Title

Novel idebenone analogs block Shc's access to insulin receptor to improve insulin sensitivity

Authors

Hui, ChunKiu
Tomilov, Alexey
Garcia, Chase
et al.

Publication Date

2020-12-01

DOI

10.1016/j.biopha.2020.110823

Peer reviewed

Biomedicine & Pharmacotherapy 132 (2020) 110823

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Novel idebenone analogs block Shc's access to insulin receptor to improve insulin sensitivity

ChunKiu Hui [a], Alexey Tomilov [a], Chase Garcia [a], XiaoSong Jiang [a], David M. Fash [b], Omar M. Khdour [b], Cristian Rosso [d], Giacomo Filippini [d], Maurizio Prato [d,e,f], James Graham [a], Sidney Hecht [b,c], Peter Havel [a], Gino Cortopassi [a,*]

[a] Department of Molecular Biosciences, 1089 Veterinary Medicine Dr., VM3B, UC Davis, CA, 95616, USA
[b] Center for BioEnergetics, Biodesign Institute, Arizona State University, 1001 S McAllister Ave, Tempe, AZ, 85287, USA
[c] School of Molecular Sciences, Arizona State University, Tempe, AZ, 85287, USA
[d] Department of Chemical and Pharmaceutical Sciences, CENMAT, Center of Excellence for Nanostructured Materials, INSTM UdR, Trieste, University of Trieste, Via Licio Giorgieri 1, Trieste, 34127, Italy
[e] Center for Cooperative Research in Biomaterials (CIC biomaGUNE), Basque Research and Technology Alliance (BRTA), Paseo de Miramón 182, 20014, Donostia San Sebastián, Spain
[f] Basque Fdn Sci, Ikerbasque, Bilbao, 48013, Spain

ARTICLE INFO

Keywords:
Drug discovery
Novel insulin sensitizing agents
Type 2 Diabetes
Shc blockers
Small molecule therapeutics

ABSTRACT

There has been little innovation in identifying novel insulin sensitizers. Metformin, developed in the 1920s, is still used first for most Type 2 diabetes patients. Mice with genetic reduction of p52Shc protein have improved insulin sensitivity and glucose tolerance. By high-throughput screening, idebenone was isolated as the first small molecule 'Shc Blocker'. Idebenone blocks p52Shc's access to Insulin Receptor to increase insulin sensitivity. In this work the avidity of 34 novel idebenone analogs and 3 metabolites to bind p52Shc, and to block the interaction of p52Shc with the Insulin receptor was tested. Our hypothesis was that if an idebenone analog bound and blocked p52Shc's access to insulin receptor better than idebenone, it should be a more effective insulin sensitizing agent than idebenone itself. Of 34 analogs tested, only 2 both bound p52Shc more tightly and/or blocked the p52Shc-Insulin Receptor interaction more effectively than idebenone. Of those 2 only idebenone analog #11 was a superior insulin sensitizer to idebenone. Also, the long-lasting insulin-sensitizing potency of idebenone in rodents over many hours had been puzzling, as the parent molecule degrades to metabolites within 1 h. We observed that two of the idebenone's three metabolites are insulin sensitizing almost as potently as idebenone itself, explaining the persistent insulin sensitization of this rapidly metabolized molecule. These results help to identify key SAR = structure-activity relationship requirements for more potent small molecule Shc inhibitors as Shc-targeted insulin sensitizers for type 2 diabetes.

1. Introduction

Type 2 Diabetes (T2D) is a serious and chronic metabolic disorder that affects 30 million Americans and over 400 million people worldwide [1–3]. Unlike type 1 diabetes (T1D) which is caused by a genetic or immune-mediated destruction of pancreatic β-cells and the therapeutic strategies were mostly immune-based [4–7], T2D is a chronic and progressive metabolic disease caused primarily by peripheral insulin resistance, accompanied by inadequate β-cell compensation, to which obesity is a major contributor. Downstream of insulin resistance is hyperglycemia, which has toxic consequences for nerves [8–10], for the circulatory system [11,12], and can cause wounds and tissue necrosis [13,14].

If an overall pathomechanistic scheme for T2D can include 1) obesity->2) peripheral insulin resistance->3) inadequate insulin secretion-> 4) hyperglycemia->5) glucose toxicity->6) T2D phenotype and diabetic complications, there has been a paucity of therapeutic agents to combat these different pathophysiological steps. For example, there has

* Corresponding author at: Molecular Biosciences, 1089 Veterinary Medicine Drive, UC Davis, Davis, CA, 95616, USA.
E-mail addresses: chui@ucdavis.edu (C. Hui), atomilov@ucdavis.edu (A. Tomilov), cagar@ucdavis.edu (C. Garcia), joyjiang@ucdavis.edu (X. Jiang), dfash@asu.edu (D.M. Fash), omar.khdour@asu.edu (O.M. Khdour), cristian.rosso@phd.units.it (C. Rosso), gfilippini@units.it (G. Filippini), prato@units.it (M. Prato), jlgraham@ucdavis.edu (J. Graham), sid.hecht@asu.edu (S. Hecht), pjhavel@ucdavis.edu (P. Havel), gcortopassi@ucdavis.edu (G. Cortopassi).

https://doi.org/10.1016/j.biopha.2020.110823
Received 19 July 2020; Received in revised form 26 September 2020; Accepted 26 September 2020
Available online 10 October 2020

not been substantial innovation in identifying novel insulin sensitizing agents to target step 2 since the discovery of metformin in the 1920s [15]. By contrast, there has been substantial therapeutic development at step 1, with GLP-1 and GIP incretins-based therapeutics that stimulate insulin secretion and inhibit glucagon secretion [16,17]. And there has been substantial development to address step 4, hyperglycemia, with the development of SGLT2 inhibitors that reduce glucose toxicity by increasing urinary spillover of glucose into the urine [18,19]. But there has been little pharmaceutical innovation in the step 2′middle ground' of T2D therapy, i.e. to find novel peripheral insulin sensitizers. Metformin, whose mechanism includes insulin sensitization, has been used since 1927 for T2D, and is still a major 'first therapeutic choice' for T2D [20,21].

We showed previously that mice with genetic reduction of p52Shc protein are more insulin sensitive and glucose tolerant [22,23]. Our high-throughput screen of 1600 drugs used in humans identified the first drug known to engage the p52Shc protein and inhibit its interaction with insulin receptor, namely idebenone. Idebenone' s insulin-sensitizing mechanism was demonstrated to proceed through engagement of the p52Shc protein drug target [24].

We and others have shown that Shc activity increases in a cell model of T2D [25], multiple animal models of T2D [26–28], and in humans with T2D [27,29–32]. Thus, it is also our hypothesis that p52Shc activity increases as a consequence of T2D in laboratory animals and humans and is part of the mechanism of insulin resistance (See Fig. 1). Thus, the overall hypothesis is that p52Shc activity increases with T2D, making peripheral tissues less insulin sensitive, and that a small-molecule Shc inhibitor, by binding and blocking p52Shc's access to the insulin receptor, reduces p52Shc activity to IR phosphotyrosine, thus increasing IRS1–>Akt activity and the peripheral glucose lowering (Fig. 1).

To identify potentially novel p52Shc binders and blockers, and also to understand why idebenone is such a potent insulin sensitizer even though it is quickly metabolized, we tested 34 novel idebenone analogs including 3 idebenone metabolites, to characterize their p52Shc binding activity, as well as their ability to block the interaction of p52Shc with insulin receptor (IR). Our hypothesis was that if a molecule both bound and/or blocked p52Shc's access to IR better than idebenone, they should be more effective insulin sensitizers than idebenone. One such potent idebenone analog, compound 11, was identified. We were puzzled why idebenone with a 10-carbon aliphatic substituent that is rapidly converted to shorter substituents QS8, QS6 and QS4, is such a potent insulin sensitizer, if its breakdown products are inactive. We observed that some of these metabolites are indeed bioactive. We believe that this study identifies some principles important for designing more potent p52Shc binders and blockers and support the search for a more potent insulin sensitizer than idebenone for treatment of T2D.

2. Materials and methods

2.1. Animals

Animals were housed and bred in the animal facility in the Department of Nutrition at the University of California, Davis, and were maintained on a 12 -hs light-dark cycle. All experimental protocols were approved and supervised by the University of California Davis Institutional Animal Care and Use Committee.

For high fat diet-treated mice, C57Bl6 mice were purchased from Jackson Laboratory (Bar Harbor, ME). Mice were equally separated and assigned to indicated groups. Control or high fat diet were prepared as previously described and were provided to the mice for 6 months [33]. Mice were sacrificed at 6 months old and liver were collected and used for traditional Western Blot. For the studies in the UCD-T2DM rat model, samples from diabetic UCD-T2DM rats and lean Sprague-Dawley rats were supplied by Dr. Peter Havel's breeding colony. The UCD-T2DM rat model is a well-characterized polygenic rat model of adult-onset T2D that combines insulin resistance with a pancreatic β-cell defect resulting

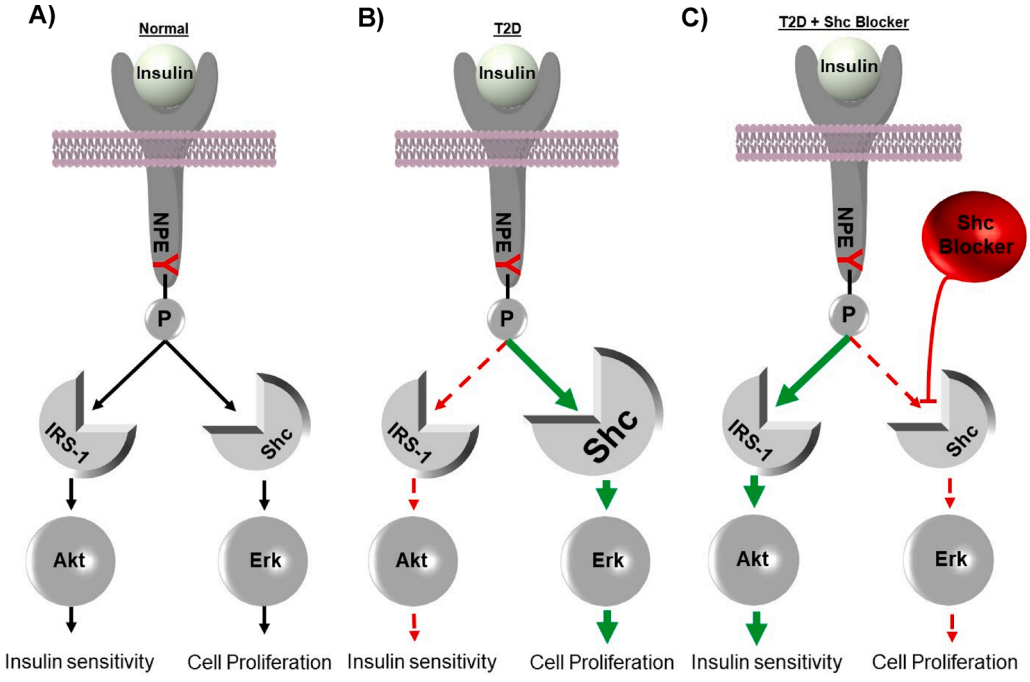

Fig. 1. A schematic of the potential mechanisms of Shc-based peripheral insulin resistance, and Shc-based insulin sensitization.s.
IRS1 and Shc compete for the identical phosphotyrosine Tyr 960 on NPEY motif of activated IR [45,46]. In a normal individual, Shc, especially p52Shc, competes normally with IRS-1 on the IR, activating both arms of the insulin signaling and resulting in normal insulin sensitivity (A). In a T2D patient, Shc protein and its activity (phospho-Shc) are increased. Therefore, in T2D Shc because of its higher activity or greater amount or both outcompetes IRS-1, reducing IRS-dependent p-Akt signaling that drives the metabolic insulin response, at the expense of increased MAPK-Erk signaling (B, green arrow). As a result less IRS-1 interacts with IR causing less Akt activation resulting in lower insulin sensitivity (B, red arrow). Based on our hypothesis, Shc blockers should rescue this situation. When idebenone is administered, binds to the PTB domain of Shc, thus blocking access of Shc's PTB to the IR phosphotyrosine. As more phosphotyrosines are available to IRS1, IRS-1mediated through phospho-Akt signaling is increased per molecule of insulin resulting in increased insulin sensitivity (C).

in inadequate insulin secretion and hyperglycemia/overt diabetes [34]. Thus, the pathophysiology of T2D in UCD-T2DM rats is more similar to that of the disease in humans than other rodent models [35] and the model been employed in a number of studies investigating the effects of pharmacological interventions for the prevention and treatment of T2D, including liraglutide [36], pioglitazone [37], and leptin [38]. UCD-T2DM rats were provided ad lib access to normal chow diet (2018 Teklad global protein rodent diets, Envigo, Hayward, CA) and were monitored bi-weekly for the onset of diabetes using a glucometer (One Touch Ultra-LifeScan, Inc., Milpitas, CA). Two consecutive non-fasting glucose readings $\geq$ 200 mg/dl were considered diagnostic of diabetes onset. At 5.5 months of age, rats were divided into two groups: pre-diabetic rats that were not hyperglycemic (glucose $<$ 200 mg/dl), and rats that had been diabetic for three months. Rats were fasted overnight and a whole blood sample was collected the next morning and frozen at −80 °C. Frozen blood samples were processed into lysate and used for Jess Western. Nondiabetic control group is the combination of both learn Sprague-Dawley rats and the prediabetic UCD-T2DM rats, and the diabetic group is the post-diabetic UCD-T2DM rats. Characteristics of the UCD-T2DM rats and their controls used were illustrated in Supp Fig. 4A. For fast food diet mice, C57Bl6 mice at the age of 7–11 months were supplied with either control chow diet or RD Western diet (Catalog #: D12079B; Research Diets Inc., NJ) for a total of 16 weeks. Mice were then equally separated and assigned to indicated groups. At week 8 after the diet was initiated, mice were introduced with drug treatments. Vehicle treated groups were receiving additional 200ul of peanut butter once daily, while the treatment groups were receiving 200ul of peanut butter mixed with 40 mg/kg idebenone powder once daily. The drug treatment was maintained until the end of the 16 weeks diet. At the end of the 16 weeks, mice were sacrificed, and blood was collected and used for Jess Western analysis. Characteristics of the fast food diet mice and their controls used were illustrated in Supp Fig. 4B.

2.2. Cell culture

Human Embryotic Kidney (HEK) 293 cell line and mouse liver hepatocyte (FL83B) cell lines were purchased from American Type Culture Collection (Manassas, VA) and were maintained at 37 °C in a humidified atmosphere with 5% CO_2. Cells were cultured in Dulbecco's Modification of Eagle's Medium/F-12 (DMEM) powder Mix (Thermo Fisher, CA) mixed with 1% 5 mM uridine (MP-Biomedicals), 1% 5 mM sodium pyruvate (Sigma-Aldrich), and 10 % fetal bovine serum (Corning, Fremont CA). For transfection, HEK293 cells were grown until >70 % confluence and transfection mix were prepared as follow: 1.9 ml of 1x Opti-MEM media (Thermo Fisher, CA) were mixed with 50ul of transfection reagents TransIT-LT1 (Mirus Bio LLC, Madison WI) and 17ug of cDNA plasmid. Transfection mix was incubated at room temperature for 30 min, and the mixture was added to the media. N-terminally biotinylated p52Shc plasmid and p52ShcδPTB plasmid were purchased from GeneCopoeia (Rockville, MD). HEK293 cells with transfection reagent were incubated at standard 37 °C and 5% CO_2 condition for 3 days. Media was removed after 3 days of incubation and cells were then removed with 1x PBS. Protein lysates were prepared with 1x cell lysis buffer (Cell signaling, CA) for further use.

2.3. Direct p52Shc vs p52ShcδPTB binding assays

Idebenone analogs and idebenone metabolites used in this experiment were listed below. Idebenone analogs were synthesized at Arizona State University [39] and were provided by Dr. Sidney Hecht from Arizona State University (Tempe, AZ). Idebenone metabolites were synthesized following the synthetic approaches reported by Okamoto et al. [34] and were provided by Cristian Rosso, Dr. Giacomo Filippini and Dr. Maurizio Prato from University of Trieste (Trieste, Italy). P52Shc and p52ShcδPTB proteins were expressed in HEK293 cells as described above. Proteins were loaded onto sets of Octet RED384 super streptavidin biosensors (Molecular Devices, CA) until loading density >10 nm followed by the blocking of non-binded streptavidin on the biosensors with 200μM biotin. The loaded sensors were used to test against different idebenone analogs and metabolites at the indicated concentration in BLI Kinetic Buffer (Molecular Devices, CA) with 0.1 % DMSO using the Octet RED384 instrument. Experimental parameters were as followed: baseline 20 s, association 30 s, dissociation 40 s. Data collected were analyzed using Octet BLI software 8.1 (Molecular Devices, CA).

2.4. Qualifying compounds inhibitory efficacy on p52Shc – IR interaction

Super streptavidin sensors were loaded with p52Shc until loading density >10 nm. The sensors were then incubated with NPEYp peptide at 6μM to test against binding towards p52Shc using BLI in the presence or absence of the idebenone analogs and metabolites mentioned above at indicated concentrations in BLI Kinetic Buffer with 0.1 % DMSO. NPEYp peptide was purchased from Biopeptide Co. Inc. (San Diego, CA) and the sequence for the peptide is: GPLYAASNPEY(PO3)LSASD-OH. Responses were recorded by the Octet 384 RED. IC50 was determined using sigmoidal dose response, three parameter model.

2.5. ELISA

FL83B cells were seeded at a concentration of 200,000 cells/well onto 24 well plates in DMEM medium. Cells were allowed to grow for 48 h at 37 °C with 5% CO_2, and the medium was replaced with DMEM without fetal bovine serum and the cells were incubated at 37 °C for 16 h. After 16 h of incubation, selected idebenone analogs at indicated concentration in PBS with 0.1 % DMSO were supplied to cells and cells were incubated at 37 °C for 1 h. Cells were then stimulated with 0.2 nM insulin for 10 min at room temperature. Cell lysate were collected and used for ELISA, Western Blot, and Protein Simple Jess Western. For ELISA, cell lysate will be analyzed with PathScan Phospho-Akt(Thr308) Sandwich ELISA Kit (Cell Signaling Technology Inc. Danvers, MA). ELISA protocol was followed as manufacturer indicated. EC50 was determined using sigmoidal dose response, three parameter model.

2.6. Western Blotting analysis

Cell lysates and drug treatment were described above. Total protein was isolated with ice-cold CellLytic MT Cell Lysis Reagant (Sigma Aldrich. St Louis, MO) containing 1 tablet of PhosSTOP phosphatase inhibitor and Complete EDTA-free mini protease inhibitor cocktail (Roche). Protein concentration was determined by Bradford assay (BioRad Laboratories) and 20 microgram of protein per line were loaded and resolved by SDS-PAGE using NuPAGE Bis-Tris Gels and the NuPAGE LDS Sample Buffer (Invitrogen Inc.) following the manufacturer instructions. Results were then transferred to nitrocellulose membrane and blocked with Odyssey Blocking Buffer (Li-Cor Biosciences), followed by hybridization with indicated primary antibodies from Cell Signaling Technology Inc. at a dilution of 1:1000: mouse monoclonal anti-Akt(pan)(40D4), rabbit monoclonal anti-phospho-Akt(Ser473)(193H12), mouse monoclonal anti-p44/42 MAPK(Erk1/2)(L34F12), rabbit monoclonal anti-phospho-p44/42 MAPK(Erk1/2)(Thr202/Tyr204)(197G2) antibody; mouse monoclonal anti-α-tubulin (Sigma Aldrich, St Louis, MO) at 1:2000 dilution was used as internal control. Membrane were developed with infrared IR-dye 700CW and 800CW labelled secondary antibodies at a dilution of 1:15,000 (Li-Cor). Blots were scanned on Li-Cor Odyssey infrared imaging instrument and results were quantified and analyzed using Odyssey 2.1 software.

2.7. Protein Simple Jess Western analysis

Western blots were also performed using Protein Simple Jess Western instrument (San Jose, CA). Cell and tissue lysates were prepared as

described above. 6ul of protein simple were mixed with 5x fluorescent master mix (Protein Simple) to achieve a finial concentration of 1x master mix buffer according to manufacturer's instructions. Samples were then denatured at 95 °C for 5 min. All materials and solutions added onto the assay plate were purchased from Protein Simple except primary antibodies.10ul of antibody diluent, protein normalizing reagent, primary antibodies, secondary antibodies, chemiluminescent substrates, 3ul of sample, and 500ul of wash buffer were prepared and dispensed into the assay plate. Assay plate was loaded into the instrument and protein was separated within individual capillaries. Protein detection and digital images were collected and analyzed with Compass software (Protein Simple) and data were reported as area under the peak, which representing the intensity of the signal. For primary antibody, mouse monoclonal anti-Akt(pan)(40D4), rabbit monoclonal anti-phospho-Akt(Ser473)(193H12), mouse monoclonal anti-p44/42 MAPK (Erk1/2)(L34F12), and rabbit monoclonal anti-phospho-p44/42 MAPK (Erk1/2)(Thr202/Tyr204)(197G2) antibody (Cell Signaling Technology, Davers, MA) were used at 1:100 dilution, and is mixed with mouse monoclonal anti-α-tubulin (Sigma Aldrich, St Louis, MO) at 1:250 dilution. For secondary antibody, anti-mouse NIR and anti-rabbit HRP secondary antibodies from Protein Simple were used.

2.8. Statistical analysis

All statistical analysis was performed with Excel Statistical Data Analysis Tool package 2007. Unless indicated otherwise, p-values were determined with 2-tails *t*-test. Sigmoidal dose response fitting analysis was completed using 3 or 4 parameters models, as indicated, the equitation used for the model was $Y = R_{min} + (R_{max}\text{-}R_{min}) / (1 + 10^{(LogEC50-LogX)*Hill-Slope)}$; for 3 parameter model Hill Slope = 1, X = concentration of compound, Log was by base 10, R_{max} = maximum response, R_{min} = minimum response, R^2 was tested for significance using standard statistical equitation.

3. Results

3.1. p52Shc is activated in multiple rodent models of T2D

3.1.1. Both phospho-p52Shc and total p52Shc increase in the UCD-T2DM rat model

Blood samples were collected from UCD-T2DM rats that had developed diabetes and remained diabetic for 3 months, and whole blood lysates were assayed for phospho-p52Shc as a measure of Shc activity, and for total p52Shc expression, by Protein Simple Jess Western quantification (Fig. 2A, B, Supp Fig. 6). The measurements demonstrated a significant and approximately 4-fold increase of phospho-p52Shc level and a mean 2-fold increase in total p52Shc level in UCD-T2DM rats after 3 months of diabetes compared with the combination of nondiabetic Sprague-Dawley rats and the prediabetic UCD-T2DM rats (Fig. 2A, B).

3.1.2. p52Shc activity increases in mouse high-fat diet T2D model

Mice consuming a high fat diet are commonly employed as an animal model of T2D [40,41], and phospho-p52Shc (i.e. activated Shc) level was compared in HFD vs. control diet mice. Phospho-p52Shc level significantly increased in mice on high fat diet when comparing to those on control diet, while total p52Shc protein did not significantly change (Fig. 2C, D).

3.1.3. Trends for increase in p52Shc activity in the mouse 'Fast Food Diet'

The 'fast food diet' FFD, has gained traction as a model of 'American unhealthy diet and metabolic disease' and animals consuming it develop significant insulin resistance [42,43]. Blood samples were collected from C57Bl6 mice fed with 16 weeks of either control diet, fast food diet, or FFD with 40 mg/kg idebenone, and the blood lysates were used for quantification of phosphorylated and total p52Shc level using Protein Simple Jess Western. FFD mice had mean increased phospho-p52Shc level by ~ 1.7 fold compared to mice fed with control diet, while mice fed with western fast food diet plus 40 mg/kg of idebenone tended to diminished this increase back to control level, but neither the rise in pShc on FFD, nor the decrease on FFD plus idebenone, were significant (Fig. 2E, F, Supp Fig. 7). Thus, there was a trend to increase Shc in the context of insulin resistance and T2D in the 3 different rodent models.

3.2. Screening of idebenone analogs for their binding affinity to p52Shc by Biolayer Interferometry

In order to identify molecules that are more biologically potent than idebenone, we screened 34 idebenone analogs including three major idebenone metabolites shown in Fig. 3 and Supp Figs. 5 and 6. The binding tests were performed using Biolayer interferometry (BLI), to identify tighter p52Shc binders than idebenone among the analogs. As a control for specificity, we compared the binding also to a p52Shc protein with p52ShcδPTB, i.e., p52Shc with its phosphotyrosine binding (PTB) domain deleted. The binding of all idebenone analogs at 1, 5, and 10μM towards p52Shc and p52ShcδPTB were performed. The relative binding response and specific binding ratio to p52Shc and p52ShcδPTB were calculated. Combined binding response were calculated by summing the relative binding response for all idebenone analogs at 3 concentrations. We ranked all idebenone analogs based on their combined binding responses towards p52Shc at 3 different concentrations (Table 1).

There were six idebenone analogs that bound p52Shc more avidly than idebenone. For example, analogue 11, a compound containing a 5-phenylpentyl substituent, bound p52shc with greater affinity than idebenone, as shown by 11 having a ~1.6-fold increase in maximum binding response towards p52Shc when compared with idebenone (Fig. 4A). Also, 11 was more specific, as it bound full length p52Shc 3.6-fold more tightly than p52Shc with its PTB removed (Fig. 4B). The binding of analog 11 to p52Shc was dissociable, as shown in the decline of the dissociation curve (Fig. 4A, B). All compounds tested were ranked by their combining binding response (Supp. Fig. 1), and the 10 best p52Shc binders with the best combining binding response were then used in a 24-point titration to determine Kd. The affinity of 11 to full length p52Shc was 1μM (Fig. 4C). Further investigation on the binding showed that in general analogs with alkyl substituents having 9–11 carbons were bound significantly better than other compounds, while modification of the benzoquinone moiety did not affect the binding of the compounds to p52Shc. Based on these results, we identified 10 idebenone analogs that bound well to p52Shc. If we conceptualize Shc inhibition as a two-step process, which requires first binding of analog to PTB-p52Shc, and subsequently blockage of p52Shc's interaction with Insulin Receptor, then Shc binding activity could be thought of as a precondition for Shc blocking activity.

3.3. Idebenone analogs and metabolites block the interaction of Shc with IR

The relative potency of the best binders to block the interaction of p52Shc and IR was investigated. The assay utilized a simalacrum of the activated IR, i.e. a 16-amino acid peptide that contains phospho-NPEY, phosphorylated on the tyrosine corresponding to phosphotyrosine 960 of the human IR [44]. This is the moiety to which p52Shc binds upon stimulation of IR by insulin [45,46]. All selected compounds were tested for their ability to block the Shc-NPEYp interaction at 10, 1, and 0.1μM. Among 34 idebenone analogs tested, 7 had a similar or better blocking efficency compared to idebenone at 100 nM. However, only 4 of the 7 compounds (analogs 5, 11, 12 and 13) blocked the Shc-IR interaction better than idebenone (Fig. 5C). While the blocking potency of analogs was not exceptional, all compounds inhibited the Shc-NPEY interaction by at least 30 % as compared to vehicle (Fig. 5C). Interestingly, the better Shc blockers such as analogs 11 or 12 generally had shorter, less bulky substituents as compared to the poor Shc blocker analog 14. Analog 11 was the most promising Shc blocker among those tested as it

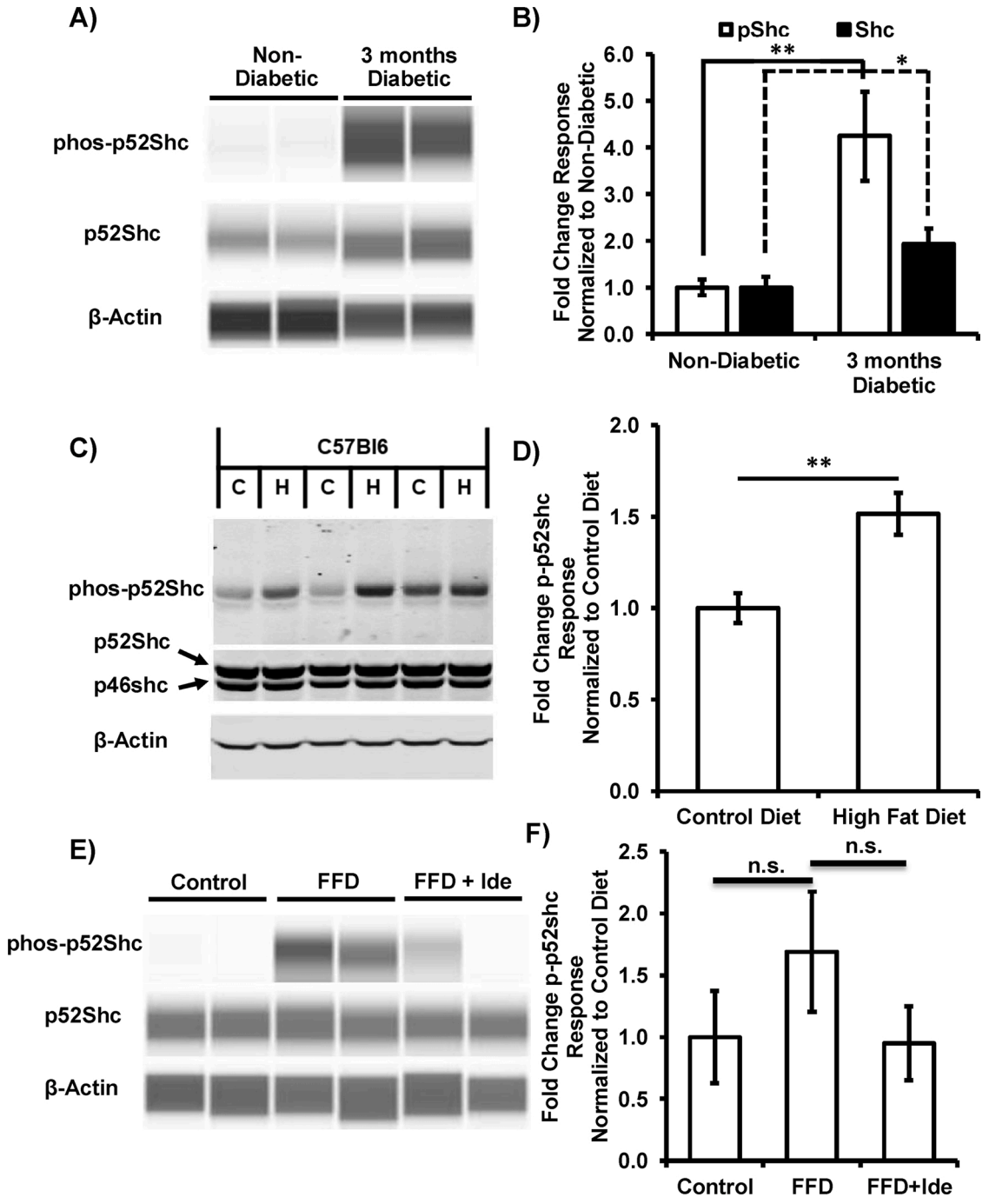

Fig. 2. phos-p52Shc increases in multiple T2D or insulin resistance rodent models, and idebenone treatment can reverse this upregulation.
Blood samples from UCD-T2DM rats were collected, and whole blood lysates were prepared as described above and were used for Jess Western. Phos-p52Shc, total p52Shc and β-actin were blotted and shown (A). Both phos-p52Shc (white bar) and total p52Shc level (black bar) were increased by ~ 4-fold and ~2-fold in 3 months after the rat had entered the diabetic states when compared to non-diabetic rat (B). Bars are showing average fold change with SEM, p-value were calculated using 2-tail *t*-test, N for each group: non-diabetic = 10; 3 months diabetic = 14, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Treatment on different mouse/rat models were described above. Liver lysate from 6 months old C57Bl6 mice on control (C) or high fat diet (H) were collected and used for traditional western blot. Phos-p52Shc, p52Shc, p46shc and β-actin were blotted and shown (C). phos-p52Shc protein expression is upregulated in C57Bl6 mice under high fat diet comparing to those on control diet (D). Bars are showing a combination of two technical replicates and average fold change with SEM were shown, p-value were calculated using 2-tail *t*-test, N = 6 for each group, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. For fast food diet (FFD) mice, 7–11 months old C57Bl6 mice were equally separated and randomly assigned to control group (Control), vehicle group (FFD), and treatment group (FFD + Ide), dosing protocol as described above. Blood were collected from animals after the end of the experiment and blood lysate were prepared and used for Jess Western. Phos-p52Shc, p52Shc and β-actin were blotted and shown (E). phos-p52Shc level were upregulated by ~1.75-fold after 16 weeks of FFD diet, and oral idebenone treatment at 40 mg/kg once daily for 8 weeks helped to rescue the upregulated phos-p52Shc level (F). Bars show the average fold change response with SEM of mice from the indicated groups, p-value were calculated using 2-tail *t*-test, N for each group: Control = 6, FFD = 7, FFD + Ide = 8.

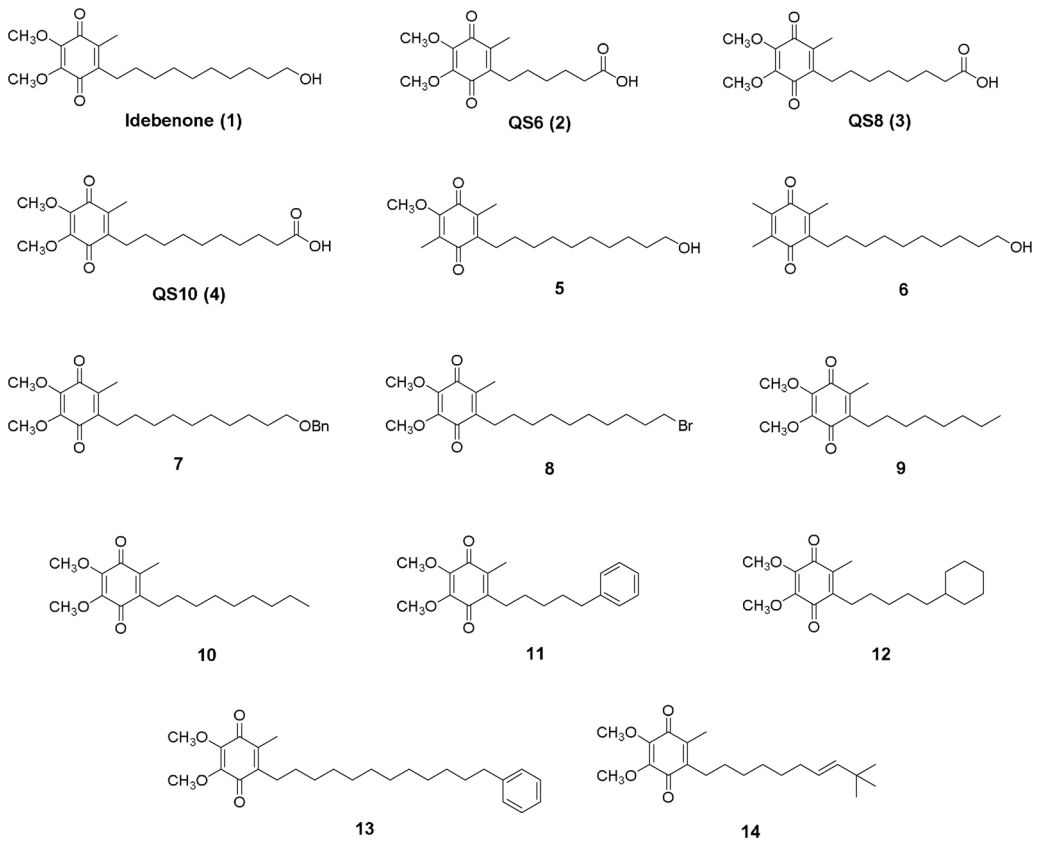

Fig. 3. Structures of the best compounds tested.
Chemical structures of the top-scoring debenone analogs and the three major idebenone metabolites tested and screened for potential insulin sensitizers and Shc blockers.

Table 1
Ranking of the best 10 p52shc binders.

Name	Combined Binding Response (pm)	Relative Ranking
6	115.6	1
8	86.5	2
12	63.8	3
11	63.1	4
14	56.7	5
5	56.6	6
Idebenone (1)	45.0	7
15	43.2	8
16	39.5	9
10	33.5	10

showed a similar blocking efficacy towards the p52Shc-NPEY(p) interaction as idebenone at 100 nM; analog 1 had a 5% better blocking efficiency towards Shc-NPEYp interaction than idebenone (Fig. 5A, C).

3.4. Could idebenone' s metabolites retain Shc-inhibitory activity?

When idebenone is metabolized by the liver, the hydrophobic idebenone substituent is broken down to the metabolites QS10, QS8 and QS6 [47]. We were puzzled why idebenone was such a potent insulin sensitizer in rodents after 8 h of administration [24], given that idebenone is metabolized to what could be inactive QS10, QS8 and QS6 metabolites within 1 h of administration to rodents [48]. Recent data suggest that some of these short-chain, catabolized idebenone molecules retain their ability to be a cytoprotective [36], and engagement of p52Shc confers both cytoprotection and insulin sensitization [24]. If these shorter idebenone metabolites retain their ability to block the p52Shc-IR interaction, then these molecules could contribute to the extended insulin sensitization potency of idebenone. Accordingly, blocking of the IR-p52Shc interaction by idebenone metabolites QS10, QS8 and QS6 was investigated (Fig. 5C). These short-chain idebenone metabolites blocked the p52Shc-IR interaction with a predictable order of efficiency, i.e. QS6 > QS8 > QS10, i.e. the shorter the idebenone metabolite the better it blocks (Fig. 5C), and this was also consistent with analog 11, which has a 5-phenylpentyl substituent, and was one of the most effective blockers.

3.5. Analog 11 is a more effective insulin sensitizer than idebenone, as judged by ELISA

Selected Shc binder and Shc blocker 'winners' from the above experiments were tested in cells *in vitro*, for their ability to stimulate the insulin-dependent phospho-Akt (pAkt) response, which is the main driver of the insulin response as shown in Fig. 1 [49]. As expected, idebenone dose-dependently increased pAkt expression as shown in pAkt ELISA with an EC50 = 5.6μM (Fig. 6A, black solid line). Analog 11 also dose-dependently increased pAkt expression in FL83B cells, and had about a 4-fold lower EC50 = 950 nM (Fig. 6A, black dashed line), i.e. it was a more potent insulin sensitizer than idebenone. We also performed the same experiment with analog 13 as it was the most effective Shc blocker from the blocking assay (Fig. 5C). However, analog 13 showed no dose-dependent improvement on pAkt expression within the concentration range tested (data not shown). Thus, analog 13 might not be a suitable drug candidate to further pursue.

3.6. Confirmation of the superiority of analog 11 over idebenone by JESS-Western

To confirm our ELISA results, we tested #11's insulin sensitization

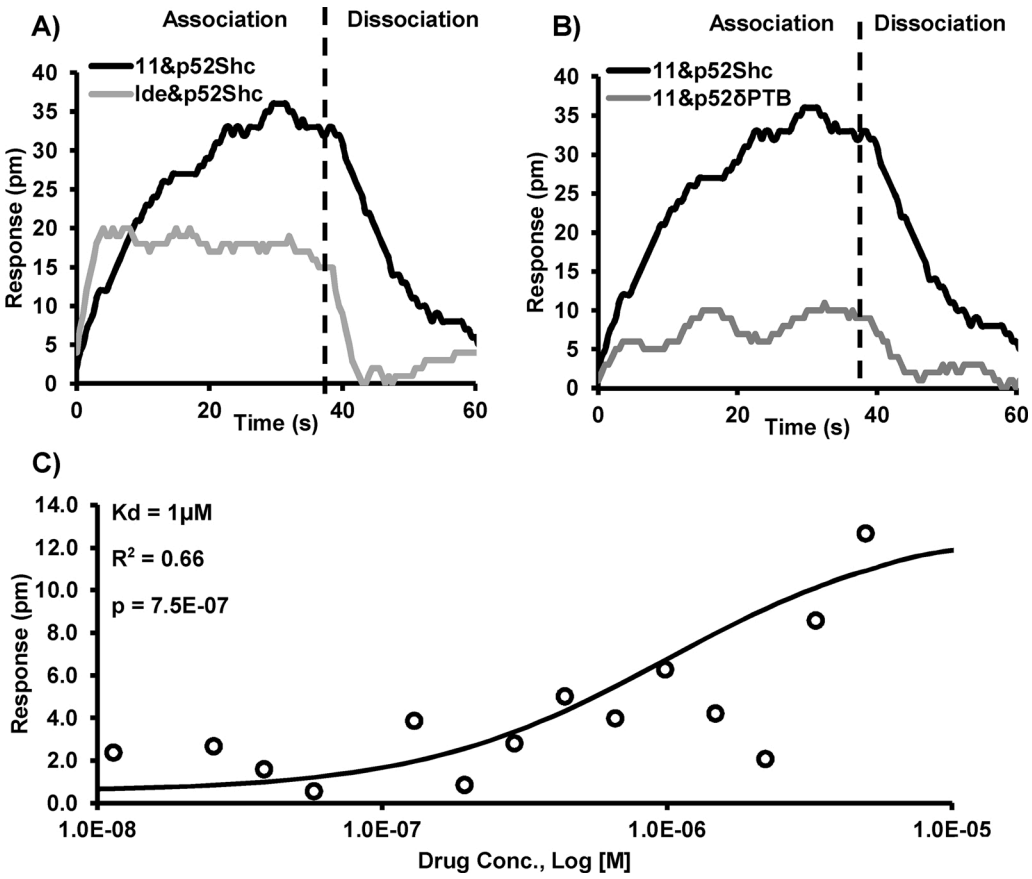

Fig. 4. Identification of Shc binders among idebenone analogs.
Responses (in picometers) of idebenone and idebenone analogs binding to full size p52Shc and p52ShcδPTB were measured in triplicate using BLI. Binding sensogram for idebenone (grey) and idebenone analog 11 (black) at 5μM to p52Shc is shown (A). In addition, the sensogram for idebenone analog 11 binding to p52Shc (black) and p52ShcδPTB (grey) are shown in (B). Compounds who are having a combined binding score at three different concentration better than idebenone were further tested for affinity to p52Shc on the PTB domain in a titration series in 4 individual experiments and concentrations are indicated. Log plot was obtained and affinity and Kd were calculated. Log plot of analog 11 binding to p52Shc is shown in (C). Fitting is done with sigmoidal dose-response three parameters model and dots are averages of four individual measurements. n = 20, R^2 = 0.66, p = 7.5E-07

potency in vitro by an independent technique, the Protein Simple Jess Western assay, an automated and capillary-based immunoassay similar to traditional westerns. In agreement with the pAkt ELISA result, idebenone dose-dependently increased pAkt expression in FL83B cells with an EC50 = 7μM (Fig. 6B, black solid line). Analog 11 again gave the same dose-dependent improvement in pAkt expression and was again about a 4-fold more insulin sensitizing than idebenone with an EC50 = 1.8μM (Fig. 6B, black dashed line). Gel image from Jess Western also showed a generally higher pAkt level in FL83B cells treated with analog 11 than idebenone at lower concentration, suggesting the possibility that analog 11 was generally more active than idebenone (Fig. 6C, Supp Fig. 8). Like the pAkt ELISA result, FL83B cells treated with analog 13 showed no improvement in pAkt expression (Supp Fig. 3). In conclusion, analog 11 was the most optimal drug candidate to be further developed as a novel insulin sensitizer and T2D therapeutic, while analog 13 was not.

3.7. Idebenone metabolites QS6 and QS8 are as insulin-sensitizing as idebenone

Idebenone is metabolized in 1 h in rodents to its QS10, QS8 and QS6 [47,48,50], but is still a potent insulin sensitizer and cytoprotector many hours after administration [24]. So, we tested the hypothesis that idebenone' s in vivo insulin sensitization potency could be 'the sum of its parts', i.e. the Shc-inhibition of the sum of idebenone itself plus its 3 breakdown products into which it is rapidly converted in vivo. The insulin sensitizing potency of metabolites QS10, QS8 and QS6 were tested in FL83B cells. We observed no insulin sensitizing ability of QS10, but QS8 and QS6 insulin sensitized with EC50 s of 37 nM and 38 nM respectively (Supp Fig. 2), about half the potency of idebenone with an EC50 = 23 nM (Supp Fig. 2). Thus two of idebenone's metabolites, QS6 and QS8, are significant insulin sensitizers, and thus could contribute in part to idebenone' s overall insulin sensitization effect in vivo. However, as single molecules, they were not superior to idebenone.

4. Discussion

4.1. Type 2 Diabetes, insulin resistance, and p52Shc: overview

Type 2 Diabetes (T2D) is a serious and chronic metabolic disorder that affects over 30 million Americans and 400 million people worldwide [1–3]. One major pathophysiological characteristic of T2D is peripheral insulin resistance, which results in hyperglycemia and leads to multiple serious to lethal consequences [51,52]. Type 1 Diabetes is often the result of immune attack of pancreatic cells, and thus therapeutic strategies focus on immunotherapy and immunoregulation. T2D therapeutic strategies tend to focus more on reducing blood glucose, as T2D-dependent hyperglycemia produces many of the comorbities of type 2 diabetes. [5–7].

If an overall pathomechanistic scheme for T2D can include 1) obesity->2) peripheral insulin resistance->3) inadequate insulin secretion-> 4) hyperglycemia->5) glucose toxicity->6) T2D phenotype and diabetic complications, there has been an unequal development of therapeutics to combat these different pathophysiological steps. For example, there has not been substantial successful innovation in identifying novel insulin sensitizers to combat step 2 since the discovery of metformin in the 1920s [15]. By contrast, there has been substantial therapeutic development at step 3, with GLP-1 and GIP incretins that increase insulin secretion [16,17]. And there has been substantial development to address step 4, hyperglycemia, with the development of SGLT2 inhibitors that reduce glucose toxicity by increasing urinary spillage of glucose through the urine [18,19].

We in this work (Fig. 2), and several groups before us have shown that Shc activity and amount is increased in multiple rodent models and

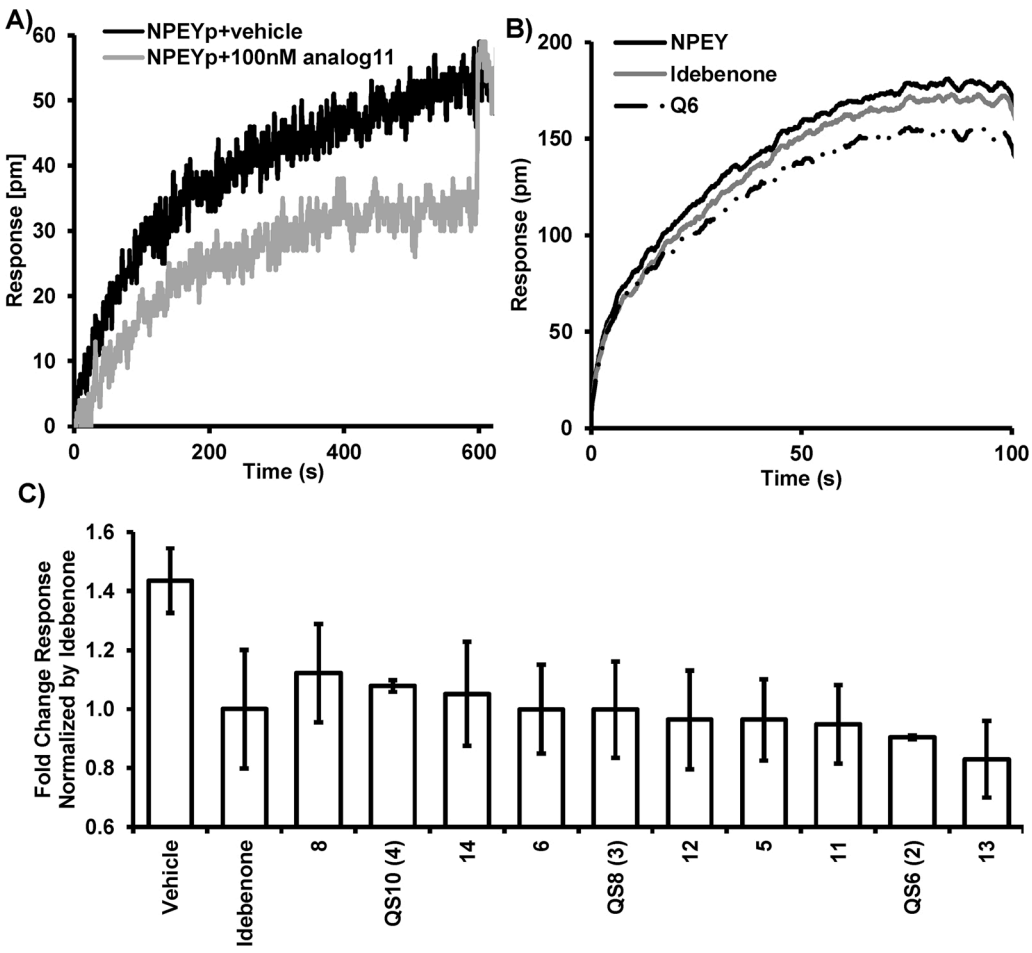

Fig. 5. Inhibitory effect of selected idebenone analogs and metabolites on Shc-NPEYp interaction.
From the 35 total idebenone analogs we selected the best 15 better p52Shc binders. All 15 compounds plus three idebenone metabolites were tested for their ability to inhibit the interaction between p52Shc and NPEY(p), a fragment of IR which contains the phosphorylated tyrosine residue 960 which known to be interacting with p52Shc; NPEY(p) sequence is given above. p52Shc was loaded onto super streptavidin biosensors for BLI analysis. Biosensors were exposed to the NPEY(p) peptide at 6μM with or without the presence of compounds at indicated concentration. The BLI sensograms of association and dissociation of p52Shc and NPEY(p) were recorded. The sensogram for idebenone analog 11 at 100 nM is shown (a), and the sensogram for idebenone metabolites QS6 at 200 nM is shown in (b). Compounds were prioritized by their ability to inhibit p52Shc-NPEY(p) interaction as indicated by BLI responses. Fold Change BLI response for all compounds tested normalized to idebenone were shown in (c). Bars are representing an average fold change of three individual blocking experiment replicates with n = 2, error bars are representing SEM.

the human conditions of obesity and diabetes [25–32], and we suggest here that this increased Shc activity in peripheral tissues may contribute to insulin insensitivity.

We and others have shown that mice with a genetic reduction of p52Shc are more insulin sensitive and glucose tolerant [22,23]. This is conceptualized in Fig. 1, i.e. that p52Shc competes for the *identical phosphotyrosine* as insulin receptor substrate-1 (IRS-1) on the insulin receptor [53–55]. Thus if p52Shc is genetically reduced or pharmacologically blocked from accessing the phosphotyrosine, then IRS1 has more access to these same phosphotyrosines, it receives more of the insulin signal, and there is a more pronounced insulin response. This concept derived from p52 Shc hypomorphic mice suggested that small molecules that block p52Shc's access to Insulin receptor with could represent novel insulin sensitizers. We previously screened 1600 human-used drugs and identified idebenone as a novel insulin sensitizer via binding to p52Shc and blocking p52Shc interaction with IR [24]. We predicted mechanistically that idebenone analogs or metabolites that are better p52Shc binders and/or blockers of the Shc-IR interaction should be even more potent insulin sensitizers and therapeutics than idebenone (Fig. 1). Although the most-used insulin sensitizer globally for T2D is metformin, there are T2D patients for whom metformin is not the best option, and so development of novel insulin sensitizers is warranted [56,57]. p52Shc inhibition is a novel therapeutic target and strategy to address the middle of the T2D pathomechanism, i.e. peripheral insulin insensitivity, and therapeutic development for T2D.

4.2. Analogue 11 is an excellent p52Shc binder, blocker of Shc-IR interaction, and insulin sensitizer

In the attempt to identify novel insulin sensitizers, we developed and investigated 34 idebenone analogs to characterize their binding affinity to p52Shc, their blocking efficacy on p52Shc-IR interaction, and their insulin sensitizing ability in mouse liver FL83B cells. Using Octet-BLI analysis we identified six out of 34 of the idebenone analogs that bind to p52Shc as well as or better than idebenone (Fig. 4, Supp Fig. 1). We then tested the best 15 Shc binders for their blocking efficiency on p52Shc-NPEYp interaction, and we identified four molecules that are similar or better Shc blockers than idebenone (Fig. 5), with molecules 11 and 13 being the two best Shc blockers. We further investigated these two molecules by performing an insulin sensitivity test by studying these two molecules and idebenone in FL83B liver cells and measuring the changes in phosphorylated Akt level in cells after the treatment (Fig. 6). We identified 11 as the best drug candidate as it performs well in all three of the screening experiments. Analog 11 has about the same number of carbon atoms in its substituent as idebenone, but the additional aromatic ring makes 11 more compact than idebenone (Fig. 3). Also, 11 lacks the hydroxide group of idebenone, making it less polar then idebenone (Fig. 3). This raises the issue of whether these structural and chemical differences are necessary for creating a better insulin Shc blocker as well as better insulin sensitizer when comparing to idebenone. To further discover the structural requirements for a better Shc blocker, we reviewed the binding and blocking data of all the idebenone analogs tested. We observed that molecules that are bulkier and have longer substituents tend to be a weaker Shc binder. Analogue 13, for example, is the best Shc blocker among the group (Fig. 5). However, due to its longer carbon tail it is not a good Shc binder (Supp. Fig. 1). Interestingly, modifying the benzoquinone head of the idebenone-like molecule did not change their binding affinity to p52Shc as much as its blocking efficacy towards p52Shc-NPEY(p) interaction; analog 5 is a good Shc binder and has a similar blocking efficiency as idebenone.

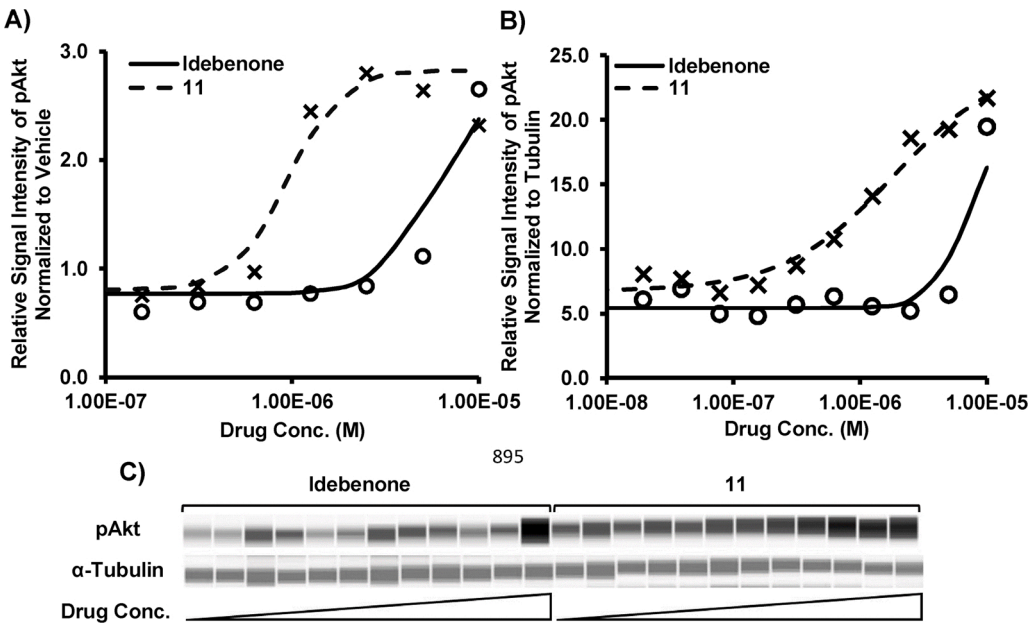

Fig. 6. Insulin sensitizing ability of idebenone analog 11.
Insulin sensitizing ability of selected idebenone analogs were tested in two different experiments – pAkt ELISA (A) and Jess Western blotted with pAkt antibody (B,C). FL83B cells treated with idebenone and analog 11 at indicated concentration followed by an administration of insulin as described above. FL83B cells lysate was used for ProteinSimple Jess Western and Cell Signaling Technology p-Akt ELISA kit to quantify the insulin sensitivity of FL83B cell after treated with compounds. Protocol for the experiments were described above. For p-Akt ELISA, FL83B cell lysate was prepared and was used with Cell Signaling Technology p-Akt ELISA kit; p-Akt fluroscence signal was measured as instructed by the company. Relative fluorescence signal normalized to vehicle were shown in (A). Fitting curve is done with sigmoidal dose-response three parameters model, dots are representing a combination of cell lysates from 2 biological replicates; idebenone: EC50 = 5.6μM, R^2 = 0.893, n = 12, p = 5.8e-7; analog 11: EC50 = 950 nM, R^2 = 0.953, n = 12, p = 6.2e-9. For Jess Western, 6ug of cell lysate were added to each well on the Jess protein normalization plate, and the indicated antibodies were supplied to the plate to create signal response collected by the Jess machine. Gel images and the dose-depending fitting curve were shown (B,C). For (B), Fitting curve is done with sigmoidal dose-response three parameters model, dots are representing a combination of cell lysates from two biological replicates; idebenone: EC50 = 7μM, R^2 = 0.891, n = 12, p = 2.0e-6; analog 11: EC50 = 1.8μM, R^2 = 0.980, n = 12, p = 3.8e-10.

(Figs. 4 and 5). However, from the perspective of creating molecules that are better Shc binders and Shc blockers than idebenone, this modification is not valuable as it does not improve the molecules' blocking efficacy towards p52Shc-IR interaction shown by analog 5 (Fig.5). Considering the values of both binding and blocking efficacy, we conclude that for an idebenone-like molecule to be a better Shc binder and blocker it should have a shorter carbon substituent, perhaps no more than 6 carbons long, while modification of the benzoquinone moiety will not affect either its binding affinity or blocking efficacy.

4.3. Multiple idebenone metabolites block Shc and are insulin sensitizing explaining the prolonged insulin sensitization of idebenone

Idebenone is a short chain benzoquinone compound is used for in treatment of Leber's Hereditary Optic Neuropathy (LHON) [58–60]. Idebenone is metabolized in rodents within one hour to its metabolites QS10, QS8 and QS6 [48], however idebenone' s insulin sensitizing effects appear to last for more than 6 h after administration [24]. In addition, idebenone's metabolites improved mitochondrial bioenergetics, indicating that idebenone metabolites might retain certain biochemical activity as idebenone [61,62]. We tested the p52Shc blocking efficacy of the idebenone metabolites and found that QS8 and QS6 were reasonable Shc blockers (Fig. 5C) and had similar insulin sensitization potency in vitro (Supp. Fig. 2). This finding supported our hypothesis that a benzoquinone metabolite molecule with shorter carbon tail has better Shc blocking efficacy, similar to molecule 11. And these data appear to explain the long-acting insulin sensitization of a rapidly metabolized parent compound.

5. Conclusions

To summarize the main points of the MS. First, we find as have others cited in the manuscript, that Shc activity or amount is increased in multiple animal models of Type 2 Diabetes. We suggest but do not claim to have proven here that Shc activation in lymphocytes could become a novel biomarker of use in human T2D, and we also suggest but do not claim to have proven that increased Shc activity in peripheral tissues may contribute to peripheral insulin resistance.

Second, we find that pharmacologically active Shc inhibitors need to have both appreciable Shc binding activity in the PTB region and must also block the PTB domain's access to insulin receptor phosphotyrosines, possibly in some kind of two-step mechanism, i.e. binding first, blocking second.

Thirdly, we suggest the reason for idebenone' s long-acting insulin

sensitization potency in rodents is the aggregate contribution of idebenone and two of its metabolites QS6 and QS8.

Lastly, we have identified SAR requirements for Shc binding and blocking and suggest that analog 11 is a starting point for further development of Shc-engaging small molecule insulin-sensitizers of the treatment of hyperglycemia in patients with type 2 diabetes.

Author contributions

Hui – Screening for novel insulin sensitizing agents, identification of analog 11 as novel insulin sensitizer; Tomilov – affinity of idebenone analogs to Shc protein; Garcia and Jiang – determination of Shc expression and activity in rodents; Fash, Khdour and Hecht – synthesize idebenone analogs; Rosso, Filippini and Prato – synthesize idebenone metabolites; Graham and Havel – develop and provide samples from UCD-T2DM rat model; Cortopassi – overseeing entire project, writing MS.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgements

We thank Kimber L. Stanhope for co-developing the UC Davis T2D rat model, and the NIHPO1AG062817 for support.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.biopha.2020.110823.

References

[1] E.L. Gonzalez, et al., Trends in the prevalence and incidence of diabetes in the UK: 1996-2005, J. Epidemiol. Commun. Health 63 (4) (2009) 332–336.
[2] National Diabetes Statistics Report, 2020, Centers for Disease Control and Prevention, 2020.
[3] Global Report on Diabetes, World Health Organization, 2016.
[4] M. Ben Nasr, et al., The rise, fall, and resurgence of immunotherapy in type 1 diabetes, Pharmacol. Res. 98 (2015) 31–38.
[5] M. Ben Nasr, et al., PD-L1 genetic overexpression or pharmacological restoration in hematopoietic stem and progenitor cells reverses autoimmune diabetes, Sci. Transl. Med. 9 (416) (2017).
[6] P. Fiorina, et al., Targeting CD22 reprograms B-cells and reverses autoimmune diabetes, Diabetes 57 (11) (2008) 3013–3024.
[7] A. Vergani, et al., A novel clinically relevant strategy to abrogate autoimmunity and regulate alloimmunity in NOD mice, Diabetes 59 (9) (2010) 2253–2264.
[8] A.J. Boulton, R.A. Malik, Diabetic neuropathy, Med. Clin. North Am. 82 (4) (1998) 909–929.
[9] J.J. Duby, et al., Diabetic neuropathy: an intensive review, Am. J. Health. Syst. Pharm. 61 (2) (2004) 160–173, quiz 175-176.
[10] J.L. Edwards, et al., Diabetic neuropathy: mechanisms to management, Pharmacol. Ther. 120 (1) (2008) 1–34.
[11] P.O. Carlsson, A. Andersson, L. Jansson, Pancreatic islet blood flow in normal and obese-hyperglycemic (ob/ob) mice, Am. J. Physiol. 271 (6 Pt 1) (1996) E990–5.
[12] J.R. Kersten, et al., Hyperglycemia reduces coronary collateral blood flow through a nitric oxide-mediated mechanism, Am. J. Physiol. Heart Circ. Physiol. 281 (5) (2001) H2097–104.
[13] D. Levigne, et al., Hyperglycemia increases susceptibility to ischemic necrosis, Biomed Res. Int. 2013 (2013), 490964.
[14] F. Gonzalez, et al., In vitro evidence that hyperglycemia stimulates tumor necrosis factor-alpha release in obese women with polycystic ovary syndrome, J. Endocrinol. 188 (3) (2006) 521–529.
[15] P.M. Bell, D.R. Hadden, Metformin, Endocrinol. Metab. Clin. North Am. 26 (3) (1997) 523–537.
[16] J.J. Holst, T. Vilsboll, C.F. Deacon, The incretin system and its role in type 2 diabetes mellitus, Mol. Cell. Endocrinol. 297 (1-2) (2009) 127–136.
[17] T. Vilsboll, J.J. Holst, Incretins, insulin secretion and Type 2 diabetes mellitus, Diabetologia 47 (3) (2004) 357–366.
[18] S. Riser Taylor, K.B. Harris, The clinical efficacy and safety of sodium glucose cotransporter-2 inhibitors in adults with type 2 diabetes mellitus, Pharmacotherapy 33 (9) (2013) 984–999.
[19] J. Rosenstock, et al., Efficacy and safety of empagliflozin, a sodium glucose cotransporter 2 (SGLT2) inhibitor, as add-on to metformin in type 2 diabetes with mild hyperglycaemia, Diabetes Obes. Metab. 15 (12) (2013) 1154–1160.
[20] B. Viollet, et al., Cellular and molecular mechanisms of metformin: an overview, Clin. Sci. 122 (6) (2012) 253–270.
[21] J.E. Gunton, et al., Metformin rapidly increases insulin receptor activation in human liver and signals preferentially through insulin-receptor substrate-2, J. Clin. Endocrinol. Metab. 88 (3) (2003) 1323–1332.
[22] A.A. Tomilov, et al., The Shc locus regulates insulin signaling and adiposity in mammals, Aging Cell 10 (1) (2011) 55–65.
[23] I. Berniakovich, et al., p66Shc-generated oxidative signal promotes fat accumulation, J. Biol. Chem. 283 (49) (2008) 34283–34293.
[24] A. Tomilov, et al., Idebenone is a cytoprotective insulin sensitizer whose mechanism is Shc inhibition, Pharmacol. Res. 137 (2018) 89–103.
[25] P. Song, et al., PKCdelta promotes high glucose induced renal tubular oxidative damage via regulating activation and translocation of p66Shc, Oxid. Med. Cell. Longev. 2014 (2014), 746531.
[26] L. Laviola, et al., Effects of streptozocin diabetes and diabetes treatment by islet transplantation on in vivo insulin signaling in rat heart, Diabetes 50 (12) (2001) 2709–2720.
[27] M. Albiero, et al., Diabetes causes bone marrow autonomic neuropathy and impairs stem cell mobilization via dysregulated p66Shc and Sirt1, Diabetes 63 (4) (2014) 1353–1365.
[28] M.H. Zhao, et al., P66Shc expression in diabetic rat retina, BMC Ophthalmol. 18 (1) (2018) 58.
[29] R. MacLaren, et al., Influence of obesity and insulin sensitivity on insulin signaling genes in human omental and subcutaneous adipose tissue, J. Lipid Res. 49 (2) (2008) 308–323.
[30] S. Ciciliot, et al., p66Shc deletion or deficiency protects from obesity but not metabolic dysfunction in mice and humans, Diabetologia 58 (10) (2015) 2352–2360.
[31] J.S. Manavalan, et al., Circulating osteogenic precursor cells in type 2 diabetes mellitus, J. Clin. Endocrinol. Metab. 97 (9) (2012) 3240–3250.
[32] X. Xu, et al., p66Shc: a novel biomarker of tubular oxidative injury in patients with diabetic nephropathy, Sci. Rep. 6 (2016) 29302.
[33] A. Tomilov, et al., p46Shc inhibits thiolase and lipid oxidation in Mitochondria, J. Biol. Chem. 291 (24) (2016) 12575–12585.
[34] K. Okamoto, et al., Synthesis of quinones having carboxy- and hydroxy-alkyl side chains, and their effects on rat-liver lysosomal membrane, Chem. Pharm. Bull. (Tokyo) 30 (8) (1982) 2797–2819.
[35] M. Kleinert, et al., Animal models of obesity and diabetes mellitus, Nat. Rev. Endocrinol. 14 (3) (2018) 140–162.
[36] B.P. Cummings, et al., Chronic administration of the glucagon-like peptide-1 analog, liraglutide, delays the onset of diabetes and lowers triglycerides in UCD-T2DM rats, Diabetes 59 (10) (2010) 2653–2661.
[37] B.P. Cummings, et al., Administration of pioglitazone alone or with alogliptin delays diabetes onset in UCD-T2DM rats, J. Endocrinol. 221 (1) (2014) 133–144.
[38] B.P. Cummings, et al., Subcutaneous administration of leptin normalizes fasting plasma glucose in obese type 2 diabetic UCD-T2DM rats, Proc. Natl. Acad. Sci. U. S. A. 108 (35) (2011) 14670–14675.
[39] D.M. Fash, Synthetic Analogues of Redox-Enabled Natural Products, Department of Chemistry, University of Virginia, Virginia, 2011.
[40] M.S. Winzell, B. Ahren, The high-fat diet-fed mouse: a model for studying mechanisms and treatment of impaired glucose tolerance and type 2 diabetes, Diabetes 53 (Suppl. 3) (2004) S215–9.
[41] A. Heydemann, An Overview of Murine High Fat Diet as a Model for Type 2 Diabetes Mellitus, J. Diabetes Res. 2016 (2016), 2902351.
[42] E. Isganaitis, R.H. Lustig, Fast food, central nervous system insulin resistance, and obesity, Arterioscler. Thromb. Vasc. Biol. 25 (12) (2005) 2451–2462.
[43] P. Lang, et al., Effects of different diets used in diet-induced obesity models on insulin resistance and vascular dysfunction in C57BL/6 mice, Sci. Rep. 9 (1) (2019) 19556.
[44] M.M. Zhou, et al., Structure and ligand recognition of the phosphotyrosine binding domain of Shc, Nature 378 (6557) (1995) 584–592.
[45] T.A. Gustafson, et al., Phosphotyrosine-dependent interaction of SHC and insulin receptor substrate 1 with the NPEY motif of the insulin receptor via a novel non-SH2 domain, Mol. Cell. Biol. 15 (5) (1995) 2500–2508.
[46] W. He, T.J. O'Neill, T.A. Gustafson, Distinct modes of interaction of SHC and insulin receptor substrate-1 with the insulin receptor NPEY region via non-SH2 domains, J. Biol. Chem. 270 (40) (1995) 23258–23262.
[47] K. Kutz, J. Drewe, P. Vankan, Pharmacokinetic properties and metabolism of idebenone, J. Neurol. 256 (Suppl. 1) (2009) 31–35.
[48] H. Torii, et al., Disposition of idebenone (CV-2619), a new cerebral metabolism improving agent, in rats and dogs, J. Pharmacobiodyn. 8 (6) (1985) 457–467.
[49] K. Ueki, et al., Potential role of protein kinase B in insulin-induced glucose transport, glycogen synthesis, and protein synthesis, J. Biol. Chem. 273 (9) (1998) 5315–5322.
[50] M. Bodmer, et al., Pharmacokinetics and metabolism of idebenone in healthy male subjects, Eur. J. Clin. Pharmacol. 65 (5) (2009) 493–501.
[51] G. Wilcox, Insulin and insulin resistance, Clin. Biochem. Rev. 26 (2) (2005) 19–39.
[52] D. Brealey, M. Singer, Hyperglycemia in critical illness: a review, J. Diabetes Sci. Technol. 3 (6) (2009) 1250–1260.
[53] T. Sasaoka, et al., Evidence for a functional role of Shc proteins in mitogenic signaling induced by insulin, insulin-like growth factor-1, and epidermal growth factor, J. Biol. Chem. 269 (18) (1994) 13689–13694.
[54] T. Sasaoka, et al., Tyrosine phosphorylation-dependent and -independent role of Shc in the regulation of IGF-1-induced mitogenesis and glycogen synthesis, Endocrinology 142 (12) (2001) 5226–5235.

[55] T. Sasaoka, M. Kobayashi, The functional significance of Shc in insulin signaling as a substrate of the insulin receptor, Endocr. J. 47 (4) (2000) 373–381.
[56] C.D. MacLean, B. Littenberg, A.G. Kennedy, Limitations of diabetes pharmacotherapy: results from the Vermont Diabetes Information System study, BMC Fam. Pract. 7 (2006) 50.
[57] J.J. Marin-Penalver, et al., Update on the treatment of type 2 diabetes mellitus, World J. Diabetes 7 (17) (2016) 354–395.
[58] G. Buyse, et al., Idebenone treatment in Friedreich's ataxia: neurological, cardiac, and biochemical monitoring, Neurology 60 (10) (2003) 1679–1681.
[59] J.C. Gillis, P. Benefield, D. McTavish, Idebenone. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in age-related cognitive disorders, Drugs Aging 5 (2) (1994) 133–152.
[60] K.A. Lyseng-Williamson, Idebenone: a review in Leber's hereditary optic neuropathy, Drugs 76 (7) (2016) 805–813.
[61] V. Giorgio, et al., The effects of idebenone on mitochondrial bioenergetics, Biochim. Biophys. Acta 1817 (2) (2012) 363–369.
[62] V. Giorgio, et al., The idebenone metabolite QS10 restores electron transfer in complex I and coenzyme Q defects, Biochim. Biophys. Acta Bioenerg. 1859 (9) (2018) 901–908.