Glucose-based spiro-oxathiazoles as in vivo anti-hyperglycemic agents through glycogen phosphorylase inhibition

David Goyard, Bálint Kónya, Katalin Czifrák, Paolo Larini, Fanny Demontrond, Jérémy Leroy, Sophie Balzarin, Michel Tournier, Didier Tousch, Pierre Petit, et al.

ARTICLE

Glucose-based spiro-oxathiazoles as *in vivo* anti-hyperglycemic agents through glycogen phosphorylase inhibition

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

David Goyard,[a] Bálint Kónya,[b] Katalin Czifrák,[b] Paolo Larini,[a] Fanny Demontrond,[a] Jérémy Leroy,[c] Sophie Balzarin,[c] Michel Tournier,[c] Didier Tousch,[c] Pierre Petit,[c] Cédric Duret,[d,e] Patrick Maurel,[d,e] Tibor Docsa,[f] Pál Gergely,[f] László Somsák,[b] Jean-Pierre Praly,[a]* Jacqueline Azay-Milhau,[c] and Sébastien Vidal[a]*

The design of glycogen phosphorylase (GP) inhibitors targeting the catalytic site of the enzyme is a promising strategy for a better control of hyperglycaemia in the context of type 2 diabetes. Glucopyranosylidene-spiro-heterocycles have been demonstrated as potent GP inhibitors, and more specifically spiro-oxathiazoles. A new synthetic route has now been elaborated through 1,3-dipolar cycloaddition of an aryl nitrile oxide to a glucono-thionolactone affording in one step the spiro-oxathiazole moiety. The thionolactone was obtained from the thermal rearrangement of a thiosulfinate precursor according to Fairbanks' protocols, although with a revisited outcome and also rationalised with DFT calculations. The 2-naphthyl substituted glucose-based spiro-oxathiazole **5h**, identified as one of the most potent GP inhibitors (K_i = 160 nM against RMGPb) could be produced on the gram-scale from this strategy. Further evaluation *in vitro* using rat and human hepatocytes demonstrated that compound **5h** is a druggable anti-hyperglycaemic compound performing slightly better than DAB used as a positive control. Investigation in Zucker *fa/fa* rat model in acute and subchronic assays further confirmed the potency of compound **5h** since it lowered blood glucose levels by ~36% at 30 mg/kg and ~43% at 60 mg/kg. The present study is one of the few *in vivo* investigations for glucose-based GP inhibitors and provides data in animal models for such drug candidates.

Introduction

Diabetes mellitus is one of the most severe global health problems of the 21st century. According to the International Diabetes Federation (IDF) 425 million people, ~9% of the adult population suffer from this disease, many of them may even remain undiagnosed. The number of diabetic patients is predicted to reach close to 700 million by 2045 with the most significant increase in low and medium income territories of Asia, Africa and South-America. In 2017 USD ~727 billion was spent for diabetes treatments representing more than 12% of the global health expenditures. Most of the diabetic patients (>90%) belong to the so-called type 2 *diabetes mellitus* (T2DM) when the organism does not produce insulin in sufficient quantities and/or the peripheral cells are more or less resistant to the insulin action to promote uptake of blood glucose. Due to the constantly high levels of glycaemia, short and especially long term complications are developed being frequent causes of death, thereby diabetes is one of the main contributors to global mortality. At present no causal therapy is known, treatment regimens aim at maintaining blood sugar levels around the normoglycemic value of ~6.1 mM by applying various medications.[1, 2]

Hepatic glucose output is elevated in T2DM patients. This glucose production consists of glycogenolytic and gluconeogenetic components and the latter is known to be cycled through the glycogen pool.[3, 4] Therefore, liver glycogen phosphorylase, the rate limiting enzyme of glycogen degradation has become a validated target to find potential new therapeutical possibilities against T2DM.[4, 5]

A broad variety of synthetic compounds and natural products have been tested as inhibitors of glycogen phosphorylase (GP)[6-12] mainly with the most easily available rabbit muscle GP*b* (RMGP*b*).[13] Many of these compounds' complexes with the GP enzyme were also studied by X-ray crystallography. From the so discovered seven GP binding sites the most investigated one is the catalytic (or active) site which can accommodate D-glucose (the physiological inhibitor of the enzyme) and a large array of glucose derivatives.[14, 15] The most

[a] *Institut de Chimie et Biochimie Moléculaires et Supramoléculaires, UMR 5246, CNRS, Université Claude Bernard Lyon 1, Bâtiment Lederer, 1 Rue Victor Grignard, F-69622 Villeurbanne, France. Fax: +33 472 448 109; Tel: +33 472 448 349; E-mail: sebastien.vidal@univ-lyon1.fr*
[b] *Department of Organic Chemistry, University of Debrecen, POB 400, H-4002 Debrecen, Hungary*
[c] *Montpellier University, EA7288, Biocommunication in cardiometabolism (BC2M), Montpellier, France*
[d] *INSERM U1040, Montpellier, France*
[e] *Montpellier University, UMR-1040, Montpellier, France*
[f] *Institute of Medical Chemistry, University of Debrecen, POB 7, Nagyerdei krt. 98, H-4012 Debrecen, Hungary*

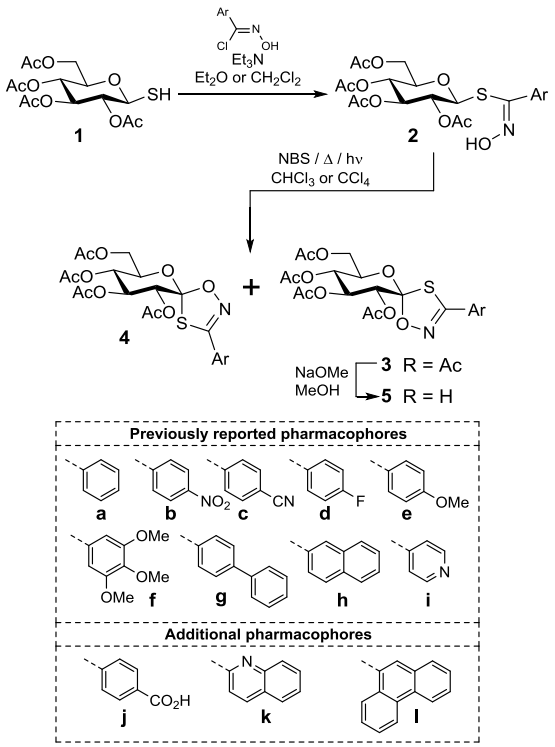
Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

efficient glucose derived inhibitors belong to three main compound categories: glucopyranosylidene-spiro-heterocycles,[14-17] *N*-acyl-*N*'-β-D-glucopyranosyl ureas,[14, 15] and *C*-β-D-glucopyranosyl heterocycles.[18-26] In each category several inhibitors have submicromolar and even low nanomolar inhibitor constants. Some of these glucose analog GP inhibitors (GPIs) were subjected to various physiological investigations to show significant blood sugar diminishing effects in streptozotocin-induced diabetic rats,[27] restoration of whole body insulin sensitivity,[28] triggering of mitochondrial oxidation and mTORC2 (mammalian target of rapamycin complex 2) signaling,[29] and improvement of pancreatic β-cell function.[30]

Among the anomeric spirocycles the most efficient inhibitors are the glucopyranosylidene-spiro-isoxazolines[31, 32] (K_i = 0.63 μM against RMGP*b* for a 2-naphthyl derivative) and the glucopyranosylidene-spiro-oxathiazoles[33] (K_i = 0.16 μM against RMGP*b* for a 2-naphthyl derivative) while their xylopyranosylidene counterparts remained inactive.[34] The glucose derived isoxazolines were shown to have significant inhibitory effects in rat and human hepatocytes, and also diminished hepatic glucose production in Zucker *fa*/*fa* rats by single dose oral administration.[35] In this paper a new synthesis of the glucopyranosylidene-spiro-oxathiazoles, their *in vitro* enzymatic evaluation, as well as cellular and *in vivo* evaluations of the best inhibitor as an antihyperglycaemic agent are described.

Synthesis

Our previously explored strategy[36] was developed for the synthesis of glycosylidene-spiro-oxathiazoles. The key step was the oxidative spirocyclization of *O*-peracetylated glycosyl hydroximothioates[37] (e.g. **2**) to glycosylidene-spiro-oxathiazoles **3** and **4** (Scheme 1), a transformation achieved readily upon treatment with NBS in refluxing halogenated solvents (CCl_4, $CHCl_3$). When the hydroximothioate moiety displayed an aryl substituent, our earlier study showed that the cyclization proceeded well whatever the configuration of the pyranose ring (D-*gluco*, D-*galacto*) or that of the anomeric centre (α/β). Moreover, the cyclization was stereoselective, yielding preferentially 1*S*-spiro-oxathiazoles in the D-*gluco*, D-*galacto* series. Later on, this methodology was applied for preparing D-*gluco*[33, 38] or D-*xylo*[34] analogues. In this last series, the cyclization was shown to occur with the opposite stereoselectivity, thus favouring formation of the 1*R*-configured spiro-oxathiazoles. The compounds studied in more details displayed the aromatic pharmacophores **a**-**i** (Scheme 1).[33, 38] For the first step of the sequence, earlier studies[37] showed that, under basic conditions, 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose **1** reacted readily with hydroximoyl chlorides to afford the corresponding glucosyl hydroximothioates **2**. For improving access to glucosinolates,[39] a procedure involving the *in situ* formation of an oximoyl chloride from the oxime using inexpensive bleach, which is then reacted directly under basic conditions with thioglycopyranose has been reported. However, comparison of these procedures showed that the conventional method to hydroximothioates resulted in higher yields.[34] Therefore, the two-step conventional method has been used to prepare new analogs in the present work (Ar = *p*-CO_2H-C_6H_4, 2-quinolinyl, 9-phenanthryl) with either hydrogen bond donors or acceptors or expanded aromatic moieties for better interactions of the aglycons in the β-pocket of the enzyme's catalytic site.



Scheme 1. Synthesis of glucose-based spiro-oxathiazoles[33, 36-38] by oxidative spirocyclization.

Glucosyl hydroximothioates precursors **2j-l** were obtained in variable yields (Table 1), their spirocyclization proceeded as generally observed, yielding preferentially the 1*S*-configured spirobicycles **3j-l**. The anomeric configurations for compounds **3j-l** were deduced from NMR spectroscopy which revealed significantly different chemical shifts for the H3 and H5 pyranosylidene protons, and the C1 spiro carbon.[33, 34, 36, 38] As noted earlier for 1*S*-configured analogs, these signals were respectively close to 5.65, 4.45, and 122.5 ppm. The protected 1*S*-spirobicylces were deacetylated in high yield under Zemplén conditions.

Enzyme kinetic studies (IC_{50} and/or K_i measurements) were then performed to evaluate whether the prepared spirobicycles were GP inhibitors. Then, the most potent candidates were further evaluated through *in vitro* and *in vivo* biological assays to demonstrate their pharmacological interest. Therefore, substantial quantities of material were needed, typically gram- to multi-gram-scale. This called for the design and development of an unprecedented route to D-glucose based spiro-oxathiazoles, with the hope that a stereoselectivity higher than that of the NBS-mediated cyclization could be achieved.

Table 1: Stereoselective cyclization of β-D-glucosyl thiohydroximates **2** to glucose-based spiro-oxathiazoles **3-4** and their deacetylation to **5**.[33, 36, 38]

	isolated yields (%)[a]			
Ar	**2**	**3**	**4**	**5**
a	90[37]	46[36]	11	90[36]
b	60[37]	33[a]	16[a]	71
c	48	15[a]	7[a]	84
d	65[37]	30[36]	16[36]	79
e	71[37]	69[a]	17[a]	79
f	85	unselective reaction		-
g	40	61[a]	18[a]	97
h	78	36[a]	16[a]	94
i	78	0	30	-
j	53	50	-	73
k	35	36	-	88
l	86	49	-	>95%

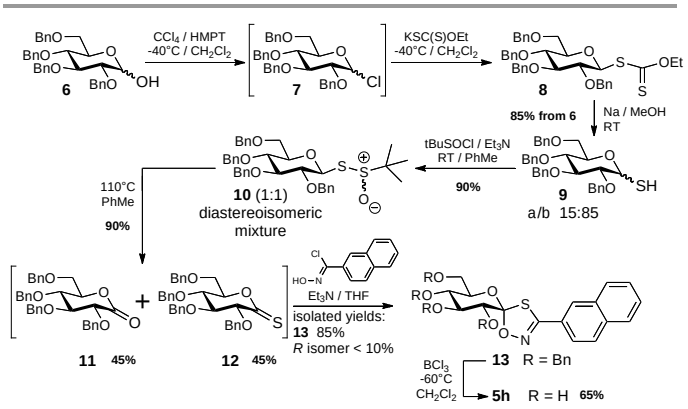
[a] Yields were calculated based on the isolated yields and on the amount of product present in the 1*RS*-mixtures (ratio measured by ^{1}H NMR) unresolved by column chromatography.

A new route to spiro-oxathiazoles by 1,3-dipolar cycloaddition of nitrile oxides to gluconothionolactone

The design of a new access to spiro-oxathiazoles was inspired by our reported synthesis of glycosylidene-spiro-isoxazolines based on 1,3-dipolar cycloaddition of nitrile oxides to *exo*-methylene glycals (pyranoid *exo*-glucals).[31, 32, 35] Concerted reactions are advantageous as regard to atom economy, experimental conditions and yield, so they are of particular interest in carbohydrate chemistry. We and others could demonstrate that such cycloadditions occurred with a high stereocontrol, the dipole attacking the *exo*-methylene carbohydrate from the α-face of the D-glucopyranosylidene ring,[40] while for steric and electronic reasons, regioselectivity was equally high, favouring attack of the anomeric carbon by the oxygen atom of nitrile oxides in all cases studied. The more complex cycloaddition of nitrones (*E* and/or *Z* configurations, *endo* and/or *exo* transition states) to pyranoid *exo*-glycals led to mixtures of cis/trans and α/β products, but the α-face approach usually predominated, or was even exclusive in some cases.[41, 42]

Based on this 1,3-dipolar cycloaddition analogy, the known D-glucono-δ-thionolactone **12**[43-45] appeared as the required precursor towards spiro-oxathiazoles. Moreover, benzylated D-glucono-δ-thionolactone **12** appeared to be a readily accessible substrate, based on the one-pot two-step synthetic route by the group of Fairbanks.[46, 47] 1-Thiosugars reacting with *tert*-butyl-sulfinyl chloride in the presence of triethylamine were converted into glycosyl *S*-*tert*-butyl thiosulfinates (within 10 min at room temperature), which upon thermolysis (10-20 min in refluxing toluene) yielded various glycono-thionolactones, and in particular D-glucono-δ-thionolactone **12** in a notable 85% yield.[47] Glycono-δ-thionolactones have been shown to react with dienes, diazoalkanes, and carbenoids,[48] and the thermolysis of the obtained dihydro-1,2,3-, and -1,3,4-thiadiazoles has been investigated.[49]

We developed this new route for synthesizing compound **5h** on the multi-gram scale considering its remarkable inhibitory properties. The direct synthesis of dithiocarbonate **8** from the commercially available hemiacetal **6** using tosyl chloride and potassium *O*-ethyl dithiocarbonate under phase-transfer conditions[50] gave in our hands poor results. However, the two-step protocol in one-pot reported by Vasella[51] (treatment of hemiacetal **6** with CCl_4 and HMPT, then with potassium *O*-ethyl dithiocarbonate at -40°C, for which basic conditions dictate the choice of base-stable protective groups) afforded the desired dithiocarbonate **8** via the intermediate chloride **7** (Scheme 2). Subsequent methanolysis delivered the 2,3,4,6-tetra-*O*-benzyl-1-thio-D-glucopyranose **9** in a 85% overall yield. Thiol **9** was reacted with *tert*-butyl-sulfinyl chloride[47] to afford the β-configured thiosulfinate **10** in 90% yield as a ~1:1 S(*S*),S(*R*) diastereoisomeric mixture. While mass spectrometry displayed a main peak as expected (*m/z* = 661 $[M+H]^+$), the formed diastereoisomers **10** were visible as two close spots on TLC plates. They were purified by column chromatography but could not be fully separated in pure form. The ^{1}H NMR spectra of this mixture displayed two singlets for the diastereotopic *tert*-butyl groups resonating at 1.40 and 1.58 ppm, respectively.



Scheme 2. New access to 1*S*-glucopyranosylidene-spiro-oxathiazole by nitrile oxide-thionolactone 1,3-dipolar cycloaddition

Following Fairbanks' procedure, thermal elimination was carried out in refluxing toluene with either the crude thiosulfinate **10** (two-step one-pot process) or the purified diastereoisomeric mixture, but disappointingly, the yields recorded for the thionolactone **12** were around 45%, significantly lower than the 85% yield reported.[47] As a byproduct, the glucono-δ-lactone **11** was identified and actually isolated in an equal amount when the reaction time was increased thus allowing for the complete conversion of both thiosulfinate diastereoisomers **10**. Formation of an undesired analogous δ-lactone was observed by Fairbanks (~20% yield) during the thermal elimination at 120°C of a *rhamno*-configured thiosulfinate.[46] Presence of di-*tert*-butylthiosulfinate as a byproduct (*vide infra*, text and Fig. 3, Fig. 4) has been noted as another limitation of the method.[47] These disappointing and intriguing observations led to repeated trials for enhancing the yield of thiolactone **12**, but without success. TLC monitoring of the reaction revealed that the more mobile thiosulfinate diastereoisomer **10** was converted faster than the other one, and while its spot decreased visually on TLC plates, that of the thionolactone **12** increased. Seemingly, after complete conversion of the more reactive thiosulfinate, there was no increase in the amount of D-glucono-δ-thionolactone **12** from the qualitative analysis of TLC, and disappointingly, the isolated yields never exceeded 45%. Analysis of MS data collected for the fractions recovered from column chromatography confirmed the formation of D-gluconolactone **11** (*m/z* $[M+Na]^+$ = 561), and showed the presence of an unidentified product (*m/z* = 607 $M+H^+$) (*vide infra*, text and Fig. 1). After inquiring about these discrepancies, Prof. A.J. Fairbanks kindly provided us with the investigator's original report. It appeared that the 85% yield reported[47] for the thionolactone **12** was overestimated as, from the report, it corresponded to a crude product « *contaminated with tert-butyl species, possibly di-tert-butyl thiosulfinate. Yields projected to approximately 54-56%* ». Presence of byproducts, allegedly mixed disulfides or disproportionation products, was also supposed, but not proven. A note clearly stated that the yield estimated by ^{1}H NMR for the thionolactone **12** were 54 and 56% (close to the 43-53% yields observed in the D-*galacto* series),[47] so that these data corroborated ours, suggesting the occurrence of at least one unwanted reaction detrimental to the yield of the desired glycono-thionolactone.

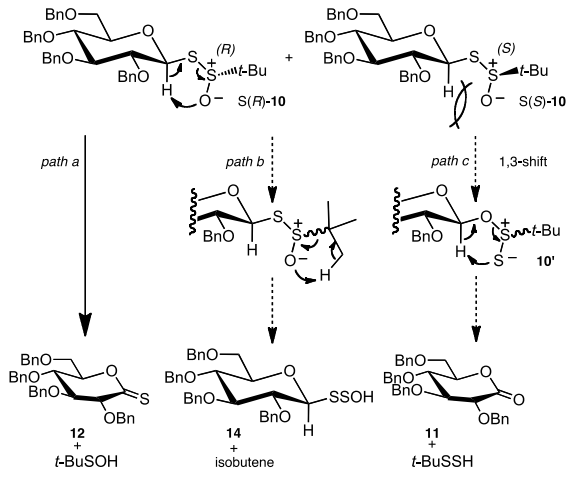
D-Glycono-thionolactones are subject to epimerization[47] at C-2 or 2,3-elimination[43] under basic conditions. Therefore, D-glucono-δ-thionolactone **12** was often used without further purification for the 1,3-dipolar cycloaddition. With the crude thionolactone **12**, this step proceeded smoothly in the presence of 2-naphthyl hydroximoyl chloride to afford the spiro-oxathiazole **13** in high yield (~95%) and stereoselectivity (9:1 *S*/*R* mixture). The diastereoisomeric mixture could be resolved by silica gel column chromatography to afford the desired spiro-oxathiazole **13** with a *S*-configuration at the spiro-anomeric center (85% isolated yield) and the minor *R*-epimer (<10% yield).

Not surprisingly, the final debenzylation step proved to be problematic, and standard hydrogenolysis in the presence of palladium did not proceed at all, while Et_3SiH/I_2[52] resulted in partial deprotection only. Treatments with bromine or NBS and water under various free radical conditions (NBS, CCl_4, hν, with or without $CaCO_3$; $NaBrO_3/Na_2S_2O_4$) resulted in complex mixtures, due to partial debenzylations,[53, 54] and/or formation of oxidized products as evidenced by mass spectrometry: in addition to the expected fragments corresponding to the partial or total debenzylation, fragments corresponding to M+14 indicated the oxidation of benzyls to benzoates, while others (M+16 and M+32) suggested oxidation to sulfoxides and sulfones. Fortunately, debenzylation could be achieved by treatment with BCl_3 at –60°C[55] with careful TLC monitoring to quench the reaction immediately after disappearance of the starting material to avoid further unwanted reactions. Purification by column chromatography delivered the desired final *O*-unprotected spiro-oxathiazole **5h** (815 mg, 65% yield).

In conclusion, the designed 1,3-dipolar cycloaddition approach between 2-naphthyl nitrile oxide and perbenzylated D-glucono-thionolactone **12** was most efficient for synthesizing the corresponding D-glucose-based 1*S*-3-(2-naphthyl)-spiro-1,4,2-oxathiazole **13**. Both regio-, and stereo-selectivities were excellent. In contrast to literature reports, the limiting step of this approach was the synthesis of tetra-*O*-benzyl D-glucono-δ-thionolactone **12** by thermolysis of glucopyranosyl *tert*-butyl sulfinate precursors **10**. The discrepancies between our results and those of Fairbanks as reported and detailed in an internal document prompted the investigation of the thermolysis mechanism by DFT calculations to better understand the possible path to the desired thionolactone.

Proposed mechanisms for the thermal elimination of thiosulfinates 10 to thionolactone 12

Various possible modes of reaction were envisaged (Scheme 3) since *tert*-butyl thiosulfinates may undergo thermal elimination by hydrogen atom abstraction through five-membered cyclic transition states (TS). Glycono-thionolactones being reportedly obtained sometimes in excellent yields,[46, 47] elimination involving the anomeric proton (*path a*) was privileged (anomeric effect), thus delivering the desired thionolactone **12** and *tert*-butyl sulfenic acid. However, elimination might also involve one of the nine equivalent hydrogen atoms of the *tert*-butyl moiety (*path b*), which would afford isobutene and the 3-D-glucopyranosyl-thiosulfoxylic acid **14**[56] (other name: 3-D-glycopyranosyl-1-oxa-trisulfane). Considering early[56] and recent[57, 58] reports on the thermolysis of di-*tert*-butylthiosulfinate (also termed di-*tert*-butyldisulfane-*S*-oxide, *t*BuS(O)S*t*Bu),[57] these speculations were reasonable, but the total weight of lactones **11** and **12** (*ca* 90%, 1:1 ratio) measured after full conversion of both thiosufinates indicated that path *b* was only a marginal process.



Scheme 3: Possible reaction paths for the thermolysis of D-glucosyl *tert*-butyl thiosulfinates **10**

The puzzling formation of D-gluconolactone **11** might be due to hydrolysis by water present in trace amount[59] or to adventitious reactions, as discussed recently,[60] but since **11** was detected on TLC plates even at the initial stage of the reaction, and as use of a purified mixture of diastereomeric thiosulfinates **10** led to similar lactone distribution, this hypothesis was uncertain. As the glucopyranosyl *tert*-butyl thiosulfinates **10** must achieve a favourable 5-membered cyclic TS for the thermal elimination to proceed, and because of probable steric clashes between the *tert*-butyl group and the 2-*O*-benzyl group for the S(*S*)-diastereoisomer, most probably the S(*R*)- and S(*S*)-*tert*-butyl thiosulfinates underwent elimination at different rates, the (*R*)-diastereoisomer reacting faster to deliver thionolactone **12** through path *a*. Still, another hypothesis was needed to explain the presence of glyconolactone **11**. The thermal rearrangement of α-configured glycopyranosyl xanthates to α-glycosyl dithiocarbonates has been reported to proceed without inversion of configuration at moderate temperatures (50-65°C).[61] This reported example of the Freudenberg-Schönberg rearrangement[62, 63] was supposed to occur by an intramolecular 1,3-shift through a 4-membered cyclic TS.[61] An analogous thermal rearrangement could occur with the less reactive S(*S*) thiosulfinate (path *c*), and the rearranged product with a *O*-glycosidic bond [OS(S) moiety] would be susceptible to afford by thermal elimination the lactone **11** and *tert*-butyl-thiosulfenic acid (*t*BuSSH). Interestingly, the relative enthalpies for thiosulfinic acid isomers [HS(O)SH, and HS(S)OH] estimated by theoretical studies at 298 K in the gas phase were found to be almost the same,[64, 65] suggesting that isomers **10** and **10'** might have similar energies. While the rearrangement of xanthates to dithiocarbonates is stability driven, the proposed pathway to gluconolactone **11** might benefit from the activation of the anomeric center (rearrangement favoured) and, in lactone **11,** the stabilization of C=O bond (162 kcal.mol^{-1}) by ca. 50 kcal.mol^{-1} compared to the C=S thiocarbonyl bond (115 kcal.mol^{-1}) in thionolactone **12**.[66] Eventually, calculations were carried out to identify the reaction pathway leading to the gluconolactone byproduct (hydrolysis *versus* thermal rearrangement followed by elimination).

DFT Calculations

As a representative model, the tetra-*O*-methyl analog of **10** was chosen (See supporting information for further details) to have reasonable computational time. The numbering of molecules applied is the same for both *O*-benzyl- (synthetic work) and *O*-methyl-protected (computational approach) analogues, but using italicized types in the last case.

Our calculations addressed first the three reaction pathways mentioned above (Scheme 3), namely the two possible direct internal eliminations (Ei) (*path a, path b*), then *path c*, in which an internal O,S-rearrangement by 1,3-shift was supposed to occur prior to the Ei elimination. The stereochemistry at the sulfur atom did not influence the calculations and the results are presented here for a single diastereoisomer at the sulfur atom.

Starting from ***10***, the two divergent internal eliminations (Ei) discussed above were envisaged (Fig. 1). Deprotonation of the anomeric position (*path a*) was found to be easy (**TS-a**, $\Delta G^{\#}$ = 21.2 kcal.mol^{-1}), leading to the D-glucono-δ-thionolactone ***12*** and formation of *tert*-butyl sulfenic acid. In the case of *path b*, which involves one of the nine hydrogen atoms of the *tert*-butyl groups, and leads to product ***14*** and isobutene, a higher activation barrier of $\Delta G^{\#}$ = 24.9 kcal.mol^{-1} (**TS-b**) was calculated. This is in agreement with the synthetic work, as the thermal δ-elimination delivered the thionolactone **12** in moderate yield while the benzyl-protected product **14** was not observed.

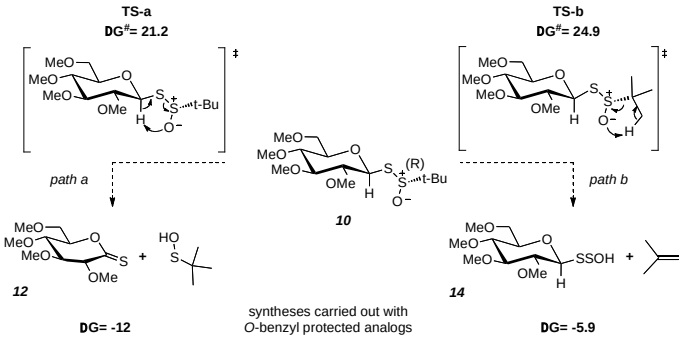


Figure 1. Calculations for Ei eliminations, involving either anomeric or *tert*-butyl protons (*paths a,b*). ΔG in kcal.mol^{-1}

As a possibility for the puzzling formation of **11** from **10**, *path c* was envisioned (Fig. 2). For this two-step pathway, a 1,3 shift of the *tert*-butyl thiosulfinate group led first to ***10'*** (**Ts-c**, $\Delta G^{\#}$ = 36.3 kcal.mol^{-1}), which subsequently could react through an Ei elimination (**TS-d**, $\Delta G^{\#}$ = 10.9 kcal.mol^{-1}) leading to ***11*** and *tert*-butyl thiosulfenic acid. Due to the high activation energy of **TS-c**, this pathway can thus be excluded.

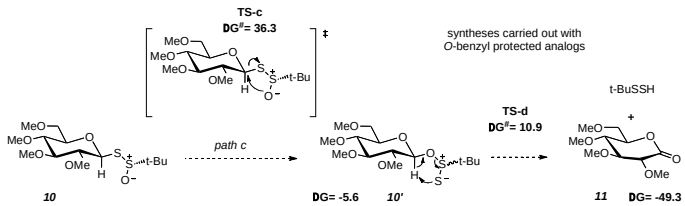


Figure 2. 1,3 Shift of the thiosulfinate group in ***10***, followed by Ei elimination (*path c*). ΔG in kcal.mol^{-1}

At this point, since the formation of the experimentally observed D-gluconolactone **11** cannot be explained through *path c*, we wondered whether lactone **11** could be formed due to hydrolysis of D-glucono-thionolactone **12**. In this event, since the reaction was conducted under anhydrous conditions, water had to be formed as the reaction proceeded. Interestingly, Block *et al*.[67] reported the dismutation of *tert*-butyl sulfenic acid, giving rise to *tert*-butyl-2-methyl-propane-2-sulfinothioate (or thiosulfinate) and water (0.5 eq. each, Fig. 3). The computed thermodynamics for the dismutation (ΔG = -4.4 kcal mol^{-1}) were in agreement with the experimental observations of Block.[67] Subsequently, water, assisted by the coordination of the thiosulfinate, can trigger the hydrolysis of compound ***12*** through **TS-e** (ΔG$^{\#}$ = 30.6 kcal.mol^{-1}), providing gluconolactone ***11*** and hydrogen sulfide (0.5 eq. each). This proposed mechanism is in agreement with the experimental formation of lactones **11** and **12** in a 1:1 ratio.

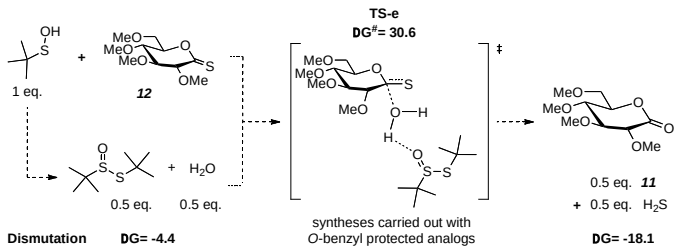


Figure 3. Dismutation of *tert*-butyl sulfenic acid, followed by hydrolysis of thionolactone ***12***. ΔG in kcal.mol^{-1}

To further confirm that lactone ***11*** came from the hydrolysis of thionolactone ***12*** (Fig. 4), we computed the attack of di-*tert*-butyl thiosulfinate on substrate ***12*** (Fig. 4). **TS-f** (ΔG$^{\#}$ = 41.5 kcal.mol^{-1}) could lead indeed to the formation of ***11*** and *tert*-butyl-2-methylpropane-2-sulfinodithioate (or dithiosulfinate) without the assistance of a water molecule. Since the ΔΔG$^{\#}$ between **TS-e** and **TS-f** was 10.9 kcal.mol^{-1} in favor of the former, water hydrolysis pathway was confirmed to be the most energetically reasonable route.

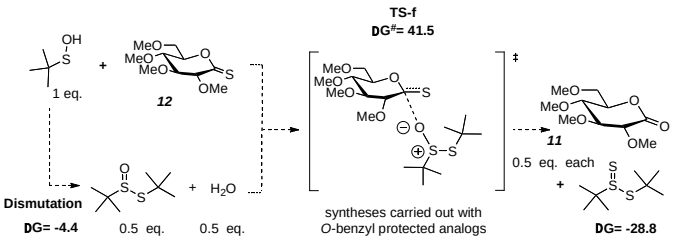


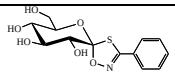
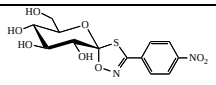
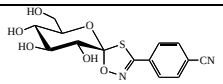
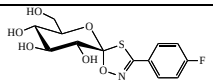
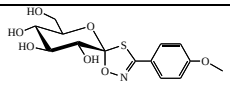
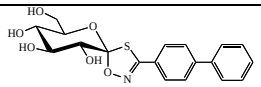
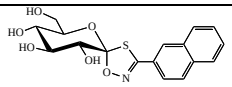
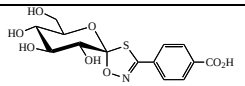
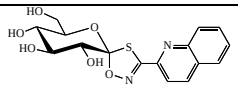
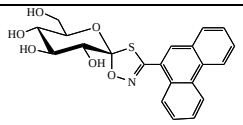
Figure 4. Dismutation of *tert*-butyl sulfenic acid, and di-*tert*-butyl thiosulfinate attacking thionolactone ***12***. ΔG in kcal.mol^{-1}

In summary, these calculations showed a good agreement with the synthetic results, as they indicated that the thermal elimination via 5-membered transition states preferentially involved the anomeric proton rather than one of the nine *tert*-butyl hydrogen atoms, thus yielding the target gluconothionolactone. Simultaneous formation of gluconolactone through a 1,3-shift of the thiosulfinate moiety, followed by Ei can be ruled out. Based on the reported dismutation of *tert*-butyl sulfenic acid which yielded di-*tert*-butyl thiosulfinate and water (0.5 eq. each), the calculations predicted that ***12*** can be hydrolysed into ***11*** (0.5 eq.). Thus, in order to optimize the yield of the target gluconothionolactone **12**, water, formed while the thermal elimination proceeds, has to be trapped by efficient means, for example by adding activated molecular sieves to the reaction medium.[46, 47]

Enzyme kinetics

Previous structure-activity relationship studies[15, 33, 38] revealed that phenyl oxathiazole **5a** was a moderate inhibitor of GP (K_i = 26 μM). Other para-substituted phenyl analogues (R = phenyl, NO_2, CN) exhibited lower inhibitory properties with IC_{50} values in the high micromolar range for **5b**,**c**,**g**. The para-fluoro and para-methoxy analogues **5d** and **5e** had similar and slightly better inhibitory potential than **5a** (K_i = 28 and 8 μM, respectively). As observed for several other classes of glucose-based GP inhibitors,[14, 15, 32, 35, 68] the 2-naphthyl oxathiazole derivative **5h** was the best inhibitor among the series of compounds studied, and one of the best among glucose-based GP inhibitors. Substitution by a para-carboxy group on the phenyl (**5j**) resulted in a good inhibitory potency (K_i = 238 μM), while the 2-quinolinyl analogue **5k** was more potent (K_i = 26 μM), although much less potent than the 2-naphthyl analogue **5h**. In spite of the size of the 9-phenanthryl residue, compound **5l** displayed moderate inhibitory properties.

Table 2: Kinetic data measured for spiro-oxathiazoles **5a-e,g,h,j-l** for the inhibition of RMGP*b*

Compound	Structure	IC_{50} or K_i values (μM)
5a		K_i = 26 ± 2.1[38]
5b		IC_{50} = 250[33]
5c		IC_{50} = 700[33]
5d		K_i = 28 ± 2.8[38]
5e		K_i = 8 ± 0.9[38]
5g		IC_{50} = 250[33]
5h		K_i = 0.16 ± 0.04[33, 38]
5j		K_i = 238.5
5k		K_i = 26.02
5l		IC_{50} = 32.4 ±1.1

In vitro pharmacological evaluations

Guided by the kinetic results and for an in depth evaluation of his inhibitory property, the glucose-based GP inhibitor **5h** with the best K_i value was assayed *in vitro* with rat and human hepatocytes in primary culture (see Table S1). The potent *in vitro* GP inhibitor 1,4-dideoxy-1,4-imino-D-arabinitol (DAB, K_i = 400 nM)[69] was selected as the reference compound for its established *in vivo* activity in a GP-dependent glycaemia study.[3, 69, 70] An evaluation of glucose release after glucagon stimulation in primary rat hepatocytes was performed. Further evaluation in human hepatocytes for the candidate **5h** was accomplished by measuring both glucose release and intracellular glycogen for an assessment of species specificity.

IC_{50} Values calculated for glucose release (product of GP-mediated glycogen depolymerisation) or intracellular glycogen content (substrate of GP-mediated glycogen depolymerisation) are similar (2-5 μM, Table 3) in human hepatocytes. In the studied cellular model, this result demonstrates that compound **5h** affected glycogenolysis via GP inhibition.

Table 3: *In vitro* IC_{50} for compound **5h** based on glucose release and intracellular glycogen content after glucagon stimulation in rat and human primary hepatocytes in primary cultures

Compound	Glucose release IC_{50} (μM)[a]		Intracellular glycogen IC_{50} (μM)[b]
	Rats	Humans	
5h	6.7	2.08 ± 1.51	4.88 ± 1.20
DAB	8	2.17 ± 1.30	1.10 ± 1.11

[a] IC_{50} values are average of three to five measurements (see supporting information).

[b] IC_{50} values are average triplicate measurements for three human hepatocyte cultures (see supporting information).

The concentration-response curves obtained for glucose release (Fig. 5) and observed intracellular glycogen content (Fig. 6) provided a basis for a comparison against DAB. Compound **5h** performed slightly better than DAB in terms of concentrations to obtain 50% inhibition on glucose release in rat and human hepatocytes. Such concentrations are compatible with potential pharmacological applications.

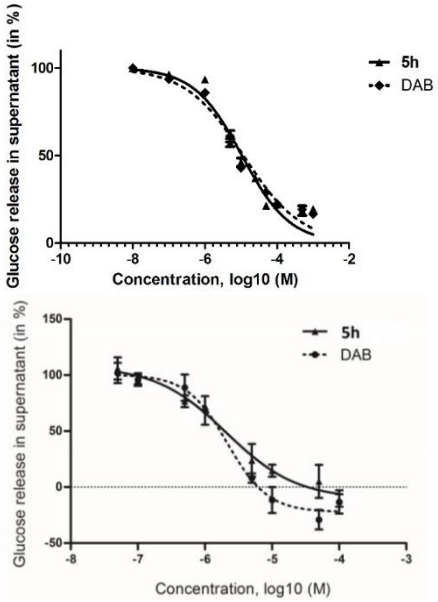


Figure 5: Glucose release from rat and human hepatocytes after glucagon stimulation *in vitro* measured in the presence of compound **5h** *versus* DAB as the reference compound. Top: Rat hepatocytes, bottom: human hepatocytes

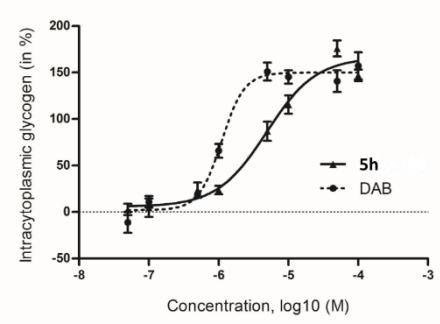


Figure 6: Intracellular glycogen content in human hepatocytes after glucagon stimulation *in vitro* measured in the presence of compounds **5h** *versus* DAB as the reference compound

In vivo pharmacological evaluations

The glucagon challenge test in the Zucker *fa/fa* rat model was performed to measure the glucose-lowering effect *in vivo* with for compound **5h**. This animal model, displaying hyperphagia and insulin resistance with hyperinsulinemia, was chosen due to its high hepatic glycogen content. We used glucagon (200 µg/kg, in a single subcutaneous "SC" administration) as hyperglycemic agent. A dose-dependent effect was clearly observed when compound **5h** was introduced orally in a single administration (Figure 7).

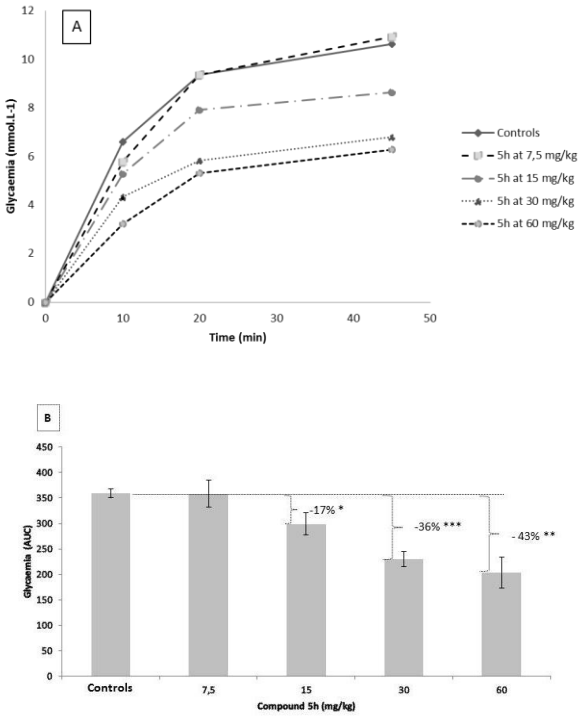


Figure 7. Hepatic glucose production of the unprotected compound 5h obtained in Zucker fa/fa rats in acute in vivo glucagon challenge. (A) Kinetics of glucose output for 45 min after acute glucagon administration in mmol/L. (B) Area Under the Curves (AUC) corresponding to glucose release for 45 min after acute glucagon administration. *p<0.05 for 15 mg/kg, ***p<0.001 for 30 mg/kg and **p<0.01 for 60 mg/kg

Liver glucose output kinetics (Fig. 7A) and their areas under the curves (AUC) for 45 min (Fig. 7B) pointed to a dose-dependent decrease in liver glucose production ranging from 7.5 to 60 mg/kg (Fig. 7B). The rate of endogenous glucose production being elevated in type 2 diabetes renders this effect on liver output relevant for therapeutic applications.[71] Compound **5h** was then selected for a subchronic oral administration with a dose corresponding to the first significantly effective in the acute *in vivo* glucagon challenge test (30 mg/kg). In a glucagon challenge test performed after 4 days of treatment, a nearly 33% reduction of hepatic glucose production ($p<0.05$) was observed (Fig. 8A-B). It is worth pointing out that the activity of glycogen phosphorylase was preserved, in spite of the subchronic treatment, since hepatic glucose production was not further reduced.

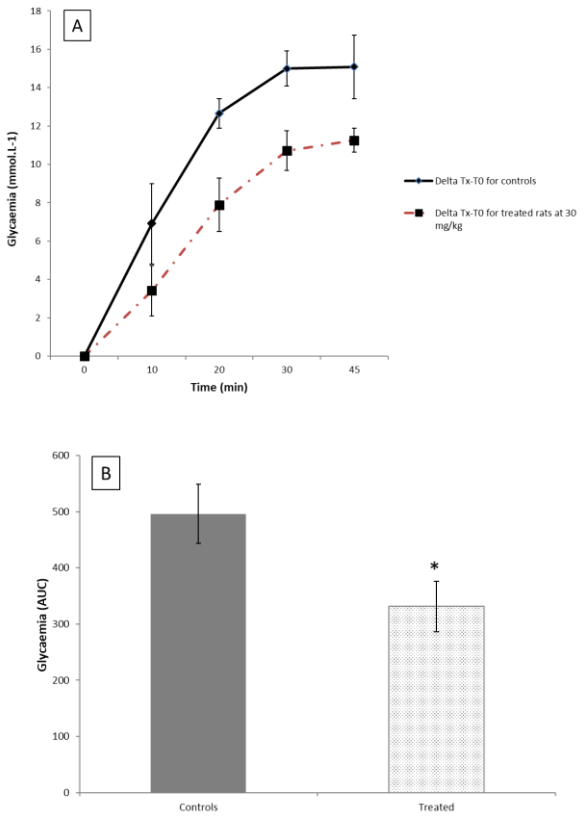


Figure 8. Plasma glucose concentration of the unprotected compound **5h** obtained in Zucker *fa/fa* rats subjected to *in vivo* glucagon challenge after subchronic administrations. (A) Kinetics of glucose output for 45 min after acute glucagon administration in mmol/L. (B) Area Under the Curve (AUC) corresponding to glucose release for 45 min after acute glucagon administration. Plasma glucose concentrations were expressed as variations (delta), meaning that the basal value of glycaemia was substracted for each individual value and for each rat.

As a summary, the *in vitro* and *in vivo* pharmacological effects of glucose-based spiro-oxathiazole **5h** were analyzed and they validated its potent GP inhibition both *in vitro*, in cell assays and in a diabetic rat model (*in vivo*). In rat and human hepatocytes, compound **5h** reduced glucagon-stimulated glucose output through glycogenolysis inhibition (Table 3, Fig.

5). No species specificity could be observed since the potency of compound **5h** for inhibition of glucose output were comparable between rat and human cells (Table 3) as previously reported.[72, 73] Compound **5h** was efficient *in vivo* in the Zucker *fa/fa* rat model of insulin resistance and a dose-dependent decrease of hepatic glucose output to a maximal 43% was observed (Fig. 7). This glucose lowering effect allows for the design of therapeutic applications due to a high rate of endogenous glucose production in type 2 diabetes.[71] Indeed, hepatic glucose production tends to match the values usually obtained in Wistar normal rat.

The complete suppression of glucagon-induced glucose output in the presence of DAB appears in sharp contrast with the moderate level of inhibition of GP.[70] The incomplete inhibition of GP by compound **5h** is advantageous for limiting potential side effects. In addition, the absence tissue-specificity between liver and muscle GP isoforms could impair exercise-mediated metabolism of muscle glycogen. Preserving a minimum level of activity will limit this risk, although the selection of a liver-specific inhibitor preferred for long-term therapy remains a valuable research challenge.[74] Even though hypoglycaemia could be a detrimental consequence of GP inhibition, hepatic glucose production was not further reduced after repeated administrations of compound **5h**, indicating a residual activity of GP thus limiting the risk of hypoglycaemia (Fig. 7).

Conclusion

Type 2 diabetes is a major public health problem and design of glycogen phosphorylase inhibitors targeting the catalytic site of the enzyme appears as a promising strategy for a better control of hyperglycaemia. An especially potent group of GP inhibitors is represented by some glucopyranosylidene-spiro-heterocycles, among them spiro-oxathiazoles. A new synthesis has now been elaborated for this valuable class of compounds by the 1,3-dipolar cycloaddition of an aryl nitrile oxide to a glucono-thionolactone affording in one step the spiro-oxathiazole moiety. The thionolactone was obtained from the thermal rearrangement of a thiosulfinate precursor according to Fairbanks' protocols, although with a revisited outcome and also based on DFT calculations. The 2-naphthyl substituted glucose-based spiro-oxathiazole **5h** has recently been identified as one of the most potent GP inhibitors with a K_i of 160 nM against RMGPb. Further evaluation *in vitro* using rat and human hepatocytes demonstrated that compound **5h** was promising as a potential anti-hyperglycaemic druggable compound as it performed better than DAB used as a positive control. Investigation in Zucker *fa/fa* rat model in acute and subchronic assays further confirmed the potency of compound **5h** since it lowered blood glucose levels by ~36% at 30 mg/kg and ~43% at 60 mg/kg. This indicates that compound **5h** can be considered as a powerful anti-hyperglycemic agent in the context of type 2 diabetes. The present study is one of the few *in vivo* investigations for glucose-based GP inhibitors and provides data in animal models for such drug candidates. This study is also an example of molecular diversity-oriented strategies to identify the fittest inhibitor of GP and from the identification of this candidate, multigram-scale preparations for *in vivo* validation called for a robust and faster synthetic route to the target glucose-based spiro-oxathiazole.

Conflicts of interest

There are no conflicts to declare

Acknowledgements

Financial support from CNRS and the Hungarian Academy of Sciences, University Claude Bernard Lyon 1, the French Agence Nationale de la Recherche (ANR-08-BLAN-0305) as well as the Hungarian National Research, Development and Innovation Office (OTKA K109450), and the EU co-financed by the European Regional Development Fund under the project GINOP-2.3.2-15-2016-00008 are gratefully acknowledged. We are grateful to Prof. A.J. Fairbanks for providing the internal report of B. Wilkinson (January 2008) on the research carried out under his supervision. CCIR-ICBMS (UCBL) is gratefully acknowledged for the allocation of computational resources.

Notes and references

1. Z. H. Israili, *Am. J. Ther.*, 2011, **18**, 117-152.
2. N. Kerru, A. Singh-Pillay, P. Awolade and P. Singh, *Eur. J. Med. Chem.*, 2018, **152**, 436-488.
3. B. Andersen, A. Rassov, N. Westergaard and K. Lundgren, *Biochem. J.*, 1999, **342**, 545-550.
4. R. Kurukulasuriya, J. T. Link, D. J. Madar, Z. Pei, J. J. Rohde, S. J. Richards, A. J. Souers and B. G. Szczepankiewicz, *Curr. Med. Chem.*, 2003, **10**, 99-121.
5. B. R. Henke, Inhibition of glycogen phosphorylase as a strategy for the treatment of type 2 diabetes mellitus in *New Therapeutic Strategies for Type 2 Diabetes: Small Molecule Approaches*, ed. R. M. Jones, RSC Drug Discovery, Dorset Press, Dorchester, UK, 2012, Chap. 12, pp. 324-365.
6. J. Hayes, A. Kantsadi and D. Leonidas, *Phytochem. Rev.*, 2014, **13**, 471-498.
7. L. Somsák, K. Czifrák, M. Tóth, E. Bokor, E. D. Chrysina, K. M. Alexacou, J. M. Hayes, C. Tiraidis, E. Lazoura, D. D. Leonidas, S. E. Zographos and N. G. Oikonomakos, *Curr. Med. Chem.*, 2008, **15**, 2933-2983.
8. M. Donnier-Maréchal and S. Vidal, *Exp. Opin. Ther. Pat.*, 2016, **26**, 199-212.
9. J. M. Fernández-Novell and M. Díaz-Lobo, *Appl. Biochem. Biotechnol.*, 2018, **184**, 909-918.
10. T. Fischer, S. M. Koulas, A. S. Tsagkarakou, E. Kyriakis, G. A. Stravodimos, V. T. Skamnaki, P. G. V. Liggri, S. E. Zographos, R. Riedl and D. D. Leonidas, *Molecules*, 2019, **24**, 1322.
11. V. Maffeis, K. Mavreas, F. Monti, M. Mamais, T. Gustavsson, E. D. Chrysina, D. Markovitsi, T. Gimisis and A. Venturini, *Phys. Chem. Chem. Phys.*, 2019, **21**, 7685-7696.
12. M. Díaz-Lobo, A. L. Concia, L. Gómez, P. Clapés, I. Fita, J. J. Guinovart and J. C. Ferrer, *Org. Biomol. Chem.*, 2016, **14**, 9105-9113.
13. E. D. Chrysina, *Mini-Rev. Med. Chem.*, 2010, **10**, 1093-1101.
14. L. Somsák, *C. R. Chimie*, 2011, **14**, 211-223.

15. J.-P. Praly and S. Vidal, *Mini-Rev. Med. Chem.*, 2010, **10**, 1102-1126.
16. K. Szabó, S. Kun, A. Mándi, T. Kurtán and L. Somsák, *Molecules*, 2017, **22**, 1760.
17. K. E. Szabó, E. Kyriakis, A.-M. G. Psarra, A. G. Karra, Á. Sipos, T. Docsa, G. A. Stravodimos, E. Katsidou, V. T. Skamnaki, P. G. V. Liggri, S. E. Zographos, A. Mándi, S. B. Király, T. Kurtán, D. D. Leonidas and L. Somsák, *J. Med. Chem.*, 2019, **62**, 6116-6136.
18. É. Bokor, S. Kun, D. Goyard, M. Tóth, J.-P. Praly, S. Vidal and L. Somsák, *Chem. Rev.*, 2017, **117**, 1687-1764.
19. S. Kun, J. Begum, E. Kyriakis, E. C. V. Stamati, T. A. Barkas, E. Szennyes, É. Bokor, K. E. Szabó, G. A. Stravodimos, Á. Sipos, T. Docsa, P. Gergely, C. Moffatt, M. S. Patraskaki, M. C. Kokolaki, A. Gkerdi, V. T. Skamnaki, D. D. Leonidas, L. Somsák and J. M. Hayes, *Eur. J. Med. Chem.*, 2018, **147**, 266-278.
20. S. Kun, É. Bokor, Á. Sipos, T. Docsa and L. Somsák, *Molecules*, 2018, **23**, 666.
21. E. Kyriakis, T. G. A. Solovou, S. Kun, K. Czifrák, B. Szőcs, L. Juhász, É. Bokor, G. A. Stravodimos, A. L. Kantsadi, D. S. M. Chatzileontiadou, V. T. Skamnaki, L. Somsák and D. D. Leonidas, *Bioorg. Chem.*, 2018, **77**, 485-493.
22. L. Somsák, É. Bokor, L. Juhász, S. Kun, L. Lázár, É. Juhász-Tóth and M. Tóth, *Pure Appl. Chem.*, 2019, **91**, 1159-1175.
23. D. Barr, E. Szennyes, É. Bokor, Z. H. Al-Oanzi, C. Moffatt, S. Kun, T. Docsa, Á. Sipos, M. P. Davies, R. Mathomes, T. J. Snape, L. Agius, L. Somsák and J. M. Hayes, *ACS Chem. Biol.*, 2019, **14**, 1460-1470.
24. E. Szennyes, É. Bokor, T. Docsa, Á. Sipos and L. Somsák, *Carbohydr. Res.*, 2019, **472**, 33-41.
25. E. Szennyes, É. Bokor, P. Langer, G. Gyémánt, T. Docsa, Á. Sipos and L. Somsák, *New J. Chem.*, 2018, **42**, 17439-17446.
26. É. Bokor, E. Kyriakis, T. G. A. Solovou, C. Koppány, A. L. Kantsadi, K. E. Szabó, A. Szakács, G. A. Stravodimos, T. Docsa, V. T. Skamnaki, S. E. Zographos, P. Gergely, D. D. Leonidas and L. Somsák, *J. Med. Chem.*, 2017, **60**, 9251-9262.
27. T. Docsa, K. Czifrák, C. Hüse, L. Somsák and P. Gergely, *Mol. Med. Rep.*, 2011, **3**, 477-481.
28. T. Docsa, B. Marics, J. Nemeth, C. Huse, L. Somsak, P. Gergely and B. Peitl, *Top. Curr. Med. Chem.*, 2015, **15**, 2390-2394.
29. L. Nagy, T. Docsa, M. Szántó, A. Brunyánszki, C. Hegedűs, J. Márton, B. Kónya, L. Virág, L. Somsák, P. Gergely and P. Bai, *PLoS ONE*, 2013, **8**, e69420.
30. L. Nagy, J. Márton, A. Vida, G. Kis, É. Bokor, S. Kun, M. Gönczi, T. Docsa, A. Tóth, M. Antal, P. Gergely, B. Csóka, P. Pacher, L. Somsák and P. Bai, *Br. J. Pharmacol.*, 2018, **175**, 301-319.
31. M. Benltifa, J. M. Hayes, S. Vidal, D. Gueyrard, P. G. Goekjian, J.-P. Praly, G. Kizilis, C. Tiraidis, K.-M. Alexacou, E. D. Chrysina, S. E. Zographos, D. D. Leonidas, G. Archontis and N. G. Oikonomakos, *Bioorg. Med. Chem.*, 2009, **17**, 7368-7380.
32. M. Benltifa, S. Vidal, D. Gueyrard, P. G. Goekjian, M. Msaddek and J.-P. Praly, *Tetrahedron Lett.*, 2006, **47**, 6143-6147.
33. V. Nagy, M. Benltifa, S. Vidal, E. Berzsényi, C. Teilhet, K. Czifrák, G. Batta, T. Docsa, P. Gergely, L. Somsák and J.-P. Praly, *Bioorg. Med. Chem.*, 2009, **17**, 5696-5707.
34. L. Somsák, É. Bokor, B. Czibere, K. Czifrák, C. Koppány, L. Kulcsár, S. Kun, E. Szilágyi, M. Tóth, T. Docsa and P. Gergely, *Carbohydr. Res.*, 2014, **399**, 38-48.
35. D. Goyard, B. Kónya, A. S. Chajistamatiou, E. D. Chrysina, J. Leroy, S. Balzarin, M. Tournier, D. Tousch, P. Petit, C. Duret, P. Maurel, L. Somsák, T. Docsa, P. Gergely, J.-P. Praly, J. Azay-Milhau and S. Vidal, *Eur. J. Med. Chem.*, 2016, **108**, 444-454.
36. J.-P. Praly, R. Faure, B. Joseph, L. Kiss and P. Rollin, *Tetrahedron*, 1994, **50**, 6559-6568.
37. L. Brochard, B. Joseph, M.-C. Viaud and P. Rollin, *Synth. Commun.*, 1994, **24**, 1403-1414.
38. L. Somsák, V. Nagy, S. Vidal, K. Czifrák, E. Berzsényi and J.-P. Praly, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 5680-5683.
39. S. E. Cobb, K. F. Morgan and N. P. Botting, *Tetrahedron Lett.*, 2011, **52**, 1605-1607.
40. C. Taillefumier and Y. Chapleur, *Chem. Rev.*, 2004, **104**, 263-292.
41. G. Enderlin, C. Taillefumier, C. Didierjean and Y. Chapleur, *Tetrahedron: Asymm.*, 2005, **16**, 2459-2474.
42. X. Li, R. Wang, Y. Wang, H. Chen, Z. Li, C. Ba and J. Zhang, *Tetrahedron*, 2008, **64**, 9911-9920.
43. M. Hürzeler, B. Bernet and A. Vasella, *Helv. Chim. Acta*, 1993, **76**, 995-1012.
44. D. Kahne, D. Yang, J. J. Lim, R. Miller and E. Paguaga, *J. Am. Chem. Soc.*, 1988, **110**, 8716-8717.
45. T. Belhadj and P. G. Goekjian, *Tetrahedron Lett.*, 2005, **46**, 8117-8120.
46. K. Chayajarus and A. J. Fairbanks, *Tetrahedron Lett.*, 2006, **47**, 3517-3520.
47. B. L. Wilkinson and A. J. Fairbanks, *Tetrahedron Lett.*, 2008, **49**, 4941-4943.
48. M. Hürzeler, B. Bernet, T. Mäder and A. Vasella, *Helv. Chim. Acta*, 1993, **76**, 1779-1801.
49. B. Bernet, T. Mäder and A. Vasella, *Helv. Chim. Acta*, 1997, **80**, 1260-1279.
50. W. Szeja and J. Bogusiak, *Carbohydr. Res.*, 1987, **170**, 235-239.
51. M. Hürzeler, B. Bernet and A. Vasella, *Helv. Chim. Acta*, 1992, **75**, 557-588.
52. A. Pastore, S. Valerio, M. Adinolfi and A. Iadonisi, *Chem. Eur. J.*, 2011, **17**, 5881-5889.
53. J. G. Riley and T. B. Grindley, *J. Carbohydr. Chem.*, 2001, **20**, 159-169.
54. M. Niemietz, L. Perkams, J. Hoffman, S. Eller and C. Unverzagt, *Chem. Commun.*, 2011, **47**, 10485-10487.
55. A. Dondoni and A. Marra, *J. Org. Chem.*, 2006, **71**, 7546-7557.
56. E. Block, *J. Am. Chem. Soc.*, 1972, **94**, 644-645.
57. H. P. Reisenauer, G. Mloston, J. Romanski and P. R. Schreiner, *Eur. J. Org. Chem.*, 2012, 3408-3415.
58. B. Mondal, D. Mandal and A. K. Das, *J. Phys. Chem. A*, 2011, **115**, 3068-3078.
59. W. M. Doane, B. S. Shasha, C. R. Russell and C. E. Rist, *J. Org. Chem.*, 1965, **30**, 3071-3075.
60. O. Jackowski, F. Chrétien, C. Didierjean and Y. Chapleur, *Carbohydr. Res.*, 2012, **356**, 93-103 and references therein.
61. J.-R. Pougny, *J. Carbohydr. Chem.*, 1986, **5**, 529-535.
62. A. Schönberg and L. v. Vargha, *Chem. Ber.*, 1930, **63**, 178-180.
63. G. C. Lloyd-Jones, J. D. Moseley and J. S. Renny, *Synthesis*, 2008, 661-689.
64. R. Steudel, Y. Drozdova, R. H. Hertwig and W. Koch, *J. Phys. Chem.*, 1995, **99**, 5319-5324.
65. M. Mikolajczyk, M. Cypryk and G. Krasiński, *J. Mol. Struct. THEOCHEM*, 2008, **863**, 105-110.
66. K. B. Wiberg and Y. Wang, *Arkivoc*, 2011, **v**, 45-56.
67. E. Block, *Angew. Chem. Int. Ed.*, 1992, **31**, 1135-1178.
68. M. Tóth, S. Kun, É. Bokor, M. Benltifa, G. Tallec, S. Vidal, T. Docsa, P. Gergely, L. Somsák and J.-P. Praly, *Bioorg. Med. Chem.*, 2009, **17**, 4773-4785.
69. K. Fosgerau, N. Westergaard, B. Quistorff, N. Grunnet, M. Kristiansen and K. Lundgren, *Arch. Biochem. Biophys.*, 2000, **380**, 274-284.

70. P. Mackay, L. Ynddal, J. V. Andersen and J. G. McCormack, *Diabetes Obes. Metab.*, 2003, **5**, 397-407.
71. M. Roden and E. Bernroider, *Best Pract. Res. Clin. Endocrinol. Metab.*, 2003, **17**, 365-383.
72. S. Freeman, J. B. Bartlett, G. Convey, I. Hardern, J. L. Teague, S. J. G. Loxham, J. M. Allen, S. M. Poucher and A. D. Charles, *Br. J. Pharmacol.*, 2006, **149**, 775-785.
73. W. H. Martin, D. J. Hoover, S. J. Armento, I. A. Stock, R. K. McPherson, D. E. Danley, R. W. Stevenson, E. J. Barrett and J. L. Treadway, *Proc. Natl. Acad. Sci. U.S.A.*, 1998, **95**, 1776-1781.
74. M. Combettes and C. Kargar, *Thérapie*, 2007, **62**, 293-310.