Patent highlights October–November 2018

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

First draft submitted: 31 December 2018; Accepted for publication: 4 February 2019; Published online: 14 March 2019

WO/2018/178304

Compounds useful as inhibitors of ALCAT1

Inventors: Shi Y, Che D, Baell J, Liu X, Fu J

Assignee: Perenna Pharmaceuticals, Inc., San Antonio, TX (USA)

ALCAT1, an endoplasmic reticulum acyltransferase which catalyzes the acylation of mono- and diacyl-cardiolipin back into cardiolipin [1], is upregulated by oxidative stress and in diet-induced obesity [1]. WO/2013/123305 (by the first-named inventor, then at the Penn State Research Foundation) identified ALCAT1 as a missing link between mitochondrial fusion defects and reactive oxygen species (ROS) production in metabolic diseases. Because fatty acyl chains are highly sensitive to oxidative damage, leading to mitochondrial dysfunction and more ROS production, a vicious cycle can be initiated that extends to many age-related diseases including hypertrophic cardiomyopathy, diabetes and neurodegeneration. The hundreds of molecules disclosed here might be the first small molecule inhibitors of ALCAT1. The preferred compound seems to be 2-ethoxy-5-ethyl-N-(2-fluoro-5-(5-(furan-2-yl)-1,3,4-oxadiazol-2-yl)phenyl)benzamide. In an enzymatic assay based on membrane fractions from insect cells expressing human ALCAT1, it causes complete inhibition at concentrations from 1 μ M upward. Such compounds could also be useful for limiting oxidative stress and cardiolipin remodeling in patients treated with thyroid hormone [2].

Published: 4 October 2018

MeSH keywords: acyltransferases/cardiolipins/free radicals/mitochondria

WO/2018/182051

IP6K inhibitors

Inventors: Terao Y, Takahashi M, Hara R, Hidaka K, Furukawa H, Yamasaki T, Kasai S

Assignee: Takeda Pharmaceutical Co. Ltd., Osaka (Japan)

Inositol is a sugar that is involved in a large number of diverse physiological processes and its many roles have been researched for decades. Yet, it receives little attention from drug developers for precisely this reason: the potential side effects of interfering with the complex inositol pyrophosphate pathway [3,4] would seem difficult to predict or control, given the many involved enzymes and the even more numerous subtypes and their function. Mammals have three inositol hexakisphosphate kinases (IP6K1, 2 and 3) of which mostly IP6K1 has been investigated. IP6K1 can attach phosphate to a range of myo-inositol phosphates that are phosphorylated at positions 1 and 5, producing a series of inositol pyrophosphates among which diphosphoinositol pentakisphosphate (IP7) is the most important. N2-(m-(trifluoromethyl)benzyl) N6-(p-nitrobenzyl)purine (TNP), an inhibitor that is not subtype-specific, is used as a tool compound; and IP6K2 inhibitors have most recently been reported [5]. Takeda now claims IP6K inhibitors, focusing on diabetes and cardiac failure – but they are also not subtype-specific: the majority inhibits all enzyme subtypes at a 100 nM concentration. When administered to Zucker diabetic rats at 0.3, 3 and 30 mg/kg p.o., (1*S*,4*S*)-4-((3, 5-dichloropyridin-2-yl) oxy)-2'-oxo-1',2'-dihydrospiro[cyclohexane-1,3'-pyrrolo[3, 2-*b*] pyridine]-5'-carboxylic acid showed a sustained improvement of glucose tolerance that correlated with a decrease in tissue

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concentrations of IP7. The same compound, and a closely related indole compound, had a life-prolonging effect in the CSQ-tg calsequestrin transgenic mouse model of cardiac failure at 10 mg/kg.

Published: 4 October 2018

MeSH keywords: diabetes mellitus/heart failure/inositol/inositol hexakisphosphate kinase

WO/2018183411

Small molecule DCN1 inhibitors & therapeutic methods using the same

Inventors: Wang S, Zhou H, Lu J, Liu L, Sun Y, Bernard D

Assignee: The Regents of the University of Michigan, Ann Arbor, MI (USA)

The Cullin-RING ubiquitin ligases, which regulate the turnover of approximately 20% of cellular proteins via the ubiquitin proteasome system, are controlled by the ubiquitin-like protein, NEDD8 through the complex neddylation cascade [6]. DCN1, a scaffold-like E3 ligase, interacts with UBC12 (a NEDD8-specific E2 enzyme) to facilitate the transfer of NEDD8 from UBC12 to cullins, the enzymatic activity of which are thereby greatly enhanced [7,8]. Small-molecule inhibitors of the DCN1-UBC12 interaction have been described [9], but none have reached the clinical development stage. This document discloses novel ones that were designed around a peptidomimetic scaffold and have micromolar IC₅₀ values in a fluorescence polarization assay using recombinant human DCN1. Such molecules might offer mechanistic variations to the anticancer proteasome inhibitors, bortezomib and carfilzomib. For the peer review companion paper see Zhou *et al.* [10] Also see WO/2018/191199 from the same applicant.

Published: 4 October 2018

MeSH keywords: CULL-RING ligase, human/Dsc1 E3 ligase, S pombe/ubiquitin-protein ligases

WO/2018/184113

Iminopiperidine compounds as inhibitors of human polynucleotide kinase phosphatase

Inventors: Hall D, Weinfeld M, Berard S, Verdelet T, Lamontagne V, Shire Z, Lavasanifar A

Assignee: The Governors of the University of Alberta, Edmonton, Alberta (Canada)

Ionizing radiation and genotoxic agents generate strand breaks with incompatible termini that must be removed in order for single and double-strand break repair pathways to function. This process, for which the bifunctional enzyme PNKP provides a polynucleotide 3'-phosphatase reaction followed by a 5'-kinase reaction, is essential for the maintenance of genome integrity. However, it is undesired in cancer radio- and chemo-therapy. Inhibitors should sensitize tumor cells [11] because PNKP cannot be replaced by other 3'-phosphatases [12]. The first such inhibitor (A12B4C3) was characterized by the applicant in 2009 from a library of polysubstituted imidopiperidines [13]. The current document presents second-generation analogs of A12B4C3, the design and synthesis of which is extensively discussed. This produced two more potent inhibitors of PNKP, A12B4C50 and A83B4C63 which had low aqueous solubility and were investigated in polymeric micelles as solubilizers. IC₅₀ and K_d values are mostly in the low micromolar and mid-nanomolar range, respectively. The micellar formulations could offer additional opportunities for tumor targeting, which the inventors demonstrated by covalently attaching a dodecapeptide (YHWYGYTPQNVI; a selective and efficient EGFR allosteric ligand) to the nanocapsule surface. For the peer review companion paper see Shire *et al.* [14]

Published: 11 October 2018

MeSH keywords: DNA repair enzymes/double-strand DNA breaks/neoplasms/PNKP protein, human/radiotherapy

WO/2018/187957

PUM1 protein as a target for virus inhibition

Inventors: Zhou G, Roizman B, Liu Y

Assignee: Shenzhen International Institute for Biomedical Research, Shenzhen, Guangdong (China)

The pumilo protein PUM1, also known as PUF1 or RBE, is a member of the PUF family of RNA-binding proteins that is widely expressed in human tissues and can repress or activate protein expression, depending on the context [15]. The inventors show that siRNA knockdown of *PUM1* in uninfected HEp-2 cells results in a significant accumulation of transcripts encoding LGP2, CXCL10, IL-6 and PKR. In a second phase, which commences after about 24 h, mRNAs encoding RIG-I, MAD5, SP100 PML and STING, IFN-β, and to a lesser extent IFN-γ, show a similar increase far beyond their normal levels. All these interferon-stimulated proteins are associated with innate

cellular immunity, and indeed protect cells against herpes simplex virus infection. Detection of IFN- β in the cell culture supernatants confirmed this as the source of the antiviral effect. The general claim for PUM1 inhibitors extends to any compound that disrupts the PUM1–LGP2 interaction. It has been reported earlier [16] that Pumilio facilitates recognition of Newcastle virus RNA by LGP2 (an RNA helicase also known as RLR-3) through its chaperon-like function. For the peer review companion papers see Liu *et al.* [17,18]

Published: 18 October 2018

MeSH keywords: chemokines/DHX58 protein, human/RNA-binding proteins/RNA helicases

WO/2018190713

Inhibitors of lysine methyltransferase for treatment of pain

Inventors: Willemsen HJLM, Eijkelkamp N

Assignee: UMC Utrecht Holding B.V., Utrecht (The Netherlands)

Building on their earlier work showing the involvement of the 5p15.2 chromosomal region in chronic widespread pain syndrome [19] the inventors identify FAM173B, a mitochondrial protein of previously unknown function, as a lysine methyltransferase that promotes chronic pain through a pathway involving ROS and/or activation of glial cells. In the C57BL/6 mouse model of transient carrageenan-induced inflammatory pain, expression of human FAM173B in sensory neurons induced ongoing nonevoked inflammatory hyperalgesia and spontaneous pain that was present 1 month after carrageenan injection. Administration of the reactive oxygen scavenger phenyl-N-t-butyl nitron at day 5 completely reversed the persistent mechanical hyperalgesia, indicating that is maintained through a pathway depending on such molecular species. Inhibitors are presented; these are peptides or peptide analogs (such as chaetocins) with a cell-penetrating domain, delivered to sensory neurons and targeted to mitochondria. FAM173B inhibitors can be combined with minocycline, an inhibitor of glial cell activation. Note that chaetocin, a lysine methyltransferase also known as SUV39H1, has already been shown to contribute to nerve injury-induced allodynia and hyperalgesia [20]. For the peer review, companion paper see Willemsen *et al.* [21]

Published: 18 October 2018

MeSH keywords: chronic pain/free radical scavengers/mitochondrial proteins

WO/2018193129

Inhibitors of BMP2 & BMP4 signaling for use in the treatment of Barrett's esophagus

Inventors: Krishnadath KK

Assignee: Academisch Medisch Centrum, Amsterdam (The Netherlands)

It has been known for more than a decade that the bone morphogenetic protein pathway can be activated in chronic esophagitis. This can induce the metaplastic changes in the squamous epithelium that are characteristic of Barrett's esophagus [22], which can progress to esophageal adenocarcinoma. The BMP pathway activation can be demonstrated in a human model of gastroesophageal reflux disease [23]. Therefore, it is not surprising if the inventors have now found that inhibitors of BMP2 and/or BMP4 can resolve the phenotypical nonmalignant histological changes (i.e., development of columnar epithelial cells) in human Barrett tissue biopsies implanted into mice. Normal multilayered epithelium of the esophagus reappeared upon BMP inhibition, expressing the squamous cell markers p63 and CK5. Preferably, the inhibitors are recombinant single chain antibodies that bind within residues 10–17, 45–56 and 69 of BMP4, which define an epitope containing a hydrophobic groove. The inventors also showed that inhibition of BMP2 and 4 drives the development of squamous epithelium in a conditional noggin knockout mouse model; noggin is a BMP-binding protein [24].

Published: 25 October 2018

MeSH keywords: Barrett esophagus/bone morphogenetic proteins/esophagitis/gastroesophageal reflux

WO/2018197708

Use of 20-hydroxyecdysone & the derivatives thereof in the treatment of myopathies

Inventors: Dilda P, Lafont R, Latil M, Serova M, Agbulut O, Veillet S

Assignee: Biophytis, Paris (France); Sorbonne Université, Paris (France)

An insect molting hormone that also is a phytosteroid that some plants produce as a developmental insecticide is not what one would normally expect as being effective in genetic muscular dystrophies. However, when the peer review literature started to offer indications for a muscle anabolic effect of 20-hydroxyecdysone (ecdysterone) that

was mediated by the β estrogen receptor [25,26], patent applications were filed claiming utility in sarcopenia: by the present assignees in WO/2015/177469, and in WO/2016/125025 by another French company, Naturex SA for plant extracts containing it. While the inventive leap to claim it for Duchenne and Becker muscular dystrophies does not seem huge, ecdysterone and its analogs could indeed offer a pharmacological treatment option with relatively few side effects that might support future gene therapies. Two derivatives, BIO101 and BIO103, were tested in the dystrophin mutation carrying mdx mouse model at 50 mg/kg/d for 12 weeks, showing improved muscle vascularization. In myocytes from Duchenne patients, both compounds activated the AKT and MAPK signaling pathways, and BIO101 also improved the differentiation of myoblasts and mitochondrial respiration of Duchenne skeletal muscle cells within 2 days at *in vitro* concentrations of 1 or 5 μ M. Also see the inventors' companion patent application, WO/2018/197731.

Published: 1 November 2018

MeSH keywords: ecdysterone/muscular dystrophies/phytosteroids

WO/2018/201074

Methods for treating atherosclerosis with γ -ketoaldehyde scavengers

Inventors: Linton MR, Oates JA, Davies SS, Roberts LJ, Amaranth V, Yancey P, Tao H

Assignee: Vanderbilt University, Nashville, TN (USA)

Peroxidation of cell membrane phospholipids by reactive oxygen species (ROS) can provide a starting point for neurodegeneration and cardiovascular diseases; polyunsaturated fatty acyl chains are the prime target for oxidative attack. The breakdown of peroxidized lipids to reactive carbonyls (e.g., malondialdehyde, isolevuglandins and 4-oxo-nonenal) can start a chain reaction. Using scavengers of reactive α -dicarbonyl species instead of antioxidants that inhibit upstream ROS formation could offer attractive options because ROS are needed as signaling molecules and as cytotoxic defense against pathogens [27]. Such selective scavengers are well known and include aminoguanidine and D-penicillamine [28]. The inventors have applied this to atherosclerosis, using salicylamine (2-hydroxybenzylamine, 2-HOBA). This is a natural compound from buckwheat seeds that has been even suggested as a nutritional supplement because of its excellent preclinical safety profile, with a no-observed-adverse-effect level ≥ 1000 mg/kg after 3 months of daily treatment [29,30]. Indeed 2-HOBA attenuated atherosclerosis without altering plasma cholesterol in LDL-receptor knockout mice that were fed a western diet for 16 weeks. Compared with treatment with either vehicle or the inactive 4-HOBA, cell death was reduced by 72.9 and 72.4% in proximal aortic lesions of 2-HOBA treated mice. An extensive range of 2-HOBA derivatives is also claimed, with no biological data disclosed.

Published: 1 November 2018

MeSH keywords: atherosclerosis/benzylamines/free radical scavengers/reactive oxygen species

WO/2018/201516

Compound & application thereof for treatment of cataract

Inventors: Rao Y, Yan Y, Yang X, Yang Z, Chen X

Assignee: Tsinghua University, Beijing (China)

Attempts at pharmacological therapy of cataract, which occurs when lens proteins misfold or are crosslinked by glycosylation, have always suffered from limited effectiveness (sufficient drug penetration to the lens is difficult to achieve) and the almost universal availability of an excellent and cheap surgical alternative (lens replacement is the most frequently performed surgical procedure worldwide). Chinese researchers are now trying again, with derivatives of lanosterol which they had identified as a lead compound earlier [31], separately confirmed by a group from India [32]. This supports a biological pathway because lanosterol synthase mutations or inhibition of the enzyme cause cholesterol deficiency-associated cataracts [33,34]. In an *in vitro* model based on the α -crystallin mutation Cryab-R120G, derivatives showed EC₅₀ values down to 5.5 nM (compound C29), compared with 1.4 μ M for lanosterol. Partition coefficients are also more promising: logP is 5.98 for C29 as opposed to 8.17 for lanosterol. Considering that the compounds are supposed to be administered as eye drops, this is very promising; dissolution of early-stage cataracts might actually become a possibility.

Published: 8 November 2018

MeSH keywords: cataract/lanosterol

WO/2018/204278**Compositions & methods related to xCT antibodies***Inventors: Pericle F, O'Rourke J, Cavallo F**Assignee: Agilvax, Inc., Albuquerque, NM (USA)*

Cancer stem cells use various mechanisms to resist common cytotoxic therapies, including improved detoxification and antioxidant ability. In triple negative breast cancer, this is in part attributable to expression of xCT (SLC7A11), the functional subunit of a cystine-glutamate antiporter that ultimately modulates glutathione synthesis [35] and is normally restricted to neurons and a subset of macrophages. xCT is inhibited by sulfasalazine, an old drug for inflammatory bowel diseases and rheumatoid arthritis which is metabolized to 5-aminosalicylic acid by the colon microbiome. However, sulfasalazine has a broad range of side effects that could be avoided by monoclonal antibodies against xCT extracellular domains; which is what the inventors have developed, targeting epitopes defined by amino acid sequences SPKGVLQNTG, RPAATAVISLAFGRYILEPFFIQC, MQLIKGQTQNFKDAF-SGRDSSITR and others. The hybridomas, developed from mice inoculated with virus-like particle vaccines bearing DNA coding for these epitopes, are extensively characterized but no tumor progression or life extension data are provided here, in contrast to the peer review companion paper [36] which shows inhibition of lung metastasis in a mouse model. Note that xCT has also been suggested as a target for alcohol addiction therapy [37].

Published: 8 November 2018

MeSH keywords: amino acid transport system y⁺/neoplastic stem cells**WO/2018/208630****Aryl-sulfonamide & aryl-sulfone derivatives as TRPML modulators***Inventors: Liang C**Assignee: Calygene Biotechnology, Camden, DE (USA)*

TRPML1, the principal lysosomal Ca²⁺ channel, is a key regulator of most membrane trafficking processes in the lysosome. Its loss-of-function mutations underlie diseases caused by lysosomal trafficking and storage defects, such as Type IV mucopolysaccharidosis (ML4), a neurodegenerative disease [38]. TRPML1 also has an essential role in autophagy [39], and it might be involved in Alzheimer's [40] and Parkinson's disease [41]. Its primary physiological activator is the low-abundance phosphatidylinositol 3,5-bisphosphate, while the 4,5-bisphosphate blocks it [42]. Disclosed here are what might be the first pharmacologically useful TRPML modulator compounds. One stereoisomer of 2-(4-chloro-2-fluorophenethyl)-7-fluoro-1-tosyl-1,2,3,4-tetrahydroquinoline showed an IC₅₀ of 0.79 μM in a lysosomal calcium imaging assay. The same compound significantly inhibited histamine-induced formation of vacuolar apical compartments in gastric acid glands (a significant indicator of activity because TRPML1 is required for gastric acid secretion [43]). Also see Calygene's associated patent application WO/2018/005713.

Published: 15 November 2018

MeSH keywords: lysosomes/MCOLN1 protein, human/phosphatidylinositols/transient receptor potential channels

WO/2018/211276**Piperidine-dione derivatives for use as contraceptives***Inventors: Sieng B, Lundvall S, Bøen CA, Hnida K**Assignee: Spermatech AS, Oslo (Norway)*

Although several pharmacological mechanisms have been suggested and tested in clinical trials, reliable and reversible male contraceptives have remained elusive. Among the most promising targets are enzymes of the glycolytic pathway in sperm cells, which lack mitochondria and rely on conversion of pyruvate into lactate for ATP generation. Lactate dehydrogenase is required for sperm motility [44], but to be acceptable as oral male contraceptives inhibitors have to be specific for the homotetrameric LDH-C4 isoform, which is mostly restricted to testes and sperm. N-Alkyl oxamates have been identified as such inhibitors [45,46] (oxamic acid itself being an LDH-A inhibitor). The oxamic acid motif is only marginally recognizable in the Spermatech compounds of this invention, which are quite drug-like; however, they do resemble the LDH-A inhibitors that Genentech disclosed in WO/2015/140133 and WO 2015/142/903 as being useful for cancer treatment. In a human sperm motility assay, 3-((2-chloro-4-fluorophenyl)thio)-6-(4-morpholinophenyl)-6-(thiophen-3-yl)piperidine-2,4-dione completely inhibited progressive motility in the presence of pyruvate at a concentration of about 8 μM, the IC₅₀ being about 2 μM. While this

clearly requires further optimization for potency (and probably for LDH subtype selectivity, which is not disclosed), the general approach seems to be a promising one.

Published: 22 November 2018

MeSH keywords: contraception/lactate dehydrogenase C4/sperm motility

WO/2018/215563

Calcineurin B homologous protein 1 inhibitors & therapeutic & nontherapeutic uses thereof

Inventors: Wirth B, Janzen E, Mendoza-Ferreira N, Hosseinibarkooie S

Assignee: Universität zu Köln, Köln (Germany)

Suppressing expression of CHP1, a calcium-binding neuronal protein and a cofactor of the Na⁺/H⁺ exchanger 1, NHE1 [47] can treat spinal muscular atrophies by improving impaired endocytosis (by increasing of CaN phosphatase activity) and by triggering the maturation of neuronal stem cells into motor neurons: this is the essence of this patent application. The mechanistic key insight is that CHP1 directly interacts with PLS3, apparently preventing this plastin protein from restoring axonal length [48]. Experiments were carried out in transgenic zebrafish, an intermediate-severity SMA mouse model resembling SMA Type II patients, murine primary motor neuron cultures, and with yeast two-hybrid pulldown assays using full-length PLS3 as the bait. Preferably, patients would receive an oligonucleotide-driven double knockdown of CHP1 and of the silencer region of the *SMN2* gene or are treated with *CHP1* knockdown and an oligonucleotide enhancing a splice enhancer of exon 7 of *SMN2*. For SMA Type I patients who carry only two *SMN2* copies, such a treatment might be a crucial addition to Biogen, Inc.'s newly approved *SMN2* splicing modulator oligonucleotide, nusinersen (Spinraza®). For the peer review, companion paper see Janzen *et al.* [49]

Published: 29 November 2018

MeSH keywords: CHP1 protein, human/muscular atrophy, spinal/plastin

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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