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Carbazole-containing amides and ureas: Discovery of cryptochrome modulators as antihyperglycemic agents

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ABSTRACT

A series of novel carbazole-containing amides and ureas were synthesized. A structure–activity relationship study of these compounds led to the identification of potent cryptochrome modulators. Based on the desired pharmacokinetic/pharmacodynamic parameters and the results of efficacy studies in db/db mice, compound **50** was selected for further profiling.

Type 2 diabetes (T2D) is a metabolic disorder that accounts for 422 million patients worldwide and the number is likely to grow to greater than 590 million by the year 2035.[1] T2D is a major cause of blindness, kidney failure, heart attacks, stroke and lower limb amputation.[2,3]

In mammals, many physiological processes such as body temperature, hormone secretion, metabolism, and sleep/wake behavior are controlled by the circadian clock in a daily rhythmic manner.[4–7] Dysregulation in the circadian system caused by variants of clock genes has been correlated with T2D and insulin resistance.[8,9] More specifically, genetic variants in *Cryptochrome* (*Cry*) genes have been linked with glucose homeostasis, hepatic lipid content and beta-cell function.[10–15]

Cell-autonomous circadian rhythms are generated through clock genes, which are orchestrated by interconnected transcriptional and translational regulatory loops. The core clock loop is constituted by two transcriptional activators, CLOCK and BMAL1, and two repressors: Cryptochrome (CRY1 and CRY2) and Period (PER1 and PER2). The CLOCK–BMAL1 heterodimer activates the transcription of Cry and Per though binding to E-box enhancer elements. Translated PER and CRY proteins dimerize and translocate into the nucleus to inhibit CLOCK–BMAL1 function, resulting in rhythmic gene expression pattern. The loop regulates a large number of clock-controlled genes in circadian physiology. This timing mechanism is regulated by post-translational modifications such as phosphorylation and ubiquitination, which tune the pace of the clock.[16,17] The SCF^{FBXL3} ubiquitin ligase complex plays an important role in CRY ubiquitination and degradation to modulate the negative feedback loop.[18–20] Recent X-ray crystallographic results revealed details of CRY regulation by its PER partners and stability by FBXL3. The F-box protein FBXL3 occupies the FAD binding pocket of CRY2 with its C-terminal tail and also covers the PER binding domain of CRY2.[21,22]

Circadian clock-deficient animals have been utilized to examine disease pathophysiology linked to the circadian clock. Mice lacking *Cry1* and *Cry2* (Cry-null mice), on a high fat diet, rapidly gain weight, become hyperinsulinemic and have elevated corticosterone and upregulated expression of lipogenic genes in white adipose tissue.[23] These mice also display a rapid progression from non-alcoholic fatty liver disease to non-alcoholic steatohepatitis, fibrosis and hepatocellular carcinoma.[24] Cry-null mice exhibit salt-sensitive hypertension due to abnormally high synthesis of the mineralocorticoid aldosterone by the adrenal gland.[25,26] Under normal salt exposure, mice show increased albumin excretion and kidney tubular injury, decreased nephrin expression and increased reactive oxygen species production in the absence of hypertension.[27]

A period-lengthening small molecule, KL001, that directly targets CRY was discovered (Fig. 1)[28] to stabilize CRY by binding to

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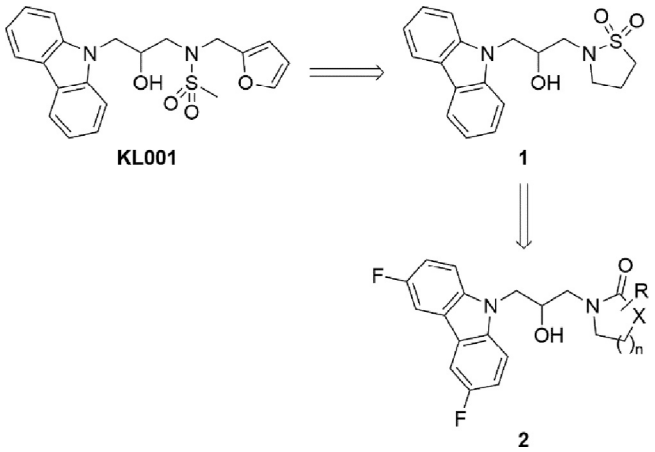


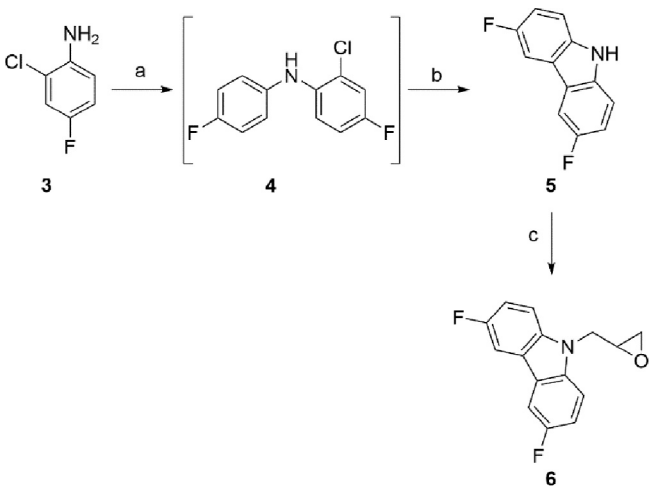
Fig. 1. KL001, initial lead **1** and new lead scaffold **2**.

the FAD-binding pocket in competition with FBXL3,[29] thereby extending the observed circadian period. KL001 was initially optimized to yield **1,** which demonstrated antihyperglycemic effects in animal models of T2D.[30] In order to further determine the structural requirements for activity and to increase in vivo exposure, we wished to synthesize compounds represented by the general structure **2** (Fig. 1). Herein, we report the synthesis, structure-activity relationships (SAR), and in vivo activity of this new class of compounds.[31]

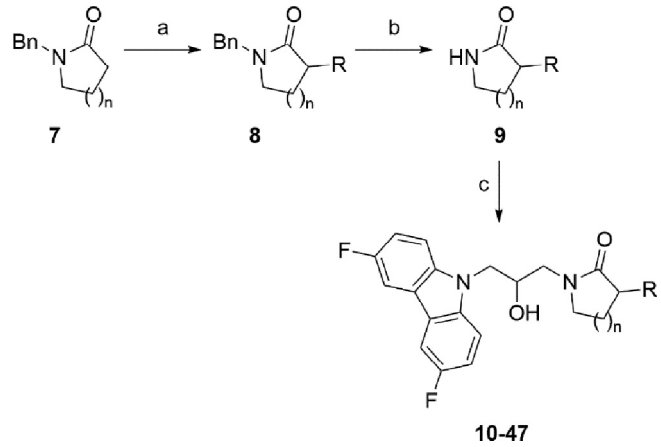
Following the early optimization to cyclic sulfonamide **1**, installation of fluorine substituents at the 3- and 6-positions of the carbazole moiety was shown to decrease in vitro intrinsic clearance without affecting potency and so this moiety was held constant going forward.

The synthetic route for the preparation of critical epoxide intermediate **6** is shown in Scheme 1. Coupling of 2-chloro-4-fluoroaniline **3** and 1-bromo-4-fluorobenzene afforded **4** in high yield and purity. This crude material was telescoped directly into the intramolecular direct arylation and yielded the desired carbazole **5** in a 64% yield over two steps. Coupling of epibromohydrin and carbazole **5** afforded epoxide **6** in excellent yield.

Lactams of various ring sizes and substitutions were produced by the synthetic route in Scheme 2. Commercially available benzyl-protected lactams **7** were deprotonated with base and then



Scheme 1. Reagents and conditions: (a) 4-F-PhBr, $Pd_2(dba)_3$, $P^tBu_3{\cdot}HBF_4$, KO^tBu, PhMe, 50 °C, 3 h; (b) $Pd(OAc)_2$, $PCy_3{\cdot}HBF_4$, K_2CO_3, DMA, 130 °C, 7 h, 64% over two steps; (c) epibromohydrin, KOH, DMF, 0 °C to rt, 16 h, 90%.



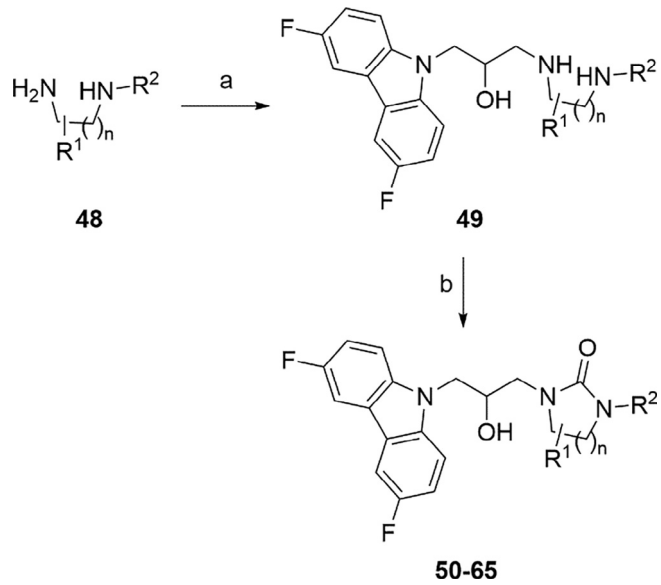
Scheme 2. Reagents and conditions: (a) LDA, THF, −78 °C, 30 min then R-Hal, −78 °C to rt, 16 h; (b) TfOH, PhMe, μW, 200 °C, 15 min to 1 h; (c) NaH, DMF, rt then **6**, 45 °C, 16 h.

treated with a variety of alkyl halides to afford substituted lactams **8**. Deprotection of **8** yielded **9**, which were then treated with sodium hydride and epoxide **6** to produce final products **10**–**23**. Repeating the deprotonation and alkylation step (*e.g.,* transformation of **7** to **8**) on compound **8**, followed by deprotection and coupling with epoxide **6** afforded final products **31**–**34**. Final products **24**–**47** were synthesized by coupling commercially available or previously reported lactams with epoxide **6**.

A variety of commercially available or previously reported cyclic carbamates and bicyclic amides were also coupled with epoxide **6** to afford final products **35**–**47**.

Attempts to further decrease lipophilicity led us to explore the tolerance and SAR for substituted ureas on the right-hand side of the chemical series. As shown in Scheme 3, commercially available diamines **48** were coupled with epoxide **6** to afford intermediates **49**. Cyclization of intermediates **49** with carbonyldiimidazole (CDI) yielded the final products **50**–**65**.

The newly synthesized compounds were evaluated for their effects on circadian rhythms in a human osteosarcoma U2OS cell line harboring a *Per2-dLuc* luciferase reporter.[28] Continuous incu-



Scheme 3. Reagents and conditions: (a) **6**, EtOH, 40 °C, 16 h; (b) CDI, THF, 0 °C to rt, 16 h.