Conference paper

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From metabolism to disease: the biological roles of glutamine:fructose-6-phosphate amidotransferase (GFAT)

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Abstract: Glutamine:fructose-6-phosphate amidotransferase (GFAT) is the rate-limiting enzyme of the hexosamine biosynthetic pathway (HBP), an important route for *de novo* synthesis of amino sugars, which are key components of prokaryotic cell walls, chitin, and complex eukaryotic glycoconjugates. GFAT also plays a major role in several pathological processes, including cancer and diabetes. It has been 60 years since GFAT was first characterized. During this time, the knowledge about the enzyme's mechanisms and biological relevance has increased considerably. We take the anniversary of GFAT's discovery as an opportunity to discuss the role of GFAT in both health and disease and explore its biotechnological potential as a target for antimicrobial and anticancer chemotherapy.

Keywords: D-glucosamine-6-phosphate synthase (GlmS); glycosylation; hexosamine biosynthetic pathway (HBP); ICS-30; inhibitors; L-glutamine:D-fructose-6-phosphate amidotransferase (GFAT); metabolic diseases; UDP-GlcNAc.

Introduction

All living cells, from bacteria to mammals, obtain activated hexosamines, through the indispensable and ubiquitous hexosamine biosynthetic pathway (HBP). The final product of this pathway, uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc), is perhaps the most indispensable monosaccharide in glycobiology, as it is a substrate for most types of glycosylation in vertebrates, including *O*- and *N*-glycosylation, glycosaminoglycan synthesis, and intracellular *O*-GlcNAcylation (Fig. 1). UDP-GlcNAc is also the precursor for activated forms of important monosaccharides, like *N*-acetylgalactosamine (GalNAc), *N*-acetylmuramic acid (MurNAc), and sialic acid (Sia) (Fig. 1). In addition, UDP-GlcNAc is a substrate for the synthesis of several essential carbohydrate polymers, including chitin in arthropods [1] and fungi [2], and peptidoglycans and lipopolysaccharides in bacteria (Fig. 1) [3–6]. Therefore, the HBP is not just a route that supplies structural moieties but rather a pathway that influences a myriad of cell functions, such as cell–cell communication, adhesion, differentiation, and cell-cycle regulation [7, 8].

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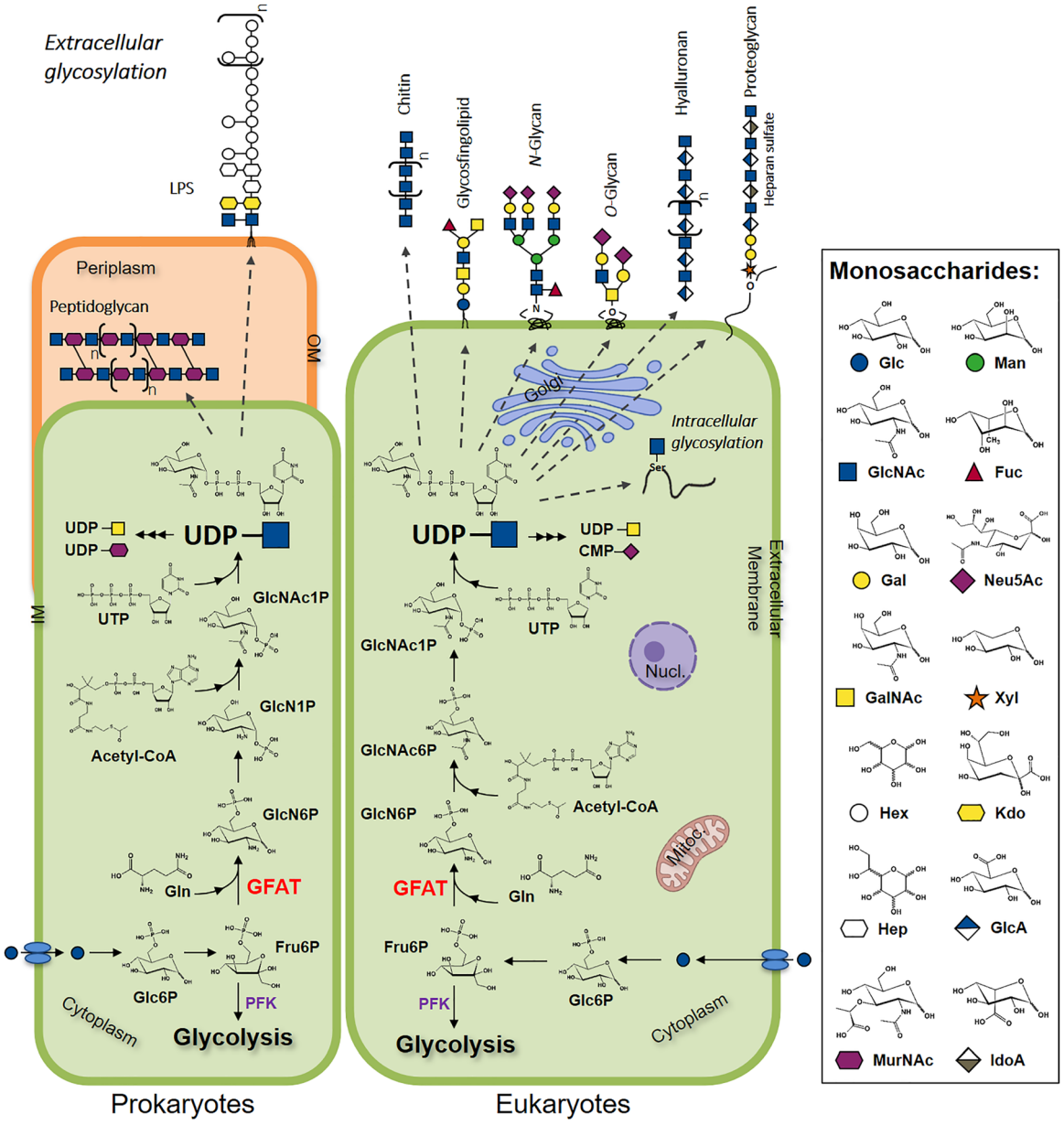

Fig. 1: GFAT's relevance for cell glycosylation. Upon its entry into the cell, glucose is phosphorylated to glucose-6-phosphate (Glc6P) and isomerized to fructose-6-phosphate (Fru6P). The fructose-6-phosphate can then be directed to either the glycolysis pathway, by the action of phosphofructokinase (PFK), or to the hexosamine biosynthetic pathway (HBP), by the action of L-glutamine:D-fructose-6-phosphate amidotransferase (GFAT). The steps of HBP differ between prokaryotic (left panel) and eukaryotic (middle panel) cells, although both end with the production of uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc). UDP-GlcNAc can be incorporated in several glycan structures of prokaryotic (left panel, represented by a gram-negative bacteria scheme) and eukaryotic cells (right panel). In addition, UDP-GlcNAc is also used in the synthesis of other activated monosaccharides, such as UDP-*N*-acetylgalactosamine (UDP-GalNAc) and UDP-*N*-acetylmuramic acid in prokaryotes (left panel), or UDP-*N*-acetylgalactosamine and cytidine monophosphate *N*-acetylneuraminic acid (CMP-Neu5Ac) in eukaryotes (middle panel). The legend on the right panel depicts the chemical structures and symbols of the highlighted monosaccharides, according to the Symbol Nomenclature for Glycans (SNFG) [19, 20]. IM, inner membrane; OM, outer membrane; LPS, lipopolysaccharide; Gln, L-glutamine; GlcN6P, D-glucosamine-6-phosphate; GlcN1P, D-glucosamine-1-phosphate; GlcNAc1P, *N*-acetyl-D-glucosamine-1-phosphate; GlcNAc6P, *N*-acetyl-D-glucosamine-6-phosphate; UTP, uridine triphosphate; nucl., nucleus; mitoc., mitochondria; Glc, D-glucose; GlcNAc, *N*-acetyl-D-glucosamine; Gal, D-galactose; GalNAc, *N*-acetyl-D-galactosamine; Hex, hexose; Hep, heptose; MurNAc, *N*-acetylmuramic acid; Man, D-mannose; Fuc, L-fucose; Neu5Ac, *N*-acetylneuraminic acid; Xyl, D-xylose; Kdo, 2-Keto-3-Deoxy-D-Mannooctanoic Acid; GlcA, D-glucuronic acid; IdoA, L-iduronic acid.

HBP is an alternative to the glycolytic pathway since it diverts from glycolysis at D-fructose-6-phosphate (Fru6P). As the first and irreversible step of HBP, Fru6P receives an amino group from L-glutamine and is isomerized to D-glucosamine-6-phosphate (GlcN6P) in a reaction catalyzed by the enzyme L-glutamine:D-fructose-6-phosphate amidotransferase (GFAT, EC 2.6.1.16). Despite GFAT being a highly conserved protein and its enzymatic reaction does not differ among species, the following sequence of HBP steps does (Fig. 1). In prokaryotes, GlcN6P is isomerized into D-glucosamine-1-phosphate (GlcN1P), followed by its *N*-acetylation using acetyl-CoA to generate *N*-acetyl-D-glucosamine-1-phosphate (GlcNAc1P). In contrast, in eukaryotes, GlcN6P is initially *N*-acetylated to form *N*-acetyl-D-glucosamine-6-phosphate (GlcNAc6P), and then isomerized to GlcNAc1P. In both cases, GlcNAc1P is then activated to UDP-GlcNAc with the expense of a uridine triphosphate (UTP) molecule. Because HBP uses molecules from all biosynthetic routes (Fru6P from carbohydrate metabolism, glutamine from protein metabolism, acetyl-CoA from lipid metabolism, and UTP from nucleotide metabolism), this pathway is considered a metabolic sensor [9] and has been implicated in an increasing number of pathological processes [10, 11].

While recent excellent reviews have explored the relevance of the hexosamine biosynthetic pathway (HBP) for specific research areas [12–16], a comprehensive review specifically dedicated to GFAT had not been conducted for over a decade [17, 18]. Therefore, there is still a lack of thorough compilation regarding the role and impact of GFAT in different organisms. In this review, we aim to address this gap by discussing the current knowledge of this remarkable enzyme in light of novel findings regarding the relevance of GFATs isoforms in the development and homeostasis of diverse organisms.

GFAT: a multifaceted enzyme involved in hexosamine biosynthesis and evolutionary divergence

The first characterization of an enzyme responsible for converting hexose-6-phosphate to GlcN6P, using glutamine as the nitrogen donor, was reported by Leloir's group in *Neurospora crassa* [21]. Leloir was awarded the 1970 Nobel Prize for his pioneering research on polysaccharide biosynthesis and the discovery of sugar nucleotides. However, it was not until seven years later that the specificity of the enzyme for Fru6P, rather than D-glucose-6-phosphate, and its naming as GFAT were documented by Ghosh *et al.* [22], who extensively purified extracts from rat liver, *Escherichia coli*, and *N. crassa*.

GFAT is a member of the glutamine amidotransferase (Gn-AT) family, which belongs to class II or Ntn (recently reviewed by Linhorst and Lübke [23]), meaning it has an N-terminal cysteine (Cys) as the catalyst nucleophile [24]. GFAT employs a two-step mechanism that involves glutamine hydrolysis, generating free ammonia, and transferring it to the acceptor substrate (Fig. 2) [25–31]. The glutamine amide transfer and isomerase domains catalyze each step of the reaction (Fig. 2). Unlike most Gn-ATs [32], GFAT cannot use exogenous ammonia as a nitrogen source to perform the amine transfer reaction within the isomerase domain [24]. The complete GFAT reaction mechanism has been proposed based on studies with *E. coli* Glucosamine-6-phosphate synthase (GlmS) and is characterized as bi–bi-ordered [31, 33, 34], with Fru6P entry inducing conformational changes that favor binding to glutamine [28]. Allosteric inhibition by UDP-GlcNAc occurs only in eukaryotic enzymes, unlike prokaryotic ones [35–38].

The GFAT gene is present in all domains of life, with structural and functional studies in various phyla. The GFAT protein is found in the cytoplasm and exists as a single isoform in prokaryotes, fungi, and *Candida elegans*. Another GFAT gene has been observed in some arthropods [1, 39], but there is insufficient data to determine if this is a pattern of the phyla. Mammals contain two copies of GFAT genes, *GFPT1* and *GFPT2*, located in different chromosomes [40–42] and with different tissue-specific expression patterns [42–44]. Interestingly, the GFAT1-coding gene undergoes alternative splicing and generates both GFAT1 and GFAT1-Alt (or GFAT-1L), with the former being longer and expressed solely in skeletal muscle, as observed in mice, pigs and humans [45–47]. The evolution of GFAT isoforms is not clear, but a recent study with vertebrates shows a strong separation of vertebrate GFAT1 and GFAT2 in two distinct branches of the phylogenetic tree, suggesting

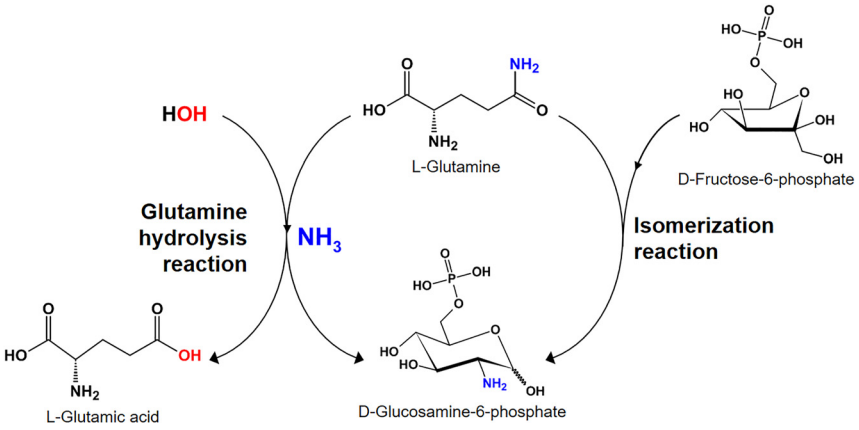

Fig. 2: Scheme of GFAT enzymatic reaction. Glutamine hydrolysis and sugar isomerization occur in different domains of the protein. The released ammonia from L-glutamine hydrolysis travels to the other GFAT domain to be used in the transamination of the D-fructose-6-phosphate during the isomerization of this sugar, yielding the formation of D-glucosamine-6-phosphate.

an ancient gene duplication event. Additionally, using a *Plasmodium* GFAT sequence as an external group suggests that GFAT2 is older than GFAT1, as it is closely related to the outgroup [48].

GFAT in prokaryotes

Bacteria are highly dependent on amino sugars such as glucosamine and GlcNAc for peptidoglycan synthesis in Gram-negative bacteria, and lipoteichoic acid production in Gram-positive bacteria [49–51]. Some bacteria can also use these monosaccharides for capsule or exopolysaccharide production [52] pointing to GlmS as an interesting target for new antibiotic development, which was reviewed recently by Wyllie *et al.* [53].

Although GlmS is a crucial enzyme in prokaryotes and plays a significant role in *E. coli* biology, there is a limited amount of research on its impact. Nonetheless, the enzyme's importance cannot be underestimated, as deleting its gene results in *E. coli* cell death [54]. In *Salmonella enterica*, a Gram-negative bacteria that causes gastroenteritis in humans, deletion of the GlmS gene severely impairs bacterial growth. Although exogenous supplementation with glucosamine can partially restore growth, the cell envelope's integrity is significantly compromised, leading to reduced ability of the bacteria to bind and invade intestinal epithelial cells [55].

The knockout of GlmS in Gram-positive bacteria also results in cell death [52, 56], which can be rescued by GlcNAc supplementation, although the outcomes vary among species. In *Staphylococcus aureus*, GlmS is essential for producing GlcNAc required for cell wall synthesis [52]. However, activation of the HBP pathway, either through GlmS expression or exposure to GlcNAc, negatively impacts the expression of virulence factors critical for biofilm formation in *Streptococcus mutans* [56]. Since *S. mutans* biofilm mainly comprises a glucose-based polymer, its synthesis competes with HBP and, consequently, with GlmS, for substrates [56].

GFAT in chitin synthesizing organisms

GFAT in fungi

A key adaptation in several fungi species is the ability to produce a cell wall, which is responsible for cell shape and protection [57]. Chitin, a β-1,4-GlcNAc homopolymer, can account for 1–30 % of cell wall dry mass depending on fungal type (filamentous or not) [58–60] and cell stress conditions [61]. Biochemical and molecular biology studies have established a correlation between cell wall stress and an increase in GFAT gene transcription, expression levels and/or enzyme activity, leading to increased chitin deposition in the cell wall for restoration of its integrity. In *Saccharomyces cerevisiae*, the promoter region of the GFAT gene includes a transcriptional regulation sequence responsive to the alpha factor, a mating pheromone that also increases chitin content [62]. In fact, the GFAT gene and chitin synthetic pathway are mutually related in *S. cerevisiae*: the GFAT gene is

activated upon the activation of the chitin synthesis pathway caused by cell-wall gene mutations, and chitin synthesis is activated by GFAT overexpression [63]. Cell wall damage induced by Congo Red, Zymolyase, or mutations in cell-wall remodeling responsive genes elicits the transcription of several genes involved in chitin synthesis, including GFAT [64, 65]. In addition, GFAT plays an important role by protecting this fungus from methylmercury accumulation [66]. GFAT is also crucial for *S. pombe* survival [54]. Cell wall stress was shown to increase both chitin content and GFAT transcripts in *Aspergillus*, and GFAT gene deletion led to cell death. Although the lethal phenotype can be rescued by glucosamine supplementation, the mutant strains have shown slower growth even in high glucosamine conditions [67, 68]. Despite being purified and characterized from other fungi, such as *N. crassa* [22, 69], *Volvariella volvacea* [70], *Proteus mirabilis* [71], GFAT's relevance for physiology of these fungi was not studied.

The dynamics of chitin synthesis and GFAT activity are particularly intriguing in fungi undergoing cell differentiation. In the life cycle of *Blastocladiella emersonii*, the correlation between chitin synthesis and GFAT activity is regulated by sensitivity to UDP-GlcNAc inhibition [72]. During the growing phase, when chitin synthesis is occurring, GFAT shows lower sensitivity to UDP-GlcNAc inhibition. However, its sensitivity increases over time during the sporulation phase when chitin synthesis is turned off [38]. Phosphorylation triggers this sensitivity both *in vitro* [73] and *in vivo* during sporulation [74].

In *Candida albicans*, an opportunist fungus, chitin synthesis is regulated during dimorphic growth [75]. The chitin content increases by 5-fold and GFAT activity increases by 4-fold during the yeast-to-mycelial transformation [76]. The essential role of GFAT in promoting *C. albicans* growth is evident as antifungal drugs targeting this enzyme induce cell agglutination, swelling, disruption of cell integrity, and inhibition of septum formation [77]. GFAT activity from *C. albicans* is also modulated by phosphorylation, and the loss of this post-translational modification resulted in reduced germination efficiency [78].

The hyphal form of *Sporothrix* is more sensitive to cell membrane stress induced by dyes compared to the yeast form. Mycelial cells from *Sporothrix schenckii* and *S. globosa* displayed a transient increase in GFAT activity following exposure to Congo Red [79]. The response in GFAT activity was directly proportional to the extent of damage caused by the tested dyes to the mycelia [79, 80]. These findings highlight the crucial role of GFAT in mediating chitin synthesis, an important mechanism of fungal protection. Consequently, this enzyme emerges as an attractive target for antifungal chemotherapy, as recently reviewed by Stefaniak *et al.* [81].

GFAT in arthropods

Chitin is the main component of the arthropod exoskeleton, and it has a key role in physical and pathogen stress. However, little is known about GFAT in insects. Its transcripts are detected in all tissues and stages of development, but its expression profile positively correlates with chitinous tissues exposed to stress, like the intestine of the shrimp *Litopenaeus vannamei* [82], the midgut of *Aedes aegypti* [1], the cuticle of *Nilaparvata lugens* [83] and *Haemaphysalis longicornis* [84]. The synthesis and regulation of chitin are important for insect development and molting, particularly following blood-feeding. GFAT mRNA levels have been shown to be upregulated in a tissue-specific manner in *H. longicornis* during chitin remodeling [84]. In ticks, knockdown of GFAT gene causes a significant reduction in body weight after blood-feeding, as well as death within 5–10 days post-engorgement [84]. A similar pattern has been observed in *A. aegypti*, where GFAT knockdown impairs chitin synthesis and peritrophic matrix formation upon blood-feeding, an effect that cannot be reversed by glucosamine supplementation [1, 85]. Downregulation of GFAT in *N. lugens* decreases mRNA levels of HBP enzymes, chitin synthesis, and degradation-related genes, leading to insect malformation (such as molting difficulties and wing deformities) and death [83]. In contrast, inhibiting chitinase in *Spodoptera frugiperda* larvae increases GFAT transcript levels and abnormal molting [86].

In *D. melanogaster*, the apparent redundancy of the GFAT isoforms was investigated using CRISPR technology. While deletions of either GFAT1 or GFAT2 were lethal, GFAT2-deficient animals could be rescued with a supplementary diet containing GlcN6P, generating fertile females depending on the sugar concentration. Interestingly, animals lacking GFAT1 could not be rescued, even with high GlcN6P concentrations [39], indicating

that GFAT isoforms are not functionally redundant. The authors speculate that GFAT1 may generate a non-canonical product. Recent cytogenetic analysis located the GFAT2 gene in euchromatin and the GFAT1 gene in heterochromatin in *Drosophila* species, showing that GFAT1 and GFAT2 mRNAs have distinct expression patterns during embryogenesis [87]. The authors also showed that both GFAT1- and GFAT2-deficient phenotypes are lethal, but in contrast to the results of Chen *et al.* [39], they could be rescued from lethality by each other transgenes, suggesting that GFAT1 and GFAT2 are functionally equivalent. During *D. melanogaster* development, GFAT1 activity is regulated on the posttranslational level. UDP-GlcNAc inhibits the activity of GFAT, and it can be stimulated by PKA [88].

Altogether, these data clearly indicate a very close cross-talk between chitin synthesis and GFAT (and consequently, HBP) activation in fungi and arthropods. Nevertheless, the potential effects of chitin disruption on other HBP-supported pathways, such as *N*-glycosylation, as well as those that compete with HBP for substrate, including glycolysis, PPP (indirectly), and beta-glucan synthesis, remain insufficiently investigated. Furthermore, our understanding of the spatial and temporal distribution of GFAT protein and its regulatory mechanisms is currently limited.

GFAT in plants

The first report describing the presence of GFAT in plants dates to the late 1950s and, since then, only a few works aiming to identify how HBP regulates different aspects of plant physiology have been published [89]. In *Arabidopsis thaliana*, a single copy of the GFAT gene is present, and it is highly expressed in mature pollen grains [90]. GFAT expression increases under endoplasmic reticulum (ER) stress conditions, and circadian analysis has indicated that GFAT can be modulated in a day-period dependent fashion, with the highest expression occurring at the end of a 12-h light period and the lowest expression occurring at the end of the night [90, 91]. In addition, the disruption of the GFAT gene by T-DNA results in male gametophytic sterility, which is rescued by exogenous supplementation of glucosamine [90].

GFAT in worms

Among worms, Nematoda stands out as the first and, to our knowledge, the only phylum in which the functional and physiological aspects of GFAT have been explored. The use of *C. elegans* as a model organism has provided valuable insights linking GFAT with protein homeostasis and longevity [92, 93]. Horn *et al.* have shown that a mutation in the GFAT1 gene sequence, resulting in enhanced enzyme activity, led to an increase in the lifespan of *C. elegans* [93]. Remarkably, this effect is independent of *O*-GlcNAcylation but instead arises from the induction of protein homeostasis through the unfolded protein response (UPR) components IRE1 and the X-box binding protein 1 (XBP1), along with autophagy [92, 93].

GFAT in mammals: in health and disease

Regulation of GFAT1 and GFAT2 expression and activity in response to stress and nutrient deprivation

GFAT1, as a critical enzyme in the hexosamine biosynthesis pathway, is regulated by multiple factors. Under hypoxic conditions, GFAT1 mRNA expression is upregulated in macrophages via the hypoxia response element (HRE) located in the GFAT promoter, but whether HIF1 regulates GFAT1 transcription in this context remains to be determined [94]. In contrast, the basal transcription of GFAT1 is mainly regulated by the transcription factor Sp1, as the GFAT promoter lacks the typical TATA box found in most “housekeeping” genes [95]. In addition to

hypoxia, GFAT1 expression is also influenced by nutrient stress, such as glucose deprivation. Under these conditions, the GCN2/eIF2α pathway induces the expression and activation of ATF4, a transcription factor that leads to increased GFAT1 levels and *O*-GlcNAcylation within cells [96]. GFAT2 transcription is also modulated by distinct transcription factors. In the retina, the nuclear receptor subfamily four group A member 1 (NR4A1) modulates GFAT2 mRNA expression [97]. NF-kB transcriptionally upregulates GFAT2 in non-small cell lung cancer (NSCLC) [98]. Furthermore, in macrophages, FoxO1 mediates the effect of lipopolysaccharides (LPS) in inducing GFAT2 expression which permits limited exacerbation of inflammation upon macrophage activation [99].

The UPR is a cellular stress response pathway that is activated when the ER is overwhelmed by unfolded or misfolded proteins. In mammals, GFAT plays a key role in linking the UPR to the HBP. The study by Wang *et al.* [100] showed that GFAT1 is regulated by the UPR through the spliced form of XBP1, a key transcription factor in the UPR pathway. XBP1s directly binds to the promoter of GFAT1 and increases its expression, leading to an increase in UDP-GlcNAc synthesis and subsequent protein glycosylation. This finding highlights the importance of GFAT1 in linking the UPR to the HBP and maintaining ER protein folding and quality control.

Mammal cells modify GFAT expression in response to stress or nutrient deprivation and regulate GFAT activity through post-translational modifications. Phosphorylation of human GFAT1 at Ser205 was shown to reduce its enzyme activity [101], while the phosphorylation of murine GFAT2 at the homologous site, Ser202, leads to an increase in enzymatic activity [102]. Moreover, GFAT1 Ser205 phosphorylation by PKA abolished the UDP-GlcNAc feedback inhibition [103]. Phosphorylation of GFAT1 at Ser243 by AMPK has been found to decrease its enzymatic activity [104]. This same study also demonstrated that glucose deprivation induces GFAT1 phosphorylation at the same site [104]. Furthermore, upon VEGF stimulation, AMPK activation leads to GFAT1 phosphorylation at Ser243 in human endothelial cells [105]. Zibrova *et al.* observed that high glucose activates GFAT1/HBP, yet impairs vascular sprouting, indicating that VEGF-induced GFAT1 phosphorylation via AMPK may serve as a mechanism to ameliorate hyperglycemia-induced vascular dysfunction [105]. In the context of nutrient deprivation, Moloughney *et al.* [106] demonstrated that glutamine and glucose limitation enhance mTORC2 activity. This, in turn, modulates GFAT1 expression through the transcription factor XBP1s [106] and sustains GFAT1 phosphorylation at Ser243 [107], thereby affecting GFAT1 activity. In a recent study by Wei *et al.* [108], a non-canonical role for GFAT1 in glucose deprivation was discovered, where it interacts with phosphorylated TAB1 protein, facilitating its glutamylation by TTLL5. TAB1 glutamylation is essential for sustaining p38 MAPK autophosphorylation, promoting cell survival under nutrient stress through autophagy. Additionally, the GFAT2:GFAT1 protein ratio can be adjusted to sustain UDP-GlcNAc levels, as observed in murine embryonic stem cells (mESC) [109], given that GFAT2 exhibits lower sensitivity to UDP-GlcNAc inhibition [102, 110]. Transamination has also been reported as a positive regulator of GFAT activity in response to increased intracellular calcium concentrations [111].

The role of GFAT in diabetes and its comorbidities

The involvement of GFAT in glucose metabolism raises the question of whether this enzyme plays a role in the development of diabetes mellitus (DM). In the series of seminal articles authored by Marshall and Traxinger, the direct involvement of GFAT in the desensitization of adipocytes to insulin promoted by the combined stimulation of glucose and insulin was observed [112–114]. In fact, hyperglycemia, a common feature of DM, combined with glutamine and insulin stimulation, was shown to desensitize the glucose transport system in an HBP-dependent mechanism [112]. In a study by Daniels *et al.*, the effects of glucose and insulin on GFAT activity were investigated in skeletal-muscle cells derived from non-diabetic and non-insulin-dependent diabetic subjects. Both groups showed increased GFAT activity in response to glucose and insulin, although the relationship between GFAT activity and glucose disposal rates measured *in vivo* differed between the two groups [115].

The activation of HBP by GFAT overexpression or glucosamine treatment results in glucose-stimulated insulin secretion and reduced expression of several β-cell specific genes in rat pancreatic islets [116]. This can lead to hyperinsulinemia, obesity, and insulin resistance in transgenic mice [117] or impaired glucose-stimulated insulin secretion in isolated rat islets. GFAT overexpression also leads to insulin resistance in muscle and adipose

tissue [118], possibly by reducing the expression of GLUT4 on the cell surface [119]. In liver cells, GFAT overexpression results in the increased expression of ER stress markers and promotes lipid accumulation as well as the activation of inflammatory pathways [120]. Cooksey and McClain conducted a study involving transgenic mice that overexpressed GFAT in specific tissues. The results revealed varied effects based on the tissue site of overexpression. Overexpression of GFAT in muscle and adipose tissue resulted in insulin resistance and hyperleptinemia. Conversely, GFAT overexpression in the liver led to hyperlipidemia, while in pancreatic β-cells, it resulted in increased insulin secretion [121].

Studies have analyzed GFAT genes looking for single nucleotide polymorphisms (SNPs) associated with diabetes. Zhang *et al.* [122] found 11 SNPs on the *GFPT2* and a variant of exon 14 linked to type 2 DM in Caucasian subjects, but Ng *et al.* [123] found no association of *GFPT1* variants with type 1 and type 2 DM in Caucasians despite using a larger sample size. Polymorphisms in the 5′-flanking region of GFAT genes and the −913 G/A SNP were also found to alter the risks of obesity and intramyocellular lipid content in male subjects [124]. However, more research is needed to confirm these findings.

In addition to its role in DM establishment, HBP activation is linked to several comorbidities associated with DM. Increased flow through the HBP (observed by glucosamine treatment and GFAT overexpression) activates the expression of genes implicated in vascular injury pathways in mesangial cells, as is often seen in diabetic nephropathy [125, 126].

GFAT plays a significant role in adipocyte differentiation, as its pharmacological or transcriptional (siRNA) inhibition results in the downregulation of key adipogenesis mediators, including C/EBPβ, PPARγ, and SREP-1, impairing lipid droplet formation [127]. This, along with the evidence of the interactions between HBP, leptin and free fatty acids in adipocytes [128], indicates a role of GFAT in obesity. Additionally, GFAT is thought to regulate critical stages in the onset and development of atherosclerotic lesions [129].

GFAT in cancer

A prominent role attributed to GFAT is the close relationship between HBP and cancer (for review, see [13]). Overexpression of GFAT was observed in human colorectal cancer (CRC) samples compared with adjacent tissue, and GFAT knockdown decreased tumor growth and metastasis in a mice xerograph model under hyperglycemic conditions [130]. GFAT is also associated with breast cancer evolution [131], and its inhibition has been shown to potentiate cisplatin-induced cell apoptosis in non-small cell lung cancer (NSCLC), suggesting that GFAT inhibition can be combined with traditional cancer chemotherapy to improve clinical outcomes [132].

Regarding isoform specificity, GFAT1 has been found to be upregulated in breast [133] and prostate [134] cancers. It has also been associated with tumor initiation by KRAS mutation in pancreatic ductal adenocarcinoma [135]. In contrast, GFAT2 levels have been observed to increase considerably in pancreatic adenocarcinoma [136]. NSCLC with double mutations in KRAS and STK11, an aggressive tumor prone to metastasis, have a high dependence on GFAT2 expression levels. The selective inhibition of GFAT2 was shown to reduce tumor growth in cell culture as well as in xenografts and genetically-modified mice, highlighting the relevance of this GFAT isoform to the aggressive NSCLC subtype [137]. High expression of GFAT2 was also reported in lung adenocarcinoma [138] and CRC, where it is a predictive factor for the poorest prognosis [139]. In light of the work of Kroef *et al.* [109], it is still an open issue whether cancer cells also exploit the regulation of GFAT2:GFAT1 ratio to match their need for UDP-GlcNAc.

GFAT overexpression is also capable of promoting the epithelial-mesenchymal transition (EMT) *in vitro*, a process required by many cancers to metastasize [140], resulting in oncofetal fibronectin glycosylation [141]. TGFβ-induced EMT increases GFAT activity, having direct effects on UDP-GlcNAc production and extracellular glycosylation [142] through an increase in GFAT stability by reducing proteasomal degradation [143]. GFAT gene silencing also leads to a decrease in hyaluronic acid synthesis in keratinocytes and the inhibition of cell migration [144], which can indicate a role of the crosstalk between HBP and glycosaminoglycans in cancer metastasis.

GFAT: linking cardiomyocyte hypertrophy and neuromuscular function

Recently, GFAT was also associated with cardiomyocyte hypertrophy [145, 146]. The knock-out of GFAT1 gene, but not GFAT2 gene, led to a decrease in stress-induced overall protein *O*-GlcNAcylation in neonatal cardiac cell preparations, indicating reduced HBP activity that is dependent on GFAT1 [43] in this tissue.

Mutations in *GFPT1* and other HBP genes cause a congenital myasthenic syndrome characterized by fatigable muscle weakness, owing to impaired neurotransmission [10, 147–152]. The results obtained with a muscle-specific GFAT1 deficient mouse model suggest a critical role for muscle-derived GFAT1 in the development of the neuromuscular junction, neurotransmission, and skeletal muscle integrity [153].

GFAT and *O*-GlcNAcylation

GFAT plays a critical role as the first step in UDP-GlcNAc synthesis, a key metabolite for intracellular *O*-GlcNAcylation. Therefore, the level of *O*-GlcNAcylation is often used as an indirect indicator of GFAT activity, and many studies have altered GFAT expression or pharmacologically inhibited its activity to modulate *O*-GlcNAcylation levels [154]. These manipulations have demonstrated the impact of this post-translational modification on circadian rhythm regulation [155], aging (for a review, see [156]), immune system modulation [99, 157, 158], and the pathophysiology of various diseases (for review, see [159]). Although this is a simplification of the GFAT/HBP impact, it highlights the relevance of the enzyme in numerous diseases and points it as an interesting target for drug development.

GFAT use in biotechnology

Aside from the direct biological relevance in understanding the role of GFAT in normal homeostasis and in diseases, there is growing interest in this enzyme in biotechnology. Recently, engineering the HBP of microbial organisms has become of biotechnological interest to increase the production of UDP-GlcNAc, glucosamine, GlcNAc, and even sialic acid [160–163] in the food, pharmaceutical, and agricultural sectors due to the relevance of this process for scaling up the production of chondroitin, hyaluronic acid, and human milk oligosaccharides (for a review, see [14]).

Inhibitors of GFAT

HBP has emerged as a promising target in the context of cancer [7, 137] and diabetes [126, 164, 165]. Additionally, the potential of GFAT as a viable target for antibacterials and antifungals is beyond doubt [166] (for an extensive review, see [53, 81]). Consequently, the development of specific, potent, and selective inhibitors of GFAT would facilitate investigations into the biological functions of GFAT1 and GFAT2 in mammals, while minimizing perturbations to their protein levels.

Since GFAT inhibition by 6-diazo-5-oxo-L-norleucine (DON) (**1**, K_i = 2.8 μM, Fig. 3), and its *N*-acetyl derivative, Duazomycin A (**2**, Fig. 3) [167], numerous glutamine analogs have been reported. Some of them, alike DON, are irreversible inhibitors that act as site-directed alkylating agents by forming covalent bonds with the sulfhydryl group of the Cys1 residue, which acts as a catalytic nucleophile in the glutaminase reaction [24, 168–170]. The stability of diazoketone inhibitors, exemplified by DON, in physiological environments stems from the presence of an electron-withdrawing carbonyl group that stabilizes the diazo dipole. This characteristic allows the inhibitor to behave as a reactive electrophile exclusively in specific circumstances, such as near a serine residue located in the active-site of the glutaminase domain. Therefore, this protonation event induces the liberation of nitrogen (N_2) [171]. Consequently, DON operates as a discerning mechanism-based inactivator of reactions that rely on glutamine, rather than functioning as a non-specific reactive intermediate.

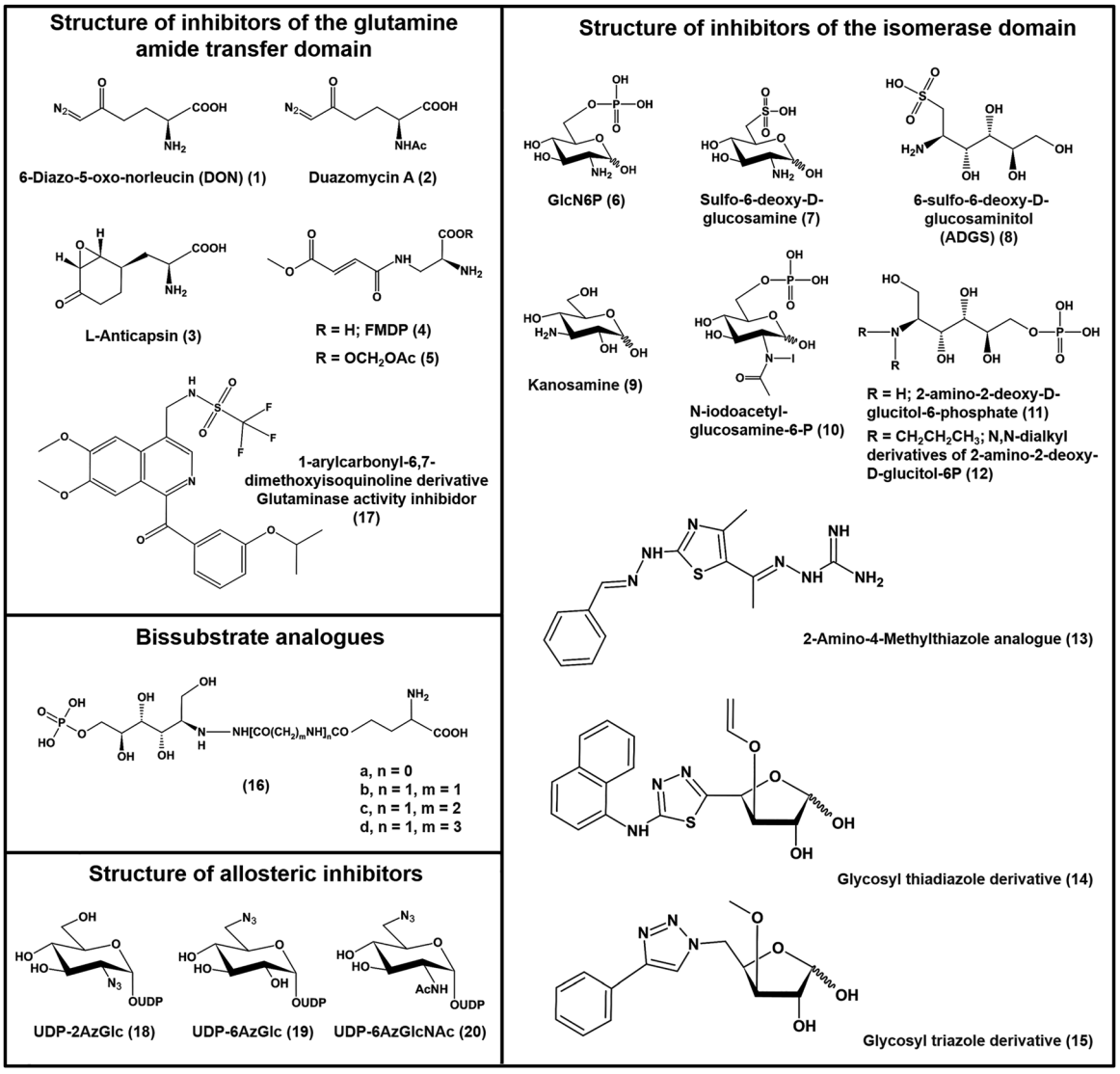

Fig. 3: GFAT inhibitors. Chemical structures of molecules described as GFAT inhibitors are separated into classes according to the targeted site or type of enzyme activity affected.

DON lacks specificity, as it also inhibits glutamine-metabolizing enzymes, including glutaminases, L-asparagine synthetase, and gamma-glutamyl transpeptidase. DON has shown promise as an anticancer therapeutic in the 1950s, but its clinical development has been hindered by dose-limiting gastrointestinal toxicities and inappropriate dosing schedules. Prodrug strategies for DON have been developed to enhance its delivery to tumor tissues, including the central nervous system [172, 173], and low daily dosing regimens have been found to be effective without significant toxicity [174, 175].

Unlike DON, the S-enantiomer of anticapsin (**3**, K_i = 0.1 μM, Fig. 3) [176], and the N3-(4-methoxyfumaroyl)-l-2,3-diaminopropanoic acid (FMDP) (**4**, K_i = 0.35 μM, Fig. 3) [177] are selective for GFAT and do not inhibit other amidotransferases [168], pointing that the catalytic site of the GFAT glutamine amide transfer domain differs in structure from other transaminases. Such differences have been explored by molecular modeling [178], the results of which suggested that selectivity against GFAT is possible due to the difference in distance between the active Cys sulfhydryl group site and the site in the active center of the enzyme which recognizes and binds

the carboxylic group of the glutamine analog. In GFAT, this distance is possibly greater than in other amidotransferases.

Due to FMDP (**4**, Fig. 3) selectivity and inhibitory activity against fungal GFAT (IC_{50} = 4.03 μM), several peptide analogs have been synthesized as antifungal agents [179–182], with no cytotoxicity against mammalian cells [183]. Those peptides function as pro-drugs, making the entry of the inhibitor into the cell feasible. Despite acceptable drug uptake by cells, the lack of cytotoxicity of FMDP-peptides in mammalian cells might be due to the poor rate of peptide cleavage by the intracellular peptidases [183]. Therefore, other modifications of the FMDP molecule, involving the formation of esters or other derivatives of the active agent to increase the penetration of compounds into the cell have been tested [180, 184]. Esters of FMDP (**5**, Fig. 3) showed greater GFAT inhibition than their amide derivatives [179, 180, 184] with IC_{50} in the order of 5 and 100 μM. Recently, a significant advancement was made in delivering FMDP to human cancer cells that overexpress GFAT. Researchers successfully achieved this delivery by utilizing polyethylene glycol-coated iron oxide/silica core-shell nanoparticles, to which FMDP was covalently attached. Remarkably, the application of these nano-encapsulated FMDP derivatives exhibited notable growth inhibition in the tested cancer cells. Furthermore, a comparative analysis of the cellular internalization of these nanoencapsulated FMDP derivatives was conducted in tumor cells and *C. albicans*. Surprisingly, it was observed that the mammalian cells displayed a high level of biocompatibility. This observation raises an important question regarding the potential toxicity in non-carcinogenic cells [181].

The isomerase domain of GFAT presents potential for the exploration of selective inhibitors. There is a growing number of reports on analogs specifically targeting the Fru6P binding site. GlcN6P (**6**, Fig. 3), the reaction product of GFAT, demonstrates inhibitory properties on the prokaryotic enzyme at millimolar concentrations [185]. It also acts as a potent competitive feedback inhibitor of human GFAT1 at micromolar concentrations, with a K_i of 6 μM [35]. Additionally, GlcN6P analogs such as 6-sulfo-6-deoxy-D-glucosamine (GlcN6S, **7**, Fig. 3) and 6-sulfo-6-deoxy-D-glucosaminitol (ADGS, **8**, Fig. 3) exhibit inhibition of the microbial enzyme at micromolar concentrations (IC_{50} of 0.21 and 0.18 mM, respectively). However, they display inhibitory effects on fungal GlcN6P *N*-acetyl transferase at millimolar concentrations, with IC_{50} values of 0.08 and 0.13 mM, respectively. *In vitro* antimicrobial tests have shown that these GlcN6P derivatives induce cell-wall destruction in both prokaryotic and fungal cells [186]. These findings underscore the variations in the isomerase domain framework of GFAT among different species, highlighting the potential of this domain as a target for structure-based drug design of novel microbicide compounds with desirable selectivity profiles. It is crucial to consider avoiding human GFAT as an unintended consequence of inhibition could lead to adverse effects.

The antibiotic kanosamine has been identified as an inhibitor of the isomerase activity of GFAT in *C. albicans* [77]. Kanosamine (**9**, Fig. 3) enters the cells through the glucose transport system and undergoes subsequent phosphorylation. The intracellular metabolism of kanosamine generates kanosamine-6-phosphate, which acts as an inhibitor of GFAT1 in *C. albicans* with a K_i of 5.9 mM. Notably, kanosamine has shown significant effects on the morphology of *C. albicans*, leading to notable alterations such as suppressed septum formation and severe cellular agglutination. Importantly, kanosamine-6-phosphate exhibits specificity towards GFAT1 as it does not affect the enzyme responsible for the subsequent metabolic step, glucosamine-6-phosphate *N*-acetylase. These observations suggest a selective impact of kanosamine-6-phosphate on GFAT1 in *C. albicans*.

The GFAT product analog, *N*-iodoacetyl-glucosamine-6-phosphate (**10**, Fig. 3), has been identified as an irreversible inactivator of the *E. coli* enzyme, with a K_i of 220 μM [187]. Additionally, analogs of the *cis*-enolamine transition state intermediate, such as 2-amino-2-deoxy-D-glucitol-6-phosphate (ADGP, **11**, Fig. 3), have demonstrated potent *in vitro* inhibition of GFAT (IC_{50} = 0.23 mM). However, these analogs exhibit low antimicrobial activity [33]. It is worth noting that these phosphorylated compounds are poorly taken up by cells, likely due to their negative charge and intracellular metabolism. Factors such as dephosphorylation by phosphatases or conversion to GlcN1P by GlcN6P isomerase (GlmM) may reduce access to the targeted GFAT [27]. To enhance the uptake of these compounds by fungal cells, various chemical modifications of ADGP aimed at increasing lipophilicity have been investigated. Hydrophobic compounds showed weak antifungal activity in the minimum inhibitory concentration assays (MIC) [166, 188]. However, *N*-alkylation (**12**, Fig. 3) of ADGP has proven to be a promising strategy, yielding novel inhibitors of fungal GFAT with higher antifungal activity compared to the

parent compound. The MIC assays of the best alkylated inhibitors ranged from 0.08 to 0.625 mg/mL [166]. These findings highlight the potential of modifying ADGP to improve its lipophilicity and enhance uptake by fungal cells, thereby leading to more effective inhibitors of fungal GFAT with increased antifungal activity.

New thiazole-carboximidamide derivatives (**13**, Fig. 3) designed as GlcN6P analogs have been tested against bacterial and fungal GFAT with MIC at micrograms per mL. Overall, the synthesized compounds were shown to be more active against Gram-positive than against Gram-negative microorganisms, and they presented low levels of activity against fungal and human cells, indicating prominent selectivity for the prokaryotic GFAT isoform. Moreover, cytotoxicity assays showed that none of the compounds presented activity against human normal cells, therefore these compounds can be considered for further drug development as antibacterial agents [189]. Molecular modeling studies have suggested that the addition of a guanidine moiety to de hydrazinecarboximidamide improves the inhibitory activity of the derivatives due to its interaction with the enzyme in the same manner as the phosphate group from GlcN6P. Glycosyl-thiadiazole (**14**, Fig. 3) and -triazole (**15**, Fig. 3) derivatives, that are structurally similar to the substrate Fru6P, inhibit fungal GFAT [190, 191].

The proximity of the glutamine amide transfer and isomerase domains makes it possible to design bisubstrate compounds. However, few compounds have been tested. Bisubstrate analogs (**16**, Fig. 3) synthesized by coupling the compound ADGP to the carboxyl function of glutamine did not exhibit significant inhibition of *E. coli* GFAT [27].

High throughput screening and subsequent hit identification and optimization have led to the synthesis of a series of 1-arylcarbonyl-6,7-dimethoxyisoquinoline derivatives, the most potent and only patented GFAT inhibitors reported so far. The most potent compound of the series (**17**, Fig. 3) was shown to inhibit GFAT with an IC_{50} of 1 μM, and it was found to interact with the glutamine amide transfer domain, as demonstrated by competition experiments. The compound showed significant efficacy in reducing the glucose levels in an oral glucose tolerance test in ob/ob mice [192] pointing it as a potential antiglycemic compound. However, more studies are needed to define its selectivity and toxicity.

GFAT inhibition by UDP-GlcNAc analogs raises concerns about the potential accumulation of UDP-sugar derivatives containing various monosaccharides with reactive groups at different positions, such as azides and alkynes [193]. Studies have demonstrated that monosaccharides with larger substitutions at the *N*-acetyl position do not inhibit GFAT, whereas UDP-glucose substituted with azide at either the 2- or 6-positions does exhibit inhibition (**18** and **19**, Fig. 3). Additionally, the substitution of the 6-hydroxyl group with an azide group on GlcNAc (**20**, Fig. 3) might slightly enhance the inhibitory effect of the UDP-sugar. In the pursuit of GFAT inhibitors, other potential compounds have been proposed through enzyme docking simulations [194–197]. These simulations provide valuable insights into the interactions between potential inhibitors and the GFAT enzyme, aiding in the identification of novel compounds with inhibitory properties.

Conclusions

Six decades after its first description, the knowledge about GFAT's enzymatic mechanism, biological relevance, evolution, and regulation complexity has increased considerably. However, there is still much to discover. The evolution pattern of the GFAT isoforms among different organisms, differences regarding the molecular structure and modulation of enzyme activity, how the post-translational modifications can affect enzyme activity, and whether GFAT has binding partners in different biological contexts, are some of the many questions that are yet to be answered. The growing evidence that GFAT isoforms play relevant roles in several human pathologies and their relevance as antimicrobial targets make them interesting targets for drug development. However, the development of selective and potent GFAT inhibitors has proven to be challenging. The complex structure of GFAT, combined with the need for high selectivity and minimal off-target effects, pose hurdles in inhibitor design and discovery. Additionally, the intricate regulation of GFAT activity and its involvement in essential cellular processes necessitate careful consideration of potential side effects and unintended consequences of inhibition. Advancements in computational methods, high-throughput screening, and structure-based drug design are contributing to the discovery of potential inhibitors. Collaborative efforts and interdisciplinary approaches are

also essential for overcoming the complexities associated with GFAT inhibition. Further research and innovation in this area are needed to unlock the full potential of GFAT inhibition and pave the way for the development of effective therapies targeting glycosylation-related disorders. Finally, the relevance of GFAT extends to several industries, including food, agriculture, and cosmetics. Therefore, continuous studies on this amazing enzyme are welcome and necessary.

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References

[1] N. Kato, R. Dasgupta, C. T. Smartt, B. M. Christensen. *Insect Mol. Biol*. **11**, 207 (2002), https://doi.org/10.1046/j.1365-2583.2002.00326.x.
[2] S. Milewski, I. Gabriel, J. Olchowy. *Yeast* **23**, 1 (2006), https://doi.org/10.1002/yea.1337.
[3] A. Typas, M. Banzhaf, C. A. Gross, W. Vollmer. *Nat. Rev. Microbiol.* **10**, 123 (2012), https://doi.org/10.1038/nrmicro2677.
[4] J. Lehrer, K. A. Vigeant, L. D. Tatar, M. A. Valvano. *J. Bacteriol.* **189**, 2618 (2007), https://doi.org/10.1128/jb.01905-06.
[5] L. A. Fernández-Herrero, M.-A. Badet-Denisot, B. Badet, J. Berenguer. *Mol. Microbiol.* **17**, 1 (1995), https://doi.org/10.1111/j.1365-2958.1995.mmi_17010001.x.
[6] C. Longzhu, M. Hiroko, K.-A. Kyoko, H. Hideaki, H. Keiichi. *Antimicrob. Agents Chemother.* **44**, 2276 (2000).
[7] A. Vasconcelos-dos-Santos, I. A. Oliveira, M. C. Lucena, N. R. Mantuano, S. A. Whelan, W. B. Dias, A. R. Todeschini. *Front. Oncol.* **5**, 138 (2015), https://doi.org/10.3389/fonc.2015.00138.
[8] P. Gagneux, T. Hennet, A. Varki. In *Essentials of Glycobiology*, A. Varki, R. D. Cummings, J. D. Esko, P. Stanley, G. W. Hart, M. Aebi, D. Mohnen, T. Kinoshita, N. H. Packer, J. H. Prestegard, R. L. Schnaar, P. H. Seeberger (Eds.), Cold Spring Harbor Laboratory Press, New York (2022), chapter 7.
[9] L. Wells, K. Vosseller, G. W. Hart. *Cell. Mol. Life Sci.* **60**, 222 (2003), https://doi.org/10.1007/s000180300017.
[10] A. P. Willems, B. G. M. van Engelen, D. J. Lefeber. *Biochim. Biophys. Acta* **1860**, 1640 (2016), https://doi.org/10.1016/j.bbagen.2015.12.017.
[11] M. G. Buse. *Am. J. Physiol. Endocrinol. Metab.* **290**, E1 (2006), https://doi.org/10.1152/ajpendo.00329.2005.
[12] G. K. Cork, J. Thompson, C. Slawson. *Front. Endocrinol.* **9**, 522 (2018), https://doi.org/10.3389/fendo.2018.00522.
[13] N. M. Akella, L. Ciraku, M. J. Reginato. *BMC Biol.* **17**, 52 (2019), https://doi.org/10.1186/s12915-019-0671-3.
[14] P. Coussement, D. Bauwens, G. Peters, J. Maertens, M. De Mey. *Biotechnol. Adv.* **40**, 107512 (2020), https://doi.org/10.1016/j.biotechadv.2020.107512.
[15] A. R. Brasier, D. Qiao, Y. Zhao. *Front. Pharmacol.* **12**, 808735 (2021), https://doi.org/10.3389/fphar.2021.808735.
[16] A. Paneque, H. Fortus, J. Zheng, G. Werlen, E. Jacinto. *Genes (Basel)* **14**, 933 (2023), https://doi.org/10.3390/genes14040933.
[17] P. Durand, B. Golinelli-Pimpaneau, S. Mouilleron, B. Badet, M.-A. Badet-Denisot. *Arch. Biochem. Biophys.* **474**, 302 (2008), https://doi.org/10.1016/j.abb.2008.01.026.
[18] S. Mouilleron, M.-A. Badet-Denisot, B. Badet, B. Golinelli-Pimpaneau. *Arch. Biochem. Biophys.* **505**, 1 (2011), https://doi.org/10.1016/j.abb.2010.08.008.
[19] A. Varki, R. D. Cummings, M. Aebi, N. H. Packer, P. H. Seeberger, J. D. Esko, P. Stanley, G. Hart, A. Darvill, T. Kinoshita, J. J. Prestegard, R. L. Schnaar, H. H. Freeze, J. D. Marth, C. R. Bertozzi, M. E. Etzler, M. Frank, J. F. G. Vliegenthart, T. Lütteke, S. Perez, E. Bolton, P. Rudd, J. Paulson, M. Kanehisa, P. Toukach, K. F. Aoki-Kinoshita, A. Dell, H. Narimatsu, W. York, N. Taniguchi, S. Kornfeld. *Glycobiology* **25**, 1323 (2015), https://doi.org/10.1093/glycob/cwv091.
[20] S. Neelamegham, K. Aoki-Kinoshita, E. Bolton, M. Frank, F. Lisacek, T. Lütteke, N. O'Boyle, N. H. Packer, P. Stanley, P. Toukach, A. Varki, R. J. Woods, T. S. D. Group. *Glycobiology* **29**, 620 (2019), https://doi.org/10.1093/glycob/cwz045.
[21] E. Cabib, L. F. Leloir, C. E. Cardini. *J. Biol. Chem.* **203**, 1055 (1953), https://doi.org/10.1016/s0021-9258(19)52376-6.
[22] S. Ghosh, H. J. Blumenthal, E. Davidson, S. Roseman. *J. Biol. Chem.* **235**, 1265 (1960), https://doi.org/10.1016/s0021-9258(18)69397-4.
[23] A. Linhorst, T. Lübke. *Cells* **11**, 1592 (2022), https://doi.org/10.3390/cells11101592.
[24] B. Badet, P. Vermoote, P. Y. Haumont, F. Lederer, F. le Goffic. *Biochemistry* **26**, 1940 (1987), https://doi.org/10.1021/bi00381a023.
[25] M.-A. Denisot, F. Le Goffic, B. Badet. *Arch. Biochem. Biophys.* **288**, 225 (1991), https://doi.org/10.1016/0003-9861(91)90188-o.
[26] B. Golinelli-Pimpaneau, F. Le Goffic, B. Badet. *J. Am. Chem. Soc.* **111**, 3029 (1989), https://doi.org/10.1021/ja00190a042.
[27] M.-A. Badet-Denisot, C. Leriche, F. Massière, B. Badet. *Bioorg. Med. Chem. Lett.* **5**, 815 (1995), https://doi.org/10.1016/0960-894x(95)00119-e.
[28] M. N. Isupov, G. Obmolova, S. Butterworth, M.-A. Badet-Denisot, B. Badet, I. Polikarpov, J. A. Littlechild, A. Teplyakov. *Structure* **4**, 801 (1996), https://doi.org/10.1016/s0969-2126(96)00087-1.
[29] A. Teplyakov, G. Obmolova, B. Badet, M.-A. Badet-Denisot. *J. Mol. Biol.* **313**, 1093 (2001), https://doi.org/10.1006/jmbi.2001.5094.

[30] A. Teplyakov, G. Obmolova, M.-A. Badet-Denisot, B. Badet, I. Polikarpov. *Structure* **6**, 1047 (1998), https://doi.org/10.1016/s0969-2126(98)00105-1.
[31] A. Teplyakov, G. Obmolova, M.-A. Badet-Denisot, B. Badet. *Protein Sci*. **8**, 596 (1999), https://doi.org/10.1110/ps.8.3.596.
[32] F. Massière, M.-A. Badet-Denisot. *Cell. Mol. Life Sci.* **54**, 205 (1998), https://doi.org/10.1007/s000180050145.
[33] S. Mouilleron, M.-A. Badet-Denisot, B. Golinelli-Pimpaneau. *J. Mol. Biol.* **377**, 1174 (2008), https://doi.org/10.1016/j.jmb.2008.01.077.
[34] S. Mouilleron, M.-A. Badet-Denisot, B. Golinelli-Pimpaneau. *J. Biol. Chem.* **281**, 4404 (2006), https://doi.org/10.1074/jbc.m511689200.
[35] K. O. Broschat, C. Gorka, J. D. Page, C. L. Martin-Berger, M. S. Davies, H. Huang, E. A. Gulve, W. J. Salsgiver, T. P. Kasten. *J. Biol. Chem.* **277**, 14764 (2002), https://doi.org/10.1074/jbc.m201056200.
[36] K. Kwiatkowska-Semrau, M. Wojciechowski, I. Gabriel, S. Crucho, S. Milewski. *Biochim. Biophys. Acta* **1866**, 1181 (2018), https://doi.org/10.1016/j.bbapap.2018.08.003.
[37] S. Kornfeld, R. Kornfeld, E. F. Neufeld, P. J. O'Brien. *Proc. Natl. Acad. Sci. U.S.A.* **52**, 371 (1964), https://doi.org/10.1073/pnas.52.2.371.
[38] C. P. Selitrennikoff, N. E. Dalley, D. R. Sonneborn. *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5998 (1980), https://doi.org/10.1073/pnas.77.10.5998.
[39] P. Chen, S. Visokay, J. M. Abrams. *Fly (Austin)* **14**, 3 (2020), https://doi.org/10.1080/19336934.2020.1784674.
[40] T. E. Whitmore, S. L. Mudri, G. L. McKnight. *Genomics* **26**, 422 (1995), https://doi.org/10.1016/0888-7543(95)80235-e.
[41] J. Zhou, J. L. Neidigh, R. Espinosa, M. M. LeBeau, D. A. McClain. *Hum. Genet.* **96**, 99 (1995), https://doi.org/10.1007/bf00214194.
[42] T. Oki, K. Yamazaki, J. Kuromitsu, M. Okada, I. Tanaka. *Genomics* **57**, 227 (1999), https://doi.org/10.1006/geno.1999.5785.
[43] A. A. Nabeebaccus, S. Verma, A. Zoccarato, G. Emanuelli, C. X. C. Santos, K. Streckfuss-Bömeke, A. M. Shah. *Biochem. Biophys. Res. Commun.* **583**, 121 (2021), https://doi.org/10.1016/j.bbrc.2021.10.056.
[44] A. G. Nerlich, U. Sauer, V. Kolm-Litty, E. Wagner, M. Koch, E. D. Schleicher. *Diabetes* **47**, 170 (1998), https://doi.org/10.2337/diabetes.47.2.170.
[45] M. Niimi, T. Ogawara, T. Yamashita, Y. Yamamoto, A. Ueyama, T. Kambe, T. Okamoto, T. Ban, H. Tamanoi, K. Ozaki, T. Fujiwara, H. Fukui, E. Takahashi, H. Kyushiki, A. Tanigami. *J. Hum. Genet.* **46**, 566 (2001), https://doi.org/10.1007/s100380170022.
[46] J. E. DeHaven, K. A. Robinson, B. A. Nelson, M. G. Buse. *Diabetes* **50**, 2419 (2001), https://doi.org/10.2337/diabetes.50.11.2419.
[47] K. Liu, G. Wang, S. H. Zhao, B. Liu, J. N. Huang, X. Bai, M. Yu. *Mol. Biol. Rep.* **37**, 2711 (2010), https://doi.org/10.1007/s11033-009-9805-y.
[48] S. Wei, R. Xu, Y. Ji, Z. Ding, Y. Zou. *Genes Genomics* **44**, 175 (2022), https://doi.org/10.1007/s13258-021-01188-8.
[49] J. A. Plumbridge, O. Cochet, J. M. Souza, M. M. Altamirano, M. L. Calcagno, B. Badet. *J. Bacteriol.* **175**, 4951 (1993), https://doi.org/10.1128/jb.175.16.4951-4956.1993.
[50] D. C. Alexander, M. A. Valvano. *J. Bacteriol.* **176**, 7079 (1994), https://doi.org/10.1128/jb.176.22.7079-7084.1994.
[51] J. Coley, M. Duckworth, J. Baddiley. *Microbiology (N Y)* **73**, 587 (1972), https://doi.org/10.1099/00221287-73-3-587.
[52] H. Komatsuzawa, T. Fujiwara, H. Nishi, S. Yamada, M. Ohara, N. McCallum, B. Berger-Bächi, M. Sugai. *Mol. Microbiol.* **53**, 1221 (2004), https://doi.org/10.1111/j.1365-2958.2004.04200.x.
[53] J. A. Wyllie, M. V. McKay, A. S. Barrow, T. P. Soares da Costa. *IUBMB Life* **74**, 1232 (2022), https://doi.org/10.1002/iub.2664.
[54] G. Wu, Y. Sun, W. Qu, Y. Huang, L. Lu, L. Li, W. Shao. *PLoS One* **6**, e17082 (2011), https://doi.org/10.1371/journal.pone.0017082.
[55] A. M. Bennett, D. C. Shippy, N. Eakley, O. Okwumabua, A. A. Fadl. *Arch. Microbiol.* **198**, 541 (2016), https://doi.org/10.1007/s00203-016-1212-x.
[56] M. Kawada-Matsuo, Y. Mazda, Y. Oogai, M. Kajiya, T. Kawai, S. Yamada, S. Miyawaki, T. Oho, H. Komatsuzawa. *PLoS One* **7**, e33382 (2012), https://doi.org/10.1371/journal.pone.0033382.
[57] R. Garcia-Rubio, H. C. de Oliveira, J. Rivera, N. Trevijano-Contador. *Front. Microbiol.* **10**, 2993 (2020), https://doi.org/10.3389/fmicb.2019.02993.
[58] F. M. Klis. *Yeast* **10**, 851 (1994), https://doi.org/10.1002/yea.320100702.
[59] F. M. Klis, P. Mol, K. Hellingwerf, S. Brul. *FEMS Microbiol. Rev.* **26**, 239 (2002), https://doi.org/10.1111/j.1574-6976.2002.tb00613.x.
[60] H. de Nobel, H. van den Ende, F. M. Klis. *Trends Microbiol*. **8**, 344 (2000), https://doi.org/10.1016/s0966-842x(00)01805-9.
[61] J. A. Ortiz-Ramírez, M. Cuéllar-Cruz, E. López-Romero. *Front. Cell Infect. Microbiol.* **12**, 976924 (2022), https://doi.org/10.3389/fcimb.2022.976924.
[62] G. Watzele, W. Tanner. *J. Biol. Chem.* **264**, 8753 (1989), https://doi.org/10.1016/s0021-9258(18)81857-9.
[63] A. Lagorce, V. Le Berre-Anton, B. Aguilar-Uscanga, H. Martin-Yken, A. Dagkessamanskaia, J. François. *Eur. J. Biochem.* **269**, 1697 (2002), https://doi.org/10.1046/j.1432-1327.2002.02814.x.
[64] A. Lagorce, N. C. Hauser, D. Labourdette, C. Rodriguez, H. Martin-Yken, J. Arroyo, J. D. Hoheisel, J. François. *J. Biol. Chem.* **278**, 20345 (2003), https://doi.org/10.1074/jbc.m211604200.
[65] R. García, C. Bermejo, C. Grau, R. Pérez, J. M. Rodríguez-Peña, J. Francois, C. Nombela, J. Arroyo. *J. Biol. Chem.* **279**, 15183 (2004), https://doi.org/10.1074/jbc.m312954200.
[66] A. Naganuma, N. Miura, S. Kaneko, T. Mishina, S. Hosoya, S. Miyairi, T. Furuchi, S. Kuge. *FASEB J*. **14**, 968 (2000), https://doi.org/10.1096/fasebj.14.7.968.
[67] A. F. J. Ram, M. Arentshorst, R. A. Damveld, P. A. vanKuyk, F. M. Klis, C. A. M. J. J. van den Hondel. *Microbiology (N Y)* **150**, 3315 (2004).
[68] H.-S. Park, Y. Man Yu, M.-K. Lee, P. Jae Maeng, S. Chang Kim, J.-H. Yu. *Sci. Rep.* **5**, 10199 (2015), https://doi.org/10.1038/srep10199.
[69] E. Akira, K. Kazuo, M. Tomomasa. *J. Bacteriol.* **103**, 588 (1970), https://doi.org/10.1128/jb.103.3.588-594.1970.
[70] C. Luo, W. Shao, X. Li, Z. Chen, Y. Liu. *Protein J*. **28**, 34 (2009), https://doi.org/10.1007/s10930-009-9163-6.
[71] B. Cifuentes, C. Vicente. *Z. Naturforsch. C. Biosci.* **37**, 381 (1982), https://doi.org/10.1515/znc-1982-5-606.
[72] J. C. D. C. Maia. *FASEB J*. **8**, 848 (1994), https://doi.org/10.1096/fasebj.8.11.8070634.

[73] P. S. Frisa, D. R. Sonneborn. *Proc. Natl. Acad. Sci. U.S.A.* **79**, 6289 (1982), https://doi.org/10.1073/pnas.79.20.6289.
[74] L. C. Etchebehere, J. da Costa Maia. *Arch. Biochem. Biophys.* **272**, 301 (1989), https://doi.org/10.1016/0003-9861(89)90223-3.
[75] C. A. Munro, D. A. Schofield, G. W. Gooday, N. A. R. Gow. *Microbiology (N Y)* **144**, 391 (1998), https://doi.org/10.1099/00221287-144-2-391.
[76] Y. Y. Chiew, M. G. Shepherd, P. A. Sullivan. *Arch. Microbiol.* **125**, 97 (1980), https://doi.org/10.1007/bf00403204.
[77] A. M. Janiak, S. Milewski. *Med. Mycol.* **39**, 401 (2001), https://doi.org/10.1080/mmy.39.5.401.408.
[78] I. Gabriel, J. Olchowy, A. Stanisławska-Sachadyn, T. Mio, J. Kur, S. Milewski. *FEMS Microbiol. Lett.* **235**, 73 (2004), https://doi.org/10.1111/j.1574-6968.2004.tb09569.x.
[79] J. F. Sánchez-López, J. González-Ibarra, J. I. Macías-Segoviano, M. Cuéllar-Cruz, A. Álvarez-Vargas, C. Cano-Canchola, E. López-Romero. *Arch. Microbiol.* **201**, 135 (2019), https://doi.org/10.1007/s00203-018-1576-1.
[80] J. A. Ortiz-Ramírez, M. Cuéllar-Cruz, E. López-Romero. *Antonie Van Leeuwenhoek* **114**, 609 (2021), https://doi.org/10.1007/s10482-021-01545-3.
[81] J. Stefaniak, M. G. Nowak, M. Wojciechowski, S. Milewski, A. S. Skwarecki. *J. Enzyme Inhib. Med. Chem.* **37**, 1928 (2022), https://doi.org/10.1080/14756366.2022.2096018.
[82] Y. Liu, D. X. Cai, L. Wang, J. Z. Li, W. N. Wang. *Ecotoxicology* **24**, 1754 (2015), https://doi.org/10.1007/s10646-015-1480-2.
[83] C.-D. Xu, Y.-K. Liu, L.-Y. Qiu, S.-S. Wang, B.-Y. Pan, Y. Li, S.-G. Wang, B. Tang. *Sci. Rep.* **11**, 5246 (2021), https://doi.org/10.1038/s41598-021-84760-2.
[84] X. Huang, N. Tsuji, T. Miyoshi, M. Motobu, M. K. Islam, M. A. Alim, K. Fujisaki. *Int. J. Parasitol.* **37**, 383 (2007), https://doi.org/10.1016/j.ijpara.2006.11.012.
[85] N. Kato, C. R. Mueller, J. F. Fuchs, V. Wessely, Q. Lan, B. M. Christensen. *Insect Biochem. Mol. Biol.* **36**, 1 (2006), https://doi.org/10.1016/j.ibmb.2005.09.003.
[86] X.-Y. Liu, S.-S. Wang, F. Zhong, M. Zhou, X.-Y. Jiang, Y.-S. Cheng, Y.-H. Dan, G. Hu, C. Li, B. Tang, Y. Wu. *Front. Physiol.* **13**, 1034926 (2022), https://doi.org/10.3389/fphys.2022.1034926.
[87] S. Cotsworth, C. J. Jackson, G. Hallson, K. A. Fitzpatrick, M. Syrzycka, A. B. Coulthard, A. Bejsovec, M. Marchetti, S. Pimpinelli, S. J. H. Wang, R. G. Camfield, E. M. Verheyen, D. A. Sinclair, B. M. Honda, A. J. Hilliker. *Cells* **11**, 448 (2022), https://doi.org/10.3390/cells11030448.
[88] H.-R. Graack, U. Cinque, H. Kress. *Biochem. J.* **360**, 401 (2001), https://doi.org/10.1042/bj3600401.
[89] W. Z. Hassid, E. F. Neufeld, D. S. Feingold. *Proc. Natl. Acad. Sci. U.S.A.* **45**, 905 (1959), https://doi.org/10.1073/pnas.45.7.905.
[90] K. Van Vu, C. Y. Jeong, T. T. Nguyen, T. T. H. Dinh, H. Lee, S.-W. Hong. *J. Exp. Bot.* **70**, 1775 (2019), https://doi.org/10.1093/jxb/erz055.
[91] H. Riegler, T. Herter, I. Grishkovskaya, A. Lude, M. Ryngajllo, M. E. Bolger, B. Essigmann, B. Usadel. *Biochem. J.* **443**, 427 (2012), https://doi.org/10.1042/bj20112071.
[92] M. S. Denzel, N. J. Storm, A. Gutschmidt, R. Baddi, Y. Hinze, E. Jarosch, T. Sommer, T. Hoppe, A. Antebi. *Cell* **156**, 1167 (2014), https://doi.org/10.1016/j.cell.2014.01.061.
[93] M. Horn, S. I. Denzel, B. Srinivasan, K. Allmeroth, I. Schiffer, V. Karthikaisamy, S. Miethe, P. Breuer, A. Antebi, M. S. Denzel. *IScience* **23**, 100887 (2020), https://doi.org/10.1016/j.isci.2020.100887.
[94] B. Manzari, J. E. Kudlow, P. Fardin, E. Merello, C. Ottaviano, M. Puppo, A. Eva, L. Varesio. *Int. J. Immunopathol. Pharmacol.* **20**, 47 (2007), https://doi.org/10.1177/039463200702000106.
[95] P. P. Sayeski, D. Wang, K. Su, I.-O. Han, J. E. Kudlow. *Nucleic Acids Res*. **25**, 1458 (1997), https://doi.org/10.1093/nar/25.7.1458.
[96] C. Chaveroux, C. Sarcinelli, V. Barbet, S. Belfeki, A. Barthelaix, C. Ferraro-Peyret, S. Lebecque, T. Renno, A. Bruhat, P. Fafournoux, S. N. Manié. *Sci. Rep.* **6**, 27278 (2016), https://doi.org/10.1038/srep27278.
[97] W. Dai, S. K. Dierschke, A. L. Toro, M. D. Dennis. *Biochim. Biophys. Acta* **1864**, 3568 (2018), https://doi.org/10.1016/j.bbadis.2018.09.006.
[98] S. J. Szymura, J. P. Zaemes, D. F. Allison, S. H. Clift, J. M. D'Innocenzi, L. G. Gray, B. D. McKenna, B. B. Morris, S. Bekiranov, R. D. LeGallo, D. R. Jones, M. W. Mayo. *Cell Commun. Signal.* **17**, 24 (2019), https://doi.org/10.1186/s12964-019-0335-5.
[99] H. Al-Mukh, L. Baudoin, A. Bouaboud, J.-L. Sanchez-Salgado, N. Maraqa, M. Khair, P. Pagesy, G. Bismuth, F. Niedergang, T. Issad. *J. Immunol.* **205**, 2499 (2020), https://doi.org/10.4049/jimmunol.2000345.
[100] Z. V. Wang, Y. Deng, N. Gao, Z. Pedrozo, D. L. Li, C. R. Morales, A. Criollo, X. Luo, W. Tan, N. Jiang, M. A. Lehrman, B. A. Rothermel, A.-H. Lee, S. Lavandero, P. P. A. Mammen, A. Ferdous, T. G. Gillette, P. E. Scherer, J. A. Hill. *Cell* **156**, 1179 (2014), https://doi.org/10.1016/j.cell.2014.01.014.
[101] Q. Chang, K. Su, J. R. Baker, X. Yang, A. J. Paterson, J. E. Kudlow. *J. Biol. Chem.* **275**, 21981 (2000), https://doi.org/10.1074/jbc.m001049200.
[102] Y. Hu, L. Riesland, A. J. Paterson, J. E. Kudlow. *J. Biol. Chem.* **279**, 29988 (2004), https://doi.org/10.1074/jbc.m401547200.
[103] S. Ruegenberg, F. A. M. C. Mayr, I. Atanassov, U. Baumann, M. S. Denzel. *Nat. Commun.* **12**, 2176 (2021), https://doi.org/10.1038/s41467-021-22320-y.
[104] S. Eguchi, N. Oshiro, T. Miyamoto, K. Yoshino, S. Okamoto, T. Ono, U. Kikkawa, K. Yonezawa. *Genes Cells* **14**, 179 (2009), https://doi.org/10.1111/j.1365-2443.2008.01260.x.
[105] D. Zibrova, F. Vandermoere, O. Göransson, M. Peggie, K. V. Mariño, A. Knierim, K. Spengler, C. Weigert, B. Viollet, N. A. Morrice, K. Sakamoto, R. Heller. *Biochem. J.* **474**, 983 (2017), https://doi.org/10.1042/bcj20160980.
[106] J. G. Moloughney, P. K. Kim, N. M. Vega-Cotto, C.-C. Wu, S. Zhang, M. Adlam, T. Lynch, P.-C. Chou, J. D. Rabinowitz, G. Werlen, E. Jacinto. *Mol. Cell* **63**, 811 (2016), https://doi.org/10.1016/j.molcel.2016.07.015.
[107] J. G. Moloughney, N. M. Vega-Cotto, S. Liu, C. Patel, P. K. Kim, C. Wu, D. Albaciete, C. Magaway, A. Chang, S. Rajput, X. Su, G. Werlen, E. Jacinto. *J. Biol. Chem.* **293**, 16464 (2018), https://doi.org/10.1074/jbc.ra118.003991.

[108] S. Wei, Q. Zhao, K. Zheng, P. Liu, N. Sha, Y. Li, C. Ma, J. Li, L. Zhuo, G. Liu, W. Liang, Y. Jiang, T. Chen, N. Zhong. *Cell Discov.* **8**, 77 (2022), https://doi.org/10.1038/s41421-022-00423-0.
[109] V. Kroef, S. Ruegenberg, M. Horn, K. Allmeroth, L. Ebert, S. Bozkus, S. Miethe, U. Elling, B. Schermer, U. Baumann, M. S. Denzel. *Elife* **11**, e69223 (2022).
[110] I. A. Oliveira, D. Allonso, T. V. A. Fernandes, D. M. S. Lucena, G. T. Ventura, W. B. Dias, R. S. Mohana-Borges, P. G. Pascutti, A. R. Todeschini. *J. Biol. Chem.* **296**, 100180 (2021), https://doi.org/10.1074/jbc.ra120.015189.
[111] B. Maffei, M. Laverrière, Y. Wu, S. Triboulet, S. Perrinet, M. Duchateau, M. Matondo, R. L. Hollis, C. Gourley, J. Rupp, J. W. Keillor, A. Subtil. *EMBO J.* **39**, e102166 (2020).
[112] R. R. Traxinger, S. Marshall. *J. Biol. Chem.* **266**, 10148 (1991), https://doi.org/10.1016/s0021-9258(18)99202-1.
[113] S. Marshall, V. Bacote, R. R. Traxinger. *J. Biol. Chem.* **266**, 10155 (1991), https://doi.org/10.1016/s0021-9258(18)99203-3.
[114] S. Marshall, V. Bacote, R. R. Traxinger. *J. Biol. Chem.* **266**, 4706 (1991), https://doi.org/10.1016/s0021-9258(19)67706-9.
[115] M. C. Daniels, T. P. Ciaraldi, S. Nikoulina, R. R. Henry, D. A. McClain. *J. Clin. Invest.* **97**, 1235 (1996), https://doi.org/10.1172/jci118538.
[116] H. Kaneto, G. Xu, K.-H. Song, K. Suzuma, S. Bonner-Weir, A. Sharma, G. C. Weir. *J. Biol. Chem.* **276**, 31099 (2001), https://doi.org/10.1074/jbc.m104115200.
[117] J. Tang, J. L. Neidigh, R. C. Cooksey, D. A. McClain. *Diabetes* **49**, 1492 (2000), https://doi.org/10.2337/diabetes.49.9.1492.
[118] L. F. Hebert, Jr, M. C. Daniels, J. Zhou, E. D. Crook, R. L. Turner, S. T. Simmons, J. L. Neidigh, J. S. Zhu, A. D. Baron, D. A. McClain. *J. Clin. Invest.* **98**, 930 (1996), https://doi.org/10.1172/jci118876.
[119] H. Chen, B. L. Ing, K. A. Robinson, A. C. Feagin, M. G. Buse, M. J. Quon. *Mol. Cell. Endocrinol.* **135**, 67 (1997), https://doi.org/10.1016/s0303-7207(97)00191-3.
[120] A. T. Sage, L. A. Walter, Y. Shi, M. I. Khan, H. Kaneto, A. Capretta, G. H. Werstuck. *Am. J. Physiol. Endocrinol. Metab.* **298**, E499 (2009), https://doi.org/10.1152/ajpendo.00507.2009.
[121] R. C. Cooksey, D. A. McClain. *Ann. N. Y. Acad. Sci.* **967**, 102 (2002), https://doi.org/10.1111/j.1749-6632.2002.tb04268.x.
[122] H. Zhang, Y. Jia, J. J. Cooper, T. Hale, Z. Zhang, S. C. Elbein. *J. Clin. Endocrinol. Metab.* **89**, 748 (2004), https://doi.org/10.1210/jc.2003-031286.
[123] D. P. K. Ng, W. H. Walker, K.-S. Chia, S. Choo, J. H. Warram, A. S. Krolewski. *Diabetes* **53**, 865 (2004), https://doi.org/10.2337/diabetes.53.3.865.
[124] C. Weigert, C. Thamer, K. Brodbeck, A. Guirguis, F. Machicao, J. Machann, F. Schick, M. Stumvoll, A. Fritsche, H. U. Häring, E. D. Schleicher. *J. Clin. Endocrinol. Metab.* **90**, 1639 (2005), https://doi.org/10.1210/jc.2004-0058.
[125] L. R. James, I. G. Fantus, H. Goldberg, H. Ly, J. W. Scholey. *Am. J. Physiol. Renal Physiol.* **279**, F718 (2000), https://doi.org/10.1152/ajprenal.2000.279.4.f718.
[126] E. D. Schleicher, C. Weigert. *Kidney Int*. **58**, S13 (2000), https://doi.org/10.1046/j.1523-1755.2000.07703.x.
[127] T.-J. Hsieh, T. Lin, P.-C. Hsieh, M.-C. Liao, S.-J. Shin. *J. Cell. Physiol.* **227**, 108 (2012), https://doi.org/10.1002/jcp.22707.
[128] D. A. McClain. *J. Diabetes Complications* **16**, 72 (2002), https://doi.org/10.1016/s1056-8727(01)00188-x.
[129] P. Raman, I. Krukovets, T. E. Marinic, P. Bornstein, O. I. Stenina. *J. Biol. Chem.* **282**, 5704 (2007), https://doi.org/10.1074/jbc.m610965200.
[130] A. Vasconcelos-dos-Santos, H. F. B. R. Loponte, N. R. Mantuano, I. A. Oliveira, I. F. de Paula, L. K. Teixeira, J. C. M. de-Freitas-Junior, K. C. Gondim, N. Heise, R. Mohana-Borges, J. A. Morgado-Díaz, W. B. Dias, A. R. Todeschini. *Oncogenesis* **6**, e306 (2017), https://doi.org/10.1038/oncsis.2017.2.
[131] C. Chokchaitaweesuk, T. Kobayashi, T. Izumikawa, N. Itano. *Cell Death Dis.* **10**, 803 (2019), https://doi.org/10.1038/s41419-019-2034-y.
[132] W. Chen, K. C. Do, B. Saxton, S. Leng, P. Filipczak, M. Tessema, S. A. Belinsky, Y. Lin. *Mol. Carcinog.* **58**, 1046 (2019), https://doi.org/10.1002/mc.22992.
[133] T. Dong, X. Kang, Z. Liu, S. Zhao, W. Ma, Q. Xuan, H. Liu, Z. Wang, Q. Zhang. *Tumor Biol.* **37**, 8159 (2016), https://doi.org/10.1007/s13277-015-4729-8.
[134] S. Ren, Y. Shao, X. Zhao, C. S. Hong, F. Wang, X. Lu, J. Li, G. Ye, M. Yan, Z. Zhuang, C. Xu, G. Xu, Y. Sun. *Mol. Cell. Proteom.* **15**, 154 (2016), https://doi.org/10.1074/mcp.m115.052381.
[135] H. Ying, A. C. Kimmelman, C. A. Lyssiotis, S. Hua, G. C. Chu, E. Fletcher-Sananikone, J. W. Locasale, J. Son, H. Zhang, J. L. Coloff, H. Yan, W. Wang, S. Chen, A. Viale, H. Zheng, J. Paik, C. Lim, A. R. Guimaraes, E. S. Martin, J. Chang, A. F. Hezel, S. R. Perry, J. Hu, B. Gan, Y. Xiao, J. M. Asara, R. Weissleder, Y. A. Wang, L. Chin, L. C. Cantley, R. A. DePinho. *Cell* **149**, 656 (2012), https://doi.org/10.1016/j.cell.2012.01.058.
[136] F. Guillaumond, J. Leca, O. Olivares, M.-N. Lavaut, N. Vidal, P. Berthezène, N. J. Dusetti, C. Loncle, E. Calvo, O. Turrini, J. L. Iovanna, R. Tomasini, S. Vasseur. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 3919 (2013), https://doi.org/10.1073/pnas.1219555110.
[137] J. Kim, H. M. Lee, F. Cai, B. Ko, C. Yang, E. L. Lieu, N. Muhammad, S. Rhyne, K. Li, M. Haloul, W. Gu, B. Faubert, A. K. Kaushik, L. Cai, S. Kasiri, U. Marriam, K. Nham, L. Girard, H. Wang, X. Sun, J. Kim, J. D. Minna, K. Unsal-Kacmaz, R. J. DeBerardinis. *Nat. Metab.* **2**, 1401 (2020), https://doi.org/10.1038/s42255-020-00316-0.
[138] W. Zhang, G. Bouchard, A. Yu, M. Shafiq, M. Jamali, J. B. Shrager, K. Ayers, S. Bakr, A. J. Gentles, M. Diehn, A. Quon, R. B. West, V. Nair, M. van de Rijn, S. Napel, S. K. Plevritis. *Cancer Res*. **78**, 3445 (2018), https://doi.org/10.1158/0008-5472.can-17-2928.
[139] L. Liu, Y. Pan, X. Ren, Z. Zeng, J. Sun, K. Zhou, Y. Liang, F. Wang, Y. Yan, W. Liao, Y. Ding, X. Liu, L. Liang. *Am. J. Cancer Res.* **10**, 2510 (2020).
[140] D. Hanahan, R. A. Weinberg. *Cell* **144**, 646 (2011), https://doi.org/10.1016/j.cell.2011.02.013.
[141] F. Alisson-Silva, L. Freire-de-Lima, J. L. Donadio, M. C. Lucena, L. Penha, J. N. Sá-Diniz, W. B. Dias, A. R. Todeschini. *PLoS One* **8**, e60471 (2013), https://doi.org/10.1371/journal.pone.0060471.

[142] M. C. Lucena, P. Carvalho-Cruz, J. L. Donadio, I. A. Oliveira, R. M. de Queiroz, M. M. Marinho-Carvalho, M. Sola-Penna, I. F. de Paula, K. C. Gondim, M. E. McComb, C. E. Costello, S. A. Whelan, A. R. Todeschini, W. B. Dias. *J. Biol. Chem.* **291**, 12917 (2016), https://doi.org/10.1074/jbc.m116.729236.
[143] Y.-Y. Liu, H.-Y. Liu, T.-J. Yu, Q. Lu, F.-L. Zhang, G.-Y. Liu, Z.-M. Shao, D.-Q. Li. *Cell Death Differ*. **29**, 861 (2022), https://doi.org/10.1038/s41418-021-00901-0.
[144] S. Oikari, K. Makkonen, A. J. Deen, I. Tyni, R. Kärnä, R. H. Tammi, M. I. Tammi. *Glycobiology* **26**, 710 (2016), https://doi.org/10.1093/glycob/cww019.
[145] D. H. Tran, H. I. May, Q. Li, X. Luo, J. Huang, G. Zhang, E. Niewold, X. Wang, T. G. Gillette, Y. Deng, Z. V. Wang. *Nat. Commun.* **11**, 1771 (2020), https://doi.org/10.1038/s41467-020-15640-y.
[146] A. Ishikita, S. Matsushima, S. Ikeda, K. Okabe, R. Nishimura, T. Tadokoro, N. Enzan, T. Yamamoto, M. Sada, Y. Tsutsui, R. Miyake, M. Ikeda, T. Ide, S. Kinugawa, H. Tsutsui. *IScience* **24**, 103517 (2021), https://doi.org/10.1016/j.isci.2021.103517.
[147] J. Senderek, J. S. Müller, M. Dusl, T. M. Strom, V. Guergueltcheva, I. Diepolder, S. H. Laval, S. Maxwell, J. Cossins, S. Krause, N. Muelas, J. J. Vilchez, J. Colomer, C. J. Mallebrera, A. Nascimento, S. Nafissi, A. Kariminejad, Y. Nilipour, B. Bozorgmehr, H. Najmabadi, C. Rodolico, J. P. Sieb, O. K. Steinlein, B. Schlotter, B. Schoser, J. Kirschner, R. Herrmann, T. Voit, A. Oldfors, C. Lindbergh, A. Urtizberea, M. von der Hagen, A. Hübner, J. Palace, K. Bushby, V. Straub, D. Beeson, A. Abicht, H. Lochmüller. *Am. J. Hum. Genet.* **88**, 162 (2011), https://doi.org/10.1016/j.ajhg.2011.01.008.
[148] S.-Y. Huh, H.-S. Kim, H.-J. Jang, Y.-E. Park, D.-S. Kim. *Muscle Nerve* **46**, 600 (2012), https://doi.org/10.1002/mus.23451.
[149] D. Selcen, X.-M. Shen, M. Milone, J. Brengman, K. Ohno, F. Deymeer, R. Finkel, J. Rowin, A. G. Engel. *Neurology* **81**, 370 (2013), https://doi.org/10.1212/wnl.0b013e31829c5e9c.
[150] V. Guergueltcheva, J. S. Müller, M. Dusl, J. Senderek, A. Oldfors, C. Lindbergh, S. Maxwell, J. Colomer, C. J. Mallebrera, A. Nascimento, J. J. Vilchez, N. Muelas, J. Kirschner, S. Nafissi, A. Kariminejad, Y. Nilipour, B. Bozorgmehr, H. Najmabadi, C. Rodolico, J. P. Sieb, B. Schlotter, B. Schoser, R. Herrmann, T. Voit, O. K. Steinlein, A. Najafi, A. Urtizberea, D. M. Soler, F. Muntoni, M. G. Hanna, A. Chaouch, V. Straub, K. Bushby, J. Palace, D. Beeson, A. Abicht, H. Lochmüller. *J. Neurol.* **259**, 838 (2012), https://doi.org/10.1007/s00415-011-6262-z.
[151] R. A. Maselli, J. Arredondo, J. Nguyen, M. Lara, F. Ng, M. Ngo, J. M. Pham, Q. Yi, J. M. Stajich, K. McDonald, M. A. Hauser, R. L. Wollmann. *Clin. Genet.* **85**, 166 (2014), https://doi.org/10.1111/cge.12118.
[152] S. Bauché, G. Vellieux, D. Sternberg, M.-J. Fontenille, E. De Bruyckere, C.-S. Davoine, G. Brochier, J. Messéant, L. Wolf, M. Fardeau, E. Lacène, N. Romero, J. Koenig, E. Fournier, D. Hantaï, N. Streichenberger, V. Manel, A. Lacour, A. Nadaj-Pakleza, S. Sukno, F. Bouhour, P. Laforêt, B. Fontaine, L. Strochlic, B. Eymard, F. Chevessier, T. Stojkovic, S. Nicole. *J. Neurol.* **264**, 1791 (2017), https://doi.org/10.1007/s00415-017-8569-x.
[153] Y. Issop, D. Hathazi, M. M. Khan, R. Rudolf, J. Weis, S. Spendiff, C. R. Slater, A. Roos, H. Lochmüller. *Hum. Mol. Genet.* **27**, 3218 (2018), https://doi.org/10.1093/hmg/ddy225.
[154] J. Ma, C. Wu, G. W. Hart. *Chem. Rev.* **121**, 1513 (2021), https://doi.org/10.1021/acs.chemrev.0c00884.
[155] X. Liu, I. Blaženović, A. J. Contreras, T. M. Pham, C. A. Tabuloc, Y. H. Li, J. Ji, O. Fiehn, J. C. Chiu. *Nat. Commun.* **12**, 4173 (2021), https://doi.org/10.1038/s41467-021-24301-7.
[156] O. Lagerlöf. *J. Bioenerg. Biomembr.* **50**, 241 (2018), https://doi.org/10.1007/s10863-018-9760-1.
[157] N. Rodrigues Mantuano, M. A. Stanczak, I. de A. Oliveira, N. Kirchhammer, A. A. Filardy, G. Monaco, R. C. Santos, A. C. Fonseca, M. Fontes, C. de S. Bastos, W. B. Dias, A. Zippelius, A. R. Todeschini, H. Läubli. *Cancer Immunol. Res.* **8**, 1262 (2020), https://doi.org/10.1158/2326-6066.cir-19-0904.
[158] N. R. Mantuano, M. C. Oliveira-Nunes, F. Alisson-Silva, W. B. Dias, A. R. Todeschini. *Pharmacol. Res.* **146**, 104285 (2019), https://doi.org/10.1016/j.phrs.2019.104285.
[159] J. C. Chatham, J. Zhang, A. R. Wende. *Physiol. Rev.* **101**, 427 (2021), https://doi.org/10.1152/physrev.00043.2019.
[160] R. Gauttam, C. K. Desiderato, D. Radoš, H. Link, G. M. Seibold, B. J. Eikmanns. *Front. Bioeng. Biotechnol.* **9**, 748510 (2021), https://doi.org/10.3389/fbioe.2021.748510.
[161] T. Niu, Y. Liu, J. Li, M. Koffas, G. Du, H. S. Alper, L. Liu. *ACS Synth. Biol.* **7**, 2423 (2018), https://doi.org/10.1021/acssynbio.8b00196.
[162] K. Wang, X. Wang, H. Luo, Y. Wang, Y. Wang, T. Tu, X. Qin, Y. Bai, H. Huang, B. Yao, X. Su, J. Zhang. *Int. J. Mol. Sci.* **23**, 773 (2022), https://doi.org/10.3390/ijms23020773.
[163] C. Liu, X. Lv, J. Li, L. Liu, G. Du, Y. Liu. *J. Agric. Food Chem.* **70**, 15859 (2022), https://doi.org/10.1021/acs.jafc.2c05994.
[164] A. Vasconcelos-dos-Santos, R. M. de Queiroz, B. da Costa Rodrigues, A. R. Todeschini, W. B. Dias. *J. Bioenerg. Biomembr.* **50**, 175 (2018), https://doi.org/10.1007/s10863-017-9740-x.
[165] K.-C. Chou. *J. Proteome Res.* **3**, 1284 (2004), https://doi.org/10.1021/pr049849v.
[166] A. Melcer, I. Łącka, I. Gabriel, M. Wojciechowski, B. Liberek, A. Wiśniewski, S. Milewski. *Bioorg. Med. Chem. Lett*. **17**, 6602 (2007), https://doi.org/10.1016/j.bmcl.2007.09.072.
[167] C. J. Bates, R. E. Handschumacher. *Adv. Enzyme Regul.* **7**, 183 (1969), https://doi.org/10.1016/0065-2571(69)90018-1.
[168] H. Chmara, R. Andruszkiewicz, E. Borowski. *Biochim. Biophys. Acta* **870**, 357 (1986), https://doi.org/10.1016/0167-4838(86)90240-2.
[169] S. Milewski, H. Chmara, R. Andruszkiewicz, E. Borowski. *Biochim. Biophys. Acta* **1115**, 225 (1992), https://doi.org/10.1016/0304-4165(92)90058-3.
[170] B. Walker, M. F. Brown, J. F. Lynas, S. L. Martin, A. McDowell, B. Badet, A. J. Hill. *Bioorg. Med. Chem. Lett.* **10**, 2795 (2000), https://doi.org/10.1016/s0960-894x(00)00565-5.
[171] K. M. Lemberg, J. J. Vornov, R. Rais, B. S. Slusher. *Mol. Cancer Ther.* **17**, 1824 (2018), https://doi.org/10.1158/1535-7163.mct-17-1148.

[172] B. A. Riggle, S. Sinharay, W. Schreiber-Stainthorp, J. P. Munasinghe, D. Maric, E. Prchalova, B. S. Slusher, J. D. Powell, L. H. Miller, S. K. Pierce, D. A. Hammoud. *Proc. Natl. Acad. Sci. U.S.A.* **115**, E12024 (2018).
[173] R. Rais, A. Jančařík, L. Tenora, M. Nedelcovych, J. Alt, J. Englert, C. Rojas, A. Le, A. Elgogary, J. Tan, L. Monincová, K. Pate, R. Adams, D. Ferraris, J. Powell, P. Majer, B. S. Slusher. *J. Med. Chem.* **59**, 8621 (2016), https://doi.org/10.1021/acs.jmedchem.6b01069.
[174] J. J. Harding, M. L. Telli, P. N. Munster, M. H. Le, C. Molineaux, M. K. Bennett, E. Mittra, H. A. Burris, A. S. Clark, M. Dunphy, F. Meric-Bernstam, M. R. Patel, A. DeMichele, J. R. Infante. *J. Clin. Oncol.* **33**, 2512 (2015), https://doi.org/10.1200/jco.2015.33.15_suppl.2512.
[175] Y. Zhang, J. Li, Y. Huang, Y. Chen, Z. Luo, H. Huang, R. E. West, T. D. Nolin, Z. Wang, S. Li. *Theranostics* **13**, 578 (2023), https://doi.org/10.7150/thno.76614.
[176] H. Chmara, H. Zähner, E. Borowski, S. Milewski. *J. Antibiot. (Tokyo)* **37**, 652 (1984), https://doi.org/10.7164/antibiotics.37.652.
[177] B. Badet, P. Vermoote, F. Le Goffic. *Biochemistry* **27**, 2282 (1988), https://doi.org/10.1021/bi00407a006.
[178] M. Wojciechowski, J. Mazerski, E. Borowski. *J. Enzyme. Inhib.* **10**, 17 (1995), https://doi.org/10.3109/14756369509021468.
[179] D. Pawlak, M. Stolarska, M. Wojciechowski, R. Andruszkiewicz. *Eur. J. Med. Chem.* **90**, 577 (2015), https://doi.org/10.1016/j.ejmech.2014.12.007.
[180] D. Pawlak, M. Schielmann, M. Wojciechowski, R. Andruszkiewicz. *Bioorg. Med. Chem. Lett.* **26**, 3586 (2016), https://doi.org/10.1016/j.bmcl.2016.06.016.
[181] A. Kertmen, Ł. Przysiecka, E. Coy, Ł. Popenda, R. Andruszkiewicz, S. Jurga, S. Milewski. *Langmuir* **35**, 5281 (2019), https://doi.org/10.1021/acs.langmuir.8b04250.
[182] M. Stolarska, K. Gucwa, Z. Urbańczyk-Lipkowska, R. Andruszkiewicz. *J. Pept. Sci.* **26**, e3226 (2020).
[183] M. Bontemps-Gracz, S. Milewski, E. Borowski. *Acta Biochim. Pol.* **38**, 229 (1991).
[184] D. Zgódka, S. Milewski, E. Borowski. *Microbiology (N Y)* **147**, 1955 (2001), https://doi.org/10.1099/00221287-147-7-1955.
[185] A. Tourian, M. Callahan, W. Hung. *Neurochem. Res.* **8**, 1589 (1983), https://doi.org/10.1007/bf00964160.
[186] K. Skarbek, I. Gabriel, P. Szweda, M. Wojciechowski, M. A. Khan, B. Görke, S. Milewski, M. J. Milewska. *Carbohydr. Res.* **448**, 79 (2017), https://doi.org/10.1016/j.carres.2017.06.002.
[187] S. L. Bearne. *J. Biol. Chem.* **271**, 3052 (1996), https://doi.org/10.1016/s0021-9258(18)97977-9.
[188] A. M. Janiak, M. Hoffmann, M. J. Milewska, S. Milewski. *Bioorg. Med. Chem.* **11**, 1653 (2003), https://doi.org/10.1016/s0968-0896(03)00049-x.
[189] A. M. Omar, S. Ihmaid, E.-S. E. Habib, S. S. Althagfan, S. Ahmed, H. S. Abulkhair, H. E. A. Ahmed. *Bioorg. Chem.* **99**, 103781 (2020), https://doi.org/10.1016/j.bioorg.2020.103781.
[190] G. Zong, H. Zhao, R. Jiang, J. Zhang, X. Liang, B. Li, Y. Shi, D. Wang. *Molecules* **19**, 7832 (2014), https://doi.org/10.3390/molecules19067832.
[191] B. Hu, H. Zhao, Z. Chen, C. Xu, J. Zhao, W. Zhao. *Molecules* **23**, 709 (2018), https://doi.org/10.3390/molecules23040709.
[192] Y. Qian, M. Ahmad, S. Chen, P. Gillespie, N. Le, F. Mennona, S. Mischke, S.-S. So, H. Wang, C. Burghardt, S. Tannu, K. Conde-Knape, J. Kochan, D. Bolin. *Bioorg. Med. Chem. Lett.* **21**, 6264 (2011), https://doi.org/10.1016/j.bmcl.2011.09.009.
[193] L. A. Walter, A. R. Batt, N. Darabedian, B. W. Zaro, M. R. Pratt. *ChemBioChem* **19**, 1918 (2018), https://doi.org/10.1002/cbic.201800280.
[194] B. Vyas, O. Silakari, M. Singh Bahia, B. Singh. *SAR QSAR Environ. Res.* **24**, 733 (2013), https://doi.org/10.1080/1062936x.2013.797493.
[195] A. Lather, S. Sharma, A. Khatkar. *BMC Chem.* **15**, 45 (2021), https://doi.org/10.1186/s13065-021-00769-8.
[196] A. Lather, S. Sharma, A. Khatkar. *Sci. Rep.* **10**, 13903 (2020), https://doi.org/10.1038/s41598-020-70895-1.
[197] A. Lather, S. Sharma, A. Khatkar. *BMC Chem.* **14**, 41 (2020), https://doi.org/10.1186/s13065-020-00693-3.