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# Synthesis and biological evaluation of lisofylline (LSF) analogs as a potential treatment for Type 1 diabetes

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**Abstract**—Lisofylline (LSF, 1-(5-*R*-hydroxyhexyl)-3,7-dimethylxanthine) is an anti-inflammatory agent that protects β-cells from Th1 cytokine-induced dysfunction and reduces the onset of Type 1 diabetes in non-obese diabetic (NOD) mice. Due to its low potency, poor oral bioavailability, and short half-life, the widespread clinical utility of LSF may be limited. Our goal has been to develop new agents based on the LSF structural motif that resolve the potency and pharmacokinetic liabilities of LSF. In this study, we have generated a focused library of LSF analogs that maintain the side chain (5-*R*-hydroxyhexyl) constant, while substituting a variety of nitrogen-containing heterocyclic substructures for the xanthine moiety of LSF. This library includes the xanthine-like (5-aza-7-deazaxanthine), as well as non-xanthine-like skeletons. The LSF analogs were evaluated in a pancreatic β-cell line for the effects on apoptosis protection and insulin release. The metabolic stability of selected compounds was also tested.


Type 1 diabetes, a common and widespread disease occurring in every part of the world, is an autoimmune disorder that results from the immune-mediated inflammatory destruction of insulin-producing β-cells in pancreatic islets. There are an estimated 500,000 to 1 million people with Type 1 diabetes in the US today. Although the specific pathogenic mechanisms in Type 1 diabetes are not defined, it is clear that activated T cells and macrophages are required for the initiation. Once activated, macrophages secrete several inflammatory cytokines, such as interleukin 1β (IL-1β), interleukin 12 (IL-12), and tumor necrosis factor α (TNF-α), and trigger interferon-γ (IFN-γ) production from activated T cells.[1] These cytokines are cytotoxic to β-cells by inducing the formation of oxygen free radicals, nitric oxide, and lipid peroxides within β-cells and enhance Th1-cell-mediated inflammatory responses, which are responsible for β-cell destruction. [2]

Lisofylline (LSF) (Fig. 1), 1-(5-*R*-hydroxyhexyl)-3,7-dimethylxanthine, is a novel anti-inflammatory compound that was originally used to reduce the incidence of graft vs. host disease and to prevent the onset of experimental autoimmune encephalomyelitis by blocking IL-12-induced T helper 1 differentiation.[3,4] It abrogated release of inflammatory cytokines during oxidative lung injury and reduced inflammatory cytokine release in response to cytotoxic cancer chemotherapy.[5] LSF also showed beneficial effects in several inflammatory disorders such as sepsis, hypoxia, and hemorrhagic organ injury.[6,7] LSF has also been shown to protect β-cells from multiple inflammatory cytokine-mediated injuries by its ability to maintain insulin secretory capability and cell viability.[2] Although not established, the mechanism of LSF-induced protection may be due to promotion of β-cell mitochondrial metabolism, normalizing mitochondrial membrane potential and stimulating energy production. This unique spectrum of activity suggests that agents such as LSF could have clinical utility in preventing β-cell damage during the development of Type 1 diabetes or after islet cell transplantation. This hypothesis was supported by studies that showed LSF could signifi-

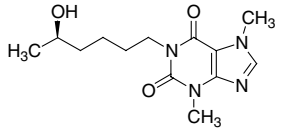


**Figure 1.** Structure of Lisofylline (LSF, (1-(5-*R*-hydroxyhexyl)-3,7-dimethylxanthine)).

*Keywords*: Type 1 diabetes; Lisofylline; LSF; LSF analogs; 5-Aza-7-deazaxanthine.
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cantly reduce spontaneous Type 1 diabetes development in the non-obese diabetic (NOD) mouse.[1]

LSF has disadvantages that may limit its clinical development: LSF exhibits low (to non-existent) oral bioavailability, has an extremely short half-life, requiring constant intravenous infusion in humans, and has relatively low potency for direct islet protective effects, requiring concentrations of 20 μM in vitro and at least 25 mg/kg body weight in rodents to see beneficial effects. In efforts directed at enhancing the potency of LSF, Klein et al.[8] prepared a library of LSF analogs based on the xanthine framework and studied the SAR for suppression of Th1 differentiation through IL-12 blockade. Despite considerable structural variation, only relatively small differences in activity were observed among the analogs studied. Importantly, it was found that the enantiomeric *S*-LSF (which possesses the *S*-alcohol configuration) was inactive, implicating a highly specific interaction of the *R* isomer with a binding point on the target site.

The aim of our investigation was to synthesize a focused library of small molecules based on the LSF structure to elucidate the SAR for potency and pharmacokinetic parameters. In one approach, we synthesized analogs of the 5-aza-7-deazaxanthine family (Fig. 2). The 5-aza-7-deazaxanthine structure is isosteric with xanthine, but has a distinct electronic character as evidenced by its lower basicity and its photophysical properties.[9] In a second approach, we substituted a series of nitrogen-containing heterocyclic substructures for the xanthine moiety of LSF.

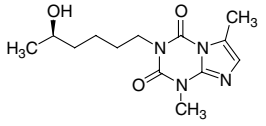
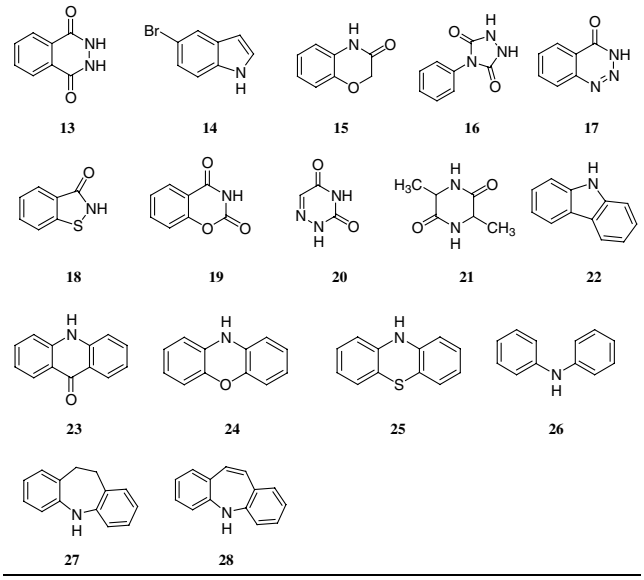
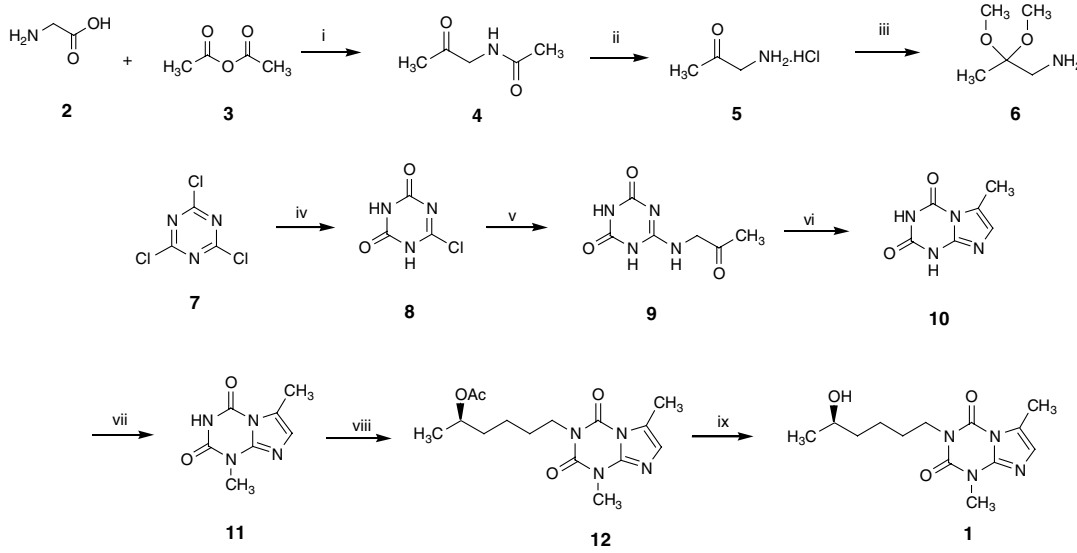


**Figure 2.** 5-Aza-7-deazaxanthine LSF (5,7-isoLSF) **1**.

The synthetic route to 5-aza-7-deazaxanthine-LSF **1** (Scheme 1) commenced with coupling of glycine **2** and acetic anhydride **3** in presence of pyridine affording acetamidoacetone **4**,[10] which was hydrolyzed in HCl to form aminoacetone hydrochloride **5**. The ketone was then transformed to its acetal by treatment with a mixture of trimethyl orthoformate and methanol in the presence of *p*-toluenesulfonic acid. The free amine **6** was obtained by treatment with sodium hydroxide.[11] Compound **6** was ready for later reactions with a protected carbonyl group and a free

**Table 1.** The selected heterocyclic compounds being investigated




**Scheme 1.** Reagents and conditions: (i) pyridine, reflux, 6 h, 78%; (ii) HCl, $H_2O$, reflux, 6 h, 60%; (iii) trimethylorthoformate, MeOH, *p*-toluenesulfonic acid monohydrate, reflux 24 h, then 3 N NaOH, 49%; (iv) NaOH, $H_2O$; (v) **6**, $H_2O$, reflux, 3 h, 17%; (vi) $H_2SO_4$, 95 °C, 1.5 h, 35%; (vii) NaOH, $CH_3I$, $H_2O$, acetone, rt, 24 h, 34%; (viii) 5-(*R*)-acetoxy-1-chlorohexane, NaH, DMSO, 70–80 °C, overnight; (ix) 1 M HCl in ether, MeOH, rt, 12 h, 60% two steps.