



Carbazole-containing sulfonamides and sulfamides: Discovery of cryptochrome modulators as antidiabetic agents



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ABSTRACT

A series of novel carbazole-containing sulfonamides and sulfamides were synthesized. A structure–activity relationship study of these compounds led to the identification of potent cryptochrome modulators. Based on the results of efficacy studies in diet-induced obese (DIO) mice, and the desired pharmacokinetic parameters, compound **41** was selected for further profiling.

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Type 2 diabetes is a metabolic disorder that accounts for 387 million patients worldwide and the number is likely to grow to greater than 590 million by the year 2035.¹ This is a complex disease and invariably type 2 diabetic patients also display cardiovascular risk factors including hypertension and dyslipidemia.^{2,3}

The circadian clock is an essential time-keeping mechanism that controls the daily rhythm of numerous physiological processes including sleep/wake behavior, body temperature, hormone secretion, and metabolism.^{4–6} Dysregulation in the circadian system caused by variants of clock genes has been correlated with type 2 diabetes and insulin resistance.^{7,8} More specifically, genetic variants in *Cryptochrome* (*Cry1* and *Cry2*) genes have been linked with glucose homeostasis and beta-cell function.^{9–12}

Cell-autonomous circadian rhythms are generated through a core set of clock genes and coordinated by interconnected transcriptional and translational regulatory loops. In the core feedback loop, the basic helix-loop-helix/Per-Arnt-Sim transcription factors CLOCK and BMAL1 activate expression of *Period* (*Per1* and *Per2*) and *Cry* genes. After translation, eventual dimerization and nuclear localization, PER and CRY proteins inhibit CLOCK-BMAL1 function, culminating in rhythmic 24-hour gene expression in almost all mammalian tissues. The stability of clock proteins is further controlled by posttranslational modification and ubiquitination.¹³

Recent X-ray crystallographic results revealed details of CRY ubiquitination and degradation by the SCF^{FBXL3} ubiquitin ligase complex.¹⁴ The F-box protein FBXL3 binds to CRY2 by occupying the FAD binding pocket of CRY2 with its C-terminal tail and also covers the PER binding domain of CRY2. FBXL3 regulation of CRY stability, therefore, plays an important role in resetting circadian period, phase and/or amplitude by modulating the negative feedback loop.^{15–17}

Circadian clock-deficient mice have been utilized as tools for examining disease etiologies linked to the circadian clock. Mice lacking *Cry1* and *Cry2* (*Cry*-null mice), on a high fat diet (HFD), rapidly gain weight (though hypophagic), are hyperinsulinemic and have upregulated expression of lipogenic genes in white adipose tissue.¹⁸ Furthermore, ablation of *Cry1*, but not *Cry2*, prevents HFD-induced obesity in mice, likely through an effect on energy expenditure.¹⁹ *Cry*-null mice also exhibit salt-sensitive hypertension due to abnormally high synthesis of the mineralocorticoid aldosterone by the adrenal gland.^{20,21} These mice display constitutive elevation of proinflammatory cytokines in a cell-autonomous manner, which once again suggests low-grade inflammation as a potential cause for chronic diseases like diabetes and obesity.²²

An unbiased cell-based circadian screen revealed a novel small molecule, KL001, that specifically interacts with CRY (Fig. 1).^{23–25} KL001 prevents ubiquitin-dependent degradation of CRY, resulting in lengthening of the circadian period. The co-crystal structure of the KL001-CRY2 complex revealed that KL001 occupies the FAD

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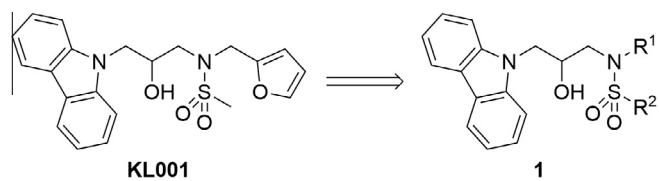


Figure 1. KL001 and chosen lead scaffold 1.

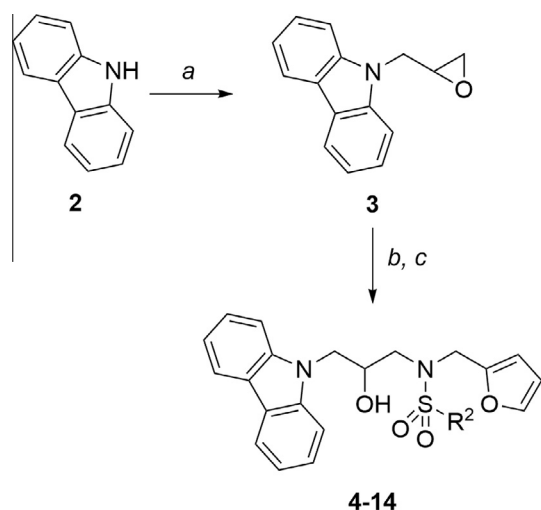
binding site of CRY2 and interferes with the binding of FBXL3 to CRY2.²⁶ In order to determine the structural requirements for KL001 activity and the molecular basis for highly potent derivatives, we wished to synthesize compounds represented by the general structure **1** (Fig. 1). Herein, we report the synthesis, structure–activity relationships (SAR), and in vivo activity of this new class of compounds.²⁷

The synthetic route for the preparation of compounds **4–14** is shown in Scheme 1. Coupling of commercially available epibromohydrin and carbazole **2** afforded **3**. Condensing furfurylamine with epoxide **3** followed by addition of a variety of sulfonyl chlorides allowed for an efficient variation of sulfonamide R² substituents giving final products **4–14**.

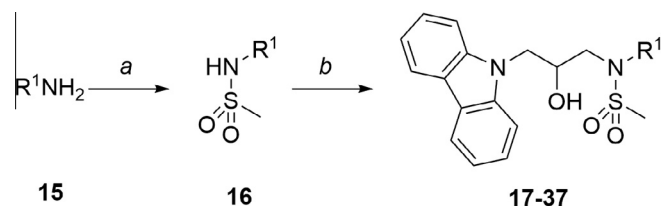
In an effort to look at the effects of variation of the amine R¹ moiety, a new synthetic route was required (Scheme 2). Methanesulfonyl chloride was added to a variety of amines **15** to afford **16**. The resulting sulfonamides **16** were then condensed with epoxide **3** under basic conditions giving final products **17–37**.

Attempts to further increase potency via conformational restriction required molecules where R¹ and R² are joined as part of a ring (Scheme 3). Commercially available sultams (**39**, X = CH₂) and synthesized cyclic sulfamides (**39**, X = NR³) were introduced to this effect. Diamines **38** were condensed with sulfamide to afford intermediates **39**. Cyclic sulfonamides and sulfamides **39** were then condensed with epoxide **3** under basic conditions giving final products **40–47**.

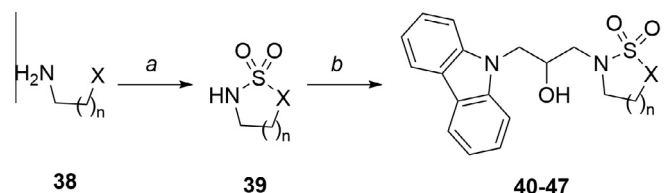
The newly synthesized compounds were evaluated for their effects on circadian rhythms in a human osteosarcoma U2OS cell line harboring a *Per2-dLuc* luciferase reporter.²³ Continuous treatment with these compounds causes period lengthening and amplitude reduction in a dose-dependent manner. The amplitude effect of these derivatives was analyzed by testing 8 points of threefold dilution series to obtain EC₅₀ values. Lipophilic efficiency (LipE)



Scheme 1. Reagents and conditions: (a) epibromohydrin, KOH, DMF, 0 °C to rt, 16 h, 71%; (b) furfurylamine, EtOH, 40 °C, 16 h, 91%; (c) R²SO₂Cl, pyridine, CH₂Cl₂, 0 °C to rt, 16 h.



Scheme 2. Reagents and conditions: (a) MeSO₂Cl, pyridine or Si-imidazole, CH₂Cl₂, rt, 16–48 h; (b) **3**, Cs₂CO₃, DMA, 100 °C, 16 h.



Scheme 3. Reagents and conditions: (a) (NH₂)₂SO₂, pyridine, reflux, 16 h; (b) **3**, NaH, DMF, 70 °C, 16 h.

was calculated in an effort to move toward a quality drug candidate with high potency, low dose and an adequate safety profile. LipE is a parameter used in drug design to evaluate the quality of compounds, linking potency and lipophilicity in an attempt to estimate druglikeness.^{28–30}

Initially, we decided to investigate the effects of varying the sulfonamide R² substituents of **1**, Table 1. Compounds **4–14** demonstrate that Per2 potency is sensitive to the size of the substituent. Small substituents yielded analogs with submicromolar potency (e.g. KL001 and compound **4**). Larger cyclic moieties (compounds **9** and **13**) resulted in significant reductions in Per2 affinity. Methyl, ethyl and 2-methoxyethyl were preferable (compounds KL001, **4** and **12**) from a LipE standpoint, due to their balance of Per2 potency and lipophilicity.

The optimal lipophilic efficiency, molecular weight and in vitro metabolism (data not shown) associated with the methyl substituent (e.g. KL001) prompted us to hold this moiety constant and make changes to the R¹ substituents (Table 2). Per2 potency for compounds **17–37** demonstrate that large changes in this region can be tolerated. Removing the R¹ substituent of KL001 (compound **17**) afforded an increase in potency and LipE. Per2 potency of ~100 nM could be achieved with two analogs (compounds **22** and **24**) although only minor increases in LipE resulted from these changes. A lipophilicity lowering strategy in this region was also successful as compounds **27**, **28** and **31** all resulted in acceptable LipE values although at the expense of Per2 potency. Interestingly, replacing a phenyl moiety (compound **32**) with a 2-pyridyl substituent (compound **33**) resulted in a significant reduction in Per2 potency possibly due to an unfavorable conformational change caused by an interaction with this new moiety and the sulfonamide functionality.

Given the increased potency and LipE associated with small R¹ and R² substituents we decided to attempt a conformational restriction strategy (Table 3). The rationale behind conformational restriction is to keep a molecule in its bioactive conformation while eliminating alternative conformations. Cyclic sulfonamides (compounds **40–43**) all showed excellent Per2 potency and LipE values. Variations in ring size (compounds **40**, **41** and **42**) resulted in no change in Per2 potency. One example of lipophilicity lowering (compound **43**) afforded very high Per2 potency and LipE. On moving from cyclic sulfonamides to cyclic sulfamides (compounds **44–47**) we also observed acceptable Per2 affinity. Within the sulfamides, we did observe a trend toward improved Per2 affinity

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