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12-Lipoxygenase and Islet β cell Dysfunction in Diabetes

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ABBREVIATED TITLE: 12-LO and β cell dysfunction

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Abstract

The insulin producing islet β cells have increasingly gained attention for their role in the pathogeneses of virtually all forms of diabetes. Dysfunction, de-differentiation, and/or death of β cells as a result of systemic and local inflammation are pivotal features in the transition from normoglycemia to hyperglycemia in both animal models of metabolic disease and humans. The lipoxygenases represent a class of enzymes that oxygenate cellular polyunsaturated fatty acids to produce lipid intermediates that directly and indirectly affect cellular function and survival. The enzyme 12-lipoxygenase is expressed in all metabolically active tissues including pancreatic islets, and has received increasing attention for its role in promoting cellular inflammation in the setting of diabetes. 12-lipoxygenase has received increasing attention in recent years, as genetic deletion models in mice reveal striking protection from metabolic disease and its complications and an emerging body of literature has implicated its role in human disease. This review focuses on the evidence supporting the proinflammatory role of 12-lipoxygenase as it relates to islet β cells, and the potential for 12-lipoxygenase inhibition as a future avenue for the prevention and treatment of metabolic disease.

Abbreviations

ER, endoplasmic reticulum

HETE, hydroxyeicosatetraenoic acid

HPETE, hydroperoxyeicosatetraenoic acid

LO, lipoxygenase

MAPK, mitogen activated protein kinase

NOD, non-obese diabetic

PP, pancreatic polypeptide

STZ, streptozotocin

T1D, type 1 diabetes

T2D, type 2 diabetes

Islet β cell dysfunction is a common feature of type 1 and type 2 diabetes.

The crude prevalence of all forms of pre-diabetes and diabetes in the US exceeds 40% (1). Worldwide, it is expected that up to 592 million people will develop diabetes by the year 2035 (2). These striking data reflect the fact that the major forms of diabetes, type 2 diabetes (T2D) and type 1 diabetes (T1D), have been increasing in incidence in recent decades. The increase in T2D is closely linked to the high prevalence of obesity and pre-diabetes (3), whereas the reasons for the increase in T1D remain elusive (4–6). Diabetes is defined as the glycemic threshold (fasting plasma glucose ≥126 mg/dl or hemoglobin A1c ≥6.5%) at which microvascular complications, such as retinopathy and nephropathy, are observed (7). By contrast, cardiovascular complications, including stroke and myocardial infarction, increase even as blood sugars rise in the pre-diabetic phase (8). Therefore the identification of drug targets that are common to all forms of diabetes is likely to have far-reaching implications for disorders of multiple organ systems. A key underlying feature of all forms of diabetes is the relative deficiency of insulin secretion from the islet β cell. T2D arises primarily in the setting of long-standing insulin resistance, wherein the magnitude of insulin secretion by the β cell fails to meet the peripheral tissue insulin demands (9). In T1D, insulin deficiency has traditionally (and perhaps too simplistically) been ascribed to autoimmune-mediated β cell destruction; however, recent studies in both rodents and humans suggest that a “prodrome” may exist in T1D, in which insulin secretory capacity is diminished even prior to overt β cell death (10–12). β cell loss, therefore, may represent a feature occurring very late in the pathogeneses of both T2D and T1D.

The β cell is unique in its ability to synthesize and secrete physiologically relevant levels of insulin in response to ambient glucose concentrations. In recent years a growing body of literature suggests that the pathways that initiate dysfunction of the β cell in T2D and T1D may be similar, if not identical (13). Of particular relevance is inflammation, which leads to the

development of oxidative stress, endoplasmic reticulum stress, and mitochondrial dysfunction in the β cell (14,15). In T2D, multiple cell types collaborate in the pathogenesis of β cell inflammation, including adipocytes, macrophages and other immune cells (dendritic cells, T cells). In the setting of high fat diets, macrophage polarization to a proinflammatory phenotype ("M1" type) within adipose tissue leads to the production of adipocytokines (e.g. IL6, TNFα), which signal systemically to β cells (16,17). This early innate immune response may give way to a later adaptive response, wherein the balance between proinflammatory, effector T cells in the fat and anti-inflammatory, regulatory T cells determine the inflammatory features of adipose tissue (13,18,19). Moreover, M1 macrophage and auto-reactive T cell trafficking into the islet itself may occur (20–23), leading to local cytokine release and cell-mediated immunity that directly trigger β cell inflammation.

The concept that β cell dysfunction is a key early feature of T1D has seen increasing attention (12,24–28). In the NOD mouse model of T1D, impaired glucose-stimulated insulin secretion—particularly first phase insulin secretion—precedes the loss of β cell mass by several weeks (11,12). Similar findings were also observed in humans with T1D, who exhibited defects in glucose-stimulated insulin release prior to the onset of diabetes (10,29). Inflammation, possibly emanating from infection or primary autoimmunity, has been implicated as underlying β cell dysfunction (26). More recently, it has been proposed that inflammation and its resultant oxidative and ER stresses act as triggers that initiate autoantigen and neoantigen exposure to drive autoimmunity (12,30,31). Taken together, these studies on β cell function in T2D and T1D suggest that pathways that promote inflammation in β cells represent potential targets to prevent or treat both diseases.

The lipoxygenase pathways

An important pathway recently implicated in diabetic inflammation involves a family of enzymes known as lipoxygenases (LOs). The LOs catalyze the oxygenation of cellular poly-unsaturated fatty acids (primarily arachidonic acid), and are classified according to both the specific carbon atom that is subjected to oxygenation (5-, 12-, and/or 15-positions) as well as the stereoselectivity of the reaction (the "S" type being the primary enantiomer) (see Fig. 1, and reviewed in (32)). The LOs are expressed in a variety of tissue types, and often given common names based on the tissue types in which they were identified. In humans, and the best studied LOs are "leukocyte" 5-LO, "platelet" 12-LO, "reticulocyte" 15-LO-1, and "epithelial 15-LO-2" (33). 5-LO, whose expression is primarily limited to bone marrow-derived cell types, has been studied in a variety of contexts with respect to inflammation, as the enzyme is required for the downstream production of proinflammatory leukotrienes (34). Accordingly, 5-LO knockout mice exhibit protection from atherogenesis and aortic aneurysms (35,36), as well as diabetes-induced retinal capillary degeneration (37). Low-level production of 5-LO products has also been described in rodent pancreatic islets (38), and the mRNA encoding 5-LO is present in human islets (39). Notwithstanding evidence for reduced atherogenesis, whole-body 5-LO knockout mice exhibit reductions in glucose-stimulated insulin secretion, and islets isolated from these mice show reduced levels of mRNAs encoding for insulin and the key β cell transcription factor Pdx1 (40). These data suggest a pleiotropic effect of 5-LO with respect to tissue-specific inflammation, with 5-LO conferring apparent protection in β cell function. Nevertheless, the role of 5-LO in glycemic homeostasis has not been extensively investigated, and a cell-autonomous role for 5-LO in β cells *in vivo* (using conditional knockout mice) is needed in follow-up studies.

In contrast to the pleiotropic effects of 5-LO, 12-LO appears to have more uniform proinflammatory effects, and is broadly expressed in virtually all metabolically active cell types, including hepatocytes, adipose tissue, islets, and macrophages/monocytes. 12-LO converts arachidonic acid to 12-hydroperoxyeicosatetraenoic acid (12-HPETE), which is subsequently

reduced to more stable 12-hydroxyeicosatetraenoic acid (12-HETE) by glutathione peroxidase. The mouse "leukocyte" 12-LO enzyme encoded by the gene *Alox15* produces a ~6:1 ratio of 12-HETE:15-HETE from arachidonic acid, and is often referred to as 12/15-lipoxygenase (33). Functionally, the mouse LO encoded by *Alox15* is closest to the human "platelet" 12-LO encoded by *ALOX12*, which produces almost exclusively 12-HETE (41). The role of 12-LO and its major product 12-HETE has been studied extensively in the context of rodent models of diabetes and in normal and diabetic human tissues; in this review, the simplified term "12-LO" will refer to the enzyme encoded by the *Alox15* and *ALOX12* genes in mice and humans, respectively.

Role of 12-lipoxygenase in inflammation and metabolic disease

The role of 12-LO in inflammation has been studied best in the immune/inflammatory responses involving monocytes and macrophages, and also more recently, mouse and human pancreatic islets. Unlike 5-LO, whose downstream products include both the HETEs and leukotrienes, the effects of 12-LO are primarily attributed to the production of 12-HETE. Activation of 12-LO has been shown to accelerate inflammation via p38 mitogen-activated protein kinase (MAPK) and nuclear factor κB (NF-κB) pathways, cause oxidation of low density lipoprotein to promote foam cell formation, and promote oxidative stress (42–50). In macrophages, 12-LO activity increases production of pro-inflammatory cytokines such as TNFα, and IL-6, and also stimulates expression of inflammatory genes such as *Cox2* (50,51). Notably, 12-LO also stimulates production of IL-12, a pivotal cytokine that mediates microbial immunity, atherosclerosis, and the Th1 autoimmune response in T1D (52–54).

The expression and activity of 12-LO are upregulated in a variety of metabolically active cell types (macrophages, adipose tissue, hepatocytes, islet β cells) in response to hyperglycemia (55–58), proinflammatory cytokines (42,59), and saturated free fatty acids (60–

63). The cause-effect relationship of 12-LO in the context of vascular disease and metabolism *in vivo* initially arose from studies of whole-body *Alox15*$^{-/-}$ mice, which exhibit no overt phenotype, but show striking protection from disease upon challenges. In the setting of the atherosclerosis-prone *Ldlr*$^{-/-}$ and *Apoe*$^{-/-}$ mouse backgrounds, the absence of 12-LO confers protection against atherosclerosis and steatohepatitis (63–65). These effects likely result from the absence of 12-LO in macrophages/monocytes, since *Apoe*$^{-/-}$ mice receiving bone marrow from *Alox15*$^{-/-}$;*Apoe*$^{-/-}$ mice were protected from the development of atherosclerosis (65) and macrophages from *Alox15*$^{-/-}$ mice have reduced ability form foam cells (43,65)

A role for 12-LO in metabolic disease has been studied in the context of obesity/T2D and T1D. Studies of Nunemaker, et al. (66) examined the effects of Western high fat diet (45% kcal from saturated fat) on *Alox15*$^{-/-}$ mice on the C57BL/6 background. Compared to control mice, the authors observed that *Alox15*$^{-/-}$ mice exhibited improved glucose tolerance, with reduced macrophage infiltration into fat, reduced proinflammatory cytokine levels (IL6, TNFα), and improved β cell function (as assessed by glucose stimulated insulin secretion *in vivo* and *in vitro*). Using a similar feeding paradigm, Sears, et al. (67) observed that high fat-fed *Alox15*$^{-/-}$ mice displayed reduced levels of proinflammatory cytokines (IL1β, IL6, IL12, TNFα) accompanied by improved whole-body insulin sensitivity as assessed by hyperinsulinemic euglycemic clamp. Although these studies point to a role for 12-LO in proinflammatory macrophages, they must be reconciled with the known expression of 12-LO in white adipocytes, particularly after exposure to high fat diets (60,61,63). 12-LO and its product 12-HETE are increased in visceral white adipose tissue of morbidly obese humans with T2D (68). High fat-fed adipose-specific *Alox15*$^{-/-}$ mice (*Alox15*$^{lox/lox}$;*Ap2-Cre*) strikingly phenocopy whole-body *Alox15*$^{-/-}$ mice, exhibiting reduced macrophage infiltration into islets, improved insulin sensitivity, and protection from glucose intolerance (69). A caveat in these studies is the known expression of the *Ap2* gene in macrophages as well as fat cells (70,71), but these studies nevertheless

raise the possibility that 12-LO in both cell types may contribute to insulin resistance and glucose intolerance seen during obesity/T2D.

Studies of 12-LO in the context of T1D are more limited, but nevertheless compelling in terms of its effect on disease outcome. In studies of McDuffie, et al. *Alox15*$^{-/-}$ mice were backcrossed onto the non-obese diabetic (NOD) background. NOD mice are the only mouse strain to exhibit spontaneous development of diabetes as a result of β cell autoimmunity (72). NOD mice exhibit immune cell infiltration into islets (insulitis) as early as 4 weeks of age (consisting mostly of macrophages at this age), with subsequent diabetes development after the age of 12 weeks (12,73). Female NOD mice generally exhibit higher rates of spontaneous diabetes compared to males, for reasons that remain unclear. *NOD-Alox15*$^{-/-}$ female mice showed nearly complete protection from T1D, whereas ~60% of control females developed diabetes; similarly *NOD-Alox15*$^{-/-}$ males were completely protected, compared to ~20% of control males that developed diabetes. A notable finding was the virtual absence of macrophage insulitis in *NOD-Alox15*$^{-/-}$ mice, a finding suggesting a possible role for 12-LO in macrophages during diabetes pathogenesis in this model. In a follow up study, Green-Mitchell, et al. (74) demonstrated that 12-LO is expressed in macrophages, but not T and B cells, of NOD mice. Splenocytes from *Alox15-/-* mice were unable to adoptively transfer T1D to recipient mice, whereas those from control mice adoptively transferred diabetes within 4 weeks. These findings suggested a primary role for macrophage 12-LO in T1D disease pathogenesis.

12-LO in the pancreatic islet

A major limitation to the global deletion models of 12-LO is the inability to definitively attribute its effects in specific tissues or cell types. Because characterization of 12-LO focused primarily on cells derived from the bone marrow, particularly cells of the macrophage/monocyte origin, much of the literature is arguably biased towards attributing effects of 12-LO in these cell

types. However, an increasing body of literature suggests that 12-LO may also play an intrinsic role in islet inflammation and dysfunction. The leukocyte isoform of 12-LO has been identified in the both the rodent islets (42,75–77) and human islets (39,42,45). Similar to macrophages and adipose tissue, 12-LO expression and/or activity are upregulated in mouse islets *in vitro* under conditions of hyperglycemia (57) and cytokine exposure (42,59,76,78), and *in vivo* following high fat diet feeding (59). In isolated human islets, 12-LO protein and activity levels are upreguated by incubation with proinflammatory cytokines (45). Notably, no expression of 12-LO in non-endocrine pancreatic cell types have been observed in these studies. Within the islet, 12-LO expression has been reported in β cells (42,59) and in α cells (79). With respect to the latter, overexpression of 12-LO in an α cell line enhanced glucagon secretion, suggesting that the promotion of glucagon secretion by 12-LO might contribute to hyperglycemia in the setting of diabetes. More recently, 12-LO expression has been documented in pancreatic polypeptide (PP) cells of human diabetic pancreas (80), an observation that may have implications for 12-LO in postnatal islet cell de-differentiation (*vide infra*).

12-LO in the islet β cell

12-LO and its products appear to affect islet β cell function, survival, and possibly differentiation. The major product of 12-LO, 12-HETE, reduces glucose-stimulated insulin secretion in human islets at low concentrations (1 nM) and induce islet death at higher concentrations (100 nM), whereas 15-HETE and the inactive form 12(R)-HETE have no effect (45). Similar findings have been observed in mouse islets (42). These findings *in vitro* suggest that the upregulation of 12-LO seen in response to cytokines exposure or hyperglycemia may correlate closely to the dysfunction of β cells observed in T2D and T1D. Recently, studies of Grzesik, et al. (80) using pancreas from donors with T2D and T1D revealed that 12-LO immunoreactivity is increased in islets of these individuals, but curiously with expression co-localizing with PP-staining cells. In light of recent provocative studies suggesting that

dedifferentiation of β cells to PP-expressing cells may underlie diabetes pathogenesis (81), the findings of Grzesik, et al. (80) suggest a potential role for 12-LO and its products in the de-differentiation of β cells in disease.

The earliest studies implicating a causative role for 12-LO in islet dysfunction *in vivo* involved low-dose streptozotocin (STZ) treatment of whole-body 12-LO knockout mice (82). STZ is a β cell toxin that is taken up via Glut2 glucose transporters (83). When given in multiple low doses, STZ results in the influx of proinflammatory leukocytes into islets, initiating a cascade of events resulting in the local release of proinflammatory cytokines, β cell dysfunction, and eventual β cell death (84–86). Bleich, et al. (82) demonstrated that whole-body 12-LO knockout mice are protected from hyperglycemia and β cell loss following multiple low-dose STZ, suggesting an inherent resistance of β cells to stress and death in the absence of 12-LO. Nevertheless, the loss of 12-LO in macrophages and other leukocytes might also have contributed to the observed phenotype. More definitive evidence supporting a causative role for 12-LO in islet dysfunction arose from recent studies of pancreas-specific *Alox15*$^{-/-}$ knockout mice (*Alox15*$^{lox/lox}$*;Pdx1-Cre*). In this model, Tersey, et al. (59) demonstrated that loss of 12-LO in the pancreas (including islets) resulted in protection from both low-dose STZ-induced hyperglycemia and high fat diet-induced glucose intolerance. Unlike whole-body knockout mice, however, the high fat diet-fed pancreas specific knockouts exhibited no protection from insulin resistance or macrophage infiltration into fat, emphasizing a previously unappreciated role for islet 12-LO in the deterioration of metabolic homeostasis. These findings suggest that phenotypes observed in whole-body 12-LO knockouts likely reflect a complexity of 12-LO action in multiple metabolically active tissue types. In the context of T1D, 12-LO enzyme levels are known to increase in islets of NOD mice in the pre-diabetic phase (74), suggesting a possible contribution of 12-LO to islet autoimmunity and dysfunction, however, a definitive role for islet 12-LO in T1D must await studies of tissue-specific knockouts on the NOD background.

Molecular mechanisms of 12-LO contributing to β cell dysfunction

The mechanisms by which 12-LO activity causes β cell dysfunction in the setting of diabetogenic stress (proinflammatory cytokines, hyperglycemia, saturated free fatty acids) remain incompletely defined, but recent evidence points to involvement of reactive oxygen species (ROS) generated by its major products 12-HPETE and 12-HETE (see Fig. 2). Islet β cells are particularly sensitive to oxidative stress, as levels of antioxidant enzymes are low in these cells relative to other metabolically active tissues (87). In addition to the previously discussed activation of stress kinases JNK and p38 MAPK by 12-HETE (42,45), 12-HETE also activates NADPH oxidase-1 (NOX-1) in mouse and human islets (88). Inhibition of 12-LO activity using specific inhibitors attenuates NOX-1 expression, reduces ROS and restores glucose-stimulated insulin secretion in response to proinflammatory cytokines (88). Studies of Tersey, et al. (59) also link 12-LO/12-HETE to the inactivation (i.e. cytoplasmic sequestration) of the Nrf2 transcription factor, which is a major transcriptional activator of antioxidant genes. Pancreas-specific 12-LO knockout mice that were fed a high fat diet exhibited greater nuclear levels of Nrf2 in β cells, with concomitant increases in antioxidant enzymes superoxide dismutase and glutathione peroxidase (59).

Excessive ROS can induce perturbations in ER homeostasis, leading to protein misfolding, β cell dysfunction, and eventual β cell death (when the ER stress cascade is initiated) (reviewed in (89)). In this respect, excessive 12-HETE (via ROS generation) leads to the development of β cell ER stress, as evidenced by increased expression of *Chop* and *spliced Xbp1*, and increased production of unprocessed proinsulin (59). These and other effects of 12-HETE may be mediated via interaction with a G-protein-coupled receptor (90). Recently, the orphan G protein-coupled receptor GPR31 was shown to interact with 12-HETE at low nanomolar concentrations (91). Activation of GPR31 receptor by 12-HETE was associated with stress kinase activation (91). However, a direct role *in vivo* for GPR31 in the pro-inflammatory effects of 12-LO, particularly in the β cell, has yet to be elucidated. Other putative HETE

receptors (though not specific for 12-HETE) include the PPARs (92) and an eicosatetraenoic receptor (93).

Apart from the production of ROS, other mechanisms of 12-LO activity have also been proposed to contribute to β cell dysfunction. Arachidonic acid levels are exceptionally high in pancreatic islets (about 30% of total islet glycerolipid fatty acid mass) (94) and is a potentiator of insulin secretion (39,95,96). In this respect, increased β cell activation of 12-LO in the setting of diabetogenic stressors may cause metabolic shunting of arachidonic acid, providing less stimulus for insulin secretion. Additionally, 12-LO has been shown to activate Cox2 in β cells, converting arachidonic acid to prostaglandin E2 (51), which is a potent inhibitor of insulin secretion (97–99). 12-HETE has been shown to induce macrophage chemoattractant protein 1 in β cells (88), promoting the influx of proinflammatory macrophages into islets as part of a non-cell autonomous role of 12-LO in inducing β cell dysfunction. Finally, in hepatocytes, it was recently demonstrated that the absence or inhibition of 12-LO leads to an increase in the appearance of autophagy (100), a finding that suggests that 12-LO may suppress a potentially protective clearing mechanism that is otherwise required during periods of stress.

Discovery and application of small molecule 12-LO inhibitors

For their role in a variety of inflammatory disorders and malignancies, the lipoxygenases have been prime targets for the development of chemical inhibitors. To date, the only FDA-approved inhibitor is targeted against 5-LO (Zileuton) for use in asthma (101). Baicalein was used in early studies as a 12-LO inhibitor, but was later shown to be non-specific and to inhibit both 12- and 15-LO (102). Early efforts to discover novel potent and selective 12-LO inhibitors through traditional medicinal chemistry (103–110), computational chemistry (111) and natural product isolation (112) were largely unsuccessful. The compounds discovered in these attempts were promiscuous and/or reductive in nature and not drug-like, chemically tractable, or selective. However, high throughput screening attempts followed by medicinal chemistry

optimization resulted in an 8-hydroxyquinoline based compound, *N*-((5-bromo-8-hydroxyquinolin-7-yl)(thiophen-2-yl)methyl)acetamide (ML127, Figure 3), which exhibits micromolar potency and over 50-fold selectivity over lipoxygenase isozymes and cyclooxygenase (113). However, a subsequent molecule, *N*-(benzo[d]thiazol-2-yl)-4-((2-hydroxy-3-methoxybenzyl)amino)benzenesulfonamide (ML355, Figure 3) exhibited slightly improved potency (sub-micromolar potency) and comparable selectivity to ML127, but is less likely to chelate metals and has improved drug-like qualities (114). Taylor-Fishwick, et al. (115) demonstrated that 8-hydroxyquinoline-based 12-LO inhibitors blocked 12-HETE production from cytokine-stimulated human islets, led to improved insulin release, and enhanced islet survival. Additionally, the authors demonstrated that one of the compounds (ML127) could reduce plasma 12-HETE levels when administered orally to mice. As such, 8-hydroxyquinoline compounds represent strong leads as clinically-tractable 12-LO inhibitors.

Conclusions and future directions

The LOs and their lipid products have been studied extensively for their roles in a variety of diseases from allergic/immunologic disorders to metabolism to cancer. 12-LO has been shown to be almost uniformly pro-inflammatory in all metabolically active tissues studied. To the extent that whole-body *Alox15*$^{-/-}$ mice exhibit no phenotype when unstressed, 12-LO represents an attractive, yet still somewhat underdeveloped target in metabolic disease. The near-parallel expression pattern and function of 12-LO in mouse and human tissues provides some level of confidence that successes with next-generation 12-LO inhibitors in mouse models will portend potential utility in human disease. Nevertheless, several crucial questions still remain unanswered with respect to the role of 12-LO in different tissue types, and precisely how 12-LO products (such as 12-HETE) exert their downstream effects and via which receptor types. Moreover, it is presently unknown if inhibition or elimination of 12-LO after the establishment of T2D or T1D will allow for reversal of disease. These and other crucial

questions can be fairly readily addressed in mouse models, since both conditional knockout mice and specific inhibitors are now available.

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Figure legends

Figure 1. Arachidonic acid metabolism. Schematic diagram showing metabolism of arachidonic acid by lipoxygenases (*LOs*) and cyclooxygenase (*COX*). *PGH_2*, prostaglandin H_2; *HPETE*, hydroperoxyeicosatetraenoic acid; *HETE*, hydroxyeicosatetraenoic acid.

Figure 2. 12-LO pathway in the β cell. The figure depicts the pathways activated by 12-LO in the islet β cell in response to elevated glucose levels, saturated free fatty acids, or proinflammatory cytokines. Activation of 12-LO leads to the production of proinflammatory lipid intermediates (12-HPETE and 12-HETE), which subsequent trigger inflammatory pathways mediated by c-Jun N-terminal kinase (*JNK*), p38 mitogen-activated protein kinase (*p38-MAPK*), and NADPH oxidase (*NOX*). 12-HETE also prevents the translocation of nuclear factor erythroid 2-related factor 2 (*Nrf2*). Collectively, these pathways lead to increased ROS (reactive oxygen species), oxidative stress, endoplasmic reticulum (*ER*) stress, and ultimately β cell dysfunction and death. *FFAR*, free fatty acid receptor; *PLA*, phospholipase A, *COX*, cyclooxygenase; *PGE_2*, prostaglandin E_2; *MCP1*, monocyte chemoattractant protein 1; *HPETE*, hydroperoxyeicosatetraenoic acid; *HETE*, hydroxyeicosatetraenoic acid.

Figure 3. Small molecule inhibitors of 12-LO. Shown are the structures of the 8-hydroxyquinoline-based inhibitors of 12-LO, ML127 and ML355.