CHAPTER 3

Central Modulation of Circadian Rhythm *via* CK1 Inhibition for Psychiatric Indications

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1. INTRODUCTION

The field of circadian biology involves the study of circadian rhythms (CRs) which are periodic behavioral and physiologic processes that cycle approximately every 24 h. CRs are primarily regulated by an organism's

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environmental photoperiod (day/night), thus CRs likely function to synchronize critical biologic processes with light/dark cycles. It has been well established that CRs in higher organisms are generated by a master clock located in the suprachiasmatic nucleus (SCN) of the brain. The master clock synchronizes central and peripheral clocks in the body regulating numerous and diverse functions including sleep–wake cycles, hormone release, and body temperature. There is a growing body of evidence linking disrupted CRs to the pathophysiology of neuropsychiatric disease including insomnia, depression, seasonal affective disorder, and bipolar disorder.

2. DESCRIPTION OF CIRCADIAN CLOCK

All organisms have an endogenous timing system which synchronizes biologic functions to exogenous daily cycles [1,2]. The circadian timing system is synchronized by the action of zeitgebers (''time-givers'' or synchronizers), the most powerful of which is light. In most animals, light is transduced into a circadian neural signal by specialized nonvisual cells in the retina, the melanopsin expressing retinal ganglion cells [3]. Signals from the retinal ganglion cells travel down the retinohypothalamic tract, a monosynaptic projection from the retina, which innervates a small paired structure within the hypothalamus called the SCN [4]. The SCN is comprised of about 20,000 cells and is generally accepted as the site of the master biological clock [4]. The SCN also receives input from the intergeniculate leaflet and serotonergic pathways in the brain that mediate the action of nonphotic zeitgebers on circadian cycles [1].

The time period it takes for one circadian cycle to occur is called tau (τ) and results from a core loop of translational, transcriptional, and post-translational events in the SCN. In the absence of light and other effective zeitgebers, the molecular events that govern clock periodicity run at an endogenous rate, a condition called ''free-running.'' The τ for animals under free-running conditions, endogenous τ, may be less than, equal to, or greater than 24 h. In mouse, endogenous τ is strain dependent and ranges from 23.3 to 23.9 h [5]. In humans, endogenous τ appears to be more variable (24–25 h) which may reflect difficulties in establishing true free-running conditions for people [6]. Since light cycles are the primary regulator of CRs, most animals are synchronized to a 24-h photoperiod by light resetting the timing of molecular events in the SCN on a daily basis.

Mammalian genetics has played a large part in defining the components of the circadian timing system. In humans, familial advanced sleep phase syndrome (FASPS) is a disease in which patients show a daily advance in the time when they go to sleep. Genetic association studies showed that these patients carry a mutation in the protein Period2 (Per2),

which removes a phosphorylation site [7]. Per2 is a member of the PAS (Per-ARNT-Sim) family of genes and is a homologue of a protein that had been identified in *Drosophila* as a component of the circadian clock. Per2 expression has been shown to be expressed in a pattern that oscillates with the circadian time. The link between Per2 phosphorylation and FASPS provided a link between Per protein phosphorylation and CR in mammals.

The first mammalian mutation linked to CR was identified in golden hamsters (*Mesocricetus auratus*). The mutation was an autosomal allele first described in the late 1980s [8] and was identified using positional cloning [9]. These animals displayed a shortened period length (20 h in homozygous animals) and were thus designated as τ mutants. Cloning revealed that the τ mutation was in casein kinase (CK) 1ε and decreased the maximal phosphorylation rates of CK1ε.

There are several genes and their respective proteins that are involved with CR and a brief description of them follows. Cryptochrome (Cry) is a flavoprotein that acts as a blue light photoreceptor, directly modulating photo-input into the circadian clock. It has been shown that Cry associates with Per proteins [7] and is homologous to the protein Timeless (tim), first identified from *Drosophila* [1]. A mouse mutation generated through chemical mutagenesis and then screened for mutations, which alter CR, was mapped and the locus cloned using positional cloning [10,11]. This protein was called Clock (Circadian Locomotor Output Cycles Kaput) and shown to be required for normal functioning of the circadian timing system. The Clock mutation in mice is a deletion of exon 19 and encodes a shorter protein [11,12]. Clock interacts with a protein called Bmal1 (Brain and Muscle Aryl hydrocarbon receptor nuclear translocator ARNT-Like 1), which contains a basic helix–loop–helix protein domain. Bmal1 interacts with the Clock protein to form a heterodimer which activates transcription from genes containing an E-box in their promoters [7].

Periodicity in the SCN is generated by cyclic transcriptional/translational positive and negative feedback-loops of which Per, Cry, Clock, Bmal1, and their respective proteins are key components. The process by which light synchronizes (entrains) SCN periodicity to light/dark cycles is summarized as follows (Figure 1). Light signals, transmitted by the retinohypothalamic tract, initiate the transcription of Per by glutamate mediated activation of calcium channels and pituitary adenylate cyclase-activating polypeptide (PACAP) activation of the vasoactive intestinal peptide receptor 1 (VIPR-1) in SCN neurons [13]. VIPR-1 activation, and Ca influx through L channels, initiates phosphorylation of CREB, which subsequently binds to a CRE in the Per gene inducing its transcription [14]. As a result, cytoplasmic Per (protein) levels accumulate during the day and heterodimerize in the cytoplasm with the protein Cry. The Per-Cry complex translocates into the nucleus and inhibits the expression of

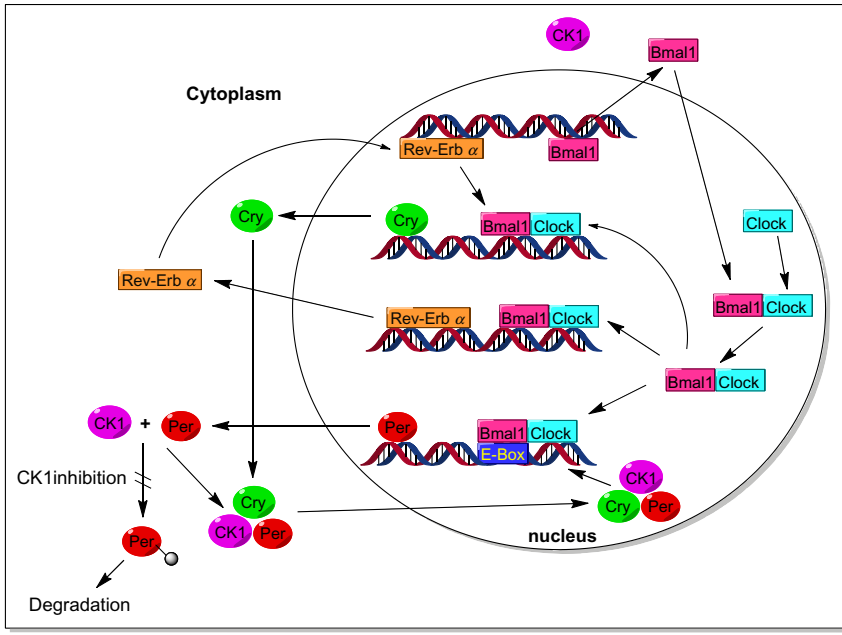


Figure 1 Clock cycle in mammalian SCN.

Clock and Bmal1. The Clock and Bmal1 proteins heterodimerize to form a complex, which activates the transcription of genes containing an E-box structure, including the genes for Per and Cry. As Per levels increase throughout the day in the cytoplasm, the protein is phosphorylated by CK1ε or CK1δ and targeted for degradation by the ubiquitin ligase system [1,15]. Blockade of CK1 prevents Per degradation and increases Per levels in the cytoplasm and the nucleus. It is this series of transcriptional, translational, and posttranslational activities that sets the timing of the endogenous clock.

A secondary loop exists and functions to stabilize the core loop [15]. The stabilizing loop is driven by expression of the nuclear receptors Rev-erb α (also known as NR1D1—Nuclear Receptor subfamily 1, group D, member 1) and RORβ (RAR-related orphan receptor β, also known as NR1F2) [15,16]. Rev-erb α functions as a negative regulator of Bmal1. Bmal1 interacts with Clock to drive expression of a set of genes with a common E-box promoter. One of these proteins is RORβ, which positively affects transcription of Bmal1, completing the stabilizing loop [15,16]. Stabilization of the core loop is thought to preserve synchronized SCN function over time periods longer than a day [15,16]. By inhibiting CK1, phosphorylation of Per could be blocked and this should alter the timing of the circadian clock, thus changing the rhythm of biological processes.

Other enzymes outside of the circadian clock also have effects on CR. Lithium, a drug used to treat bipolar disorder, has been shown to lengthen τ in a variety of systems [17]. While the molecular mechanism whereby lithium exerts its therapeutic effect is unclear, it is known to be an inhibitor of the enzyme glycogen synthase kinase 3beta (GSK-3β) [17]. Lithium at therapeutic concentrations has been shown to inhibit GSK-3β and this subsequently inhibits the phosphorylation of Rev-erb α [18].

3. CK1 INHIBITORS

3.1. Structure and function of CK1 enzymes

There are seven mammalian isoforms of CK1 (α, α2, γ1, γ2, γ3, δ, and ε) that belong to the superfamily of serine/threonine protein kinases [19]. CK1δ and CK1ε are closely related with respect to their sequence; they are predicted to be nearly identical within the kinase active site and share greater than 90% sequence identity within the kinase domain. In contrast to many other protein kinases, the CK1 isoforms do not require phosphorylation of the kinase activation segment by an upstream kinase to maintain the kinase in catalytically active form. CK1δ and CK1ε activity in the cell is regulated by a complex set of molecular events including phosphorylation on their respective carboxy-terminal regulatory domains [20,21]. Subcellular localization [22] and protein–protein interactions are also involved in the regulation of CK1δ and CK1ε activity and their coordination with the circadian cycle. Finally, an additional level of regulation for CK1δ and CK1ε activity is their strong preference for a primed or acidic substrate [22].

Chemical proteomics studies have shown that several other protein kinases are involved in setting and maintaining daily clock cycle. Interestingly, gene knockdown experiments show that suppression of CK1δ and CK1ε protein expression only induced a delay in sleep onset of a few hours while treating mice with a multitargeted kinase inhibitor like longdaysin **33** shifted the clock by more than 12 h [23]. Other protein kinases that may be involved in modulating CRs are CK1α, GSK3β, and p38 kinase.

3.2. Mechanism of action of CK1 inhibitors

The first structural determination of the kinase domain of human CK1δ occurred in 1996 [24]; however since then, there have been few other reported structural studies of the CK1 isoforms [25,26]. Therefore, the unliganded structure of CK1δ was used to enable ligand docking studies and structure-guided design of CK1 inhibitors [24]. CK1δ, like most protein kinases, retains the highly conserved features of a smaller

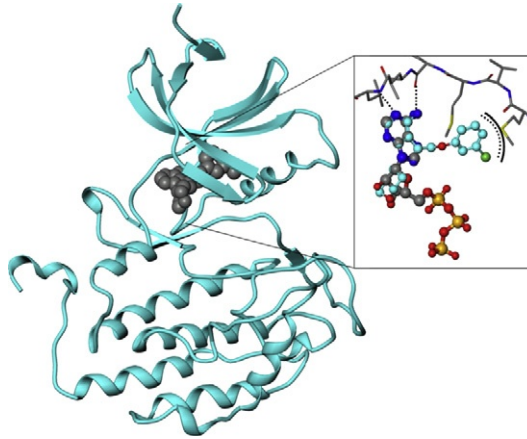


Figure 2 Model of PF-4800567 (**35**) bound in the ATP-binding site of CK1δ.

primarily β-strand amino-terminus lobe and a larger primarily α-helical carboxyl-terminal lobe, linked by a hinge region containing the kinase active site [27]. Figure 2 shows human CK1δ with PF-4800567 (**35**) modeled into the conserved kinase ATP-binding site demonstrating the ATP-competitive nature of the compound. This binding mode is fully supported by biochemistry experiments and displacement assays. The modeling studies suggest that the CK1 inhibitor binds primarily through H-bonding interactions with the kinase hinge region, with multiple hydrophobic interactions in the CK1δ active site as well. An overlay of **35** with ATP from the crystal structure of CK1i [26] demonstrates that the interactions of the ligand with the kinase are likely to be highly similar to those utilized by the adenine moiety of ATP with the exception of the inhibitor *m*-methoxy phenyl group which binds in a hydrophobic pocket adjacent to the CK1δ gatekeeper residue Met82 (Figure 2).

3.3. CK1 inhibitor chemical scaffolds

The disclosed CK1 chemical matter generally resides in chemical space favorable for optimization of small molecule drug candidates. Figure 3 demonstrates the distribution of CNS multiparameter optimization (CNS MPO desirability) score for patented CK1 inhibitors [28]. A significant fraction of the exemplified compounds possess a profile typical of CNS-penetrant drugs. While kinase targets are generally considered difficult for CNS indications, in part due to the significant number of polar functional groups and hydrogen bond donors required for efficient binding in the ATP site, this analysis of brain availability, in general, bodes well for development of neurotherapeutic CK1 inhibitors. Figure 4 provides a

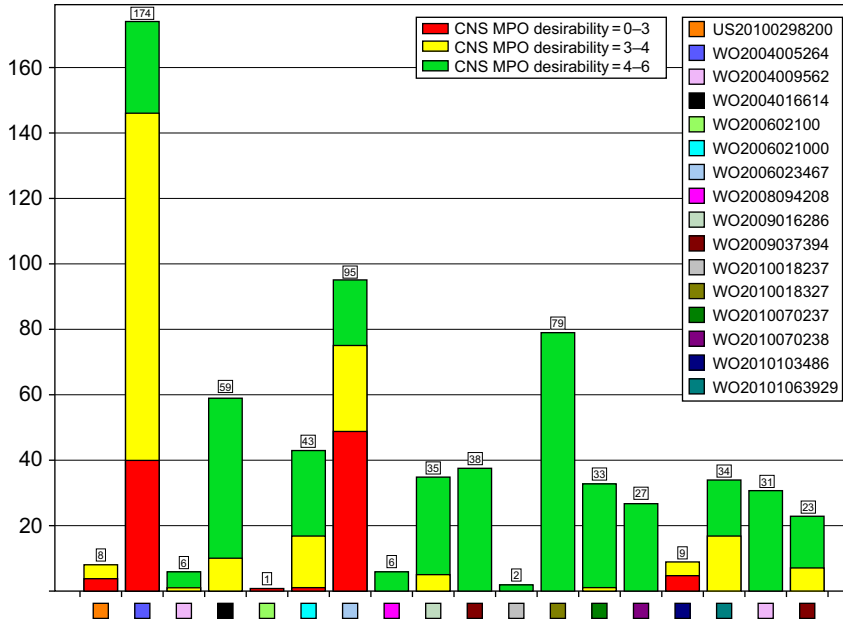


Figure 3 CNS MPO desirability analysis of 703 compounds representing CK1 patents (2004–2010).

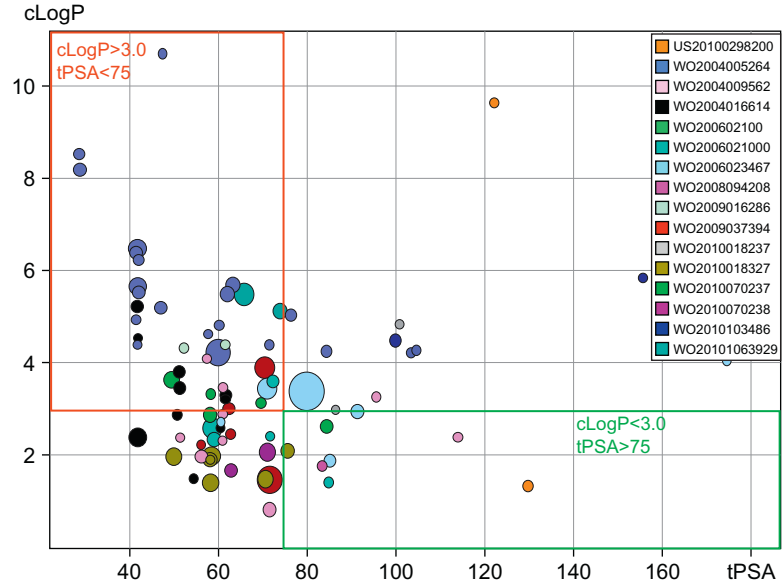
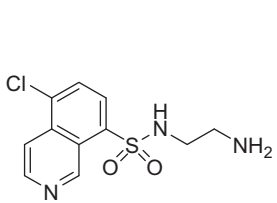


Figure 4 Toxicity plot (probability of *in vivo* toxicological findings) for 82 cluster centroids representing CK1 patent chemical space (2004–2010) sized by number of compounds in a cluster.

clustered view of claimed CK1 inhibitors in regard with the probability of general *in vivo* organ toxicological findings, not addressing issues related to kinase selectivity. The CK1 chemical space does not appear to be overly biased toward high lipophilicity/low polar surface area which is a profile associated with higher incidence of preclinical *in vivo* toxicity [29]. Of the 82 clusters analyzed 11% fell into the quadrant of lowest safety risk (clogP < 3 and tPSA > 75). Ligand efficiency (LE) [30] and lipophilic efficiency (LLE) [31] trends of CK1 inhibitors disclosed in 2004–2010 are presented in Figure 5. Such an analysis highlights chemotypes with higher potential for medicinal chemistry optimization efforts [32]. From this perspective, CK1 can be viewed as a target which is very amenable to small molecule drug discovery. Correlation between highly LE and LLE inhibitors and CNS MPO desirability score is also noteworthy.

Compound CK1-7 **1** appears to be the first reported CK1 inhibitor as identified by screening a series of isoquinolinesulfonamides [33]. Selectivity for CK1 over CK2 determined for **1** was found to be 8.2-fold with a $K_i = 8500$ nM for CK1. An inhibitor of IL-1 biosynthesis, SB-202190 **2**, was disclosed [34] and later found to have CK1 activity [35]. This compound displayed improved *in vitro* potency, relative to **1**, with an $IC_{50} = 600$ nM (enzyme assay). A final, early entry into the field of CK1 inhibitors with a unique chemical scaffold was IC261 **3** [36]. It was found to be equipotent at CK1δ and CK1ε with IC_{50}s $= 1000$ nM. The ATP-site binding interaction was confirmed by X-ray crystallography [25].



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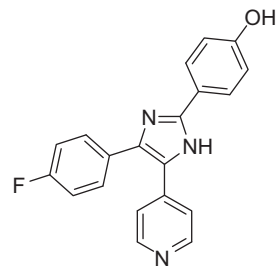

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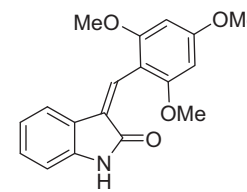


3

The triarylimidazole scaffold was identified by broad kinase screening activities of inhibitors from other disease indications. Thus, the ALK5 inhibitor [37] SB-431542 (ALK5 $IC_{50} = 94$ nM) led to the identification of D4476 **4** as a relatively selective CK1 inhibitor ($IC_{50} = 300$ nM), in addition to the ALK5 activity [38]. The selectivity of these CK1 inhibitors was profiled against a panel of 70–80 kinases [39]. CK1δ inhibition by **4** was 20- to 30-fold more potent than PKD1 or p38a MAPK, whereas **1** and **3** were 5- to 10-fold less potent inhibitors of CK1 and also inhibited several other kinases, including PIM1 and PIM3, while **1** also hit ERK8, MNK1, AMPK, and SGK1. Anthraquinones were later identified as CK1

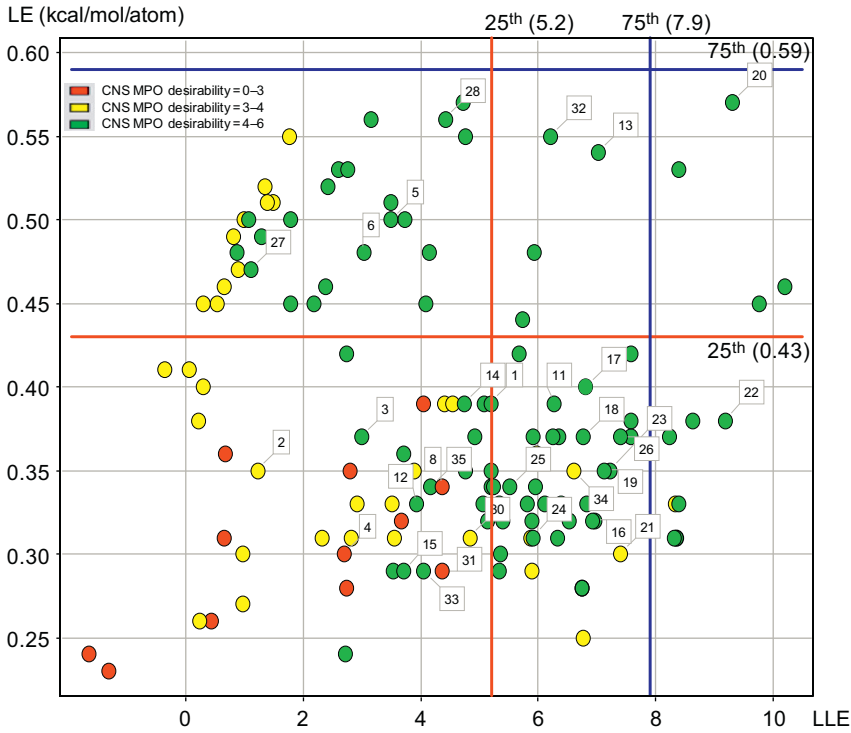
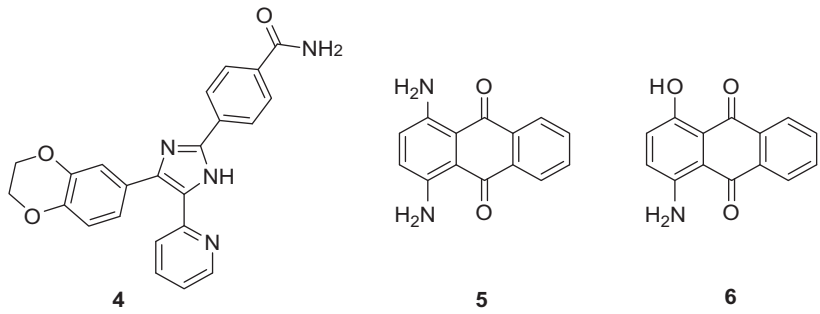


Figure 5 Ligand and lipophilic efficiency of CK1 inhibitors, using the LE and LLE 25th and 75th percentiles defined by marketed drug analysis [31]. Markers flagged by compound numbers in the body of the text.

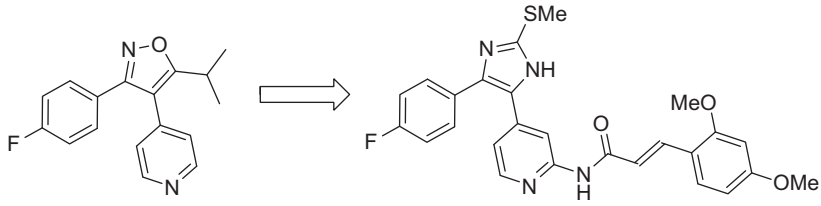
inhibitors from a structure-based virtual screen method [40]. This approach showed that **5** (CK1δ IC_{50} = 330 nM, CK1γ_1 IC_{50} = 34,000 nM, and CK1α IC_{50} = 4000 nM) and **6** (CK1δ IC_{50} = 660 nM, CK1γ_1 IC_{50} = 26,200 nM, and CK1α IC_{50} = 4000 nM) were potent at CK1δ.



4 **5** **6**

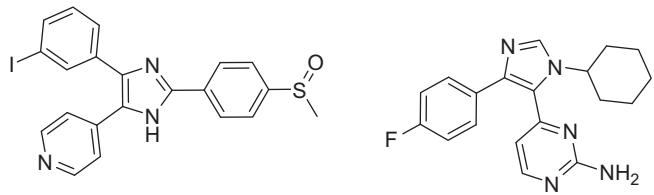
CK1 inhibitors containing the imidazole core were also identified from profiling p38α kinase inhibitors derived from an isoxazole [41]. Scaffold

hopping and subsequent elaboration of the isoxazole hit **7** (% inhib at 10 μM p38α = 80; CK1δ = 94) gave imidazole **8** (p38α IC_{50} = 19 nM; CK1δ-KD IC_{50} = 4 nM; CK1δ-GST IC_{50} = 5 nM; CK1ε IC_{50} = 73 nM).



7 **8**

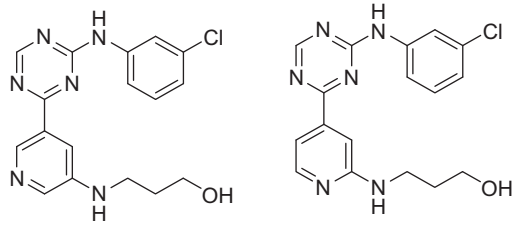
Related disclosures revealed substituted imidazoles as effective inhibitors of CK1α, CK1δ, and CK1ε, but the targeted indication was for hepatitis C viral (HCV) infections [42]. Compound **9** was reported to be safe by the authors, in a cell viability assay up to 10 μM and found to have an IC_{50} = 9 μM in a phenotypic whole cell assay of viral activity.



9 **10**

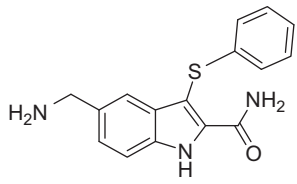
The design of imidazoles with aligned physicochemical properties to maximize brain penetration led to the disclosure of PF-670462 **10** [43]. This compound was very potent against the enzyme (CK1ε IC_{50} = 7.7 nM), exhibited potent cell-based activity (EC_{50} = 290 nM) in a Per nuclear translocation assay, and produced phase shifts in cynomolgus monkeys (see Section 4).

Triazine derivatives, initially focused on inhibition of CDK, GSK, and VEGF, were shown to also possess CK1 inhibition [44]. Derivatives **11** (CDK1 IC_{50} = 39 nM; GSK IC_{50} = 5 nM; VEGF IC_{50} = 2560 nM; CK1 IC_{50} = 115 nM) and **12** (CDK1 IC_{50} = 16 nM; GSK IC_{50} = 17 nM; VEGF IC_{50} = 2570 nM; CK1 IC_{50} = 1410 nM) illustrated how the CK1 SAR could be modulated to effect potency.



11 **12**

With the validation of CK1 as an authentic target to pursue, discovery programs were established to specifically develop inhibitors affecting the human circadian system. Substituted indoles were assessed against CK1δ/CK1ε and in an *in vivo* rat model of CR by measuring the mean body temperature circadian shifts. Assessment of the daily displacement of core body temperature in animals exposed to a constant dark cycle (0/24 h light/dark), which removes light as a zeitgeber, provided a measure of the compounds effectiveness in resetting τ ($EC_{\Delta\tau+1h}$). Two examples [45] of these structures are illustrated with **13** (CK1ε $K_i = 7$ nM; $EC_{\Delta t+1h} = 440$ nM) and **14** (CK1ε $K_i = 340$ nM; $EC_{\Delta t+1h} = 3340$ nM).



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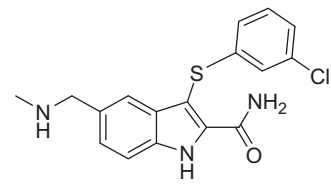

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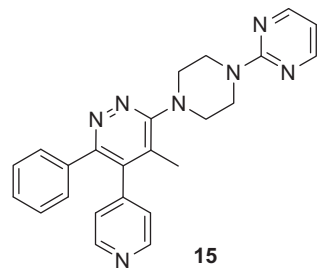
Transforming the known imidazole core into an aminopyridazine scaffold afforded dual p38α/CK1δ inhibitors [46]. Compound **15** (p38α $IC_{50} = 800$ nM; CK1δ $IC_{50} = 300$ nM) is a representative from this chemical series.



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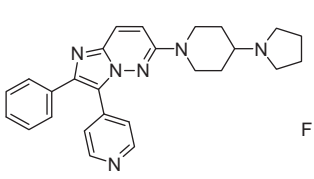
Multiple variations on the imidazopyridazine scaffold have been reported. The initial disclosure revealed the pyridyl derivatives as the hinge binding moiety [47]. Example **16** (CK1ε $IC_{50} = 69$ nM; CK1δ $IC_{50} = 52$ nM) illustrates that this template is equipotent at the two CK1 isoforms of interest, while **17** (CK1δ $IC_{50} = 5$ nM; $EC_{\Delta t+1h} = 28$ nM) and **18** (CK1ε $IC_{50} = 8$ nM; $EC_{\Delta t+1h} = 6$ nM) possess high enzyme potency which translated into excellent *in vivo* activity.



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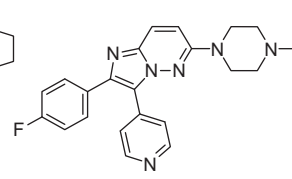

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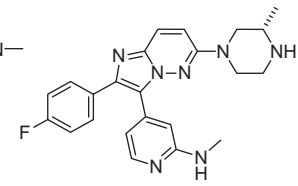

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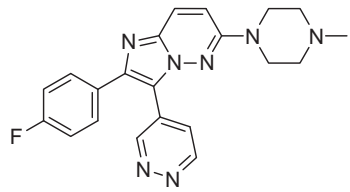
The utility of this template was probed with several modifications. The modulation of the hinge interaction and physicochemical properties were examined by transforming the pyridine ring to a pyridazine [48]. Compound **19** (CK1ε $IC_{50} = 24$ nM; CK1δ $IC_{50} = 48$ nM; $EC_{\Delta t+1h} = 5$ nM) showed that CK1 potency could be maintained without eroding *in vivo* function. Additionally, there are examples where *in vivo* potency is maintained through the evaluation of potentially subtype-selective compounds [49]. Subtype selectivity, at least at the enzyme level, of approximately 60-fold could be achieved by **20** (CK1ε $IC_{50} = 57$ nM; CK1δ $IC_{50} < 1$ nM; $EC_{\Delta t+1h} = 20$ nM) with excellent effects on CR. Exposure data were not disclosed, thus a complete assessment of subtype selectivity on CR was not possible.



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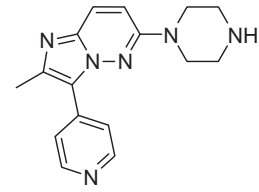

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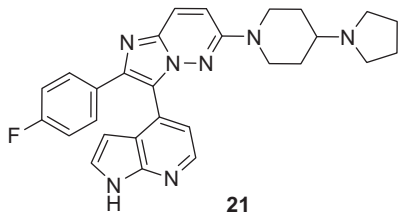
Additional hinge binding interactions, as exemplified by an indole moiety **21** (CK1ε $IC_{50} = 7$ nM; CK1δ $IC_{50} = 19$ nM; $EC_{\Delta t+1h} = 4$ nM), retained potency and functional activity [50]. Compound **22** (CK1ε $IC_{50} = 15$ nM; $EC_{\Delta t+1h} = 17$ nM) showed that increased polarity in the hydrophobic pocket, by making the fluorophenyl to furan substitution, could be tolerated [51].



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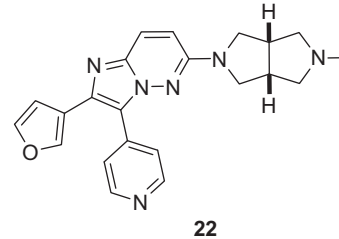

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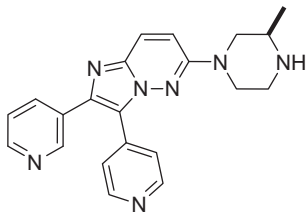
However, positioning pyridyl-substituents in the hydrophobic pocket, as with **23** (CK1ε IC_{50} = 160 nM; CK1δ IC_{50} = 107 nM) and **24** (CK1ε IC_{50} = 290 nM; CK1δ IC_{50} = 287 nM), clearly demonstrated too much polarity would not be tolerated [52].



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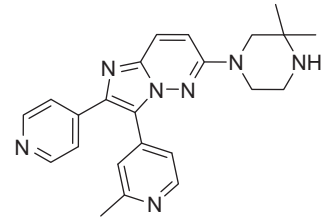

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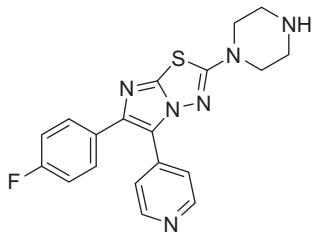
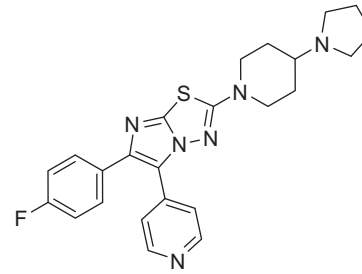
From a synthetic standpoint, these imidazopyridazines can be prepared using a four-step synthetic route. Each of the steps, organometallic deprotonation and substitution, bromination, cyclization, and S_NAr reaction, has been fully automated and can be run using flow microreactors. Using this technology, a collection of 20 diverse analogs was prepared [53].

Further modification of this scaffold core to imidazothiazoles, while maintaining similar substituents, showed that the SAR was partially transferable to this new template [54]. Compound **25** (CK1ε IC_{50} = 40 nM; CK1δ IC_{50} = 300 nM) showed a modest subtype selectivity (7.5-fold) favoring CK1ε, while **26** (CK1ε IC_{50} = 155 nM; CK1δ IC_{50} = 4 nM) displayed a distinct preference (39-fold) for CK1δ.

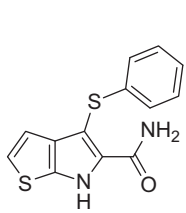


25



26

The structurally related pyrrolothiophenes and pyrrolothiazoles provide access to a novel template with CK1 activity [55]. Compound **27** (CK1ε IC_{50} = 811 nM; $EC_{\Delta t+1h}$ = 874 nM) exhibited weak enzyme potency and *in vivo* activity, while **28** (CK1ε IC_{50} = 10 nM; $EC_{\Delta t+1h}$ = 662 nM) had good enzyme potency, it also displayed weak *in vivo* activity.



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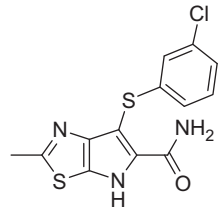
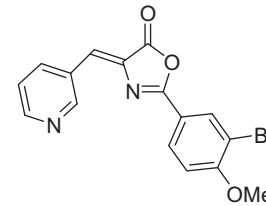


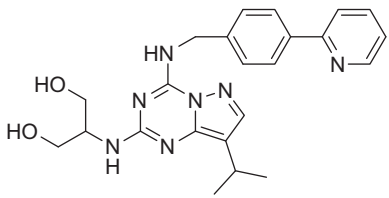
28



29

Recently disclosed [56], derivatives of 5-oxazolones, such as **29**, exhibited 94% and 99% inhibition for CK1δ and CK1ε, respectively, in an assay where the kinase concentration was 300 ng/mL.

Pyrazolotriazines, first identified with CDK activity, have now been shown to also possess activity at CK1 [57]. Compound **30** (CDK1/cyclin B $IC_{50} = 70$ nM; CDK2/cyclin A $IC_{50} = 62$ nM; CDK5/p25 $IC_{50} = 53$ nM; CDK9/cyclin T $IC_{50} = 68$ nM; CK1 $IC_{50} = 92$ nM) and **31** (CDK1/cyclin B $IC_{50} = 42$ nM; CDK2/cyclin A $IC_{50} = 42$ nM; CDK5/p25 $IC_{50} = 44$ nM; CDK9/cyclin T $IC_{50} = 53$ nM; CK1 $IC_{50} = 120$ nM) displayed a mixed profile of kinase activities including CK1 that appeared to be equipotent at all the targets. The ability to separate these activities to generate selective inhibitors will be challenging and would be required to achieve a safe compound for a CNS indication.



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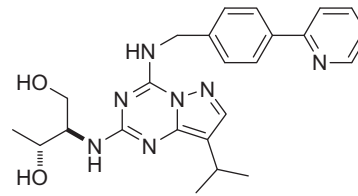
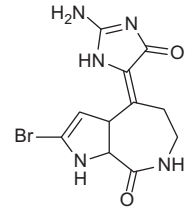


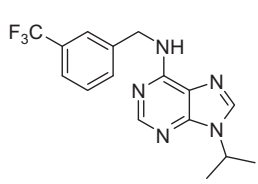
31

A novel pyrroloazepinone scaffold was identified when the natural product hymenialdisine, **32** (CK1 $IC_{50} = 35$ nM; CDK1/cyclin B $IC_{50} = 22$ nM; CDK5/p25 $IC_{50} = 28$ nM; GSK3 $IC_{50} = 10$ nM), was isolated from a marine sponge [58]. While it was very potent at CK1, it also showed equal potency at three additional kinases (CDK1, CDK5, and GSK3).

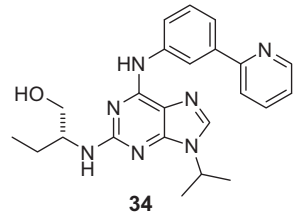


32

Imidazopyrimidines were identified by conducting a circadian screen of 120,000 compounds on human cells [59]. The compound longdaysin, **33**, (CK1δ IC_{50} = 8800 nM; EC_{50} = 9700 nM; CK1α IC_{50} = 5600 nM; EC_{50} = 9200 nM), was identified as lengthening the period of a variety of cells and was found to interact with CK1δ and CK1α by affinity chromatography.



33



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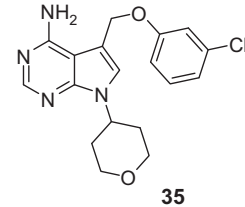

35

A structurally related compound **34** (CK1 IC_{50} = 14 nM; CDK5 IC_{50} = 80 nM) was found to display CDK5 activity in addition to CK1 [60].

CK *in vitro* subtype selectivity has been reported with pyrazolopyrimidine templates exemplified by PF-4800567, **35**, [61]. This CK1ε over CK1δ preferring compound was measured against the enzyme (CK1ε IC_{50} = 32 nM, CK1δ IC50 = 711 nM) and in a whole cell assay (CK1ε IC_{50} = 130 nM, CK1δ IC50 = 2650 nM). This pharmacological tool compound was used to elucidate the isoform *in vitro* and *in vivo* function with respect to circadian function. The CK1ε preferring compound **35** minimally altered circadian period when compared to the non-subtype-selective CK1ε/CK1δ compound **10** (see Section 4).

4. PRECLINICAL ANIMAL MODELS

The pharmacologic inhibition of CK1ε/CK1δ by PF-670462 **10** has consistently shown modulation of circadian activity in rat, mouse, and nonhuman primate models. Under 12:12 light:dark conditions, **10** (10 and 30 mg/kg) administered at zeitgeber time (ZT) 11 generated robust dose-dependent phase delays in rat [62]. In the same study, Sprouse *et al.* also demonstrated that administration of **10** at ZT 6 produced a smaller phase shift which was not significantly different from controls at the 10 mg/kg dose [62]. These results are consistent with the phase response curve reported for **10** [43] and suggest that the pharmacodynamics of CK1 inhibition follows cyclic changes in period protein (PER) levels [63]. These data further suggest that if used in patients, the clinical efficacy of a CK1ε/CK1δ inhibitor may be influenced by the timing of drug administration.

The effects of **10** on circadian activity in socially housed *Macaca fasicularis*; a diurnal nonhuman primate species with a circadian period similar to humans has been investigated. Administration of **10** (10 and 32 mg/kg) at ZT 10:30 under 12:12 light:dark conditions elicited dose-dependent phase delays in *M. fasicularis* [64]. These data are consistent with studies in singly house monkeys [65] and suggest that CK1 inhibition would induce phase delays in other diurnal species, including humans.

The aforementioned models demonstrate the effects of CK1 inhibition in animals with normal CRs. Although these observations are important, central to the development of CK1 inhibitors as therapeutic agents for psychiatric indications is the ability to modulate circadian activity in arrhythmic or disrupted circadian paradigms. Meng *et al*. have established that **10** can restore circadian rhythmicity in two different mouse models of circadian asynchrony [66]. The vasoactive intestinal peptide receptor 2 knockout mouse (*Vipr2*$^{-/-}$) exhibits an arrhythmic or weakly rhythmic circadian phenotype due to disrupted signal transduction in the SCN [67,68]. Robust entrainment of *Vipr2*$^{-/-}$ mice can be induced and sustained by daily **10** treatment [65]. Meng *et al*. also demonstrated that treatment with **10** prevented constant light-induced circadian asynchrony in wild-type mice [66].

In summary, inhibition of CK1ε/CK1δ produces phase delays in both nocturnal (rodents) and diurnal (nonhuman primate) species and restores rhythmicity under conditions that disrupt SCN function. These lines of evidence support the contention that a CK1ε/CK1δ inhibitor would be of therapeutic benefit in neuropsychiatric conditions that are associated with disrupted circadian function.

5. CONCLUSIONS

The depth of understanding of CR has greatly increased over the past decade. Confirmation of CK1 as a drug-able target has been validated by identification of chemical matter consistent with drug-like attributes, including good LE, LLE, and brain availability. Moreover, these compounds have provided the *in vivo* translation of the science and have shown CR entrainment and phase shifts in nonhuman primates, in addition to rodents. In particular, the dissection of CK1 subtype selectivity was demonstrated with **35** (CK1ε selective), with **10** (CK1ε/CK1εδ dual inhibitor) exhibiting *in vivo* efficacy at modulating of CR. Selective CK1 tool compounds have enabled a greater understanding of the role this kinase plays in modulating CR. Although no CK1 compound has been reported in the clinic; clinical evaluation of the CR hypothesis using selective CK1 inhibitors is now within the realm of possibility.

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