and this is the usual therapeutic target for which inhibitors of FAK and the homologous kinase Pyk2 are being developed. For a review of FAK inhibitors for cancer see Lv *et al.* [18]. However, both the activation of FAK and its signaling can take many paths, and recently there have been links to Type 2 diabetes. In 2012 a paper from the University of Toronto reported that FAK is critical for pancreatic β -cell viability and function through regulation in insulin signaling, actin dynamics and granule trafficking [19]. Endocannabinoid receptor couple to FAK to regulate insulin release [20]. It is very remarkable if the inventors have now found that an FAK inhibitor (PF562271, a discontinued Pfizer candidate compound) increases the number of functional islet cells in the pancreas and decreases blood glucose levels in the standard rodent model of streptozotocin-induced diabetes when administered at 50 mg/kg for 3 weeks. Administering an FAK inhibitor also results in the conversion of pancreatic acinar cells into insulin producing β -cells that embed themselves within the microenvironment of the pre-existing islets of Langerhans that survived the streptozotocin treatment. The acinar-derived insulin-positive cells also no longer expressed amylase but did express Glut2, suggesting that they share features with mature insulin-producing cells. Considering the intimate involvement of FAK with so many essential physiological processes, restricted local delivery might offer an attractive option for diabetes therapy.

Published: 16 August 2018

MeSH keywords: Diabetes Mellitus/Focal Adhesion Protein-Tyrosine Kinases

WO/2018/152134**Proline-based neuropeptide FF receptor modulators***Inventors: Zhang Y, Nguyen T*

Assignee: Research Triangle Institute, Research Triangle Park, NC (USA)

Neuropeptide FF belongs to the group of RFamide peptides which share a common Arg-Phe-NH₂ motif at their C-terminus. It binds to two orphan receptors of the rhodopsin family, GPR147 (NPFF1) and GPR74 (NPFF2). Both receptors are widely expressed in the brain, while only NPFF2 seems to be present in the spinal cord. It has long been recognized that the modulation of the opioid system is among the many functions of the NPFF system, although the peptide has no affinity for opiate receptors [21]. Together with observations that neuropeptide FF precipitates a nicotine withdrawal syndrome, and that blockade of receptors decreases amphetamine withdrawal anxiety in rats [22], inhibition of the neuropeptide FF system appears to be a viable approach to the therapy of pain and addiction. The inventors conducted a high throughput screen of a GPCR-oriented library of 22,000 compounds, which identified a compound with a proline-based scaffold as a promising lead. A focused compound library was prepared for structure–activity studies using this lead as a seed. These produced a molecule with a 4-nitrophenethyl substituent as the most potent analog at NPFF1, with approximately threefold preference over the NPFF2 receptor and reasonably drug-like properties. Two compounds were tested in a rat model of fentanyl-induced hyperalgesia. The baseline mean paw withdrawal threshold, which had decreased from 24.2 ± 1.8 g to 5.7 ± 0.8 after fentanyl treatment, was essentially restored by both candidate compounds at doses of 10–32 mg/kg. For the peer review companion paper see Nguyen *et al.* [23]

Published: 23 August 2018

MeSH keywords: neuropeptide FF receptor/Pain/Substance-Related Disorders

WO/2018/152414**Small molecule inhibition of micro-RNA-210 reprograms an oncogenic hypoxic circuit***Inventors: Disney, MD*

Assignee: The Scripps Research Institute, CA (USA)

The miR-210 has been identified as one of the major micro-RNA species induced by hypoxia. It is a regulator of several cellular functions (e.g., angiogenesis, neurogenesis after stroke and induction of erythrocyte development), including many that are not directly related to hypoxia [24]. Overexpression of miR-210 in breast cancer correlates

with aggressiveness and shorter time to distant metastasis [25]. This document describes the identification of Targapremir-210, a non-nucleotide small molecule that binds to the Dicer site of the miR-210 hairpin precursor. It can engage its RNA target even among other abundant transcripts. This interaction inhibits production of the mature miRNA, de-represses glycerol-3-phosphate dehydrogenase 1-like enzyme (a hypoxia-associated protein negatively regulated by miR-210), decreases expression of HIF-1 α and triggers apoptosis of triple negative breast cancer cells; however, this occurs only under hypoxic conditions. The inventors used two-dimensional combinatorial screening (2DCS), a library-versus-library screen that quickly defines the preferred RNA motifs for a given small molecule. The data from two-dimensional combinatorial screening were compiled into a database that was mined against folded RNA structures within the human transcriptome to identify potentially druggable RNA targets. When MDA-MB-231 breast cancer cells were pretreated with Targapremir-210 (200 nM) or anti-miR-210 antagomir (500 nM) and then implanted into mouse mammary fat pads; significantly reduced tumor growth was observed after 21 days. For the peer review companion paper see Costales *et al.* [26]

Published: 23 August 2018

MeSH keywords: MicroRNAs/Neoplasms

WO/2018/152937

Use of programmed cell death 4 as therapeutic target of antidepressant &/or anti-anxiety medicament

Inventors: Zhang L, Li Y, Wang Q, Guo C, Zhu F

Assignee: Shandong University, Shandong (China)

Programmed cell death 4 (Pcd4), an inhibitor of transcription and translation, acts as a tumor suppressor and its downregulation in various types of cancer (e.g., by miR-21) is an independent prognostic factor [27,28]. While Pcd4 is therefore intimately involved with many forms of cellular stress, the inventors' finding that its increased expression is a significant factor causing depression and anxiety during mental stress has not been reported before. Apart from glioblastoma, there seems to be no literature on Pcd4 in the brain. Here it is reported that the protein is expressed in neurons and microglia, but rarely in astrocytes. Data from classical mouse models (tail suspension and open field paradigms) show good antidepressant effect of anti-Pcd4 siRNA in immobilization-stressed animals, and no discernible effect on the normal physiological state. Mice injected with a lentiviral vector expressing Pcd4 showed depression-like behavior, which can also be abrogated by the siRNA.

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MeSH keywords: Depressive Disorder/PDCD4 protein, human/RNA-Binding Proteins

WO/2018/157244

Granzyme B inhibitor compositions & methods for the prevention &/or treatment of skin blistering &/or peeling

Inventors: Granville DJ, Russo V, Shen Y

Assignee: The University of British Columbia, Vancouver (Canada)

Granzymes are serine proteases that act as integral cytolytic effectors during apoptosis of cancerous and pathogen-infected cells. Granzyme B is present at low levels in healthy skin but is elevated in aging skin, and dramatically elevated in inflammatory skin disorders, including diabetic ulcers, hypertrophic scarring, autoimmune skin disorders and cutaneous leishmaniasis [29]. It cleaves fibronectin into fragments that increase the expression of collagen-degrading matrix metalloproteinase-1 in fibroblasts [30]. The involvement of granzyme B in the pathogenesis of the blistering skin disease, toxic epidermal necrolysis, has been strongly suspected [31]. The inventors have found that its accumulation in the skin can indeed promote separation of the epidermis from the dermis similar to what occurs in blistering. It is also demonstrated that granzyme B cleaves key ligand regions of important junctional proteins (such as collagen VII) in the dermal epidermal junction, where it accumulates in many interface dermatoses. There are also inhibitors presented, in particular 4-(((2S,3S)-1-((2-((S)-5-(((2H-tetrazol-5-yl)methyl)carbamoyl)-3-cyclohexyl-2-oxoimidazolidin-1-yl)-2-oxoethyl)amino)-3-methyl-1-oxopentane-2-yl)amino)-4-oxobutanoic acid. This compound effectively prevented blistering in a diabetic mouse-burn injury model when injected subcutaneously or applied as a topical gel. Formulations for intradermal delivery are also discussed. For the peer review companion paper see Russo *et al.* [32]

Published: 7 September 2018

MeSH keywords: Blister/Granzymes/Skin Diseases, Vesiculobullous

WO/2018/160067

Specific increase of dopamine synthesis through targeting of the guanylate cyclase 2c receptor in the treatment of Parkinson's disease

Inventors: Van der Heide LP

Assignee: Universiteit van Amsterdam, Amsterdam (The Netherlands)

Motor symptoms of Parkinson's disease become manifest when an advanced loss of neurons in the *substantia nigra pars compacta* results in a lack of dopamine release into the striatum. The classical pharmacological countermeasures are dopaminergic drugs, which produce severe side effects (such as levodopa-induced dyskinesias) by their extrastriatal actions. What is claimed here is a truly novel alternative: agonism at the membrane-bound cGMP-synthesizing receptor guanylate cyclase 2C (GUCY2C), an enzyme which regulates electrolyte and water transport in intestinal and renal epithelia, and has been found to form an appetite-regulating endocrine axis with the satiety mediator, uroguanylin [33]. The modest effects of uroguanylin on energy and glucose homeostasis are not mediated by brain guanyl cyclase C, as administration of agonists to the CNS has shown [33]. However, modulation of brain GUCY2C signaling in dopaminergic cells can increase dopamine production by phosphorylating tyrosine hydroxylase at Ser40 [35]. The GUCY2C agonist achieving this according to the invention is a member of the guanylin family of the peptides, preferably guanylin, lymphoguanylin, uroguanylin or a functional derivative thereof, such as linaclotide. The inventors demonstrate this in HEK cells co-transfected to express GUCY2C and an agonist peptide. Potential pathways by which GUCY2C activation can lead to increased tyrosine hydroxylase activity include the direct activation of protein kinase G by cGMP, and indirect activation of protein kinase A via the cGMP-mediated inhibition of phosphodiesterase-3. See WO/2013/016662 (Memorial Skloan-Kettering Cancer Center) for guanylin-conjugates targeting an L-Dopa payload to the brain.

Published: 7 September 2018

MeSH keywords: Enterotoxin-Guanylin Receptors/GUCY2C protein, human/Parkinson Disease

WO/2018/165465

DNA origami nanostructures for treatment of acute kidney injury

Inventors & Applicants: Yan H, Jiang D, Ge Z, et al.

In addition to its traditional role of incorporating and replicating genetic information, DNA can be used to assemble itself, alone or with inclusion of various molecules and nanomaterials, in a programmable fashion. The oldest constructs of this class are DNA origamis [36–38]. The inventors have created rectangular, triangular and tubular DNA origamis that showed renal targeting properties in acute kidney injury mice at different time points after initiation of the model by intramuscular injection of 50% glycerol. Excretory function and nitrogen metabolism were re-established and renal histopathology was very much improved, by administering rectangular origamis (10 µg/mouse) 2 h after injury, based on the PET imaging with ⁶⁸Ga-EDTA as a tracer. Treatment efficacy resembled that of N-acetyl cysteine (a widely applied antioxidant for contrast-induced acute kidney injury) at 4.2 mg/mouse. Recombinant expression vectors are disclosed comprising oligonucleotide helper/staple strands; they may include a polynucleotide scaffold strand that generates a scaffolded nucleic acid origami upon expression of the vector within the organism or cell. There is no information provided concerning the potential mechanism, or whether the DNA origamis have been rationally designed.

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MeSH keywords: Acute Kidney Injury/DNA

WO/2018/167147

Azaindoles & inhibitors of HPK1

Inventors: Heffron T, Malhotra S, Wei BQ, et al.

Assignees: F. Hoffmann-La Roche AG, Basel (Switzerland); Genentech, Inc., CA (USA)

HPK1 is a MAP4K family kinase that is expressed predominantly by hematopoietic cells including early progenitors. It is a negative regulator of dendritic cell activation, and of T and B cell responses. Loss of HPK1 function enhances T-cell receptor signaling and cytokine secretion in a T-cell-intrinsic manner [39]. Its inhibitors can enhance immune responses to tumors [40], especially because HPK1 can be activated by prostaglandin E2 [41], which tumors often

secrete, contributing to their escape from the immune system. However, HPK1 expression seems to be associated with longer overall survival in some breast cancers [42]. Also note that HPK1 levels are consistently decreased in peripheral blood mononuclear cells and T cells from patients with autoimmune disorders [43], accompanying T-cell overactivation. The most active inhibitors presented here (e.g., 2-amino-5-(3-ethyl-2-methyl-1*H*-pyrrolo[2,3-*b*]pyridine-5-yl)-(N,N)-dimethylbenzamide) have *K_i* values for HPK1 in the low nanomolar range. The presented data do not allow conclusions regarding specificity; the rather restricted expression profile of this kinase might lessen respective concerns. Isoquinoline inhibitors of HPK1 are disclosed by Genentech, Inc., in WO/2018/183964; for pyrazolopyridines see Incyte Corp.'s WO/2018/152220.

Published: 20 September 2018

MeSH keywords: hematopoietic progenitor kinase 1/Neoplasms

WO/2018/175480

Methods of inhibiting fungal ceramide synthase for treatment of *Cryptococcus neoformans* infection

Inventors: Del Poeta M, Munshi M, Hanaraballi K, You K, Ojima I

Assignee: The Research Foundation for the State University of New York, NY (USA)

Ceramide synthases couple sphinganine to a long-chain fatty acid to form a dihydroceramide, which is subsequently converted to the respective ceramide by a desaturase. Ceramides are component lipids of sphingomyelin and are essential as structural membrane elements as well as for various cellular signaling processes. *Cryptococcus neoformans*, a leading cause of pulmonary infections and fungal meningoencephalitis, has three ceramide synthases [44]; a specific structure of glucosylceramide is a major regulator of membrane permeability that is required for its pathogenicity [45]. The inventors deleted and reconstituted each of the three genes, characterized them biochemically after recombinant expression in *S. cerevisiae* using mass spectroscopy of ceramides and performed virulence studies of *C. neoformans* strains expressing only one type of synthase. Ceramide synthase 1 (Cer1) showed preference for C18 fatty acid acyl CoA and sphingosine as substrates and was proven to be the most relevant subtype for *C. neoformans* virulence. A series of high throughput screenings using assays that target fungal but not human ceramide synthases yielded 50 small molecules that inhibited ceramide synthase 1 (85–95%) at a concentration of 1 mM. These experiments seem to have opened entirely new ground.

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MeSH keywords: Ceramides/*Cryptococcus neoformans*/Invasive Fungal Infections/Sphingosine N-Acyltransferase

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