Published in final edited form as:
ACS Chem Biol. 2010 August 20; 5(8): 729–734. doi:10.1021/cb100129d.

Small-molecule suppressors of cytokine-induced beta-cell apoptosis

Danny Hung-Chieh Chou[1,2], **Nicole E. Bodycombe**[1], **Hyman A. Carrinski**[1], **Timothy A. Lewis**[1], **Paul A. Clemons**[1], **Stuart L. Schreiber**[1,2], and **Bridget K. Wagner**[1]

[1] Chemical Biology Program, Broad Institute, 7 Cambridge Center, Cambridge, MA 02142

[2] Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA 02138

Abstract

Pancreatic beta-cell apoptosis is a critical event during the development of type-1 diabetes. The identification of small molecules capable of preventing cytokine-induced apoptosis could lead to avenues for therapeutic intervention. We developed a set of phenotypic cell-based assays designed to identify such small-molecule suppressors. Rat INS-1E cells were simultaneously treated with a cocktail of inflammatory cytokines and a collection of 2,240 diverse small molecules, and screened using an assay for cellular ATP levels. Forty-nine top-scoring compounds included glucocorticoids, several pyrazole derivatives, and known inhibitors of glycogen synthase kinase-3β. Two compounds were able to increase cellular ATP levels, reduce caspase-3 activity and nitrite production, and increase glucose-stimulated insulin secretion in the presence of cytokines. These results indicate that small molecules identified by this screening approach may protect beta cells from autoimmune attack, and may be good candidates for therapeutic intervention in early stages of type-1 diabetes.

Type-1 diabetes is caused by the autoimmune destruction of insulin-producing beta cellsin the pancreas. Beta-cell apoptosis involves a set of signaling cascades initiated by interleukin-1β (IL-1β), interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α) (1–3). IL-1β and TNF-α induce NFκB expression, and downstream activation of gene expression is thought to occur through nitric oxide (NO) signaling, which both increases endoplasmic reticulum stress-response pathways and decreases beta cell-specific functions (4,5). NO is a highly reactive molecule that inhibits the electron-transport chain, leading to decreases in glucose oxidation rates, ATP generation, and insulin production (6); cellular nitrite is more stable and serves as a surrogate marker for NO. NFκB activation and IFN-γ-induced STAT-1 signaling work together to effect beta-cell apoptosis, mainly involving the intrinsic apoptotic pathway in both rodents and humans (7). The downstream effector of this cascade, caspase-3, results in apoptosis and the loss of the ability to secrete insulin in response to glucose stimulation.

Small molecules that increase beta-cell survival in the presence of cytokines could be of potential clinical benefit to early-stage type-1 diabetic patients. Previous studies have described small molecules with protective effects in the presence of cytokines (8,9); most of these molecules were discovered because of their antioxidant or anti-inflammatory effects. For example, resveratrol in the presence of cytokines results in restoration of viability (10),

*Corresponding author bwagner@broadinstitute.org.

Conflict of interest statement

The corresponding author declares there are no conflicts of interest.

Supporting Information Available: This material is available free of charge *via* the Internet.

possibly by SIRT1 activation (11). Further, Larsen *et al.* demonstrated that small-molecule inhibition of histone deacetylases (HDACs) with trichostatin A (TSA) or suberoylanilide hydroxamic acid (SAHA) prevents cytokine-induced beta-cell death, presumably by decreasing NFκB transactivation (12). Therefore, multiple mechanisms can serve to protect beta cells from cytokine-induced apoptosis.

Here, we describe a phenotypic screening approach to systematically discover small molecules that increase beta-cell viability and function in the presence of cytokines. Using the rat beta-cell line INS-1E, we screened 2,240 diverse compounds for the ability to alter cellular ATP levels in the presence of cytokines. We used secondary assays measuring caspase-3 activation, cellular nitrite production, and glucose-stimulated insulin secretion (GSIS) to detect complementary aspects of beta-cell biology. We identified a number of small molecules that increase beta-cell viability, including several glucocorticoids, novel pyrazole derivatives, and glycogen synthase kinase 3β (GSK-3β) inhibitors. These compounds enhanced cellular ATP levels and reduced caspase-3 activity in a dose-dependent manner. The pyrazole derivatives and one GSK-3β inhibitor, alsterpaullone, also reduced cellular nitrite production and increased GSIS in the presence of cytokines. These results suggest that small-molecule screening may provide useful compounds for therapeutic intervention in type-1 diabetes.

We sought to use a physiologically relevant model of the development of type-1 diabetes (Figure 1, panel a) by using the rat INS-1E insulinoma cell line. Two-day treatment with a cytokine cocktail of IL-1β, IFN-γ, and TNF-α resulted in a two-fold decrease in ATP levels (Figure 1, panel b). We also confirmed that the HDAC inhibitors TSA and SAHA partially suppress cytokine effects in beta cells (Figure 1, panel b), at concentrations consistent with their enzymatic inhibition of HDACs in cells (12).

We performed a pilot screen of 2,240 compounds to identify small-molecules suppressors of this cytokine cocktail on cellular ATP levels in INS-1E cells. Compounds were considered “hits” if they increased ATP levels by three standard deviations relative to the mock-treatment (DMSO) distribution (Figure 1, panel c). Using this criterion, we identified 49 “hits”, including 21 bioactives, 9 compounds synthesized by diversity-oriented synthesis (13), 5 natural products, and 14 compounds from commercial vendors. Chemical similarity analysis of the 49 “hits” revealed four clusters containing similar compounds by inspection (Figure 2, panel a). These clusters included two virtually identical compounds (alsterpaullone and kenpaullone), several pyrazole derivatives from commercial vendor libraries, nine compounds from diversity-oriented synthesis, and eight glucocorticoid derivatives.

We decided to focus initially on a set of commercially available compounds (Figure 2, panels b–d). Alsterpaullone, annotated as a GSK-3β inhibitor (14), completely restored beta-cell ATP levels in a dose-dependent manner (Figure 3, panel a). Similarly, three pyrazole derivatives increased ATP levels to >90% of untreated controls (Figure 3, panel b). Dexamethasone, chosen as a representative glucocorticoid, was slightly less potent in enhancing ATP levels, to approximately 80% of untreated levels (Figure 3, panel c).

Caspase-3 is a downstream effector of the apoptotic pathway, and its activity is increased by a 48-hour exposure of INS-1E cells to the cytokine cocktail. This increase in activity was suppressed more than 50% by 1 μ*M* alsterpaullone (Figure 3, panel d). The pyrazole derivatives also reduced caspase-3 activity in a dose-dependent manner (Figure 3, panel e), but dexamethasone was only partially effective at reducing this activity (Figure 3, panel f). These results indicate that screening for an increase in ATP levels can identify small

molecules capable of halting the apoptotic process in the presence of inflammatory cytokines.

IL-1β induces expression of inducible nitric oxide synthase (iNOS), an effect potentiated by IFN-γ and TNF-α (4); the subsequent formation of NO drives beta-cell death. Cellular production of nitrite, a stable oxidized product of NO used as a surrogate for NO levels, increased 3.5-fold after cytokine treatment, and was completely inhibited by 2 μ*M* alsterpaullone (Figure 4, panel a). The pyrazole derivatives were also effective in reducing nitrite production, though less so than alsterpaullone (Figure 4, panel b). Interestingly, dexamethasone had no effect on cytokine-induced nitrite production (Figure 4, panel c).

Finally, we examined the effects of these compounds on GSIS in INS-1E cells. Under normal conditions, stimulation with 15 m*M* glucose leads to a 3.6-fold increase in insulin secretion relative to low-glucose (2 m*M*) conditions (Figure 4, panel d). Cytokine treatment reduced GSIS to 1.4-fold. This loss of response was largely suppressed by the addition of 4 μ*M* alsterpaullone to the cytokine cocktail, with stimulation elevated to 3.2-fold (Figure 4, panel d). Treatment with the pyrazole derivative SPB07503 resulted in ~50% enhancement of GSIS, while dexamethasone had no effect (Figure 4, panel d). Together, these results suggest that cellular nitrite levels are correlated with GSIS in INS-1E cells; compounds that are capable of reducing nitrite production in the face of cytokine treatment also restore GSIS.

Another GSK-3β inhibitor, Ro 31-8220, was, like alsterpaullone, among the top-scoring compounds. Although these kinase inhibitors likely interact with several targets, we reasoned that GSK-3β could be a relevant target accounting for the protective effect on beta cells. Ro 31-8220 increased ATP levels, decreased caspase-3 activity, and reduced cellular nitrite production (Supplementary Figure 1). Similarly, the selective GSK-β inhibitors lithium chloride (15) and CHIR99021 (16) increased ATP levels in the presence of cytokines (Supplementary Figure 1). However, lithium chloride completely abolished nitrite production, while CHIR99021 only decreased nitrite by 20% (Supplementary Figure 1). To evaluate the specificity of these responses, we knocked down GSK-3β in INS-1E cells with small-interfering RNA (siRNA) constructs. Knock-down of *GSK3B* resulted in ATP levels that were 75% that of the mock- transfected control, in a complete reduction of caspase-3 activity, and in a 20% reduction in nitrite production (Supplementary Figure 2). These results were similar to those achieved by CHIR99021, and suggest that selective inhibition of GSK-3β is only partially protective of cytokine-treated INS-1E beta cells.

Inhibition of GSK-3β has been reported to protect beta cells against glucolipotoxicity and endoplasmic reticulum stress-induced beta-cell death (17,18). There are key differences between these mechanisms and cytokine-induced apoptosis, so the fact that GSK-3β inhibitors could also suppress cytokine-induced beta-cell apoptosis is not intuitively obvious. For example, the expression of iNOS and IκBα is upregulated by cytokines, but not by high glucose concentrations (19,20). Further, fatty acids such as oleate and palmitate do not activate the NFκB pathway in either INS-1E or rat islets, and fatty acid-induced beta-cell death is independent of iNOS or nitrite production (5). Because we observed a correlation between cellular nitrite production and GSIS (Figure 4), it is likely that inhibition of GSK-3β alone is insufficient to enhance beta-cell function lost due to inflammatory cytokines. These results suggest that alsterpaullone acts through multiple mechanisms, including GSK-3β inhibition, to protect beta cells from cytokine-induced apoptosis.

Glucocorticoids are a class of steroid hormones that bind to nuclear hormone receptors, which in turn translocate to the nucleus and upregulate the expression of anti-inflammatory proteins (21). Although glucocorticoids are generally detrimental to beta-cell development

and insulin secretion (22), a recent study showed that high doses of dexamethasone increase beta-cell proliferation in rat islets (23). Here, we demonstrate that low-micromolar concentrations of dexamethasone increased cellular ATP levels and reduced caspase-3 activity in the presence of cytokines. However, dexamethasone neither reduced cytokine-induced nitrite production nor restored GSIS. These results indicate that glucocorticoids can only partially increase beta-cell viability in this system.]

Finally, the novel pyrazole derivatives were obtained from commercially available libraries. We observed that these compounds protected cells against the detrimental effects of cytokines in all assays tested. To our knowledge, there have been no previous reports of the biological activities of these or related structures. These data suggest that the pyrazole derivatives in this study could protect beta cells by an as-yet unknown mechanism.

Because of the importance of inflammatory cytokines to the beta cell, many efforts have been made to identify genetic or small-molecule approaches to protect beta cells from cytokine-induced death (8–10,12,24). We have demonstrated the feasibility of cell-based screening to identify small molecules that prevent loss of beta-cell viability in the presence of cytokines. Although screening efforts to increase beta-cell number in the basal state have been described (25), to our knowledge, a systematic approach to discover small molecules that can prevent cytokine-induced beta-cell death has yet to be reported. We also find that reduction of cytokine-induced caspase activity and nitrite production appear to be prerequisites for enhancing physiological beta-cell function. The cytokines used here have numerous effects on beta cells, but we believe the use of this cocktail is a relatively fair model of the development of type-1 diabetes; further studies in mice will be necessary to confirm these conclusions. This study represents a proof of concept for the discovery of novel targets and compounds for the treatment of type-1 diabetes.

Methods

Cell culture and reagents

INS-1E cells (generously provided by C. Wollheim and P. Maechler, University of Geneva) were maintained in RPMI 1640 containing 11 m*M* glucose, 10% fetal bovine serum, 10 m*M* HEPES, 50 μ*M* 2-mercaptoethanol, 1 m*M* sodium pyruvate, cultivated at 37C with 5% CO_2 in a humidified atmosphere, and split every week. Recombinant rat IL-1β and recombinant mouse TNF-α were purchased from R&D Systems. Recombinant mouse IFN-γ, Griess reagent, and dexamethasone were purchased from Sigma. CellTiter-Glo and Caspase-Glo 3/7 reagents were purchased from Promega. Alsterpaullone and Ro 31-8220 were purchased from EMD Biosciences. The pyrazole derivatives were purchased from Maybridge. CHIR99021 was synthesized as described (26).

High-throughput screening for compounds affecting cellular ATP levels

INS-1E cells were seeded at 10,000 cells/well using a Multidrop Combi (Thermo Labsystems) in white optical 384-well plates (Corning Life Sciences). After overnight incubation, medium was removed and 50 μL RPMI containing 1% FBS and a combination of cytokines (10 ng mL^{-1} IL-1β, 50 ng mL^{-1} IFN-γ, 25 ng mL^{-1} TNF-α) was added to every well. Using libraries of compounds dissolved in DMSO and a CyBi-Well pin-transfer robot (CyBio Corp.), 0.1 μL of each compound was added. After 48 hr, medium was removed and 20 μL CellTiter-Glo reagent was added. Luminescence was measured after 10-min incubation using an EnVision plate reader (PerkinElmer).

Screening data analysis

Instrument output files were processed using Pipeline Pilot (Accelrys) and input to MATLAB (The MathWorks) for data normalization. Compound performance scores relative to a distribution of mock-treated (DMSO) wells were calculated using a revised version of the scoring system underlying *ChemBank* (27). The role of replicate treatments was further developed as follows: first, mock-treatment distributions were modeled using all mock-treated wells measured on a single day, regardless of their nominal replicate; second, per-compound scores weighted each in-plate background-subtracted measurement by the uncertainty in that measurement, using the method of maximum likelihood (28). The uncertainty in a single background-subtracted measurement was estimated using the number of mock-treated wells on the plate and, as a measure of the assay noise, the standard deviation of the per-day mock-treatment distribution. The signal, a weighted average of differences, was scaled by the noise, the standard deviation of the mock-treatment distribution.

Chemical similarity analysis

Cluster analysis was performed using Pipeline Pilot extended connectivity fingerprints (unfolded ECFP_4s). Bits representing substructures present in more than 10%, and less than 90%, of the 49 compounds were selected to generate 96-bit representations for each structure. Pairwise Tanimoto (T) similarity scores were computed among all compounds, after which “hits” were clustered hierarchically (complete linkage), both using MATLAB. Maximum common substructures for each group with similarities apparent by inspection were determined using Pipeline Pilot.

Measurement of cellular nitrite production

INS-1E cells were seeded and treated as described for high-throughput screening. After treatment with cytokine and compounds for 48 hr, 10 μL modified Griess reagent (1:1 mixture of 1% sulfanilamide in 30% acetic acid and 0.1% *N*-(1-naphthyl) ethylenediamine dihydrochloride in 60% acetic acid) was added to each well. After 5-min incubation at room temperature, the absorbance at 540 nm was measured using an Envision plate reader.

Caspase-3 activity assay

INS-1E cells were seeded at 5,000 cells/well in white optical 384- well plates and treated as described for high-throughput screening. After treatment with cytokines and compounds for 48 hr, medium was removed and 20 μL Caspase-Glo 3/7 reagent was added. Luminescence was measured after 2-hr incubation using an Envision plate reader.

Glucose-stimulated insulin secretion

INS1-E cells were seeded in 96-well plates at 20,000 cells/well and incubated for 48 hr in 100 μL fresh RPMI containing 1% FBS and the cytokine cocktail, in the presence or absence of compounds. Cells were washed and incubated for 2 hr in KRBH (135 m*M* NaCl, 3.6 m*M* KCl, 5 m*M* $NaHCO_3$, 0.5 m*M* NaH_2PO_4, 0.5 m*M* $MgCl_2$, 1.5 m*M* $CaCl_2$, 10 m*M* HEPES, pH 7.4, 0.1% BSA) buffer without glucose. Cells were subsequently incubated with KRBH buffer containing 2 m*M* or 15 m*M* glucose for 1 hr. The supernatant was taken for measurement of released insulin, and 100 μL acidified ethanol added to each well for extraction and measurement of cellular insulin content. Insulin was measured with a rat insulin ELISA kit (Alpco).

One-sentence summary

Phenotypic cell-based screening was performed to identify small-molecule suppressors of cytokine-induced beta-cell apoptosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank C. Wollheim and P. Maechler, University of Geneva, for the INS-1E cell line, and S. Kubicek, D. Fomina, and A. Shamji for their helpful suggestions. This work was funded by the National Institute of Diabetes and Digestive and Kidney Diseases (Type 1 Diabetes Pathfinder Award to B.K.W.), the Juvenile Diabetes Research Foundation (S.L.S., B.K.W.), and in part with funds from the National Institutes of Health's Molecular Libraries Probe Production Centers Network (HG-005032 awarded to S.L.S.) and the National Cancer Institute's Initiative for Chemical Genetics (contract no. N01-CO-12400). S.L.S. is a Howard Hughes Medical Institute Investigator. The content of this publication does not necessarily reflect the views or policies of the US Department of Health and Human Services, nor does the mention of trade names, commercial products or organizations imply endorsement by the US government.

References

1. Cnop M, Welsh N, Jonas JC, Jorns A, Lenzen S, Eizirik DL. Mechanisms of pancreatic beta-cell death in type 1 and type 2 diabetes: many differences, few similarities. Diabetes. 2005; 54(Suppl 2):S97–107. [PubMed: 16306347]
2. Fornoni A, Pileggi A, Molano RD, Sanabria NY, Tejada T, Gonzalez-Quintana J, Ichii H, Inverardi L, Ricordi C, Pastori RL. Inhibition of c-jun N terminal kinase (JNK) improves functional beta cell mass in human islets and leads to AKT and glycogen synthase kinase-3 (GSK-3) phosphorylation. Diabetologia. 2008; 51:298–308. [PubMed: 18066521]
3. Soldevila G, Buscema M, Doshi M, James RF, Bottazzo GF, Pujol-Borrell R. Cytotoxic effect of IFN-gamma plus TNF-alpha on human islet cells. J Autoimmun. 1991; 4:291–306. [PubMed: 1909137]
4. Darville MI, Eizirik DL. Regulation by cytokines of the inducible nitric oxide synthase promoter in insulin-producing cells. Diabetologia. 1998; 41:1101–1108. [PubMed: 9754830]
5. Kharroubi I, Ladriere L, Cardozo AK, Dogusan Z, Cnop M, Eizirik DL. Free fatty acids and cytokines induce pancreatic beta-cell apoptosis by different mechanisms: role of nuclear factor-kappaB and endoplasmic reticulum stress. Endocrinology. 2004; 145:5087–5096. [PubMed: 15297438]
6. Mandrup-Poulsen T. The role of interleukin-1 in the pathogenesis of IDDM. Diabetologia. 1996; 39:1005–1029. [PubMed: 8877284]
7. Grunnet LG, Aikin R, Tonnesen MF, Paraskevas S, Blaabjerg L, Storling J, Rosenberg L, Billestrup N, Maysinger D, Mandrup-Poulsen T. Proinflammatory cytokines activate the intrinsic apoptotic pathway in beta-cells. Diabetes. 2009; 58:1807–1815. [PubMed: 19470609]
8. Kim EK, Kwon KB, Song MY, Han MJ, Lee JH, Lee YR, Ryu DG, Park BH, Park JW. Flavonoids protect against cytokine-induced pancreatic beta-cell damage through suppression of nuclear factor kappaB activation. Pancreas. 2007; 35:e1–9. [PubMed: 18090225]
9. Matsuda T, Ferreri K, Todorov I, Kuroda Y, Smith CV, Kandeel F, Mullen Y. Silymarin protects pancreatic beta-cells against cytokine-mediated toxicity: implication of c-Jun NH2-terminal kinase and janus kinase/signal transducer and activator of transcription pathways. Endocrinology. 2005; 146:175–185. [PubMed: 15459112]
10. Lee JH, Song MY, Song EK, Kim EK, Moon WS, Han MK, Park JW, Kwon KB, Park BH. Overexpression of SIRT1 protects pancreatic beta-cells against cytokine toxicity by suppressing the nuclear factor-kappaB signaling pathway. Diabetes. 2009; 58:344–351. [PubMed: 19008341]

11. Pacholec M, Chrunyk BA, Cunningham D, Flynn D, Griffith DA, Griffor M, Loulakis P, Pabst B, Qiu X, Stockman B, Thanabal V, Varghese A, Ward J, Withka J, Ahn K. SRT1720, SRT2183, SRT1460, and resveratrol are not direct activators of SIRT1. J Biol Chem.
12. Larsen L, Tonnesen M, Ronn SG, Storling J, Jorgensen S, Mascagni P, Dinarello CA, Billestrup N, Mandrup-Poulsen T. Inhibition of histone deacetylases prevents cytokine-induced toxicity in beta cells. Diabetologia. 2007; 50:779–789. [PubMed: 17265033]
13. Nielsen TE, Schreiber SL. Towards the optimal screening collection: a synthesis strategy. Angew Chem Int Ed Engl. 2008; 47:48–56. [PubMed: 18080276]
14. Leost M, Schultz C, Link A, Wu YZ, Biernat J, Mandelkow EM, Bibb JA, Snyder GL, Greengard P, Zaharevitz DW, Gussio R, Senderowicz AM, Sausville EA, Kunick C, Meijer L. Paullones are potent inhibitors of glycogen synthase kinase-3beta and cyclin-dependent kinase 5/p25. Eur J Biochem. 2000; 267:5983–5994. [PubMed: 10998059]
15. Klein PS, Melton DA. A molecular mechanism for the effect of lithium on development. Proc Natl Acad Sci U S A. 1996; 93:8455–8459. [PubMed: 8710892]
16. Ring DB, Johnson KW, Henriksen EJ, Nuss JM, Goff D, Kinnick TR, Ma ST, Reeder JW, Samuels I, Slabiak T, Wagman AS, Hammond ME, Harrison SD. Selective glycogen synthase kinase 3 inhibitors potentiate insulin activation of glucose transport and utilization in vitro and in vivo. Diabetes. 2003; 52:588–595. [PubMed: 12606497]
17. Mussmann R, Geese M, Harder F, Kegel S, Andag U, Lomow A, Burk U, Onichtchouk D, Dohrmann C, Austen M. Inhibition of GSK3 promotes replication and survival of pancreatic beta cells. J Biol Chem. 2007; 282:12030–12037. [PubMed: 17242403]
18. Stukenbrock H, Mussmann R, Geese M, Ferandin Y, Lozach O, Lemcke T, Kegel S, Lomow A, Burk U, Dohrmann C, Meijer L, Austen M, Kunick C. 9-cyano-1-azapaullone (cazpaullone), a glycogen synthase kinase-3 (GSK-3) inhibitor activating pancreatic beta cell protection and replication. J Med Chem. 2008; 51:2196–2207. [PubMed: 18345612]
19. Cardozo AK, Kruhoffer M, Leeman R, Orntoft T, Eizirik DL. Identification of novel cytokine-induced genes in pancreatic beta-cells by high-density oligonucleotide arrays. Diabetes. 2001; 50:909–920. [PubMed: 11334433]
20. Eizirik DL, Kutlu B, Rasschaert J, Darville M, Cardozo AK. Use of microarray analysis to unveil transcription factor and gene networks contributing to Beta cell dysfunction and apoptosis. Ann N Y Acad Sci. 2003; 1005:55–74. [PubMed: 14679040]
21. Rhen T, Cidlowski JA. Antiinflammatory action of glucocorticoids--new mechanisms for old drugs. The New England journal of medicine. 2005; 353:1711–1723. [PubMed: 16236742]
22. Lambillotte C, Gilon P, Henquin JC. Direct glucocorticoid inhibition of insulin secretion. An in vitro study of dexamethasone effects in mouse islets. The Journal of clinical investigation. 1997; 99:414–423. [PubMed: 9022074]
23. Rafacho A, Cestari TM, Taboga SR, Boschero AC, Bosqueiro JR. High doses of dexamethasone induce increased beta-cell proliferation in pancreatic rat islets. American journal of physiology. 2009; 296:E681–689. [PubMed: 19158320]
24. Eldor R, Yeffet A, Baum K, Doviner V, Amar D, Ben-Neriah Y, Christofori G, Peled A, Carel JC, Boitard C, Klein T, Serup P, Eizirik DL, Melloul D. Conditional and specific NF-kappaB blockade protects pancreatic beta cells from diabetogenic agents. Proc Natl Acad Sci U S A. 2006; 103:5072–5077. [PubMed: 16551748]
25. Wang W, Walker JR, Wang X, Tremblay MS, Lee JW, Wu X, Schultz PG. Identification of small-molecule inducers of pancreatic beta-cell expansion. Proc Natl Acad Sci U S A. 2009; 106:1427–1432. [PubMed: 19164755]
26. Nuss JM, Harrison SD, Ring DB, Boyce RS, Brown SP, Goff D, Johnson K, Pfister KB, Ramurthy S, Renhowe PA, Seely L, Subramanian S, Wagman AS, Zhou XA. A preparation of aminopyrimidines and -pyridines as glycogen synthase kinase 3 inhibitors. PCT Int Appl. 1999:115–116.
27. Seiler KP, George GA, Happ MP, Bodycombe NE, Carrinski HA, Norton S, Brudz S, Sullivan JP, Muhlich J, Serrano M, Ferraiolo P, Tolliday NJ, Schreiber SL, Clemons PA. ChemBank: a small-molecule screening and cheminformatics resource database. Nucleic Acids Res. 2008; 36:D351–359. [PubMed: 17947324]

28. Bevington, PR.; Robinson, DK. Data Reduction and Error Analysis for the Physical Sciences. 3. McGraw-Hill; Boston, MA: 2003. p. 51-61.

29. Zhang JH, Chung TD, Oldenburg KR. A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. J Biomol Screen. 1999; 4:67–73. [PubMed: 10838414]

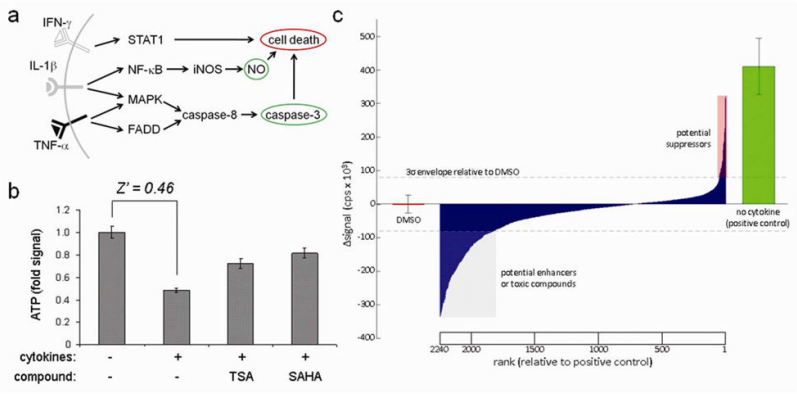

Figure 1. Assay development and screening for cellular ATP levels in the presence of inflammatory cytokines

a) Summary of the signaling pathways induced by cytokines and assay measurements used for primary (red circle) and secondary (green circles) screens. **b)** Rat INS-1E insulinoma cells were treated for 48 hr with inflammatory cytokines (IL-1β, IFN-γ, TNF-α) in the absence or presence of HDAC inhibitors (50 n*M* trichostatin A (TSA) or 1 μ*M* suberoylanilide hydroxamic acid (SAHA)). ATP levels were measured and normalized to the mean of untreated controls. Data are represented as the mean ± standard deviation of 12 independent wells. The assay Z' factor (29) was calculated based on the means and standard deviations of untreated and cytokine-treated wells. **c)** Cellular ATP levels were assessed after treatment with each of 2,240 compounds. The signal change induced by compound treatment ("Δsignal") represents the value for each compound normalized to mock-treated wells. The means and standard deviations of mock-treated (DMSO) wells and positive-control (no cytokine) wells are shown. Potential suppressors (shaded pink) were identified as those resulting in Δsignal three standard deviations above the DMSO distribution.

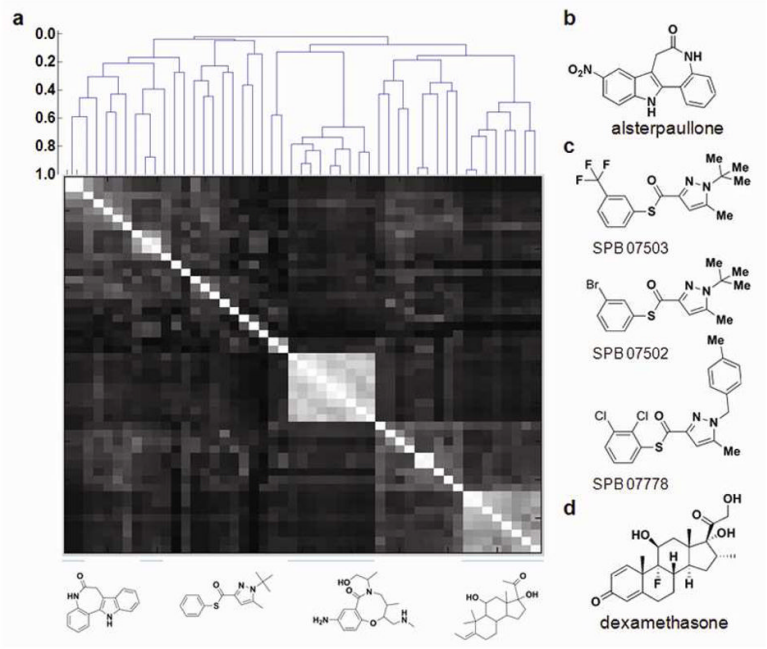

Figure 2. Chemical similarity among screening "hits"

a) Forty-nine compounds exceeding the "hit" threshold (*c.f.*, Figure 1) were selected for cluster analysis (see Methods). Pairwise Tanimoto (T) similarity scores were computed among all compounds (heat map; T=0, black; T=1, white; linear grayscale), after which "hits" were clustered hierarchically (dendrogram). For groups with visually apparent similarities (four white/light blocks in heat map; blue bars at bottom), the maximum common substructure for each group is depicted. Structures of the compounds tested in this study: **b)** alsterpaullone, **c)** pyrazole derivatives, **d)** dexamethasone.

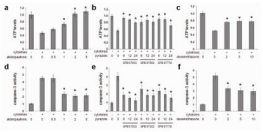

Figure 3. Suppression of cytokine-induced beta-cell damage

INS-1E cells were treated with cytokine cocktail in the presence of increasing concentrations of alsterpaullone **(a, d)**, pyrazole derivatives **(b, e)**, or dexamethasone **(c, f)**, all expressed as micromolar concentrations. Cellular ATP levels (a, b, c) and caspase-3 activities (d, e, f) were measured and normalized to untreated controls. Data are represented as the mean ± standard deviation of 24 independent wells. *, $p<0.01$ relative to cytokine-treated cells.

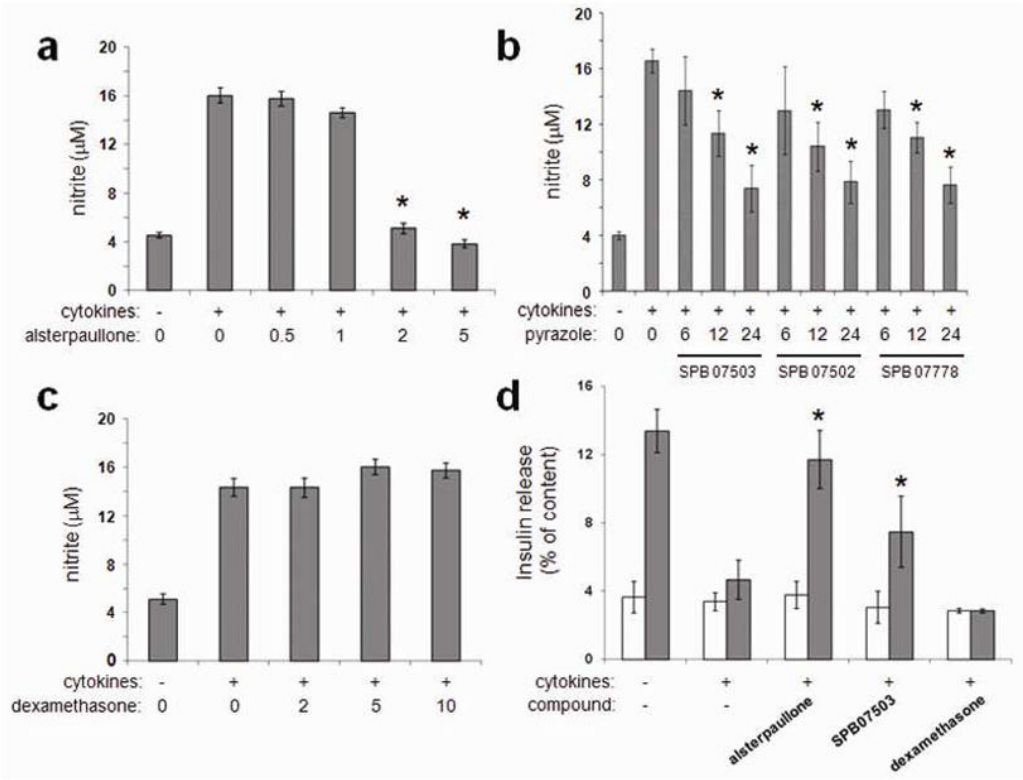

Figure 4. Reduction of nitrite production correlates with restoration of glucose-stimulated insulin secretion

Cellular nitrite production was measured after treatment with cytokine cocktail and increasing concentrations of **a)** alsterpaullone, **b)** pyrazole derivatives, or **c)** dexamethasone. Data represent the mean ± standard deviation of 24 independent wells. **d)** Glucose-stimulated insulin secretion was measured in the presence of 2 m*M* glucose (white bars) and 15 m*M* glucose (gray bars) in the absence or presence of cytokines and alsterpaullone (4 μ*M*), the pyrazole derivative SPB07503 (12 μ*M*), or dexamethasone (10 μ*M*). Data are represented as the mean ± standard deviation of 8 independent wells. *, $p<0.01$ relative to cytokine-treated cells.