Intervention of Hepatic Glucose Production. Small Molecule Regulators of Potential Targets for Type 2 Diabetes Therapy

Tjeerd Barf*

Department of Medicinal Chemistry, Biovitrum AB, Box 6443, SE-751 37 Uppsala, Sweden

Abstract: Excessive hepatic glucose production is thought to be a major contributor to the type 2 diabetic state. Drug discovery efforts have yielded small synthetic inhibitors for gluconeogenic and glycogenic regulators of this pathway. The most advanced targets are outlined in this mini-review and include: the glucocorticoid receptor, 11β-hydroxysteroid dehydrogenase type 1, fructose 1,6-bisphosphatase, the glucagon receptor, glycogen phosphorylase, glycogen synthase kinase-3, and glucose-6-phosphatase.

Keywords: glucocorticoid receptor, 11β-HSD1, F16BPase, glycogen phosphorylase, glucagon receptor, GSK-3, G6Pase.

INTRODUCTION

The number of diagnosed Type 2 diabetes mellitus patients amounts to more than 200 million today, and the prevalence is predicted to escalate to a staggering 300 million subjects worldwide by the year 2025 [1]. Type 2 diabetes is a metabolic abnormality that is strongly associated with overweight, lack of physical activity, and genetic predisposition [2]. The disease is expressed through impaired insulin secretion and insulin resistance of target tissues, progressing through various stages. This phenomenon leads to abnormally high circulating glucose levels - hyperglycaemia. Excessive production of hepatic glucose is believed to be one of the contributors to the diabetic state, and pharmacological intervention through the underlying mechanisms controlling glucose levels could be key to successful management of Type 2 diabetes. The antidiabetic efficacy of one of the marketed drugs - metformin - has often been attributed to reduction of hepatic glucose output. The exact mechanism of action remains to be elucidated, and metformin also suffers from side effects such as lactic acidosis [3]. It is desirable to regulate single hepatic targets with selective small molecules so that the outcome can be predicted, and the potential non target-related adverse events can be minimized. This emerging approach has been of considerable interest for the majority of the pharmaceutical companies, active in the field of Type 2 diabetes.

Hepatic glucose production is largely derived from *de novo* synthesis of glucose (gluconeogenesis) from three-carbon synthons such as lactate and alanine, (Fig. **1**). The equally important breakdown of the polymer glucose-depot glycogen (glycogenolysis) constitutes the second major part of glucose production. The rate-limiting enzymes of these processes - phosphoenolpyruvate carboxykinase (PEPCK), fructose 1,6-bisphosphatase (F16BPase) and glucose-6-phosphatase (G6Pase) - are upregulated in the type 2 diabetic state, causing an excessive gluconeogenic efflux [4]. Consequently, development of small molecule inhibitors for some of these enzymes is an excellent opportunity for medicinal chemists. In addition, inhibition of targets that positively regulate these gluconeogenic enzymes has potential as future antidiabetic therapies. For instance, there is substantial evidence that inactivation of the hepatic glucocorticoid pathway by blockade of glucocorticoid receptors (GR) or the 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) enzyme downregulates PEPCK gene expression and hence activity [5]. F16BPase inhibition will partly shut down gluconeogenesis due to accumulation of glucose precursor fructose-1,6-phosphate (F16P). Glucagon receptor (GlucR) antagonism and glycogen phosphorylase (GP) inhibition are putative ways of achieving glycaemic control, acting through blockade of the glycogenolysis pathway [6, 7]. Conversely, glycogen synthesis can be stimulated by inhibition of glycogen synthase kinase-3 (GSK-3), a negative regulator of glycogen synthase (GS) [8]. All of the above mentioned concepts aim to partially reduce the hepatic glucose output, with the minimal risk for hypoglycaemia. G6Pase inhibition theoretically is the most robust way of reducing hepatic glucose production, since G6Pase mediates the final step of both gluconeogenesis and glycogenolysis by converting glucose-6-phosphate (G6P) to glucose before it is released from the liver [9].

This mini-review focuses on the therapeutic approaches towards reduced hepatic glucose production that have advanced the furthest. The role of each drug target in the liver, the structural features of their inhibitors/antagonists, and apparent status for each of those intervention pathways will be highlighted.

1. Gluconeogenesis

1.1. Blockade of Liver Glucocorticoid Action

Glucocorticoids play a crucial role in the normal development and maintenance of basal and stress-related events. The GRs belong to the superfamily of nuclear hormone receptors, which function as ligand-activated transcription factors. The endogenous glucocorticoid cortisol activates GRs, thereby regulating a wide range of physiological parameters [10]. However excessive levels of circulating glucocorticoids can induce metabolic complications, such as insulin resistance and obesity [11]. In the liver, glucocorticoids oppose the action of insulin by up-regulating key enzymes like PEPCK and G6Pase, thus increasing hepatic glucose production.

*Address correspondence to this author at the Department of Medicinal Chemistry, Biovitrum AB, Box 6443, SE-751 37 Uppsala, Sweden; E-mail: Tjeerd.Barf@biovitrum.com

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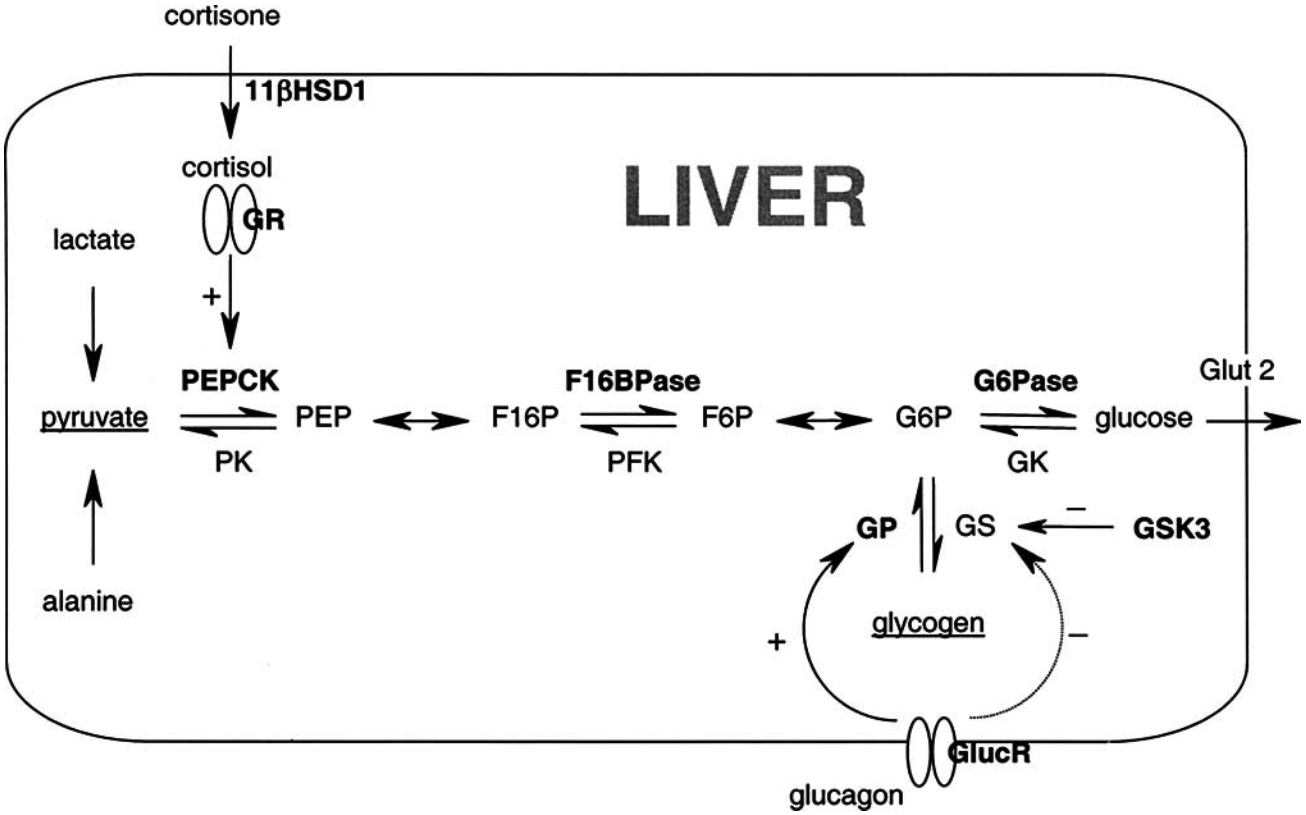


Fig. (1). A schematic and simplified overview of possible intervention points for reduced hepatic glucose output (in bold). **11β-HSD1** = 11β-hydroxysteroid dehydrogenase type 1, **GR** = glucocorticoid receptor, PEPCK = phosphoenolpyruvate carboxykinase, PK = pyruvate kinase, PEP = phosphoenolpyruvate, F16P = fructose-1,6-bisphosphate, **F16BPase** = fructose 1,6-bisphosphatase, PFK = phosphofructokinase, F6P = fructose-6-phosphate, **GP** = glycogen phosphorylase, GS = glycogen synthase, **GSK-3** = glycogen synthase kinase-3, **GlucR** = glucagon receptor, G6P = glucose-6-phosphate, **G6Pase** = glucose-6-phosphatase, GK = glucokinase, Glut 2 = hepatic glucose transporter.

1.1.1. Glucocorticoid Receptor (GR) Antagonism

The first synthetic compound found to possess GR antagonistic properties is the progesterone antagonist RU-486 (mifepristone), which has been used extensively in elucidating glucocorticoid-related events (Fig. **2**). RU-486 was able to attenuate circulating blood glucose level by 50% in *db/db* mice following an oral dose of 25 mg/kg [12]. In this experiment, a number of hepatic genes involved in glucose production were downregulated, including PEPCK and G6Pase. The anti-diabetic potential of RU-486 was also shown in *ob/ob* mice [13]. A hypercortisolemic state causes the Cushing's syndrome, a disease that manifests itself in severe obesity and impaired glucose intolerance [11]. Acute administration of RU-486 has been demonstrated to normalise fasting blood glucose at 10 mg/kg in a Cushing's disease patient [14]. However, chronic use at similar doses was shown to activate the hypothalamo-pituitary-adrenal (HPA) axis in healthy male volunteers resulting in increased circulating cortisol and ACTH levels [15].

While the synthetic steroidal human GR antagonist RU-486 (K_i = 0.24 nM) is non-selective over some other steroid receptors, a selective analogue RU-43044 (K_i = 0.10 nM) emerged. However, this compound is not functionally active *in vivo* [16]. The first literature reports have appeared on the development of non-steroidal human GR antagonists (Fig. **2**). Potent and selective examples from Pfizer are CP-394531 (K_i = 0.10 nM) and CP-409069 (K_i = 0.17 nM) that exhibit functional antagonism *in vitro* in the low nanomolar range [17]. Another recent GR antagonist is D06 and is based on a tri-aryl methane core structure [18]. D06 displays competitive GR binding with a K_i of 210 nM and is capable of antagonizing glucocorticoid-mediated transcriptional events. Little cross-reactivity against related steroid receptors was observed (K_i > 3000 nM).

The success of the GR antagonistic approach is presumably dependent on the liver specificity of the compounds, avoiding the systemic hypothalamic-pituitary-adrenal (HPA) axis activation seen with RU-486 treatment. Abbott researchers have presented *in vivo* acute and sub-chronic anti-diabetic effects of the liver-specific bile acid conjugated GR antagonist, A-348441 [19]. The bile acid has been linked through the N-aryl fragment in RU-486. The cholic acid moiety of A-348441 facilitates selective uptake by specific hepatocyte transporters. A-348441 retains high binding affinity for the human GR with an IC_{50} of 1.2 nM, and displays full antagonist activity, with an IC_{50} of 10 nM. In a euglycemic hyperinsulinemic clamp study in Zucker *fa/fa* rats, oral dosing of A-348441 for 7 days gave a dose-dependent 25-60% reduction in hepatic glucose output at 3, 10, 30 and 100 mg/kg/day [20]. These observations showed a strong correlation with the reduction in endogenous glucose production. No effects on the HPA axis were observed at doses up to 300 mg/kg (qd, po) after 5 days. A-348441 is in the pre-clinical phase.

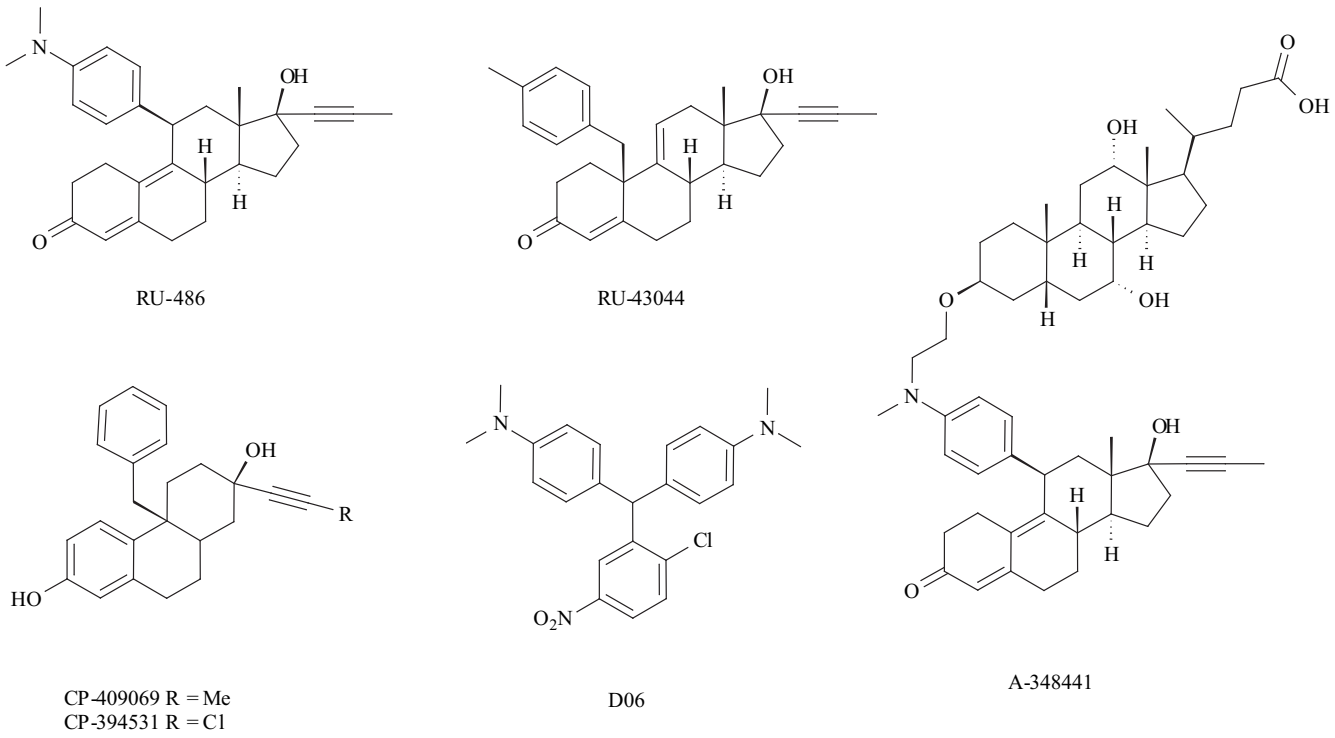


Fig. (2). Chemical structures of GR antagonists.

1.1.2. 11β-Hydroxysteroid Dehydrogenase Type 1 (11β-HSD1) Inhibition

The glucocorticoid activity at the tissue level is determined by pre-receptor metabolism of the glucocorticoids by two 11β-HSD isozymes, shuttling the glucocorticoids between hormonally active cortisol and inactive cortisone (corticosterone and dehydrocorticosterone in rodents, respectively) (Fig. **3**). This intracellular glucocorticoid shuttle is responsible for the carbon-11 keto-reduction (11β-HSD1) and the alcohol-dehydrogenation (11β-HSD2). In the liver, the membrane bound 11β-HSD1 enzyme controls the level of cortisol available for receptor activation. Accordingly, loss of hepatic 11β-HSD1 activity in rodents improves insulin sensitivity through a reduction in cortisol-induced gluconeogenesis and hepatic glucose output. Clinical data suggest that inhibition of 11β-HSD1 increases hepatic insulin sensitivity along with decreased glucose production [21]. However, the settings of this study were less than ideal due to the non-selective character of the 11β-HSD inhibitor carbenoxolone, leading to dose limiting 11β-HSD2 inhibitory activity. Nevertheless this study shows that selective inhibition of 11β-HSD1 has the potential to reduce blood glucose levels and increase insulin sensitivity in type 2 diabetic patients.

Several natural compounds have been investigated in the search for selective inhibitors of 11β-HSD1, and included licorice-derived compounds [22]. Diederich *et al* identified selective inhibitors in the micromolar range, such as chenodeoxycholic acid (IC_{50} = 4.4 μM), and confirmed the potency of carbenoxolone (IC_{50} = 60 nM), a derivative of licorice [23]. Recently, Biovitrum was the first to disclose totally synthetic small molecule inhibitors of human 11β-HSD1 [24]. These arylsulfonamidothiazoles, exemplified by BVT.14225 (IC_{50} = 52 nM), showed more than 200-fold selectivity over human 11β-HSD2 (Fig. **4**). The mouse 11β-HSD1 selective inhibitor BVT.2733 attenuated blood glucose levels in hyperglycemic KKA^{y} mice [25]. Hepatic 11β-HSD1 activity was reduced following a single oral dose of BVT.2733 (100 mg/kg), and both PEPCK and G6Pase mRNA were lowered after 7 days of continuous subcutaneous administration (167 mg/kg/day). These findings in rodents suggest that inhibition of 11β-HSD1

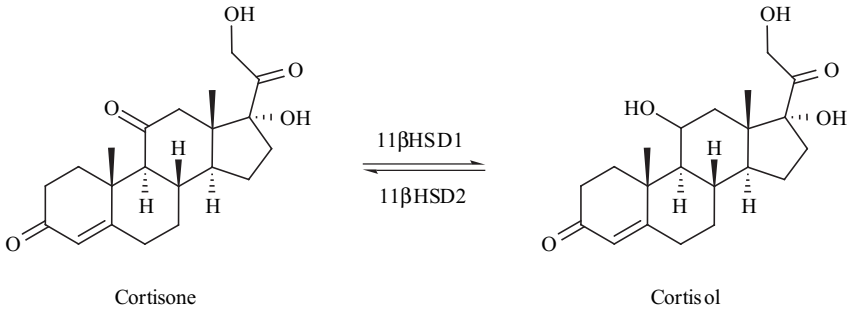
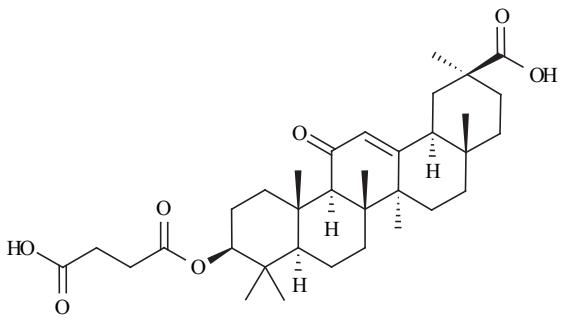
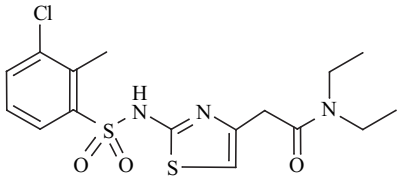


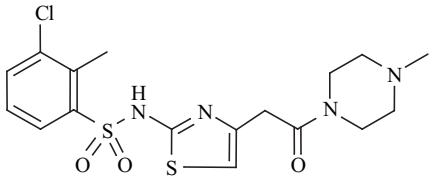
Fig. (3). Shuttling of cortisone and cortisol by 11β-HSD Type 1 and 2 Enzymes.



Carbenoxolone



BVT.14225



BVT.2733

Fig. (4). Chemical structures of 11β-HSD1 inhibitors

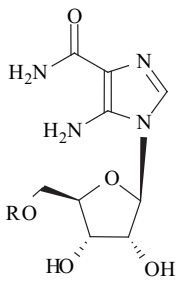
could be a viable route for the treatment of type 2 diabetes. A human selective 11β-HSD1 inhibitor, BVT.3498, recently entered phase II clinical trials.

1.2. Fructose-1,6-Bisphosphatase (F16BPase) Inhibition

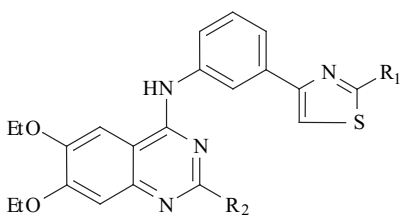
F16BPase is a cytosolic enzyme that is predominantly expressed in the liver and kidney, and is one of the key regulators of gluconeogenesis (Fig. **1**). The physiologically relevant form of F16Bpase is a homotetramer, which can exist in two distinct conformational states (T and R), depending on the relative concentrations of active site and allosteric site ligands [26]. The enzyme is subject to competitive substrate inhibition by fructose-2,6-bisphosphate [27] and to allosteric inhibition by adenosine 5'-monophosphate (AMP) [28]. Fructose-2,6-bisphosphate and AMP synergistically stabilize the T state, which is the inactive form of the enzyme, while the substrate fructose-1,6-bisphosphate stabilizes the kinetically active R state. The regulation and the molecular basis of the F16BPase activity have been deduced from crystallographic studies utilizing recombinant human F16BPase protein [29]. A F16BPase inhibitor should reduce hepatic glucose output and lower blood glucose by reducing excess gluconeogenesis in diabetic patients.

The naturally occurring ZMP (the phosphorylated analogue of 5-aminoimidazole-4-carboxamide riboside; AICAR; Fig. **5**) has been shown to display efficacy in a number of rodent diabetes models [30], which could be largely attributed to inhibition of gluconeogenesis [31].

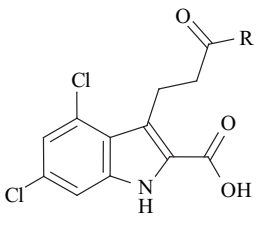
Recently, novel allosteric site inhibitors were disclosed. These anilinoquinazoline derivatives have been co-crystallized with porcine kidney F16BPase [32]. Interestingly, two molecules of **1** fill the pocket by stacking against one another in a head-to-tail mode, making key interactions with Lys50 and His55. Analogues were evaluated against the human F16BPase and IC_{50} values down to 0.22 μM for analogue **2** were reported. The same group from Pfizer identified MDL-29951 as an inhibitor of human F16BPase with an IC_{50} value of 2.5 μM [33]. Modest SAR efforts resulted in analogues with similar potencies (**3**, IC_{50} 1.7 μM), and a carboxylic acid in the indole 2-position seems to be essential for activity. An X-ray co-crystal structure with porcine kidney F16BPase in the



AICAR; R = H
ZMP; R = $PO(OH)_2$



(**1**); $R_1 = CH_2OH$; R_2 = H
(**2**);$R_1 = CH_3$; $R_2 = CH_2CH_2NH_2$



MDL-29951; R = OH
(**3**); R = NMe_2

Fig. (5). Chemical structures of allosteric site F16BPase inhibitors.

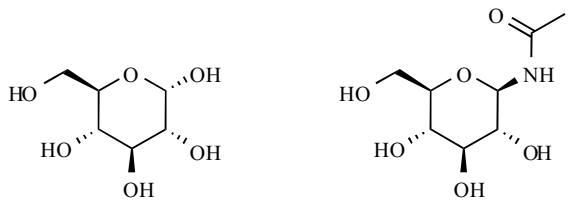
T form, shows that MDL-29951 occupies the allosteric AMP binding site. The two carboxyl groups drive the binding by making hydrogen bond contacts with Gly28 (backbone NH) and Tyr113, the latter normally making direct interactions with the phosphate moiety of AMP. These compounds could serve as leads for future optimisation. The F16BPase inhibitor CS-917, co-developed by Metabasis Therapeutics and Sankyo, seems to have advanced furthest and in the fall of 2002 was reported to be in phase II clinical studies [34].

2. Glycogenolysis

2.1. Glycogen Phosporylase (GP) Inhibition

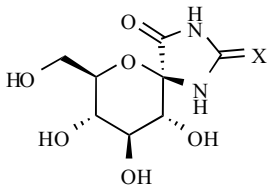
GP catalyses the degradation of glycogen to glucose-1-phosphate, which is converted by phosphoglucomutase to glucose-6-phosphate. The latter undergoes phosphorolysis with the aid of G6Pase to glucose (Fig. **1**) [35]. High glucose levels result in the conversion of GP from its active *a* form (phosphorylated) to its inactive *b* form (non-phosphorylated), and this is part of the negative feedback mechanism to maintain glucose homeostasis. Both the *a* and *b* form can exist in an active (R state) and an inactive (T state) conformation. The enzyme is reported to have a dimeric or possibly a tetrameric structure with several binding sites. At present, five different binding sites have been identified and most of them have been targeted for inhibitor development: the catalytic (active) site, the purine nucleoside (or inhibitor) site, the AMP allosteric site, the glycogen storage site, and the recently discovered allosteric (or indole) site.

Three known GP isoforms are found predominantly in the brain, the muscle and the liver, respectively, and share approximately 80% in amino acid identity [36]. Reduction of hepatic glucose output could be achieved by inhibition of the liver isoform suggesting this as a suitable drug target. However, the muscle isoform mediates glycogen breakdown as an important energy source during muscle exercise. Thus tissue specificity might be key to success. GP is an extensively studied target and the inhibitory activity has been assessed using different species variants (mostly rabbit and human) in *a* and *b* forms. This makes the comparison between the various inhibitor classes difficult, also because the degree of inhibition is often dependent on allosteric ligands such as glucose and AMP.



α-D-glucose

β-N-Acetyl-glucosamine



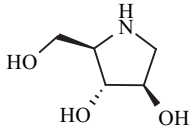
Spirohydantoin; X = O
Spirothiohydantoin; X = S

Fig. (6). Chemical structures that bind in the catalytic site of GP.

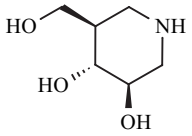
2.1.1. Catalytic Site Inhibitors

α-D-glucose with a K_i of 2 mM against rabbit muscle GP*b* and glucose-1-phosphate stabilise GP in the T state inactive conformation by binding to the catalytic site [37]. A boost in potency as compared to α-D-glucose is observed with β-N-acetyl-glucosamine, arriving at a K_i of 32 μM for rabbit muscle GP*a* and *b* [38]. Spirohydantoin and the sulfur analogue are the most potent catalytic site inhibitors against rabbit muscle GP*b* to date with K_i values of 3 and 7 μM, respectively (Fig. **6**) [39]. No *in vivo* data of catalytic site inhibitors has been reported.

A related family of compounds are the so-called azasugars, exemplified by 1,4-dideoxy-1,4-imino-D-arabinitol (DAB) [40] and isofagomine (Fig. **7**) [41], that possess inhibitory properties against pig liver GP*a* in the low micromolar range (IC_{50}'s of 0.2 and 0.7 μM, respectively). Attempts to optimise isofagomine did not result in more potent inhibitors and confirmed that the (3R, 4R, 5R)-configuration is optimal [42]. Interestingly, the compounds from this class do not seem to occupy the same binding site as for instance α-D-glucose, in spite of the close structural resemblance.



DAB

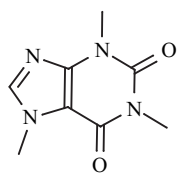


Isofagomine

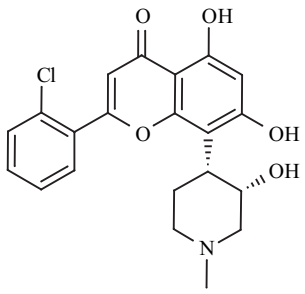
Fig. (7). Azasugars as GP inhibitors.

2.1.2. The Purine Nucleoside Site

This site has not been extensively investigated and the few known ligands that bind to the purine nucleoside site display synergism with glucose, *i.e.* the affinity increases with higher concentration of glucose. Caffeine with a K_i of about 1 mM is a relatively weak inhibitor of rabbit liver and muscle GP*a* and stablises the T state conformation (Fig. **8**)



Caffeine



Flavopiridol

Fig. (8). Chemical structures of purine nucleoside inhibitors.

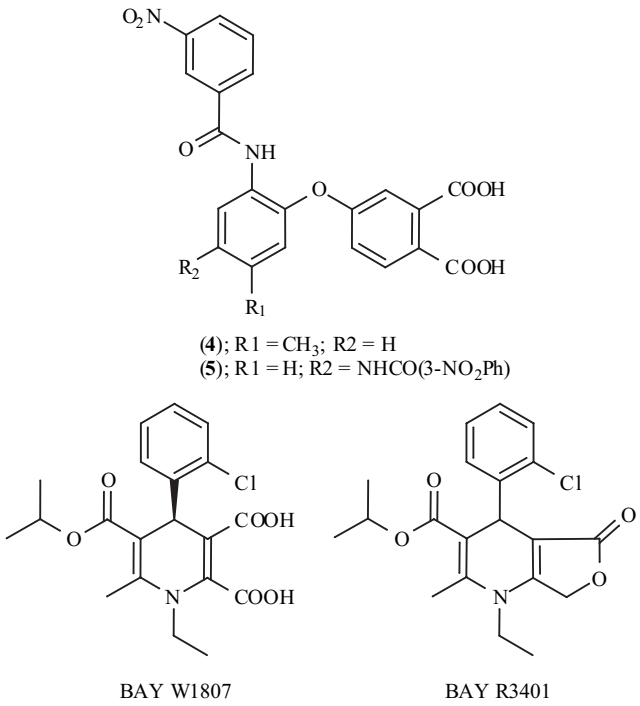


Fig. (9). Chemical structures of AMP site inhibitors.

[43]. The larger and more potent flavopiridol, initially developed in an anticancer program, inhibits both rabbit muscle GP*a* (K_i = 1 μM) and GP*b* (K_i = 10 μM) [44]. These purine nucleoside site inhibitors are hypothesised to bind by stacking the flat ring systems with aromatic amino acid residues.

2.1.3. AMP Allosteric Site Inhibitors

The AMP allosteric site binds beside AMP a range of other phosphorylated compounds, such as ATP and glucose-6-phosphate, and seems to have a preference for synthetic dicarboxylic acids. This was nicely demonstrated by a co-crystal structure of GP and one of Novo Nordisk's [2-(benzoylamine)-phenoxy]-phthalic acid derivatives with IC_{50} values down to 79 nM (Fig. **9**) [45]. The phthalic acid moiety was shown to interact with Arg81 and Arg310, and this dicarboxylic acid motif is also part of BAY W1807. The latter compound is the most potent allosteric inhibitor reported to date and blocks rabbit muscle GP*a* and GP*b* with Ki values of 10.8 and 1.6 nM, respectively [46]. The prodrug BAY R3401 is metabolised to BAY W1807 *in vivo* and reduces blood glucose levels in rats [47], showing that this inhibitor also probably targets liver GP and is thus not tissue specific. In addition, BAY R3401 administration was shown to attenuate basal and glucagon-stimulated glycogenolysis in dogs [48].

2.1.4. New Allosteric Site Inhibitors

This novel binding site is situated at the dimer interface of the GP enzyme and was identified by Pfizer researchers. It is also referred to as the indole site since all inhibitors occupying this site are based on the 5-chloroindole-2-carboxamide scaffold (Fig. **10**). A crystal structure of CP-403700 (IC_{50} = 45 nM) with human liver GP*a* was resolved showing that the 5-chloroindole moiety was buried in a lipophilic pocket, while the indole NH interacts with Lys191 [49]. The 2-carboxamide makes hydrogen bonds to the backbone carbonyl of Thr38 and a water molecule. Since two molecules of CP-403700 bound identically in the monomer subunits, bis-indole CP-526423 was prepared and co-crystallized. It was nicely demonstrated that CP-526423 spans the two inhibitor sites, and with an IC_{50} of 6 nM is the most potent GP allosteric site inhibitor reported to date.

Earlier compounds in this class demonstrated *in vitro* binding in the submicromolar range. Two of these derivatives, CP-91449 and CP-320626, have been subjected to in-depth proof-of-principle studies [50]. Oral administration of CP-91149 to *ob/ob* mice almost normalized plasma glucose levels at 25 and 50 mg/kg, and this effect was mainly attributed to inhibited glycogenolysis

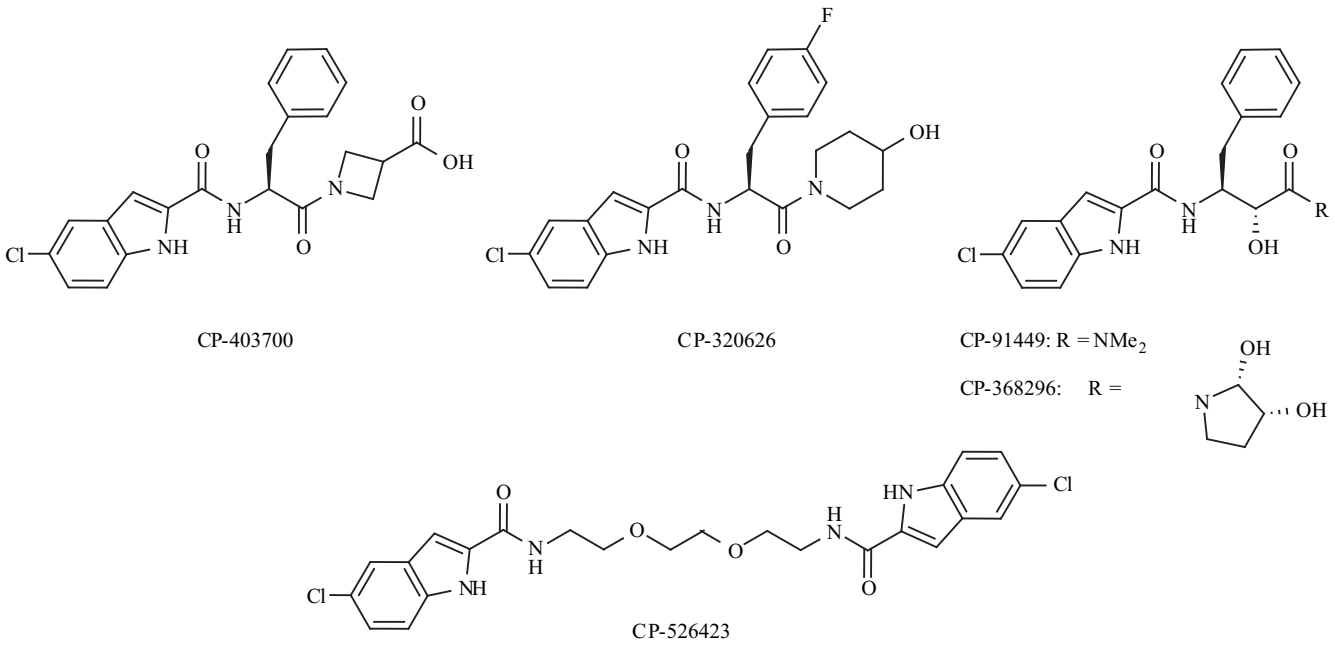


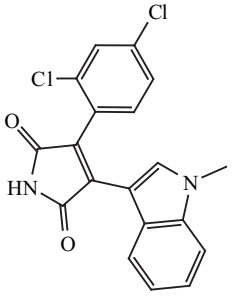
Fig. (10). Chemical structures of frequently used GP new allosteric site inhibitors.

as measured by the liver ^{14}C-glycogen content [51]. In a two-week study, longer acting CP-320626 (10 mg/kg, b.i.d.) gave a sustained fed-state glucose reduction in *ob/ob* mice, whereas the fasting glucose levels were not affected. Glycogen accumulation occurred in both liver and muscle, underlining the unselective profile of these compounds for the various GP tissue forms [6].

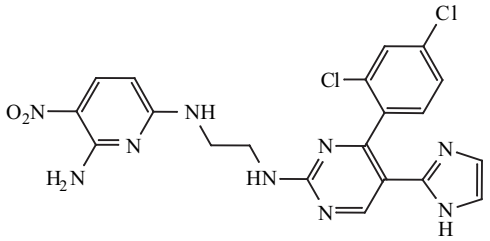
The functional activity of this compound class is glucose-dependent and the physiological relevance of this remains yet to be seen, but the upside would be clearly a reduced risk for hypoglycemia. CP-368296 (Ingliforib) with an IC_{50} value of 52 nM for human liver GP has been evaluated in phase II clinical trials, but development has been discontinued. The rather unselective profile of CP-368296 over other GPs may have contributed to the halt in its development (human muscle GP 352 nM; human brain GP 150 nM) [52]. It is likely that a related compound is currently in clinical phase development.

2.2. Glycogen Synthase Kinase-3 (GSK-3) Inhibitors

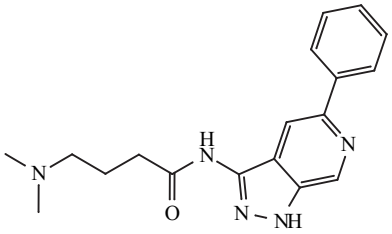
GSK-3 is an ubiquitously expressed serine/threonine protein kinase involved in the regulation of a variety of physiological processes. In a number of tissues its role is to phosphorylate and inactivate GS, the regulatory enzyme of insulin-stimulated glycogen synthesis. Consequently, hepatic GSK-3 inhibition has potential in attenuating the hepatic glucose output. It is well known that both the type 2 diabetic and overweight states are associated with impaired GS activity and glycogen synthesis, and that overactivity of GSK-3 could limit insulin-mediated biological responses [8]. Chir 98014 inhibits human GSK-3β with a K_i value of 0.87 nM against the ATP-binding site, and was > 500-fold selective versus 20 other protein kinases (Fig. **11**) [53]. This translates to functional GS activation in primary rat hepatocytes with an EC_{50} of 107 nM. *Db/db* mice treated with Chir 98014 (30 mg/kg) showed a reduction in fasting hyperglycemia and improved glucose disposal during an intraperitoneal glucose tolerance test. Reduced hepatic glucose output can be anticipated, although muscle may have been the main contributing tissue to the observed effect. Indeed, increased net hepatic glycogen synthesis and decreased hepatic glucose output were found following treatment of Zucker diabetic fatty rats with the structurally related Chir 98023 [54]. Arylindolemaleimides SB-216763 and SB-415286 inhibited human GSK-3α with IC_{50} values of 34 and 78 nM, respectively, in an ATP competitive manner [55]. Both compounds stimulated glycogen synthesis in a human liver cell line in the low micromolar range, and the activation observed was additive to insulin. In rat hepatoma cells, it was demonstrated that these GSK-3 inhibitors caused a reduction of the expression levels of PEPCK and G6Pase [56]. Low nM range ATP-binding site GSK-3 inhibitors have been reported by GlaxoSmithKline, but no *in vivo* data has been disclosed [57]. Recently, a novel hydrophilic class of ATP competitive GSK-3 inhibitors with IC_{50} values down to 0.1 μM was reported by Novo Nordisk. A 3-fold stimulation of glycogen synthesis in rat soleus muscle (10 μM) was achieved with the best compounds [58]. The field await the first results from clinical trials, and there are some questions that need to be answered. For one, there are concerns about selectivity over other kinases, but the data presented so far look promising.



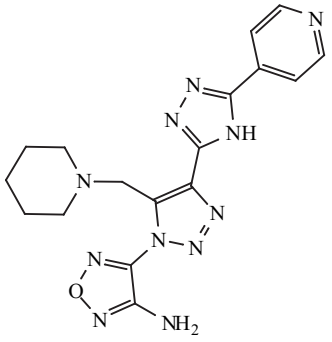
SB-216763



Chir 98014



(**6**) Inhibitor from GlaxoSmithKline



(**7**) Inhibitor from Novo Nordisk

Fig. (11). Published structures of GSK-3 inhibitors.

2.3. Glucagon Receptor (GlucR) Antagonism

The 29-amino acid glucagon is a hormone that activates the glucagon receptor (GlucR), a family B type 7 transmembrane G-protein coupled receptor. Glucagon counterbalances insulin action in the regulation of hepatic glucose production through cAMP-mediated stimulation of gluconeogenesis and glycogenolysis [59]. The central role for glucagon and the GlucR in glucose homeostasis fuels the search for antagonists against hepatic insulin resistance which is of great interest in the treatment of Type 2 diabetes.

During the last decade, some of the tremendous efforts to find non-peptidic small molecule antagonists for the GlucR have emerged, and many excellent review papers covering this area have appeared [60, 61, 62]. Early efforts struggled with the species difference of the GlucR, or compound series with sub-optimal preclinical properties as was demonstrated by studies with L-168,049 [63, 64]. Although potent *in vitro* GlucR antagonists for the human receptor evolved over the years, it is only recently that convincing *in vivo* functional effects in animal models of diabetes have been obtained. A collaboration of Novo Nordisk and Agouron (now Pfizer) led to the discovery of NNC 25-2504 belonging to the class of alkylidene hydrazides (Fig. **12**) [65, 66]. This highly potent and non-competitive antagonist of the human GlucR (IC_{50} = 2.3 nM) inhibited glucagon-stimulated glucose production in rat hepatocytes with a K_i of 14 nM. NNC 25-2504 normalized glucagon-induced blood glucose levels in normal rats at a dose of 10 mg/kg given intravenously. At a recent conference, Novo Nordisk presented a urea based library from which low nanomolar GlucR antagonists were identified. One of these compounds, exemplified by the β-alanine derivative **8,** was shown to attenuate blood glucose levels in normal rats (3 mg/kg) after a glucagon challenge [67]. In addition, the urea analogue reduced the glucose levels in conscious postprandial rats at a 10 mg/kg (iv) dose. A compound from this series was reported to be in phase I clinical trials.

Bayer researchers have shown a series of low molecular weight biphenyl and 4-phenylpyridyl compounds to be potent antagonists, and were the first to demonstrate clinical efficacy of a GlucR antagonist with the biphenyl analogue Bay 27-9955 [68]. This compound, that competitively blocks the human GlucR with an IC_{50} value of 110 nM [69], inhibited the hyperglycemic period evoked by exogenous glucagon administration in healthy human subjects. Oral treatment with Bay 27-9955 was well tolerated at the maximal 200 mg/kg dose, but inspite of this further development has been discontinued. The equipotent 4-phenylpyridine analogue **9** may stand a better chance. Introduction of a hydroxyl group on the 2-position of the phenyl ring boosted potency to an IC_{50} of 10 nM for the most active isomer **10** [70, 71].

3. Glucose-6-phosphatase (G6Pase) Inhibition

Hepatic G6Pase is a multiprotein complex comprising a number of transporter proteins and a catalytic unit, and is responsible for the terminal step of hepatic glucose production. The G6P derived from either glycogenolysis or gluconeogenesis is looped through the G6Pase complex to generate glucose. The G6P translocase T1 component facilitates G6P uptake from the cytosol into the endoplasmatic reticulum, after which the catalytic subunit converts G6P into glucose and phosphate. These are subsequently returned to the cytosol by transporter proteins T2 and T3, respectively [72].

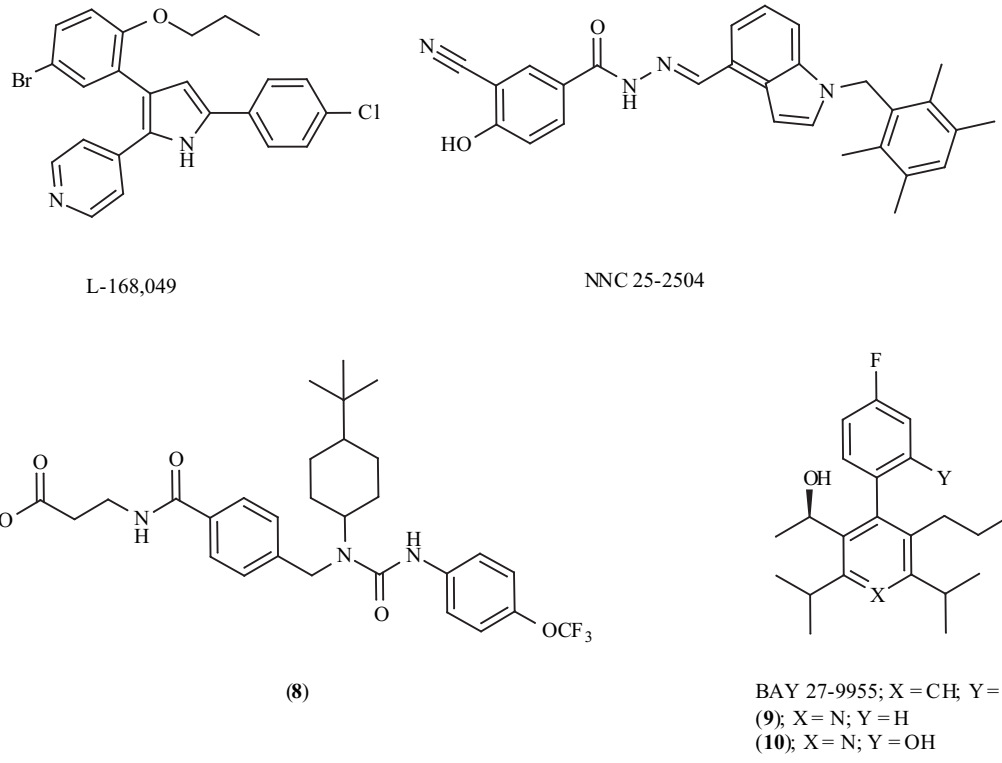


Fig. (12). Non-peptide glucagon receptor antagonists.

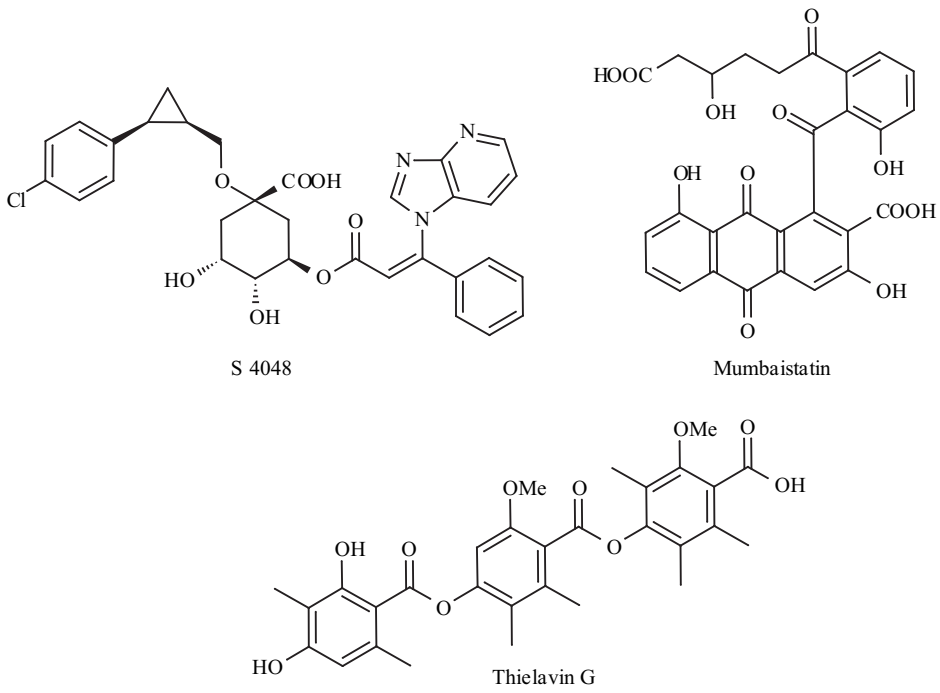


Fig. (13). G6P translocase inhibitors.

3.1. G6P Translocase Inhibition

A number of pharmaceutical companies have targeted the development of inhibitors of G6P translocase. Aventis (formerly Hoechst) identified chlorogenic acid derivatives that were very potent translocase inhibitors exemplified by S 4048 with IC_{50} = 2 nM (Fig. **13**) [73]. When the close analogue S 3483 was infused continuously (50 mg/kg/h iv) into rats, fasting blood glucose levels were lowered, suggestive of attenuated hepatic gluconeogenesis [74]. In another study it was shown that fasting blood glucose levels in rats and *ob/ob* mice were dose-dependently reduced following administration of S 4048 (1-100 mg/kg, ip) [75]. Although the effect was relatively short-lived, it confirmed that reduction of hepatic glucose output can be achieved with pharmacologically induced suppression of the hepatic G6Pase activity. Mumbaistatin has also been shown to be a G6P translocase inhibitor with an IC_{50} of 5 nM [76]. Recently, a number of thielavins were found to possess inhibitory activity against G6Pase in glucagon-stimulated rat hepatocytes, thielavin G being the most potent with an IC_{50} of 0.33 μM [77]. It was not specifically stated whether the thielavins target the translocase, or an other part of the G6Pase complex.

3.2. G6Pase Catalytic Enzyme Inhibition

Novo Nordisk looked at an alternative approach to inactive the G6Pase system and aimed to find G6Pase catalytic enzyme inhibitors, and reported on two distinct compound classes. Synthetic small molecules such as the tetrahydrothienopyridines derivative **11** inhibited the G6Pase catalytic protein in the submicromolar range (Fig. **14**) [78]. Also, a number of benzylidenehydrazines with G6Pase catalytic site inhibitory properties were disclosed, exemplified by the 5-chlorothienyl derivative **12** with an IC_{50} value of 170 nM [79]. Compounds from the former thienopyridine class were able to reduce glucose production from cultured rat hepatocytes, but no *in vivo* data has been reported [80].

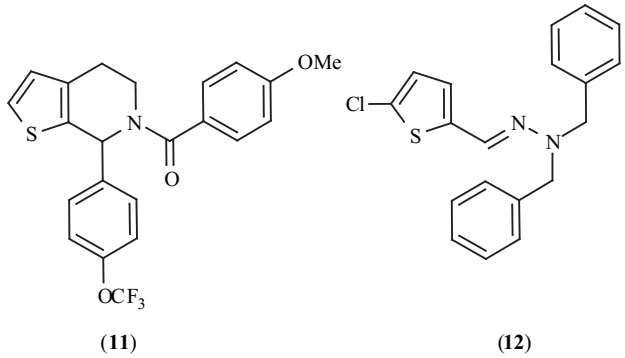


Fig. (14). G6Pase catalytic site inhibitors

Although blockade of the G6Pase system is a validated approach, a number of side effects with G6Pase inhibitors have been observed in the preclinical stage. Major concerns are the dramatic increases of lactate levels observed, and the apparent risk for hypoglycemia. In addition, hepatic steatosis due to upregulation of lipogenic genes has been associated with pharmacological inhibition of G6Pase [81]. These are the most likely explanations that no clinical data have yet been reported.

CONCLUDING REMARKS

Inhibition of targets that share the common theme of diminishing hepatic glucose output, are currently being explored for their usefulness in Type 2 diabetes treatment. These approaches have yielded promising small molecules that demonstrated proof-of-principle in a variety of diabetes animal models. Many of those have been made available to

the public, but it is clear that the most promising drug candidates are still hidden in filed patent applications and are yet to be reported. Importantly, many of these potential anti-diabetic agents are predicted to have additional effects in extrahepatic target tissues, which could be either beneficial or detrimental. This makes it very difficult to compare the various mechanisms of action and their potential. Many of the targets have been validated by means of animal models of diabetes, and clinical efficacy has been reported for GR antagonist RU-486 and GlucR antagonist Bay 27-9955. Small molecular regulators that have advanced the most are GP inhibitor CP-368296, 11β-HSD1 inhibitor BVT.3498, and F16BPase inhibitor CS-917. These clinical candidates have been, or are in phase II clinical development. The interest for G6Pase inhibitors seems to have declined considerably, presumably due to the side effects observed following G6Pase inactivation. On the other hand, the preclinical data generated in the GSK-3 inhibitory field looks encouraging. Other targets that address inappropriate hepatic glucose production are in the literature. The future will tell whether any of the above approaches will some day benefit type 2 diabetic patients. In the face of the growing epidemic in diabetes and the limitations of the drugs presently available there is certainly a demand for safer and more efficacious anti-diabetic drugs.

LIST OF ABBREVIATIONS

ACTH = Adrenocorticotropic hormone

AICAR = 5-Aminoimidazole-4-carboxamide riboside

AMP = Adenosine 5'-monophosphate

ATP = Adenosine 5'-triphosphate

DAB = 1,4-Dideoxy-1,4-imino-D-arabinitol

F16BPase = Fructose 1,6-bisphosphatase

F16P = Fructose-1,6-bisphosphate

F6P = Fructose-6-phosphate

GK = Glucokinase

GlucR = Glucagon receptor

Glut 2 = Hepatic glucose transporter

GP = Glycogen phosphorylase

G6P = Glucose-6-phosphate

G6Pase = Glucose-6-phosphatase

GR = Glucocorticoid receptor

GS = Glycogen synthase

GSK-3 = Glycogen synthase kinase-3

HPA = Hypothalamo-pituitary-adrenal

11β-HSD1 = 11β-Hydroxysteroid dehydrogenase type 1

PEP = Phosphoenolpyruvate

PEPCK = Phosphoenolpyruvate carboxykinase

PFK = Phosphofructokinase

PK = Pyruvate kinase

SAR = Structure activity relationship

ZMP = 5-Aminoimidazole-4-carboxamide ribonucleotide monophosphate

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