İsmail Celil HASKOLOĞLU

BEYOND CONVENTIONAL THERAPIES: POTENTIAL THERAPEUTIC STRATEGIES FOR ALZHEIMER'S,

Ph.D.THESIS

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NEAR EAST UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

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Approval

We certify that we have read the thesis submitted by İsmail Celil Haskoloğlu titled **"Beyond Conventional Therapies: Circadian Rhythm-Based Potential Therapeutic Strategies for Alzheimer's Disease, Amyotrophic Lateral Sclerosis and Pain"** and that in our combined opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Ph.D. in Pharmacology Department. Thesis defence was held online. The Jury members declared their acceptance verbally which is recorded.

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Declaration of Ethical Principles

I hereby declare that all information, documents, analysis and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of Institute of Graduate Studies, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

İsmail Celil Haskoloğlu

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İsmail Celil Haskoloğlu

Özet

Geleneksel Tedavilerin Ötesinde: Alzheimer Hastalığı, Amiyotrofik Lateral Skleroz ve Ağrı İçin Sirkadiyen Ritim Temelli Potansiyel Terapötik Stratejiler

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Sirkadiyen ritimler, saat genleri tarafından düzenlenerek biyolojik işlevlerin çevresel uyaranlarla senkronizasyonunu kontrol eder. Bu ritimlerdeki bozulmalar, nörodejeneratif hastalıklar, kronik ağrı gibi birçok hastalığın patogenezinde önemli bir rol oynamaktadır. Bu tez, sirkadiyen ritimlerin farmakolojik olarak modüle edilme potansiyelini inceleyerek benzoksazolon türevleri, melatonin ve onaylanmış ilaçların rollerine odaklanmaktadır. Çalışmanın ilk bölümünde, 5-substitüe benzoksazolon türevlerinin CLOCK, BMAL1, PER ve CRY gibi önemli sirkadiyen proteinler üzerindeki etkileri incelenmiştir. Moleküler kenetleme, moleküler dinamik simülasyonlar ve MM/PBSA hesaplamaları gibi analizler, elektron çekici gruplar içeren türevlerin daha yüksek bağlanma afinitesi ve stabilite gösterdiğini ortaya koymuştur. İkinci bölümde, melatoninin amyotrofik lateral skleroz (ALS) bağlamında saat proteinleri üzerindeki düzenleyici etkileri incelenmiştir. Melatonin, NR1D1 proteini ile etkileşimlerinde üstün bağlanma afinitesi ve stabilite göstermiş ve kronoterapötik yaklaşımlar aracılığıyla mevcut ALS tedavilerini iyileştirme potansiyelini ortaya koymuştur. Son bölümde, Alzheimer hastalığı (AD)'de BMAL1 ekspresyonunun modülasyonu incelenmiş, melatonin ve FDA onaylı AD ilaçlarının kombinasyonu sirkadiyen homeostazı yeniden sağlama ve nörodejenerasyonu hafifletme potansiyeli göstermiştir. Bu tez, sirkadiyen ritim modülasyonunun terapötik potansiyelini vurgulamakta ve hesaplamalı analizler ile farmakolojik yenilikler yoluyla sirkadiyen bozukluklarla ilişkili hastalıklar için hedefe yönelik tedavilerin geliştirilmesini desteklemektedir.

***Anahtar kelimeler**: melatonin, sirkadiyen ritim, nörodejeneratif hastalıklar, benzoksazolon türevleri*

Abstract

Beyond Conventional Therapies: Circadian Rhythm-Based Potential Therapeutic Strategies for Alzheimer's Disease, Amyotrophic Lateral Sclerosis and Pain

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PhD, Department of Pharmacology

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Circadian rhythms, regulated by clock genes, control the synchronization of biological functions with environmental stimuli. Disruptions in these rhythms play a significant role in the pathogenesis of various diseases, such as neurodegenerative disorders and chronic pain. This thesis explores the pharmacological modulation potential of circadian rhythms, focusing on the roles of benzoxazolone derivatives, melatonin, and approved drugs. In the first section, the effects of 5-substituted benzoxazolone derivatives on key circadian proteins, including CLOCK, BMAL1, PER, and CRY, were investigated. Computational analyses, such as molecular docking, molecular dynamics simulations, and Molecular Mechanics/Poisson-Boltzmann Surface Area (MM/PBSA) calculations, revealed that derivatives containing electron-withdrawing groups demonstrated higher binding affinity and stability. The second section examined melatonin's regulatory effects on clock proteins in the context of amyotrophic lateral sclerosis (ALS). Melatonin exhibited superior binding affinity and stability in interactions with the NR1D1 protein, highlighting its potential to enhance current ALS treatments through chronotherapeutic approaches. The final section focused on modulating BMAL1 expression in Alzheimer's disease (AD). The combination of melatonin with FDA-approved AD drugs demonstrated potential to restore circadian homeostasis and mitigate neurodegeneration. This thesis underscores the therapeutic potential of circadian rhythm modulation and supports the development of targeted treatments for diseases associated with circadian disruptions through computational and pharmacological innovations

Keywords: melatonin, circadian rhythm, neurodegenerative diseases, benzoxazolone derivatives

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List of Abbreviations

AA: Arachidonic Acid

AD: Alzheimer’s Disease

ALS: Amyotrophic Lateral Sclerosis

CADD: Computer-Aided Drug Design

COX: Cyclooxygenase

HD: Huntington's Disease

LO: Lipoxygenase

LTA: Leukotriene A4

MM/PBSA: Molecular Mechanics/Poisson-Boltzmann Surface Area

MS: Multiple Sclerosis

NSAID: Non-Steroidal Anti-Inflammatory Drug

PD: Parkinson's Disease

PGH2: Prostaglandin H_2

SCN: Suprachiasmatic Nucleus

CHAPTER I

1.1. Introduction

Circadian rhythms are internal timers that regulate the functioning of biological systems in 24-hour cycles. These rhythms evolved as organisms adapted to daily environmental changes, especially light and dark cycles. Circadian rhythms regulate sleep-wake cycles, body temperature, hormonal secretions, metabolic processes, and immune responses. In mammals, the primary circadian cycle is regulated by the suprachiasmatic nucleus (SCN), a structure within the hypothalamus that aligns the body's internal rhythms with environmental light cues (**Figure 1**). This central clock works with peripheral clocks to provide harmonious timing in all biological systems of the organism (Wong et al., 2022; Zhang et al., 2022).

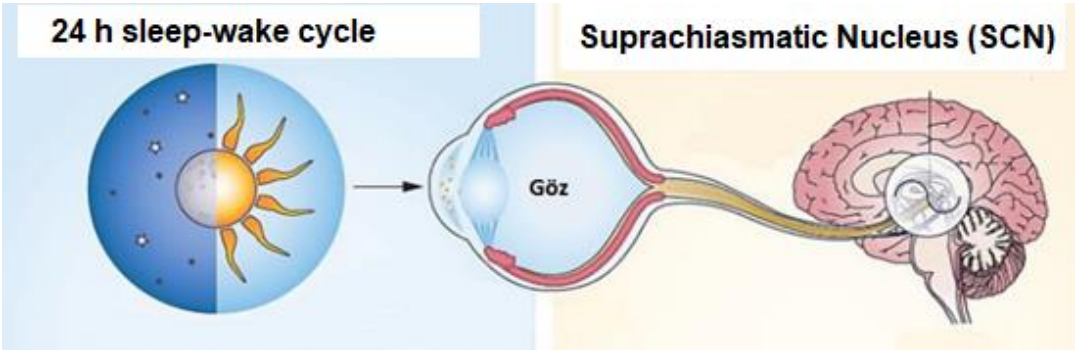


Figure 1: Light/dark cycle and suprachiasmatic nucleus (Memiş, 2019).

Clock genes are the genes that form the molecular basis of circadian rhythms. These genes regulate the production of proteins essential for circadian rhythm control (Juliana et al., 2023). Core clock genes such as CLOCK, BMAL1, PER1, PER2, CRY1, and CRY2 regulate circadian rhythms through feedback loops. CLOCK and BMAL1 proteins function as the main drivers of the circadian rhythm, and these proteins initiate the expression of clock genes, thus initiating the circadian cycle. PER and CRY proteins accumulate in the cell nucleus and inhibit the CLOCK and BMAL1 complex, thus ensuring the completion of the cycle and the beginning of a new cycle. This cycle repeats over approximately 24 hours, ensuring the continuity of the circadian rhythm (Juliana et al., 2023). Therefore, clock genes stand out as important targets both in the regulation of basic biological processes and for potential pharmacological interventions (Fan et al., 2022). The regular functioning of circadian

rhythms is critical for the healthy functioning of organisms. These rhythms regulate energy use and hormone levels to ensure optimal physiological and behavioral functioning of the organism throughout the day. For example, the level of stress hormones such as cortisol reaches its highest level in the early morning hours, preparing the organism for wakefulness; hormones such as melatonin, which facilitate the transition to sleep, increase in the evening hours (Ayyar & Sukumaran, 2021).

Circadian rhythm disruptions can lead to various health problems. For example, circadian rhythm disorders have been associated with serious conditions such as sleep disorders, depression, obesity, diabetes, cardiovascular diseases, and even cancer (Fifel & Videnovic, 2021). Health problems seen in individuals working night shifts are a direct result of circadian rhythm disruption (Canever et al., 2023). Likewise, the role of circadian rhythm disruptions in neurodegenerative diseases has led to increased research on the pharmacological targeting of these genes (Ahmad et al., 2022). Therefore, maintaining circadian rhythms regularly and modulating these rhythms by pharmacological means is of great importance in preventing or treating such health problems (Fan et al., 2022).

Molecules that regulate circadian rhythms, such as melatonin, play an important role in the modulation of clock genes. Melatonin release in the body varies within 24 hours (Figure 2). Melatonin synchronizes sleep-wake cycles by regulating the circadian rhythm, and deficiency of this hormone has been associated with various sleep disorders and mood disorders (Srinivasan et al., 2020). Pharmacological interventions on melatonin receptors can increase the effect of this hormone on clock genes and thus can be used in the treatment of circadian rhythm disorders (Liu et al., 2022). In addition, recent studies aim to develop new strategies for disease treatment by modulating these genes by examining the effects of certain chemical structures on clock genes (Comai & Gobbi, 2022).

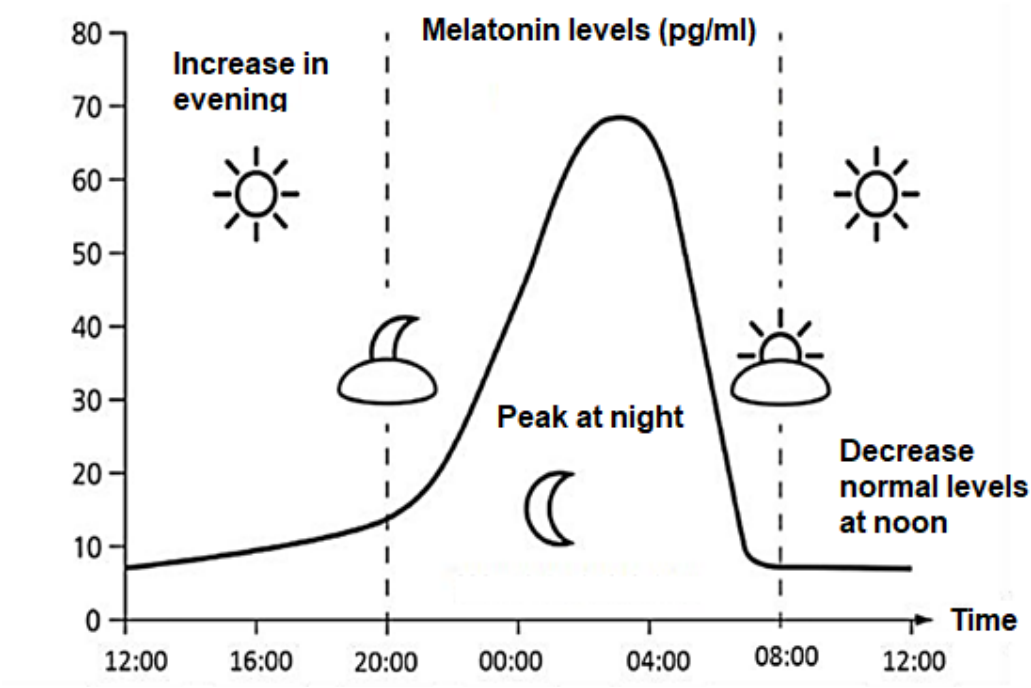


Figure 2: 24-hour physiological melatonin levels (Amanpour et al., 2021)

In recent years, research on the usability of clock genes in drug targeting has increased. These studies support the idea that diseases can be treated by pharmacologically modulating circadian rhythms. Targeting clock genes is considered a potential treatment strategy, especially in conditions such as neurodegenerative diseases, inflammatory disorders, and chronic pain (Rosato et al., 2021).

The studies conducted in this thesis focus on developing new pharmacological approaches in disease treatment through the modulation of circadian rhythms and clock genes. The studies conducted in the thesis are divided into three subsections. In the first subsection, the effects of benzoxazolone derivatives on clock genes are examined through molecular docking and dynamic simulations. In this study, the effects of benzoxazolone derivatives on clock genes were investigated, suggesting these compounds could help regulate circadian rhythms and manage pain. In particular, the high binding affinities of benzoxazolone derivatives on clock proteins such as CRY1 and CRY2 indicate that these molecules could be potential therapeutic agents if pharmacologically targeted (Juliana et al., 2023).

The second subsection examines the effects of melatonin on clock genes. This study investigates how melatonin may be effective in the treatment of neurodegenerative

diseases through clock genes. It has been suggested that modulating clock genes such as melatonin and NR1D1, especially in diseases such as ALS, may slow down the progression of the disease (Fan et al., 2022). The study emphasizes the high binding affinity of melatonin on NR1D1 and the regulatory role of this gene in inflammation and neurodegeneration (Rosato et al., 2021).

The third subsection focuses on circadian rhythm disorders associated with AD and examines how regulation of the BMAL1 gene may affect the disease. In this context, the importance of circadian rhythm and the BMAL1 gene in the treatment of AD is emphasized and how modulation of this gene can be used to decrease the pathological development of the condition is examined. The study suggests that the interaction between melatonin and BMAL1 should be examined in further clinical studies and reveals the potential of such combinations to be used in the treatment of AD (Wang et al., 2020).

The studies discussed in this thesis support the potential of developing new pharmacological approaches to the treatment of diseases that may be caused by circadian rhythm disorders through the modulation of clock genes. The aim is to show the functions of biological clocks and the clock genes in biological processes, that these genes can be used as pharmacological targets, and that this targeting can open new horizons in disease management.

CHAPTER II

This section includes conceptual explanations, definitions, and information about the research in the literature and previous research.

2.1. Conceptual Foundations

2.1.1. Biological Significance of Circadian Rhythms

Circadian rhythms are biological clock mechanisms that operate in 24-hour cycles and serve as internal timers that allow organisms to adapt to environmental changes. These rhythms regulate many biological processes, such as sleep-wake cycles, hormone secretion, body temperature, metabolism, and immune responses. In mammals, circadian rhythms are mainly controlled by the SCN located in the hypothalamus. The SCN receives light signals via the retina and synchronizes this information with the internal clock, regulating the organism's daily rhythm (Takahashi, 2017; Wong et al., 2022).

Circadian rhythms have a significant effect on sleep patterns and hormone secretion. For example, the level of the hormone cortisol peaks in the early morning hours, which helps the body transition to a state of wakefulness. In the evening hours, the hormone melatonin is released, which facilitates the transition to sleep (Cajochen et al., 2003; Zisapel, 2018). Circadian rhythms also play a role in the timing of metabolic processes. For example, the pancreas' insulin secretion and the liver's glucose metabolism are more active at certain times of the day, which helps maintain energy homeostasis (Bass & Takahashi, 2010; Ayyar & Sukumaran, 2021).

The regular functioning of circadian rhythms is critical for the healthy functioning of the organism. Disruption of these rhythms can lead to serious health problems such as sleep disorders, depression, metabolic syndrome, cardiovascular diseases, and even cancer (Foster & Kreitzman, 2014; Fifel & Videnovic, 2021). For example, increased cancer risk in people working night shifts has been associated with disruption of circadian rhythms (Stevens, 2009; Canever et al., 2023).

2.1.2. Structure and Function of Clock Genes

The molecular basis of circadian rhythms is formed by a set of genes known as clock genes. These genes encode proteins that control the functioning of the biological clock mechanism. The best-known clock genes are CLOCK, BMAL1, PER (PER1, PER2, PER3), and CRY (CRY1, CRY2). These genes regulate each other's expression through intracellular feedback loops, thus creating an approximately 24-hour cycle (Partch et al., 2014; Zhang et al., 2022).

BMAL1 and CLOCK proteins are essential for starting the circadian rhythm. They combine in the cell nucleus and bind to specific DNA sequences known as E-boxes, which trigger the transcription of the CRY and PER genes. As the CRY and PER proteins accumulate, they return to the nucleus and block the CLOCK complex, thereby stopping their transcription. This cycle repeats itself over approximately 24 hours, thus ensuring the continuity of circadian rhythms (Takahashi, 2017; Juliana et al., 2023).

Clock genes function not just in the central nervous system, but additionally in numerous external organs, including the liver, heart, kidneys, and immune cells (Buhr & Takahashi, 2013; Mendoza-Viveros et al., 2023). These circadian clocks in peripheral tissues work in synchrony with the central clock, thus ensuring coordination between different parts of the organism. For example, the daily rhythm in the liver regulates glucose metabolism and fatty acid synthesis, while the clock in the muscles optimizes energy use (Zhang et al., 2022).

2.2. Related Research

2.2.1. Disruption of Clock Genes and Its Relationship with Diseases

Disruption of clock genes can lead to various health problems. Circadian rhythm disorders have been associated with sleep disorders, mental health problems, metabolic disorders, pain, inflammation, neurodegenerative diseases, and cancer (Savvidis & Koutsilieris, 2012; Alexander et al., 2020). In particular, mutations in clock genes or changes in the expression of these genes can lead to serious irregularities in the sleep-wake cycle (Patke et al., 2017).

Neurodegenerative diseases are one of the most prominent effects of circadian cycle abnormalities. Circadian rhythm disturbance is widespread in neurodegenerative illnesses, including AD, and may contribute to disease development. The decrease in melatonin levels and frequent sleep disturbances in AD patients suggest that circadian clocks may play an important role in the pathogenesis of this disease (Musiek & Holtzman, 2016; Hoyt & Obrietan, 2022). Similarly, circadian cycle disturbances are also common in Parkinson's disease (Videnovic et al., 2014).

Metabolic diseases are another important consequence of circadian rhythm disorders. In particular, obesity, type 2 diabetes, and cardiovascular diseases are closely related to irregularities in circadian rhythms. For example, disruptions in metabolic processes regulated by clock genes in the liver can lead to conditions such as insulin resistance and hyperglycemia (Panda, 2016; Alexander et al., 2020). Furthermore, disturbance of the circadian cycles may result in increased inflammatory processes, which can cause the development of atherosclerosis along with additional cardiovascular disorders (Zhang et al., 2022; Fan et al., 2022).

The link between clock genes and susceptibility to cancer has also been supported by various studies. The timing of processes such as DNA repair, cellular proliferation, and apoptosis are regulated by circadian clocks. Disruptions in clock genes can disrupt these critical processes, leading to the accumulation of cellular damage and ultimately the development of cancer (Savvidis & Koutsilieris, 2012; Canever et al., 2023). The increased risk of cancer in individuals working the night shift demonstrates the relationship between circadian rhythm disruption and this disease (Stevens, 2009).

2.2.2. Effects of Circadian Rhythm Disorders on Pain

Circadian rhythms are internal clocks that regulate various biological processes in the body, and pain perception is also part of these processes. The effects of circadian rhythms on pain indicate that the pain threshold may vary at different times of the day. It is known that pain perception is subject to circadian regulation and therefore circadian rhythm disorders may negatively affect pain management (Martinez et al., 2021).

Pain is a complex biopsychosocial experience, and circadian rhythms can modulate this experience in several ways. For example, the expression of opioid receptors and the release of endogenous opioids are regulated by circadian rhythms. This explains why the pain threshold varies throughout the day (Nader et al., 2021). However, circadian rhythm disturbances can prevent these endogenous mechanisms from functioning properly and increase pain perception. Increased pain perception in individuals working the night shift or experiencing jet lag may be associated with circadian rhythm disturbances (Morton et al., 2020).

Circadian rhythm disorders also have important effects on chronic pain syndromes. For example, disruption of circadian rhythms has been frequently reported in patients with chronic pain syndromes such as fibromyalgia (Cudney et al., 2022). Studies on how sleep disorders and pain intensity affect each other in these patients suggest that restoring circadian rhythms may be a potential therapeutic strategy in pain management (Cudney et al., 2022).

2.2.3. Effects of Circadian Rhythm Disorders on Inflammation

Circadian rhythms also play a critical role in regulating inflammatory responses. The release of inflammatory cytokines, the activity of white blood cells, and other molecular components of inflammation are timed by circadian clocks. Therefore, circadian rhythm disturbances lead to disruption of inflammatory processes and chronic inflammation (Nader et al., 2021).

The relationship between circadian rhythm disorders and inflammation has been observed in various chronic diseases. In particular, increased inflammation has been reported in circumstances like obesity, type 2 diabetes, and cardiovascular diseases, where circadian rhythms are disrupted (Opperhuizen et al., 2019). In these diseases, the uncontrolled increase in inflammatory processes is directly related to the improper functioning of circadian rhythms. This emphasizes the importance of regulating circadian rhythms in the management of inflammation (Opperhuizen et al., 2019).

Neuroinflammation is another important area associated with circadian cycle disturbances. In particular, in AD, disruption of circadian rhythms leads to increased

neuroinflammation. This may contribute to the pathological progression of the disease, and reregulating circadian rhythms may be a potential therapeutic approach to reduce neuroinflammation (Musiek & Holtzman, 2016).

2.2.4. Clinical Applications and Therapeutic Strategies for Pain and Inflammation

The effects of circadian rhythm disorders on pain and inflammation can be attenuated by treating these disorders. Circadian rhythm regulators, such as melatonin, are being evaluated as potential therapeutic tools in the management of pain and inflammation (Zisapel, 2018). Melatonin may alleviate the symptoms of these disorders by regulating inflammatory responses and modulating pain perception (Srinivasan et al., 2020).

In chronic pain syndromes, modulation of the circadian cycle can reduce the severity of pain and improve the quality of life of patients. For example, melatonin treatment has been reported to improve pain and sleep disorders in fibromyalgia patients (Cudney et al., 2022). This suggests that the reregulation of circadian rhythms may play an important role in the treatment of such chronic pain syndromes.

Furthermore, the role of circadian rhythm regulators in the management of inflammation is also important in the treatment of various chronic diseases. Regulation of circadian rhythms may help control inflammatory responses, which may be beneficial in the management of conditions such as obesity, diabetes, and cardiovascular diseases (Opperhuizen et al., 2019).

Both pharmacological and behavioral interventions can be used in the treatment of circadian rhythm disorders. Medications such as melatonin restore the circadian rhythm, while behavioral strategies such as sleep hygiene and regular sleep-wake cycles can also contribute to the treatment process (Reiter et al., 2022). Such multidisciplinary approaches may be effective in reducing the negative effects of circadian rhythm disorders on pain and inflammation.

In conclusion, circadian rhythm disorders may negatively affect pain and inflammation processes, and management of these disorders plays a critical role in

controlling pain and inflammation. Restoration of circadian rhythms is considered an important strategy in the treatment of such conditions.

2.2.5. Alzheimer's Disease and Circadian Rhythm Disorders

AD is one of the neurodegenerative diseases in which circadian rhythm disorders are most frequently observed. In patients with AD, circadian rhythm disorders are generally characterized by irregular sleep-wake cycles, reduced nighttime sleep duration, and daytime sleepiness (Musiek & Holtzman, 2016). These disorders become more pronounced with the progression of AD and negatively affect the quality of life of patients. Disruption of circadian rhythms may also worsen AD pathologies such as amyloid-β (Aβ) accumulation, as these deposits occur more at certain times of the day (Musiek & Holtzman, 2016).

Among the treatment strategies, the use of circadian rhythm regulators such as melatonin has been widely investigated. It has been shown that melatonin can help restore the sleep-wake cycle and reduce Aβ accumulation (Wang et al., 2020). In addition, light therapy and implementation of regular sleep-wake routines can also alleviate circadian rhythm disturbances and support cognitive functions in patients with AD (Wu et al., 2019).

2.2.6. Amyotrophic Lateral Sclerosis and Circadian Rhythm Disorders

ALS is a neurodegenerative disease that affects motor neurons and causes progressive muscle weakness. Circadian rhythm disorders are common in ALS patients, which can negatively affect their sleep quality and general health (McClelland et al., 2020). These disorders may also be associated with dysregulation of inflammatory processes associated with the pathophysiology of ALS (McClelland et al., 2020).

Therapeutic strategies recommended for restoring circadian rhythms in ALS include melatonin supplementation and non-pharmacological interventions. Melatonin may have neuroprotective effects and improve sleep quality due to its antioxidant properties (Rosato et al., 2019). In addition, behavioral interventions such as light therapy are also recommended to maintain sleep patterns in ALS patients (Pimentel-Coelho et al., 2020).

2.2.7. Parkinson's Disease and Circadian Rhythm Disorders

PD is a neurodegenerative disease characterized by degeneration of dopaminergic neurons and associated with motor symptoms as well as circadian rhythm disorders. Sleep disorders, frequent awakenings during the night, daytime sleepiness, and REM sleep behavior disorder are common in PD patients (Videnovic et al., 2014). These disorders become more pronounced with the progression of PD. Thus, patients' well-being and daily life are negatively impacted.

Strategies used to treat circadian rhythm disorders in PD include melatonin and dopaminergic drugs. Melatonin may be particularly useful in treating sleep disorders and may have neuroprotective effects (Margaritelis et al., 2018). Additionally, non-pharmacological approaches such as light therapy and sleep hygiene may contribute to the restoration of circadian rhythms in PD patients (Videnovic & Golombek, 2013).

2.2.8. Multiple Sclerosis and Circadian Rhythm Disorders

MS is a chronic disease that affects the central nervous system and is characterized by inflammatory attacks. In MS patients, circadian rhythm disorders manifest themselves with symptoms such as decreased sleep quality, daytime sleepiness, and fatigue (Manouchehri et al., 2020). These disorders are closely related to the inflammatory pathophysiology of MS and may negatively affect the course of the disease.

Regulation of circadian rhythms in MS patients is seen as an important strategy to alleviate the symptoms of the disease. Melatonin is an agent investigated in the treatment of MS due to its anti-inflammatory properties and may also be effective in improving sleep patterns (Reuter et al., 2020). In addition, behavioral interventions such as maintaining a regular sleep-wake cycle and light therapy have also been shown to alleviate circadian rhythm disorders in MS patients (Reuter et al., 2020).

2.2.9. Huntington's Disease and Circadian Rhythm Disorders

HD is a genetic neurodegenerative disease characterized by motor, cognitive, and psychiatric disorders. Circadian rhythm disorders are common in HD patients,

leading to problems such as sleep disturbances, frequent awakenings during the night, and daytime sleepiness (Morton et al., 2019). These disorders increase with the progression of the disease and negatively affect the general health status of patients.

The use of melatonin and other circadian rhythm regulators is recommended for the treatment of circadian rhythm disorders in Huntington's disease. Melatonin can improve sleep patterns and has neuroprotective effects (Morton et al., 2019). In addition, behavioral interventions such as light therapy and regular sleep habits have been found to help alleviate circadian rhythm disorders in HD patients (Morton et al., 2019).

2.2.10. Circadian Rhythm-Focused Treatment Strategies in Neurodegenerative Diseases

Both pharmacological and non-pharmacological approaches are used in the treatment of circadian rhythm disorders associated with neurodegenerative diseases. Melatonin is recommended in circadian rhythm-focused treatment strategies as it can help regulate circadian rhythms in many neurodegenerative diseases and may have neuroprotective effects (Rosato et al., 2019). In addition, non-pharmacological approaches such as light therapy, sleep hygiene, and regular physical activity also play an important role in alleviating circadian rhythm disorders (Wu et al., 2019).

These treatment strategies are designed to alleviate the symptoms of neurodegenerative diseases and improve the quality of life of patients. Regulation of circadian rhythms may also contribute to slowing down pathological processes in neurodegenerative diseases (Musiek & Holtzman, 2016). Therefore, multidisciplinary approaches are important in the treatment of circadian rhythm disorders associated with neurodegenerative diseases.

2.2.11. Use of Clock Genes as Pharmacological Targets

The critical roles of clock genes in biological processes allow these genes to be evaluated as potential targets for pharmacological interventions. Reorganization of circadian rhythms through modulation of clock genes may be used in the treatment of various diseases. The effectiveness of circadian rhythm regulators such as melatonin

in the treatment of conditions such as sleep disorders and depression demonstrates the potential of this approach (Arendt, 2006; Ahmad et al., 2022).

In recent years, research has increased on the treatment of diseases such as neurodegenerative diseases, metabolic disorders, and cancer through the modulation of clock genes. Such approaches aim to target the pathological processes of diseases through circadian rhythms (Sahar & Sassone-Corsi, 2009; Fan et al., 2022). For instance, modulation of the BMAL1 in AD has the potential to slow down the progression of the disease (Musiek & Holtzman, 2016; Fan et al., 2022).

Such pharmacological interventions aim to achieve optimal timing of biological processes by targeting the regulation of circadian rhythms through the modulation of clock genes. These approaches may not only alleviate symptoms but also correct the underlying mechanisms of the disease (Hirota & Kay, 2015).

In conclusion, circadian rhythms and clock genes are of great importance in biological processes and modulation of these genes may open new horizons in the treatment of various diseases. Studies on the biological functions of clock genes and health problems that occur due to the disruption of these functions show that these genes can be used as pharmacological targets.

2.2.12. Melatonin and Circadian Rhythm

Melatonin is a hormone produced by the pineal gland that plays a critical role in regulating circadian rhythms. Melatonin is often referred to as the “hormone of darkness” because its secretion increases significantly during the night and decreases under the influence of daylight. The main function of this hormone is to signal to the body that it is nighttime and that it should prepare for sleep (Reiter et al., 2020). Melatonin helps to regulate circadian rhythms by synchronizing the rhythm of sleeping and waking and corresponding the biological rhythms of the body with natural light and dark cycles (Zisapel, 2018).

The impact of melatonin on the circadian cycle is related to its effects on the SCN. The SCN, the body's internal circadian timer, is susceptible to the effects of this hormone via melatonin receptors. Increased melatonin levels during the night affect neurons in the SCN, regulating the transition to sleep and the sleep-wake cycle

(Wong et al., 2022). Melatonin is also widely used in the treatment of circadian rhythm disorders. In circadian rhythm disorders such as jet lag and shift work, melatonin is used as a treatment tool to reset the body's biological clock and stabilize sleep patterns (Zisapel, 2018).

Pharmacological applications of melatonin range from sleep disorders to chronotherapy. Especially in conditions such as jet lag, melatonin helps reset the circadian cycle and improves the condition of sleep (Srinivasan et al., 2020). Melatonin helps regulate the circadian rhythm while also synchronizing the timing of various biological processes, which has positive effects on overall health (Reiter et al., 2022).

2.2.13. Melatonin and Clock Genes

The effect of melatonin on circadian rhythm is mediated by clock genes. Clock genes are genes that regulate the circadian cycle and can be altered by melatonin. Specifically, melatonin receptors (MT1 and MT2) trigger cellular signaling pathways that act on clock genes in the SCN (Comai & Gobbi, 2014).

Melatonin regulates the expression of clock genes, ensuring the stability of circadian rhythms. One of the most important of these genes, BMAL1, is modulated by melatonin, and this modulation is one of the basic building blocks of the circadian cycle (Li et al., 2020). BMAL1 works with other clock genes to form transcriptional feedback loops that control the timing of biological processes. Melatonin's increase in BMAL1 expression ensures the stability and harmony of the circadian rhythm (Liu et al., 2022).

Melatonin can also affect the expression of negative feedback clock genes such as CRY1 and CRY2. These genes inhibit the activity of BMAL1 and CLOCK proteins and contribute to the completion of the circadian cycle. By modulating these negative feedback loops, melatonin maintains the circadian rhythm (Comai & Gobbi, 2014). This modulation plays an important role in the resetting of the circadian rhythm, especially in conditions such as jet lag or shift work (Srinivasan et al., 2020).

The interaction between melatonin and clock genes may also be important in various pathological conditions, such as neurodegenerative diseases. In AD, decreased

melatonin levels may contribute to circadian rhythm disturbances and the progression of these diseases. In such cases, melatonin supplementation may slow disease progression through clock gene upregulation (Musiek & Holtzman, 2016).

2.2.14. Clinical Applications of Melatonin on Clock Genes

Melatonin is widely used in clinical practice in the treatment of circadian rhythm disorders. Conditions such as jet lag, sleep disorders, shift work, and old age can cause disruption of the circadian rhythm and melatonin is used as an effective agent in the treatment of these conditions (Zisapel, 2018). The effects of melatonin on clock genes form the basis of therapeutic mechanisms in such clinical conditions.

Melatonin production decreases, especially in older individuals, and this can lead to disruption of the circadian rhythm. This disruption can lead to problems such as poor sleep quality, depression, and decreased cognitive functions (Srinivasan et al., 2020). Melatonin supplementation can help restore circadian rhythm by regulating the expression of clock genes in these individuals (Wong et al., 2022).

Furthermore, the antioxidant properties and neuroprotective effects of melatonin have therapeutic potential in neurodegenerative disorders. In AD, melatonin may affect disease pathogenesis and slow disease progression through modulation of BMAL1 and other clock genes (Li et al., 2020). This suggests that melatonin regulates the circadian cycle and may help regulate neurodegenerative processes (Musiek & Holtzman, 2016).

The clinical use of melatonin is supported by numerous studies showing that it is effective in the treatment of sleep disorders and circadian rhythm disorders. For example, in the treatment of jet lag, melatonin resynchronizes the circadian rhythm and improves sleep quality (Zisapel, 2018). In shift workers, melatonin improves nighttime sleep and increases daytime alertness, which positively affects work performance and general health (Srinivasan et al., 2020).

Melatonin regulates the circadian cycle and modulates circadian genes. These effects make melatonin a potential therapeutic tool in clinical conditions such as circadian rhythm disorders and neurodegenerative diseases. The impacts of melatonin on circadian cycles and clock genes highlight the central role of this hormone in the regulation of biological clocks.

2.2.15. Computer-Based Approaches to the Treatment of Circadian Rhythm Disorders

Circadian rhythm disorders can lead to serious health problems such as neurodegenerative diseases, metabolic disorders, and cardiovascular diseases. Computer-aided drug design (CADD) methods play an important role in the treatment of these disorders. Computer-aided drug design methods consist of different stages **(Figure 3)**. Computer-driven approaches such as molecular docking, pharmacophore analysis, ADMET (absorption, distribution, metabolism, excretion, toxicity) prediction, molecular dynamics simulations, and MM/PBSA are critical tools in the development of new therapeutic molecules. These methods are widely used to identify and optimize potential drug candidates for the treatment of circadian rhythm disorders.

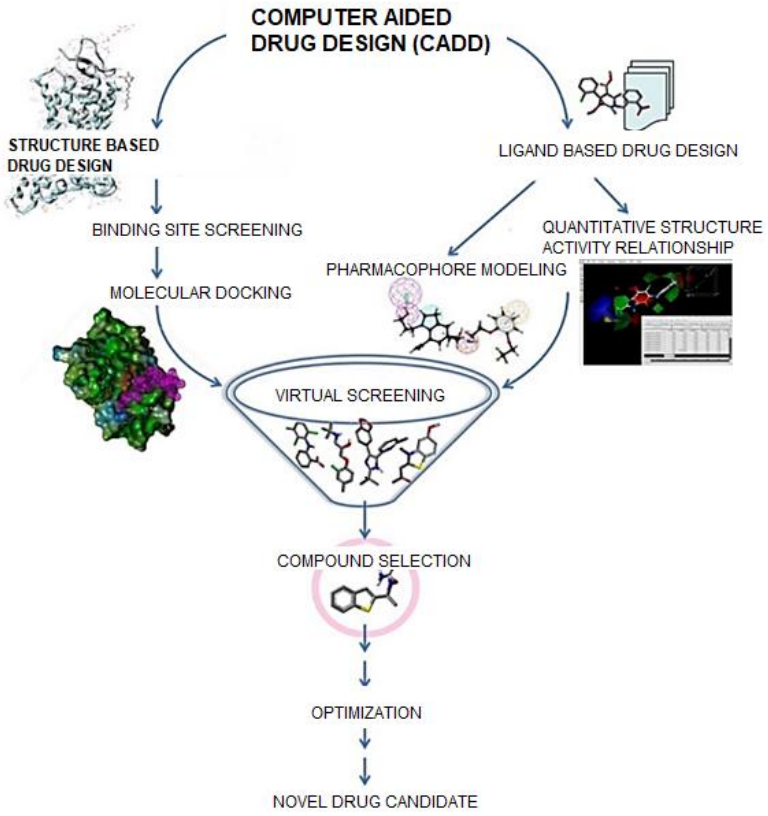


Figure 3: The diagram illustrates the workflow of CADD. Key steps involve molecular docking, pharmacophore modeling, and virtual screening to identify potential drug candidates. Selected compounds undergo optimization to develop a novel drug candidate.

2.2.15.1. Molecular Docking

Molecular docking is a method used to predict how small molecules might bind to binding sites on biomolecules (e.g. proteins) **(Figure 4)**. This technique helps to evaluate binding affinities and inhibitory potentials by simulating how potential drug candidates would interact with target proteins (Meng et al., 2021). Molecular docking is widely used for the treatment of circadian rhythm disorders, especially for the discovery of molecules that interact with targets such as clock proteins CRY, PER, BMAL1, and CLOCK (Patel et al., 2021).

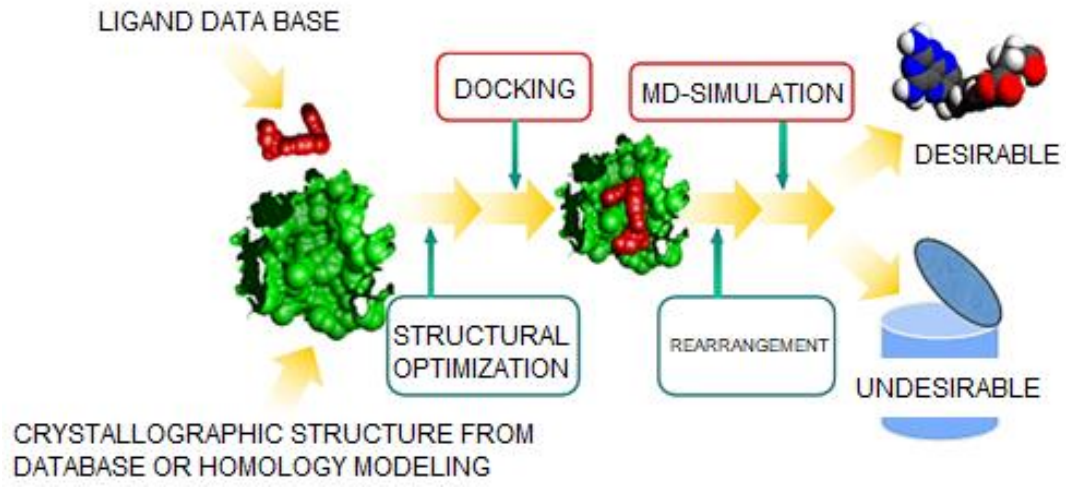


Figure 4: Molecular docking and simulation methods (Ortaakarsu, 2020)

For example, potential inhibitors or activators targeting the BMAL1 protein can be discovered by molecular docking methods. In this process, a large library of chemical compounds is screened using virtual screening techniques and the molecules with the best binding scores are selected. This approach is critical for the development of new drugs that can modulate circadian rhythms (Fan et al., 2022).

2.2.15.2. Pharmacophore Analysis

Pharmacophore analysis is a method used to identify the properties of biologically active molecules and to discover other molecules that share these properties. A pharmacophore model is an abstraction of the chemical properties required to bind to the active site of a biomolecule and has a vital role in the creation of possible drug candidates (Güner, 2020).

In the treatment of circadian rhythm disorders, pharmacophore analysis is used to design molecules that target clock proteins. For example, pharmacophore analysis on molecules that inhibit CRY1 and CRY2 proteins sheds light on the discovery of new compounds that can effectively interact with these proteins. This approach allows the development of new molecules that can be used in the treatment of circadian rhythm disorders (Liu et al., 2022).

2.2.15.3. ADMET Prediction

ADMET prediction is a computer-aided method to predict the pharmacokinetic and toxicological properties (absorption, distribution, metabolism, excretion, and toxicity) of a compound. This technique plays a critical role in the preclinical evaluation of potential drug candidates and is used to predict possible side effects or toxicities (Daina et al., 2017).

In the treatment of circadian rhythm disorders, ADMET predictions are used to understand the behavior of discovered molecules in the human body. In particular, the pharmacokinetic profiles and possible toxic effects of compounds targeting circadian clock proteins, such as CRY1/CRY2 inhibitors, are evaluated by this method. ADMET analyses are considered an important step in pharmaceutical optimization to improve the efficacy and safety of drug candidates (Patel et al., 2021).

2.2.15.4. Molecular Dynamics Simulations

Molecular dynamics (MD) simulations are a method of studying how biomolecules move over time at the atomic level. These simulations are used to understand the dynamic nature of protein-ligand interactions, conformational changes, and atomic details of biomolecular processes (Hollingsworth & Dror, 2018).

MD simulations are used to study the structural properties of proteins that are critical for the treatment of circadian rhythm disorders and to assess the stability of drug candidates that interact with these proteins. In previous studies, the dynamic behaviors of the BMAL1-CLOCK heterodimer and the binding stability of molecules interacting with this complex were investigated using MD simulations (Fan et al., 2022). Such analyses are important for improving the efficacy of molecules in the drug design process.

2.2.15.5. MM/PBSA Method

The MM/PBSA method is a technique used to perform free energy calculations of molecule-molecule interactions. This method is integrated with molecular dynamics simulations to calculate the binding free energy of a protein-ligand complex and is used to evaluate the binding affinities of potential drug candidates (Genheden & Ryde, 2015).

In the treatment of circadian rhythm disorders, the MM/PBSA method can be used to calculate the binding free energies of molecules that specifically target clock proteins. This technique, when combined with virtual screening and molecular dynamics simulations, allows the selection of molecules with the most suitable binding properties. For example, calculating the binding free energies of inhibitors interacting with CRY1 and CRY2 proteins has been used to determine the activities and potential pharmacological values of these molecules (Wang et al., 2021). Computer-aided drug design (CADD) methods are widely used to discover and optimize potential drug candidates in the treatment of circadian rhythm disorders. Methods such as molecular docking, pharmacophore analysis, ADMET prediction, molecular dynamics simulations, and MM/PBSA play a critical role in the development of new therapeutic molecules. These approaches allow the development of effective and safe drugs for the treatment of health problems caused by circadian rhythm disorders.

CHAPTER III

Method

3.1. Computer-Aided Drug Design and Simulations

3.1.1. Ligand Selections and Pharmacophore Mapping

For the core structure of 5-methoxyindole, which is present in both melatonin and indomethacin, through pharmacophore modeling. Ligands were selected from benzoxazolone derivatives that included modifications at the fifth position, specifically 5-chloro-, 5-bromo-, 5-fluoro-, and 5-nitro-2-benzoxazolone, due to their potential bioisosteric effects (Lima & Barreiro, 2005). ChemDraw Pro 12.0 and LigandScout 4.4.6 software (Wolber & Langer, 2005) were used to construct chemical structures.

The ligands capable of upregulating NR1D1, including STL1267 and SR9011, which were selected as positive controls (Wolff et al., 2020; Murray et al., 2022). FDA-approved treatments for ALS, such as riluzole, dextromethorphan, quinidine, sodium phenylbutyrate, edaravone, and melatonin, were selected for their anti-glutamate and antioxidant characteristics (Yıldırım et al., 2023). Their molecular structures were obtained from the PubChem database and formatted into mol2 files. Ligands underwent refinement processes such as water and heteroatom removal, addition of polar hydrogens, and calculation of Gasteiger charges to prepare for molecular docking.

Furthermore, FDA-approved AD drugs, including Tacrine, Donepezil, Rivastigmine, Galantamine, Memantine, and the monoclonal antibody Lecanemab, were selected alongside CLK8. Ligands were retrieved from the PubChem and ZINC database for molecular docking. Structures were similarly refined by removing heteroatoms and water molecules, adding polar hydrogens, and calculating Gasteiger charges.

3.1.2. Pharmacophore Mapping

Pharmacophore mapping was carried out using PharmMapper (Liu et al., 2010), aligning the ligands with target protein pharmacophore models derived from the Protein Data Bank (PDB). Genetic algorithms (GA) were employed for optimizing ligand-target fit values through triangulation, pairwise alignment, and other refinement processes (Wang et al., 2016).

3.1.3. Molecular Docking Studies

The docking targets for benzoxazolone derivatives included the main circadian proteins CRY1, CRY2, PER1, PER2, and CLOCK: BMAL1. Three-dimensional structures of these proteins were obtained from the PDB with identifiers 4F3L, 4DJ2, 3GDI, 7DLI, and 7V8Y, all derived from *Mus musculus*. Ligands were prepared by removing water molecules and heteroatoms, followed by energy minimization using Universal Force Field (UFF) parameters. PyRx 0.8 software facilitated molecular preparation and conversion to PDBQT format (Kerstjens & De Winter, 2022). Active site analysis, conducted with CASTp, identified key amino acid residues critical for docking interactions. These residues included ARG126, PHE141, SER143, GLU146, and LYS220 for CLOCK; ARG300, ARG312, TYR313, and PRO473 for PER1; TYR354, LEU359, and ARG399 for PER2; LYS11, PHE257, ARG256, and LEU386 for CRY1; and LEU39, VAL188, and CYS196 for CRY2 (Tian et al., 2018). Binding poses and affinities (ΔG, kcal/mol) were calculated using AutoDock Vina, while Discovery Studio Visualizer 2021 was used to generate interaction plots (Forli et al., 2016). All docking tests used a uniform grid box size of 29 × 29 × 29 Å.

Similarly, docking simulations were carried out with AutoDock Vina to investigate ligand interactions with the DNA-binding domain of RevErbα (NR1D1). The protein structure, sourced from the PDB database (entry 1GA5), had a resolution of 2.40 Å. A uniform grid box size of 25 × 25 × 25 Å was utilized for the docking process. Biovia Discovery Studio Visualizer 2021 was employed to analyze and visualize interaction diagrams and binding free energies. Same process and grid box size were repeated for the crystal structure of the CLOCK: BMAL1 dimer (PDB ID: 4F3L) for docking studies.

3.1.4. Protein-Protein Docking for Lecanemab

The interactions of Lecanemab with CLOCK:BMAL1 were analyzed using the ClusPro 2.0 protein-protein docking tool. The PIPER algorithm was employed to calculate binding energies, integrating factors such as shape complementarity, electrostatic interactions, and solvation contributions.

3.1.5. Molecular Dynamics Simulations

A 100-nanosecond (ns) MD simulation were performed for all complexes using GROMACS 5.1.4 with GROMOS96 54A7 force field parameters (Hollingworth & Young, 2014). SPC water molecules were added within a cubic simulation box, maintaining a 1.5 nm minimum distance from the system boundaries. Neutralization was achieved by introducing Na^+ and Cl^- ions, maintaining a 0.15 M NaCl concentration. The Particle Mesh Ewald (PME) approach computed long-range electrostatic forces (Yuet & Blankschtein, 2010). The steepest descent approach was used for initial energy reduction, proceeded by an NVT ensemble-based progressive heating strategy. Simulations ran at 300 K under constant pressure (1 bar) with a Parrinello-Rahman barostat (Parrinello & Rahman, 1981). RMSD values were calculated, and stability was confirmed for ligand-protein complexes over the simulation period.

3.1.6. MM/PBSA Free Energy Calculations

The binding free energies ($\Delta G_{binding}$) of ligand-protein interactions were determined using the MM/PBSA technique (Rastelli et al., 2010). $\Delta G_{binding}$ was calculated as the difference between the total free energy of the complex ($\Delta G_{complex}$) and the sum of free energies for the isolated protein and ligand ($\Delta G_{target\ protein} + \Delta G_{ligand}$). Binding-free energies were analyzed during the final 15 ns of the MD simulations to ensure stability.

3.1.7. Virtual Screening

A virtual screening approach identified small molecules capable of binding the CLOCK:BMAL1 dimer. Approximately 2 million compounds were screened, narrowing down to CLK8, a molecule reported to enhance BMAL1 expression and

circadian rhythm amplitude (Doruk et al., 2020). CLK8 served as the positive control ligand.

3.1.8. Statistical Analyses

Docking scores and MM/PBSA results were presented as mean ± standard deviation. Statistical analyses were conducted using GraphPad Prism 8.4.2. For each ligand, binding energies were averaged across five docking poses, and one-way ANOVA with post hoc Dunnett's test was applied to compare ligands against control groups. Statistical significance was set at $p < 0.05$.

CHAPTER IV

Results and Discussion

In this thesis, the results and discussion for studies including the mechanisms targeted in drug treatment through circadian rhythm and molecular dynamics of clock proteins are divided into three subsections.

4.1. First Subsection

4.1.1. Exploring the Role of Benzoxazolone Derivatives in Circadian Rhythm Modulation

Inflammation and pain are among the most common reasons for medical consultations (Alotaibi et al., 2022). These conditions arise from a cascade of biological events beginning with the enzymatic transformation of arachidonic acid (AA). Cyclooxygenase (COX) enzymes convert AA into prostaglandin H2 (PGH2), while 5-lipoxygenase (5-LO) metabolizes it into leukotriene A4 (LTA4). These intermediates are further processed by specific synthetase enzymes to produce active lipid mediators, including prostaglandin E2 (PGE2), which is vital in inflammatory pathways (Ricciotti and FitzGerald, 2011).

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for their ability to address inflammation, reduce fever, and relieve pain. Their mechanism of action involves inhibiting COX enzymes, which results in decreased PGE2 production and the alleviation of inflammation-related symptoms (Gunaydin and Bilge, 2018; Bacchi et al., 2012).

Pain intensity and frequency have been observed to exhibit daily variations, influenced by factors such as light and dark cycles (Bumgarner et al., 2023). Emerging evidence suggests a connection between circadian rhythms and inflammatory cytokines (Kim et al., 2019; Wang and Li, 2021). The circadian clock regulates biological activities over 24 hours in the SCN and a distal clock in every cell. Key components, such as the CLOCK and BMAL1, work inside feedback loops regulated by inhibitory proteins such as CRY and PER (Pilorz et al., 2020). These regulatory mechanisms result in daily fluctuations in immune functions and hormone levels, influenced by circadian rhythms (Al-Waeli et al., 2020; Tordjman et al., 2017).

The lipophilic hormone melatonin is essential for regulating the body's circadian cycles. To encourage sleep, its synthesis rises at night in response to light exposure (Masters et al., 2014). Studies indicate that melatonin diffuses passively across SCN cell membranes, independent of specific transporters (Artime-Naveda et al., 2023; Gao et al., 2023). This diffusion enables melatonin to regulate the CLOCK: BMAL1 dimer and other master clock proteins, influencing transcriptional activities and post-translational modifications (Brzezinski et al., 2021). Thus, melatonin aligns the internal biological clock with environmental light-dark cues, stabilizing circadian rhythms and enhancing their functionality (Rodríguez-Santana et al., 2023).

Pain sensitivity is greatly influenced by circadian rhythms, and disturbances, such as those brought on by shift work or sleep disorders, exacerbate pain (Chu et al., 2023). Melatonin's regulatory role in master clock proteins also modulates inflammatory pathways and central sensitization, factors implicated in chronic pain conditions (Vriend and Reiter, 2015; Warfield et al., 2021). Evidence indicates that melatonin administration normalizes pain thresholds reduced by continuous light exposure, underscoring its dual role in pain management and circadian regulation (Bumgarner et al., 2023; Warfield et al., 2021).

Indomethacin, a potent NSAID, demonstrates high efficacy in treating specific headaches, such as hemicrania continua (Summ et al., 2021). Due to its structural similarity to indomethacin, melatonin has been proposed as a viable alternative with fewer gastrointestinal side effects (Rozen, 2006). Investigating compounds that mimic the chemical structures of indomethacin and melatonin holds promise for minimizing NSAID-related adverse effects while enhancing chronotherapeutic efficacy (Tai and McAlindon, 2021; Peres et al., 2001). Identifying pharmacophores that offer bioisosteric effects comparable to the 5-methoxyindole nucleus in these compounds is crucial.

Benzoxazolone, a heterocyclic scaffold with established anti-inflammatory and analgesic activity, has garnered attention in drug development (Poupaert et al., 2005; Gökhan-Kelekçi et al., 2009). Similar to NSAIDs, benzoxazolone derivatives exert their effects by inhibiting COX enzymes, thereby reducing prostaglandin production (Kaur et al., 2018). Although direct interactions with molecular clock proteins remain unexplored, these derivatives' anti-inflammatory properties may influence

circadian systems given the interplay between inflammation and circadian rhythms (Jerigova et al., 2022).

Although benzoxazolone derivatives have been extensively studied for their pharmacological properties, their role in influencing circadian rhythms has not been thoroughly explored (Loksha and Abd-Alhaseeb, 2020). This research seeks to evaluate the impact of 5-substituted benzoxazolone derivatives on key circadian proteins, including CLOCK, BMAL1, PER, and CRY. Insights gained from this computational analysis aim to establish a preliminary basis for subsequent experimental investigations, shedding light on the potential interaction between benzoxazolone derivatives and circadian gene modulation, along with their therapeutic implications.

Pharmacophore modeling indicated that the 2-benzoxazolone ring has substantial structural similarities compared to the 5-methoxy indole ring found in melatonin and indomethacin. Substituents with electron-withdrawing properties, such as $-NO_2$, and halogen substituents at the 5th position, were found to enhance binding activity. This observation was supported by molecular docking results, where derivatives such as 5-fluoro-, 5-nitro-, and 5-chloro-2-benzoxazolone indicated the highest affinities for clock proteins. Binding energy calculations for the CLOCK: BMAL1 complex varied from -5.0 to -5.8 kcal/mol, with 5-fluoro-2-benzoxazolone exhibiting the strongest binding (-5.8 kcal/mol). Moreover, significant differences in binding affinities were noted for specific ligands at PER1 and PER2 binding sites, with statistical significance values of $p<0.05$ and $p<0.0001$, respectively. These results highlight the potential of certain benzoxazolone derivatives to modulate clock protein activity effectively.

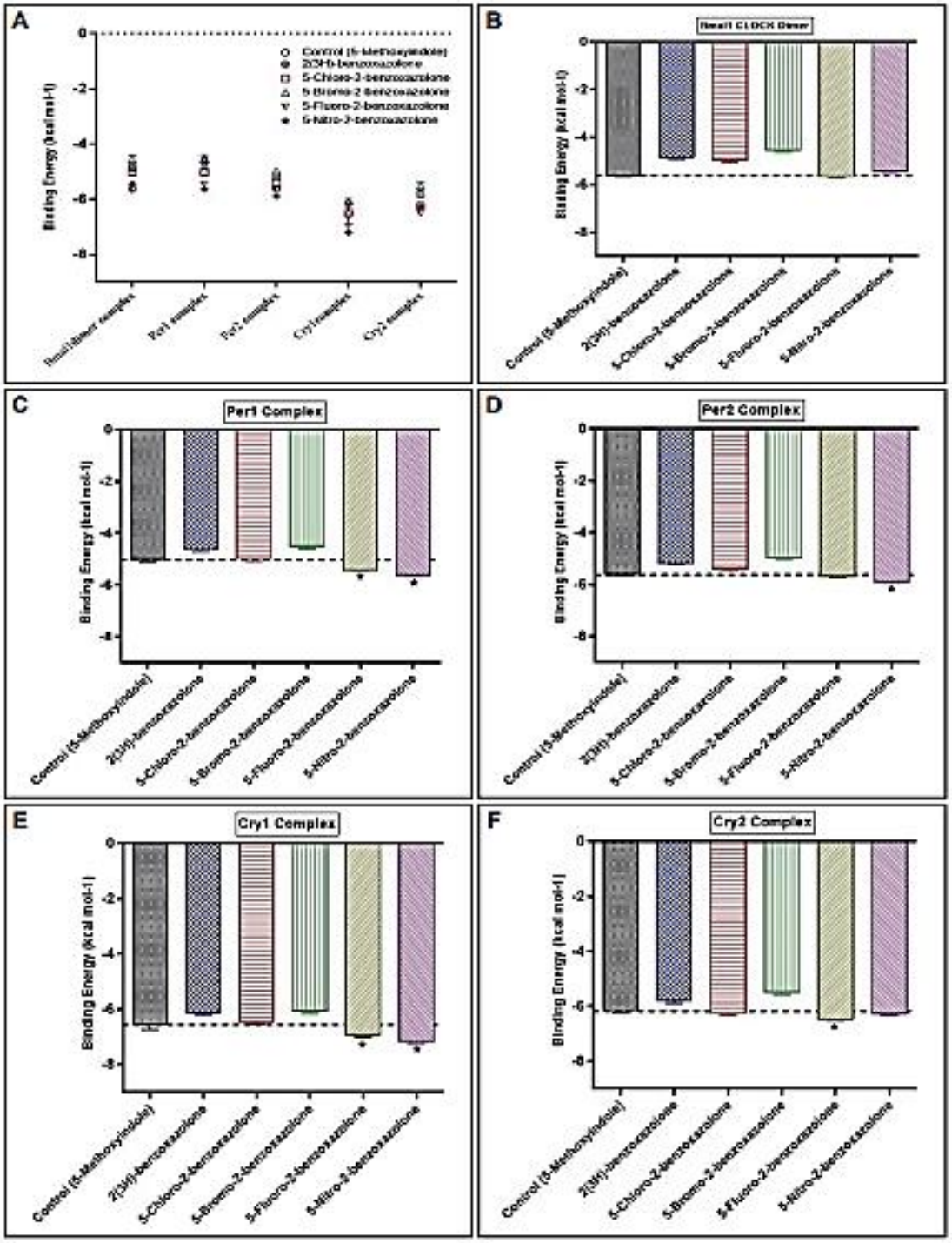


Figure 5: Variance analysis of docking scores for ligands in different clock gene binding sites was performed. Mean binding energies and standard deviations were summarized (A). Ligand binding energies in CLOCK:BMAL1 (B), PER1 (C), PER2 (D), CRY1 (E), and CRY2 (F) were statistically compared to the control (5-methoxyindole). *Indicates ligands with more negative binding energies than control.

Within the CRY1 binding site, 5-fluoro and 5-nitro derivatives of 2-benzoxazolone demonstrated significantly higher binding affinities in contrast to the reference ligand, achieving statistical significance at $P<0.05$ and $P<0.0001$, respectively. Regarding CRY2, although both 5-chloro-2-benzoxazolone and 5-nitro-2-benzoxazolone demonstrated improved binding energies compared to the control, a statistically significant difference was observed exclusively with 5-fluoro-2-benzoxazolone ($P<0.001$).

Binding energy calculations for CLOCK: BMAL1 dimer-ligand complexes using the MM/PBSA method. Among the tested ligands, 5-fluoro-2-benzoxazolone exhibited the most favorable binding energy (-176.34 ± 2.51 kJ/mol), followed by 5-nitro-2-benzoxazolone (-171.15 ± 2.42 kJ/mol) and 5-chloro-2-benzoxazolone (-169.05 ± 2.21 kJ/mol). Interaction analysis revealed that consistent electrostatic pi bonds were formed with residues ARG126 and GLU146, mirroring the interactions seen with the reference molecule 5-methoxyindole. Additional stabilization was achieved through hydrogen bonds with LYS220, with residues such as PHE141, SER143, and ARG126 playing critical roles in ligand anchoring.

For PER1, 5-nitro-2-benzoxazolone displayed the strongest affinity (-5.8 kcal/mol), followed by 5-fluoro-2-benzoxazolone (-5.6 kcal/mol) and 5-chloro-2-benzoxazolone (-5.3 kcal/mol), respectively. The MM/PBSA method calculated binding energies for PER1-ligand complexes in the range of -197.02 ± 3.53 kJ/mol to -242.72 ± 2.24 kJ/mol, which was notably stronger compared to those observed for CLOCK: BMAL1. Key interaction features included T-shaped stacking bonds, pi-alkyl interactions, and hydrogen bonding with TYR313, ARG312, PRO473, and ARG430, respectively. In addition, ARG300 was critical in carbon-hydrogen bonding, highlighting the importance of the benzoxazolone core in ligand stability. For PER2, the highest binding affinity (-6.1 kcal/mol) and MM/PBSA energy (-249.63 ± 2.25 kJ/mol) were observed with 5-nitro-2-benzoxazolone. The interaction profile was similar to that of PER1, featuring significant T-shaped pi-stacking interactions involving the TYR354 residue, which played a crucial role in the ligand-receptor interactions. The binding affinities for CRY1 and CRY2 were significantly higher than other circadian proteins. In CRY1 complexes, 5-nitro-2-benzoxazolone

demonstrated the strongest affinity (-7.4 kcal/mol), surpassing all other ligands, including the reference compound.

In CRY2, 5-fluoro-2-benzoxazolone exhibited the highest binding energy (-6.7 kcal/mol), outperforming the reference molecule (-6.4 kcal/mol). MM/PBSA calculations for CRY1 revealed binding energies ranging from -253.12 ± 2.41 kJ mol^{-1} to -352.36 ± 2.34 kJ mol^{-1}, with the reference molecule at -318.41 ± 3.52 kJ mol^{-1}. Across all clock proteins, 5-nitro and 5-fluoro substitutions consistently showed superior binding energies. MM/PBSA energy variance analysis indicated that 5-fluoro-2-benzoxazolone and 5-nitro-2-benzoxazolone had higher negative interaction energy values inside CLOCK: BMAL1 complexes than the conventional ligand. The interaction of 5-fluoro-2-benzoxazolone was statistically significant ($P<0.0001$), but 5-nitro-2-benzoxazolone did not show significant results.

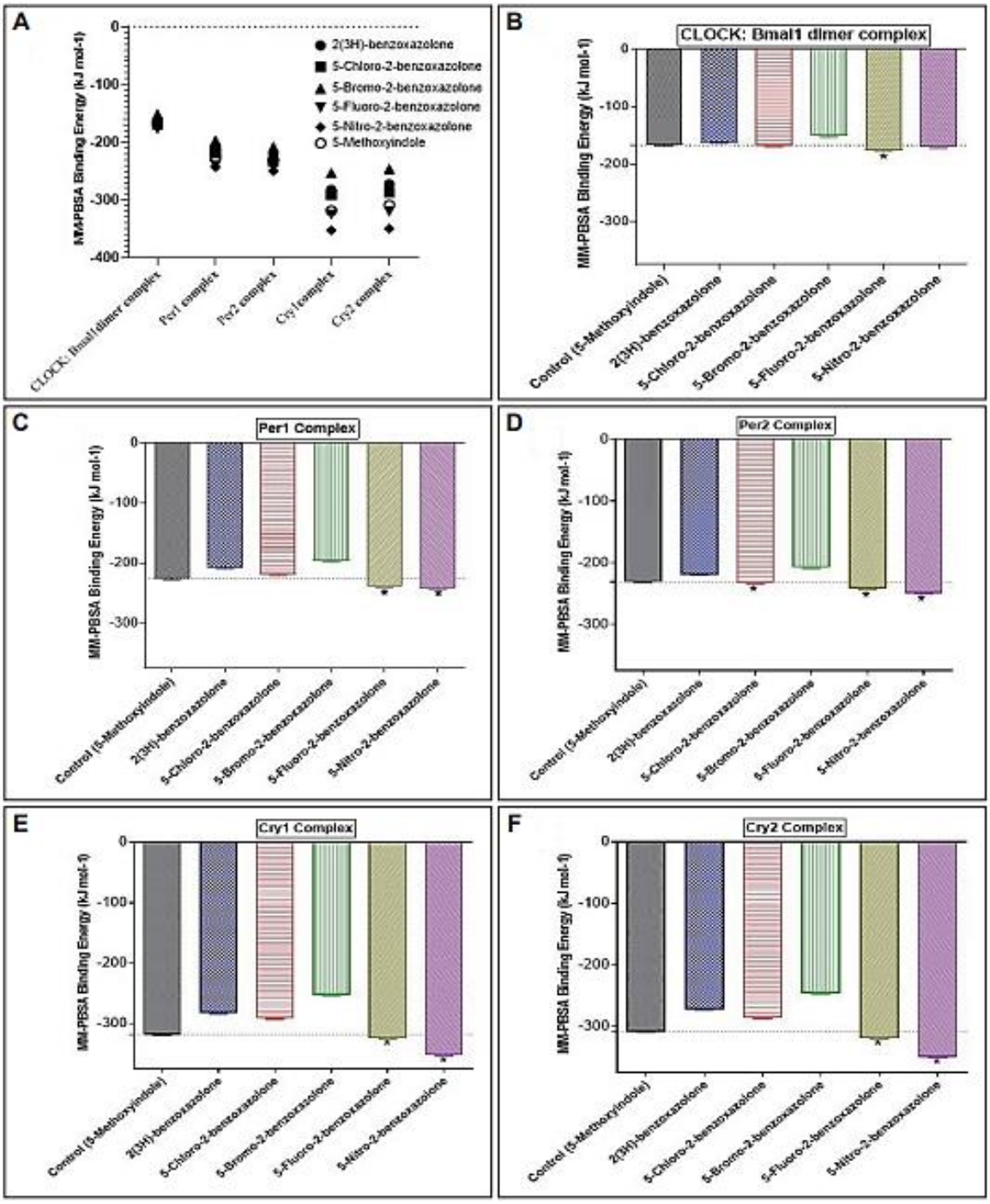


Figure 6: Variance analysis of MM/PBSA binding energies for clock gene-ligand complexes was conducted. Mean binding energies with standard deviations were summarized (A). Binding energies in CLOCK:BMAL1 (B), PER1 (C), PER2 (D), CRY1 (E), CRY2 (F), and ligand groups were statistically compared to the control (5-methoxyindole). *Indicates ligands with more negative binding energies than control.

Similar to results observed in CLOCK: BMAL1 complexes, PER1-ligand interactions indicated that 5-substituted fluoro and nitro derivatives of 2-benzoxazolone showed superior negative interacting energy values compared to the reference ($P<0.0001$). This trend persisted in PER2 complexes, where these ligands again exhibited statistically superior binding energies ($P<0.0001$).

In cryptochrome (CRY) protein interactions, fluoro and nitro-substituted derivatives consistently demonstrated significantly enhanced negative docking scores for both CRY1 and CRY2 proteins ($P<0.0001$ across all comparisons). These findings underline the consistently strong affinities of these ligands across multiple clock protein targets.

A comparative analysis of molecular interactions revealed shared binding patterns among all tested derivatives. Common amino acids such as PHE257, LYS11, LEU55, ARG256, and LEU386 were involved in interactions that predominantly included electrostatic interactions (T-shaped pi stacking, alkyl, pi-alkyl), and carbon-hydrogen bonds. However, benzoxazolone derivatives exhibited distinct features, forming unique hydrogen bonds and halogen (fluorine) bonds with residues like ARG10, ARG51, and ALA388. These interactions contributed significantly to the elevated binding affinities observed for CRY1 proteins, as depicted in Figures 9–11.

In the analysis of the CRY2-ligand complex, VAL188, MET193, and CYS196 emerged as key residues, consistently appearing in interaction profiles for all tested derivatives and the control compound. Notably, benzoxazolone derivatives uniquely demonstrated frequent interactions with leucine residues, including LEU39, LEU84, and LEU87, which further distinguished their binding profiles and contributed to their strong affinity for CRY2.

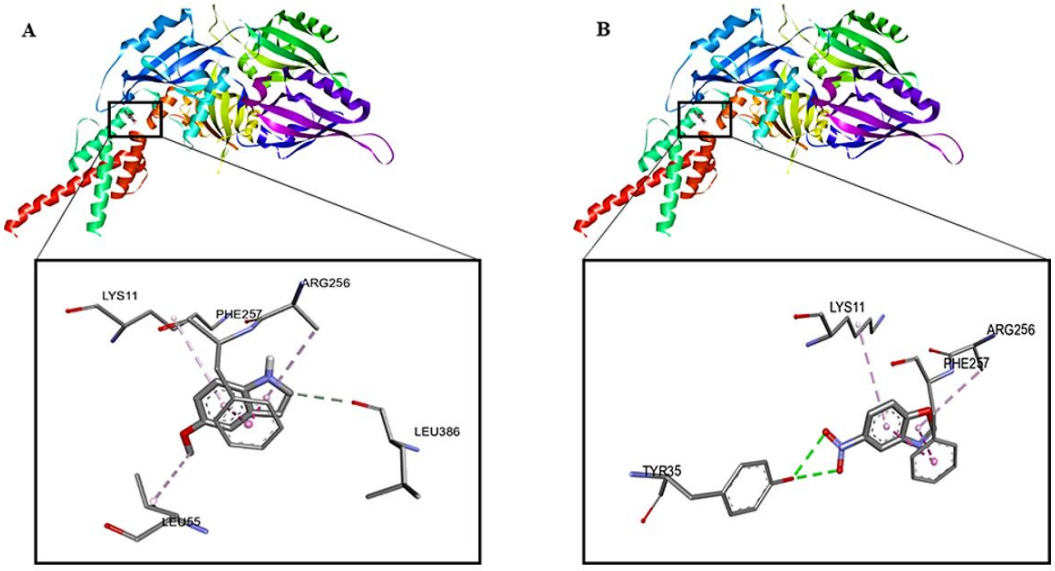


Figure 7: A comparative analysis of three-dimensional interactions within the CRY1 binding site revealed shared amino acid residues interacting with both the reference molecule, 5-methoxyindole (A), and the ligand with the highest binding affinity, 5-nitro-2-benzoxazolone (B).

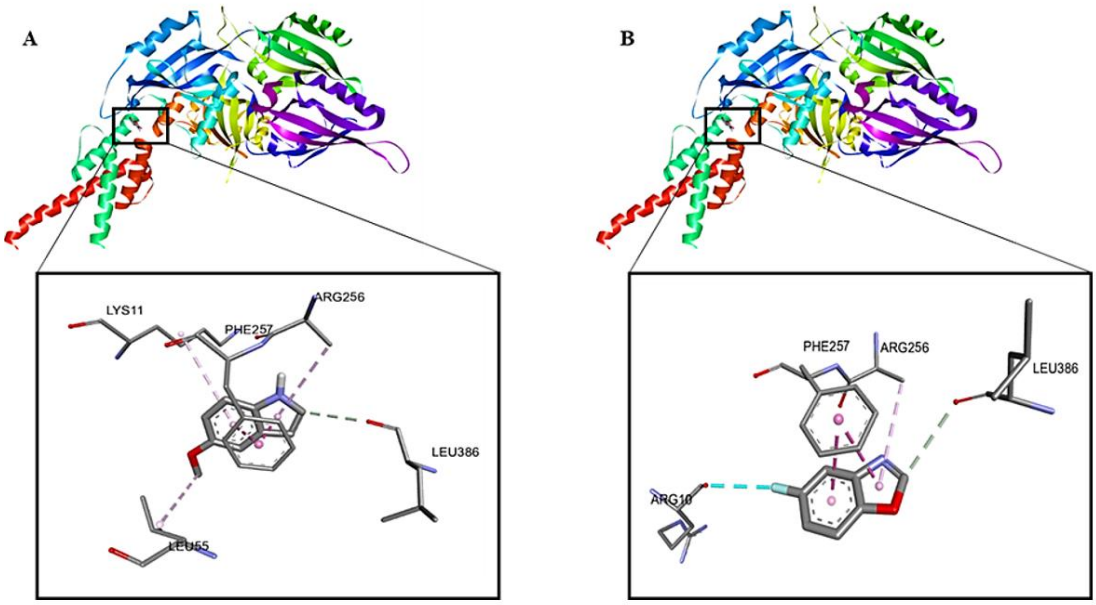


Figure 8: The 3D presentation of three-dimensional interactions within the CRY1 binding site between the reference compound, 5-methoxyindole (A), and 5-fluoro-2-benzoxazolone (B).

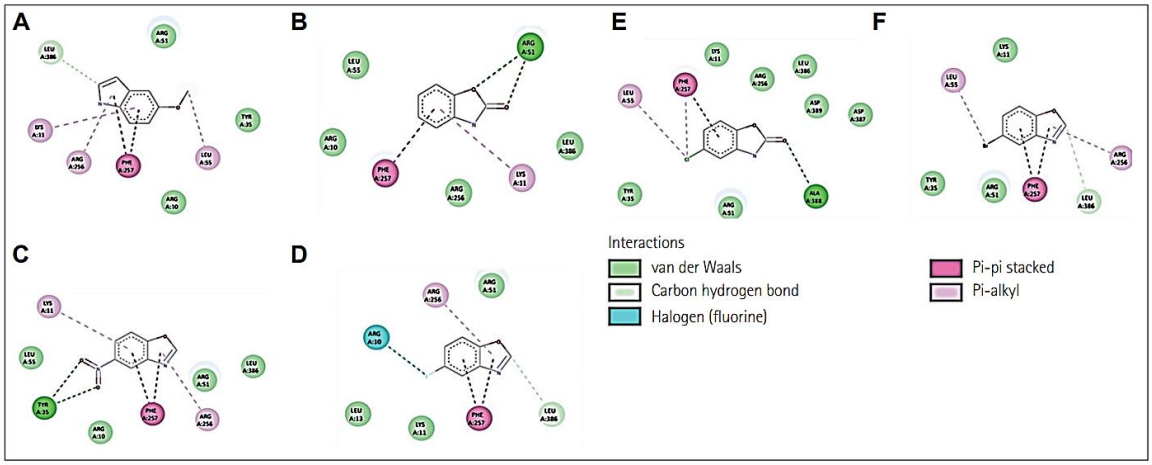


Figure 9: The two-dimensional interaction profiles of ligands within the CRY1 binding pocket reveal diverse interaction types, including hydrogen bonds, pi-pi T-shaped bonds, pi-anion/cation bonds, alkyl/pi-alkyl interactions, and halogen bonds. Ligands analyzed include 5-methoxyindole (A), 2(3H)-benzoxazolone (B), and its 5-substituted derivatives: 5-nitro- (C), 5-fluoro- (D), 5-chloro- (E), and 5-bromo-2-benzoxazolone (F).

This research investigated the binding interactions of all evaluated ligands, including the reference compound 5-methoxyindole, with key amino acid residues within CLOCK: BMAL1 and PER protein complexes. Key residues, including tyrosine, proline, lysine, leucine, serine, glutamate, and arginine, were consistently engaged in ligand-receptor interactions within these systems.

The computational findings revealed that the 2-benzoxazolone scaffold indicated binding energies and interaction patterns comparable to the control compound. Notably, the introduction of substituents, such as -NO_2, and halogens at the 5th position of the benzoxazolone ring significantly improved the interaction dynamics due to their electron-withdrawing properties. These substitutions not only enhanced the binding affinities but also resulted in distinct interaction profiles with specific residues across the circadian clock protein complexes. The resilience of all complexes was thoroughly evaluated through 100 ns molecular dynamics simulations. Across all tested complexes, minimal fluctuations were observed during the equilibration phase, and stable configurations were maintained in the final 30 ns of the simulation period. This stability underscores the robustness of the interactions

between the ligands and their target proteins, reinforcing the validity of the computational predictions.

The circadian clock, a complex and essential biological timing system, orchestrates physiological and behavioral processes to align with the 24-hour day-night cycle. Central to this mechanism are the CLOCK and BMAL1 (ARNTL) genes, which encode proteins characterized by basic helix-loop-helix PER-ARNT-SIM (bHLH-PAS) domains (Fribourgh & Partch, 2017). The interaction between these proteins forms the CLOCK:BMAL1 heterodimer, a key driver of circadian gene expression. In the promoter domains of all Per and Cry proteins including, this complex attaches to E-box regulatory sequences, starting RNA production throughout the day (Takahashi et al., 2008).

As PER and CRY proteins accumulate throughout the day, they translocate to the nucleus, where they inhibit the activity of the CLOCK: BMAL1 complex. This negative feedback loop, occurring during the night, ensures the precise regulation of circadian rhythms and maintains the cycle's continuity (Takahashi et al., 2008). The synchronized interplay between these genes and proteins orchestrates various physiological functions, including hormone secretion, metabolic regulation, and behavioral rhythms, underscoring the critical importance of circadian homeostasis in overall health.

This study’s findings highlight the potential of benzoxazolone derivatives as promising candidates for modulating circadian clock mechanisms, offering insights into their therapeutic potential in circadian-related disorders. The enhanced binding affinities and stable interactions observed with specific derivatives provide a strong foundation for future experimental investigations, with the ultimate goal of developing innovative chronopharmacological strategies.

Therapeutic approaches targeting the circadian clock have the potential to address circadian rhythm-related disorders, including pain. Studies have shown that pain sensitivity varies throughout the day, peaking at night, with approximately 80% of these fluctuations regulated by the circadian system (Daguet et al., 2022). Master clock genes have been found to interact with pain-modulating genes, such as ADAM11, CACNA1B, and CALCA, highlighting the role of circadian regulation in

pain perception (Chu et al., 2023). Additionally, melatonin, a natural circadian regulator with structural similarities to indomethacin, has demonstrated anti-inflammatory and antinociceptive properties, offering a safer alternative to traditional pain medications (Chen et al., 2016; Rozen, 2015).

The identification of pharmacophores analogous to the 5-methoxyindole core, the structural foundation of melatonin and indomethacin, represents a pivotal step in designing innovative therapeutic strategies that target circadian rhythms. In this study, pharmacophore mapping analyses underscored benzoxazolone derivatives as promising candidates for modulating clock gene activity. These findings align with prior research that has established the 2(3H)-benzoxazolone core as a versatile pharmacophore with significant analgesic and anti-inflammatory potential (Ucar et al., 1997; Tang et al., 2021).

Docking studies and molecular dynamics simulations revealed notable improvements in binding efficiency for certain benzoxazolone derivatives. Specifically, compounds with electron-withdrawing substituents at the 5th position displayed significantly increased binding energies than the reference compound, 5-methoxy indole. These derivatives formed robust interactions with essential circadian proteins, including CLOCK: BMAL1 and CRY1/CRY2, highlighting their enhanced performance.

The enhanced binding affinities observed for these derivatives may be attributed to their ability to form additional hydrogen bonds, pi-interactions, and electrostatic bonds with critical amino acid residues within the active sites of clock proteins. For instance, the nitro group in 5-nitro-2-benzoxazolone facilitated interactions with residues involved in stabilizing ligand-protein complexes, while the fluorine substituent in 5-fluoro-2-benzoxazolone enhanced polar interactions and contributed to the formation of halogen bonds.

These findings position benzoxazolone derivatives as promising molecular tools for influencing circadian rhythms. By modulating clock gene expression through their interactions with core circadian proteins, these compounds hold potential for therapeutic applications in circadian rhythm-related disorders. The superior binding profiles of 5-nitro- and 5-fluoro-2-benzoxazolone further highlight their suitability for experimental validation in future chronopharmacological studies.

This study reinforces the pharmacological significance of benzoxazolone derivatives and provides a foundational framework for their application in the development of circadian modulators. Through targeted molecular design and further experimental investigation, these derivatives could contribute to innovative treatments addressing disruptions in circadian regulation and associated pathologies.

Structural analysis of clock proteins, such as the CLOCK: BMAL1 transcriptional activator complex, and their interactions with ligands provided insights into the molecular basis of binding (Huang et al., 2012). This study focused on the function of residual amino acids including methionine, arginine, leucine, and phenylalanine in ligand binding. The nitro and fluorine substituents of the benzoxazolone ring were shown to strengthen binding through interactions like hydrogen bonds, pi-anion, pi-cation, and halogen bonding. These characteristics played a pivotal role in the enhanced affinities exhibited by the 5-nitro and 5-fluoro derivatives of benzoxazolone.

The role of cryptochromes in suppressing the activity of CLOCK dimer during the circadian cycle further underscores their potential as therapeutic targets (Ye et al., 2014). Melatonin's ability to regulate clock gene expression has been well-documented, and this study suggests that benzoxazolone derivatives with structural similarities to melatonin may exert similar regulatory effects on CRY proteins, thereby influencing circadian rhythms and pain modulation (Brzezinski et al., 2021).

Comparative analyses of binding affinities across all ligand-protein complexes demonstrated that 5-fluoro- and 5-nitro-substituted benzoxazolone derivatives consistently exhibited superior binding energies compared to 5-methoxy indole, with statistically significant differences. These findings position these derivatives as promising candidates for the development of circadian rhythm modulators and pain management therapies.

While computational methods provide valuable insights, the limitations of in silico analyses, such as the inability to replicate complex in vivo systems, should be addressed through experimental validation. Further research should broaden the range of ligands and their targets investigated to develop a deeper understanding of the curative possibilities offered by benzoxazolones. Nevertheless, this study

establishes a foundation for exploring the dual role of these compounds in regulating circadian rhythms and modulating pain perception.

4.2. Second Subsection

4.2.1. The Role of NR1D1 (RevErbα) Clock Protein and Melatonin in Amyotrophic Lateral Sclerosis Chronotherapy

ALS is a progressive neurodegenerative disorder marked by the gradual loss of motor neuron functionality, leading to symptoms such as muscle weakness, cramps, rigidity, dysphagia, and speech impairments (Bali and Miller, 2013; Le Gall et al., 2020). While 90–95% of ALS cases are sporadic, a smaller proportion (5–10%) arises from autosomal dominant inheritance, with a higher prevalence among males (Chen et al., 2013). Several pathological mechanisms are implicated in ALS, including oxidative stress, mitochondrial dysfunction, disrupted axonal signaling, RNA processing abnormalities, and glutamate excitotoxicity (Le Gall et al., 2020). Excessive glutamate levels contribute to excitotoxicity, damaging motor neurons and triggering neuroinflammation, a hallmark of ALS pathology.

Circadian rhythms, regulated by an internal clock system, influence numerous physiological and behavioral functions. These rhythms are governed by the primary clock in the suprachiasmatic nucleus, or SCN, and regional oscillators of other tissues (Meléndez-Fernández et al., 2023). Clock genes, including NR1D1 (RevErbα), regulate circadian cycles through the transcription of genes. NR1D1, in particular, is a master repressor of genes linked to metabolic and inflammatory processes, with studies demonstrating its involvement in glial activation and neuroinflammation in ALS models (Pourcet et al., 2018; Killoy et al., 2021). This highlights NR1D1's significance in linking circadian rhythms to neuroinflammatory processes.

Melatonin, a hormone that peaks during nighttime, regulates circadian rhythms and exhibits antioxidative and anti-inflammatory properties (Serin and Tek, 2019; Tarocco et al., 2019). Previous studies suggest that melatonin may reduce glutamate excitotoxicity and neuroinflammation, potentially offering neuroprotective benefits in ALS (Wolff et al., 2020). This research focuses on assessing the interactions of melatonin and existing therapies for ALS with NR1D1, advocating for a synergistic chronotherapy strategy to improve therapeutic outcomes.

Docking analysis showed that the binding free energies (ΔG) of ligands interacting with the NR1D1 DNA-binding domain ranged between -6.0 kcal/mol and -8.5 kcal/mol. For comparison, the positive control compounds STL1267 and SR9011 displayed ΔG values of -7.7 kcal/mol and -7.1 kcal/mol, respectively. Melatonin, quinidine, and edaravone demonstrated higher affinities (-8.5, -8.0, and -7.5 kcal/mol) compared to the controls, with melatonin showing the strongest binding affinity among all tested ligands. Notably, nucleic acid chains DT630, DG614, and DG629 consistently interacted with high-affinity ligands, including melatonin and quinidine. The amino acid residue PHE73 was a common interaction site for most ligands, except riluzole and sodium phenylbutyrate. These results indicate the potential of melatonin to modulate NR1D1 activity and its promise as a component of ALS chronotherapy.

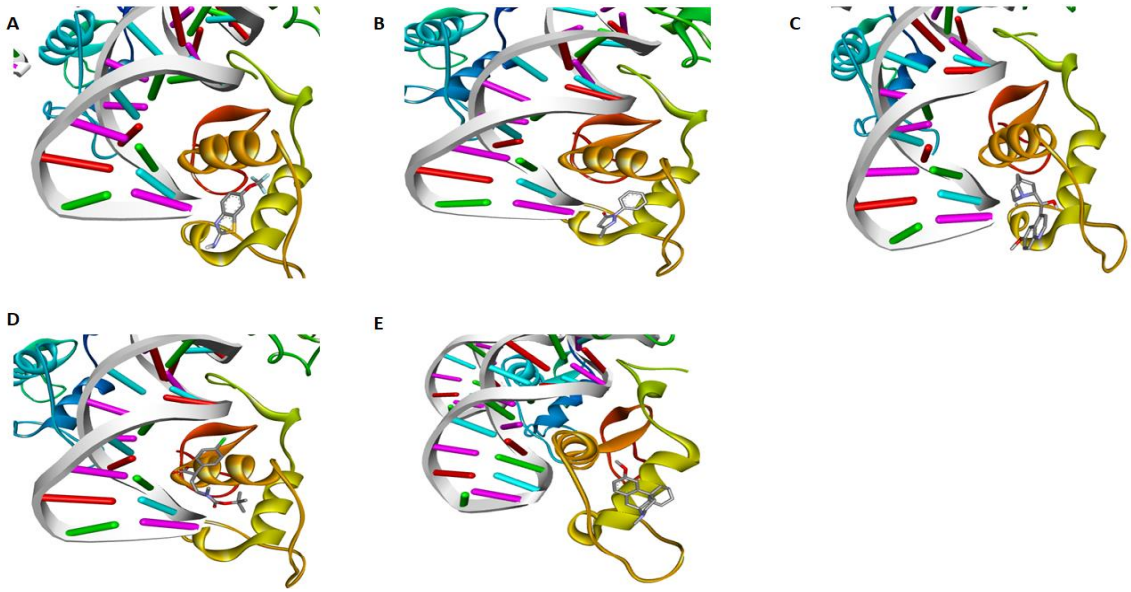


Figure 10: The interactions of ligands with amino acid residues and nucleic acid chains in the NR1D1 binding site: (A) Riluzole, (B) Edaravone, (C) Quinidine, (D) Sodium phenylbutyrate, (E) Dextromethorphan.

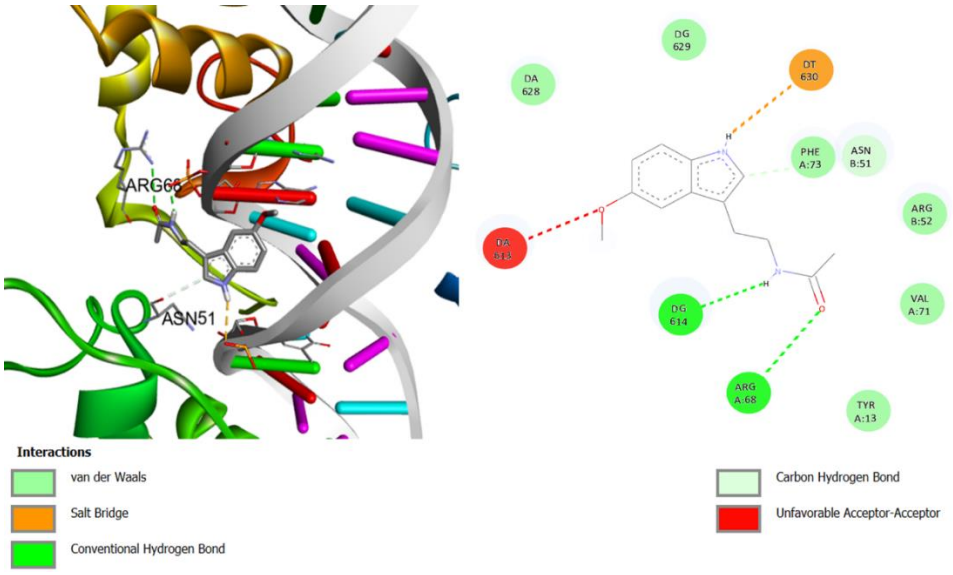


Figure 11: The interactions of melatonin with NR1D1 binding site residues.

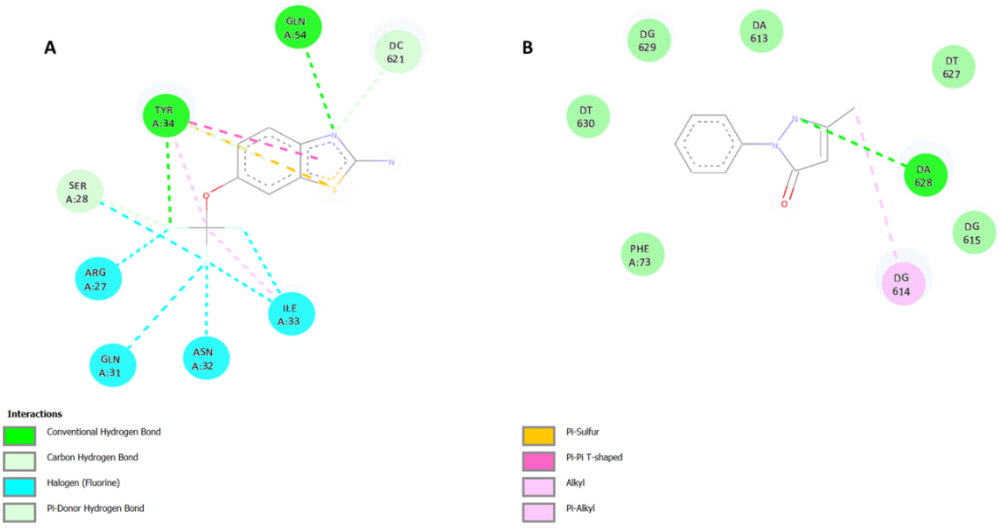


Figure 12: 2D interactions of NR1D1-ligand complexes: (A) NR1D1-Riluzole, (B) NR1D1-Edaravone.

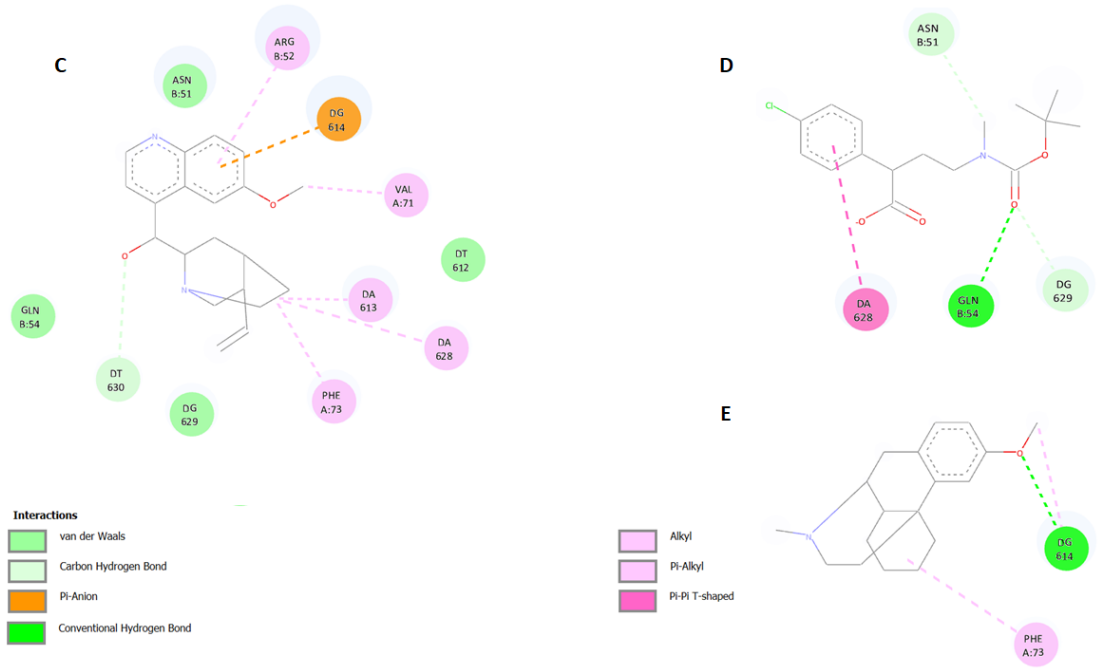


Figure 13: The interactions of NR1D1-ligand complexes: (C) Quinidine, (D) Sodium phenylbutyrate, (E) Dextromethorphan.

Edaravone demonstrated poorer London forces with PHE73 than the other ligands. In contrast, dextromethorphan and quinidine have greater electrostatic contact with PHE73 due to their heterocyclic moieties. Riluzole's binding to NR1D1 has a novel interaction profile, involving the GLN54 residue shared by the reference drug SR9011 but deviating greatly from other ligands. Quinidine demonstrated interaction contacts with nucleic acid chains DT612, DG614, and DA613, as well as DT630, DG629, and DA628, and its quinualidine group allowed for particular electrostatic interactions with residues ASN51, ARG52, GLN54, VAL71, and PHE73. These interactions comprised alkyl and pi-alkyl linkages, which contributed to the high interaction energies. Furthermore, quinidine and DG614 need a pi-anion interaction to stabilize the ligand-protein complex.

Melatonin created a hydrogen bond between its N-acetyl group and ARG68 in the protein binding site, which likely increased the protein-ligand binding energy. This discovery highlights the importance of ARG68 in the interaction process. Melatonin and quinidine were the most comparable amino acids and nucleic acid chains to the control ligands SR9011 and STL1267, as shown in **Table 1.**

Table 1: The binding energies of drugs which engage with the DNA-binding site of NR1D1 (PDB Entry: 1GA5) highlight their relative affinities, with more negative values indicating stronger interactions.

Name of Drug Molecules	**Binding Energy, ΔG (kcal/mol)**	**Interacting Residues**
Edaravone	-7.5	Nucleic Acid Chains: DT630, DG629, DA628, DT627, DG615, DA613 Amino Acid Residues: PHE73
Dextromethorphan	-6.0	Nucleic Acid Chains: DG614 Amino Acid Residues: PHE73
Sodium phenylbutyrate	-6.6	Nucleic Acid Chains: DG629, DA628 Amino Acid Residues: ASN51, GLN54
Riluzole	-6.5	Nucleic Acid Chains: DC621 Amino Acid Residues: ARG27, SER28, GLN31, ASN32, ILE33, TYR34, GLN54
Quinidine	-8.0	Nucleic Acid Chains: DT630, DG629, DA628, DG614, DA613, DT612 Amino Acid Residues: ASN51, ARG52, GLN54, VAL71, PHE73
STL1267	-7.7	Nucleic Acid Chains: DT630, DG614, DA613, DA628, DA636 Amino Acid Residues: ARG52, CYS53, ARG68, VAL71, PHE73
Melatonin	-8.5	Nucleic Acid Chains: DT630, DG629, DG614, DA613, DA628 Amino Acid Residues: TYR13, ASN51, ARG52, ARG68, VAL71, PHE73
SR9011	-7.1	Nucleic Acid Chains: DT630, DG614, DG629, DA613, DA628 Amino Acid Residues: ASN51, GLN54, ARG68, VAL71, PHE73

Melatonin had the lowest binding energy in docking tests and the best stability in MM/PBSA calculations, with a value of -211.28 ± 3.32 kJ/mol^{-1}. Aside from melatonin, edaravone and quinidine demonstrated notably high binding energies of -184.72 ± 3.27 kJ/mol^{-1} and -182.02 ± 3.45 kJ/mol^{-1}, respectively. Among the reference ligands, SR9011 exhibited a binding energy of -176.50 ± 2.25 kJ/mol^{-1}, while STL1267 showed a value of -181.65 ± 2.50 kJ/mol^{-1}. Melatonin, edaravone, and quinidine exhibited greater binding energies at the NR1D1 binding site than the positive controls, whereas the remaining medicines had lower energies, owing to the MM/PBSA results. **Table 2** presents the comprehensive findings from MM/PBSA analysis based on MD simulations.

Table 2: The binding energy values of drug complexes, determined through the MM/PBSA method, are reported as averages with corresponding standard deviations.

Name of Drugs	**Binding Energy (kJ mol^{-1})**
Edaravone	-184.72± 3.27
Dextromethorphan	-153.48± 4.19
Sodium phenylbutyrate	-114.72± 2.56
Riluzole	-172.32± 4.22
Quinidine	-182.02± 3.45
SR9011	-176.50± 2.25
Melatonin	-211.28± 3.32
STL1267	-181.65± 2.50

Melatonin maintained consistent stability in its interactions with the target protein over the 100 ns simulation period. **(Figure 14).**

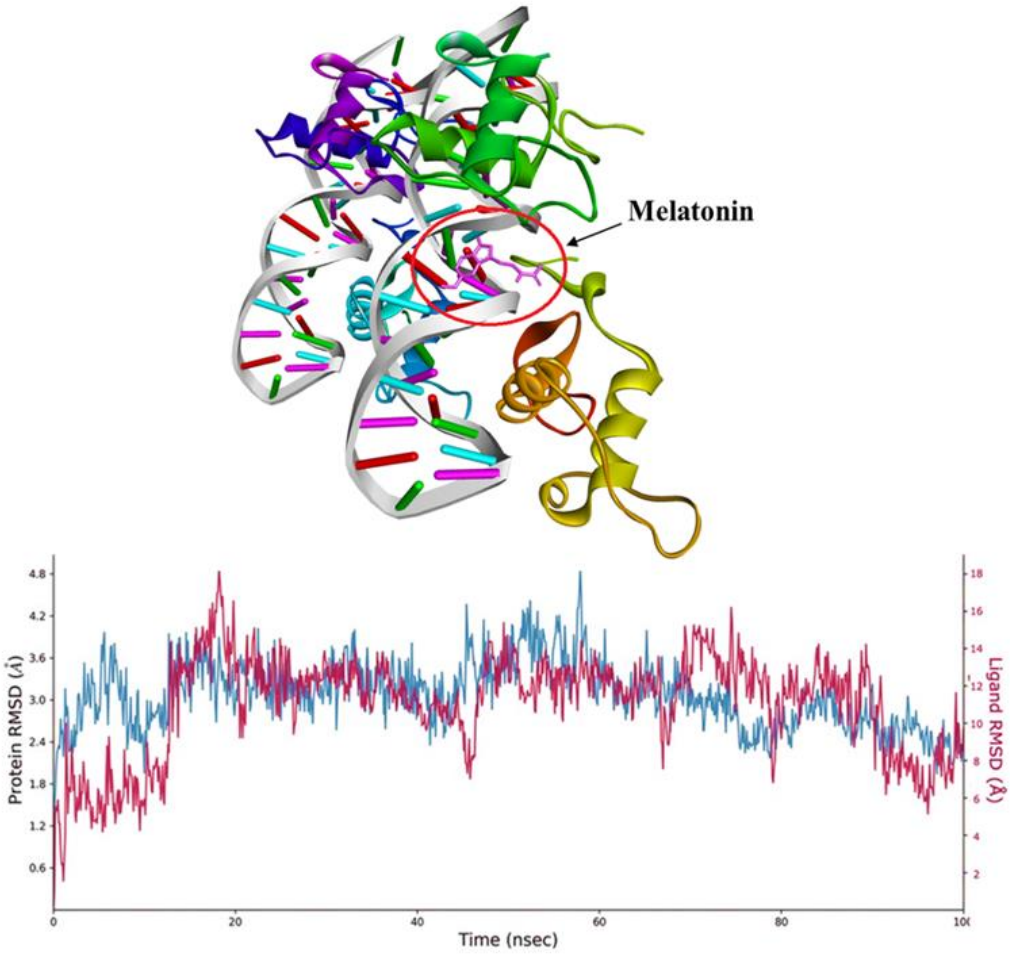


Figure 14: RMSD plot of the NR1D1-melatonin complex over 100 ns MD simulation (melatonin in pink). Initial fluctuations (0–20 ns) reflect ligand adaptation, followed by a stable complex.

Studies have explored various mechanisms to alleviate ALS symptoms, primarily by targeting glutamate excitotoxicity and oxidative stress (Soares et al., 2023; Verma et al., 2022). Excessive glutamate levels lead to calcium dysregulation, mitochondrial dysfunction, and neuronal death, while oxidative stress contributes to neurodegeneration. FDA-approved drugs for ALS aim to mitigate these effects but often fail to deliver optimal therapeutic outcomes, prompting the investigation of combination therapies (Neupane et al., 2023).

NR1D1, a clock gene critical for circadian regulation, metabolism, and neuroinflammation, has shown potential as a therapeutic target in neurodegenerative diseases, including ALS (Killoy et al., 2021). As reported recently, SR9009 reduces proinflammatory signaling and NLRP3 inflammasome activation, supporting its role in managing ALS-associated neuroinflammation (Kou et al., 2022).

This study highlights the superior binding affinity of melatonin to the NR1D1 active site, facilitated by interactions such as hydrogen bonding with ARG68 and direct bridging with the DT630 nucleic acid chain. MD simulations confirmed melatonin's stability within the binding site over 100 ns, despite initial fluctuations during the adaptation phase. These findings suggest that melatonin enhances the activity of FDA-approved ALS drugs by improving their interaction with NR1D1.

The study underscores the therapeutic potential of NR1D1-mediated interventions in ALS, with melatonin emerging as a promising modulator. While in silico results provide valuable insights, future in vitro and in vivo studies are essential to validate these findings and optimize chronotherapy strategies. Additionally, combination therapies targeting glutamate excitotoxicity and oxidative stress, complemented by melatonin's regulatory effects, may offer enhanced efficacy in ALS treatment.

4.3 Third Subsection

4.3.1. Evaluation of FDA-Approved Drugs in Modulating BMAL1 Expression for Alzheimer’s Disease

AD is a progressive neurodegenerative disorder characterized by beta-amyloid plaques, tau protein abnormalities, and widespread neuronal degeneration (Rajmohan and Reddy, 2017). Symptoms typically begin with memory loss and eventually lead to cognitive impairment, language difficulties, and behavioral changes. Although current treatments aim to alleviate symptoms, no cure exists, prompting ongoing research into alternative therapies targeting neurotransmitter systems, inflammation, oxidative stress, and neuronal connectivity (Yiannopoulou and Papageorgiou, 2020).

Emerging evidence highlights the critical role of circadian rhythm disruptions in neurodegenerative diseases like AD. This internal clock system, governed by the SCN, regulates 24-hour cycles of physiological and behavioral processes, including sleep-wake cycles, hormone release, and metabolism (Ayyar and Sukumaran, 2021). Dysregulation of circadian rhythms has been associated with inflammation, oxidative stress, and mitochondrial dysfunction, accelerating neurodegeneration (Lananna and Musiek, 2020; Canever et al., 2023).

BMAL1, a core circadian clock gene, plays a pivotal role in maintaining rhythmicity and regulating critical biological functions. Dysfunctional BMAL1 expression has been linked to neurodegenerative diseases, including AD, by contributing to the accumulation of toxic proteins, inflammation, and cellular damage (Fan et al., 2022). As a transcription factor, BMAL1 forms a heterodimeric complex with CLOCK to regulate target gene expression and maintain circadian rhythms (Zhang et al., 2022). Given its regulatory role in metabolic, inflammatory, and redox processes, BMAL1 is a promising therapeutic target for AD.

Melatonin, a circadian rhythm-regulating hormone, has shown potential in AD treatment due to its antioxidant properties and ability to modulate beta-amyloid deposition (Poeggeler et al., 2001; Lin et al., 2013). However, current FDA-approved AD treatments, such as cholinesterase inhibitors and NMDA receptor antagonists, may disrupt circadian rhythms and require combination strategies involving melatonin (Grossberg, 2003). This study explores the interactions between melatonin, FDA-approved drugs, and BMAL1 to evaluate their potential chronotherapeutic applications in AD.

All ligands demonstrated varying degrees of interaction with BMAL1, with melatonin exhibiting the most favorable binding energy. This was attributed to its ability to form hydrogen bonds with key residues such as ARG68 and interactions with the DT630 nucleic acid chain. CLK8, the positive control, also displayed strong binding, validating the docking approach. FDA-approved drugs like Donepezil and Rivastigmine showed moderate binding affinities, while Lecanemab exhibited distinct interactions due to its monoclonal antibody structure.

MD simulations confirmed the stability of all ligand-protein complexes, with melatonin and CLK8 showing consistent interactions throughout the 100 ns simulation period. MM/PBSA calculations highlighted melatonin’s superior binding free energy, supporting its potential to regulate BMAL1 expression effectively.

This study suggests that melatonin can complement FDA-approved AD drugs by restoring circadian rhythm through BMAL1 modulation. Its antioxidant properties and ability to interact with critical residues further enhance its therapeutic potential. Combining melatonin with drugs targeting cholinergic and glutamatergic systems could mitigate side effects like insomnia and optimize AD treatment outcomes.

The binding affinity and energy values of all medications indicated for AD treatment were analyzed. The results are provided in **Tables 3-4**.

Table 3. The energy values for ligand binding were assessed using the crystal configuration of the binding region within the BMAL1 dimer.

Name of Drugs	**Binding Energies, ΔG (kcal/mol)**
Melatonin	-7.0
Tacrine	-6.2
Galantamine	-6.8
Rivastigmine	-6.4
Memantine	-5.3
Donepezil	-6.6
CLK8 (Referans)	-6.9

Table 4. The binding energies of tested molecules in the binding pocket of the BMAL1 dimer are presented as mean values with their corresponding standard deviations.

Name of Drugs	**Binding Energies (kJ mol^{-1})**
Melatonin	-191.13± 2.28
Tacrine	-156.82± 2.56
Galantamine	-185.50± 2.25
Rivastigmine	-164.72± 3.17
Memantine	-112.41± 3.27
Donepezil	-178.29± 3.24
CLK8 (Referans)	-187.16± 2.45

According to Table 4, memantine exhibited the weakest interaction. Melatonin, galantamine, and donepezil were the drugs with the largest negative binding affinity

and the strongest interaction with BMAL1. Binding affinities for all investigated medicines varied from -5.3 to -7.0 kcal/mol. The MM/PBSA study supported the docking findings, confirming the superior activity of melatonin against all tested medications and the standard, CLK8.

Both two-dimensional and three-dimensional representations of the types of bonding between BMAL1 and its residues in the binding site were used (**Figures 15–22).** Prominent residues that participated in a variety of bonding types, including pi-cation and pi-anion interactions, conventional hydrogen bonds, carbon-hydrogen bonds, and pi-alkyl and alkyl interactions, were proline, arginine, serine, aspartic acid, and glutamic acid. These results highlight the intricate nature of the interactions between BMAL1 and its ligands.

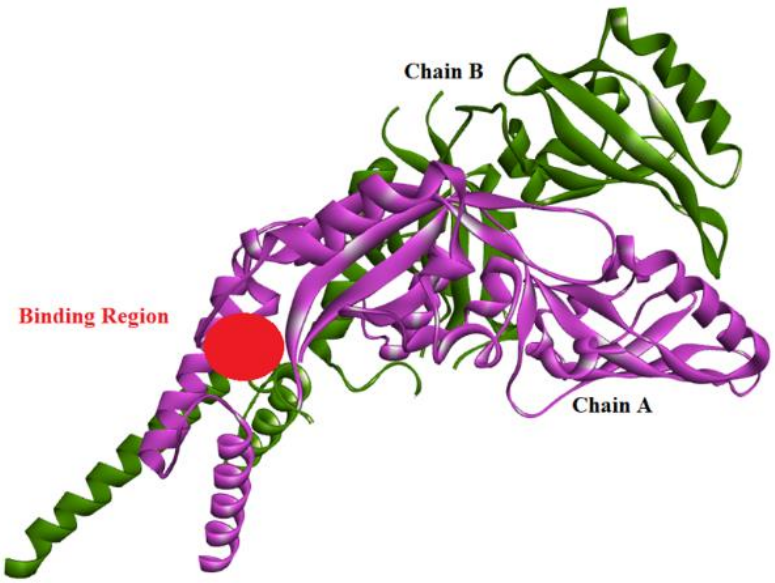


Figure 15: The 3D helical structure of BMAL1 is depicted. Chain A is represented in purple, while Chain B is shown in green. The identified binding site of the complex is marked with a red circle.

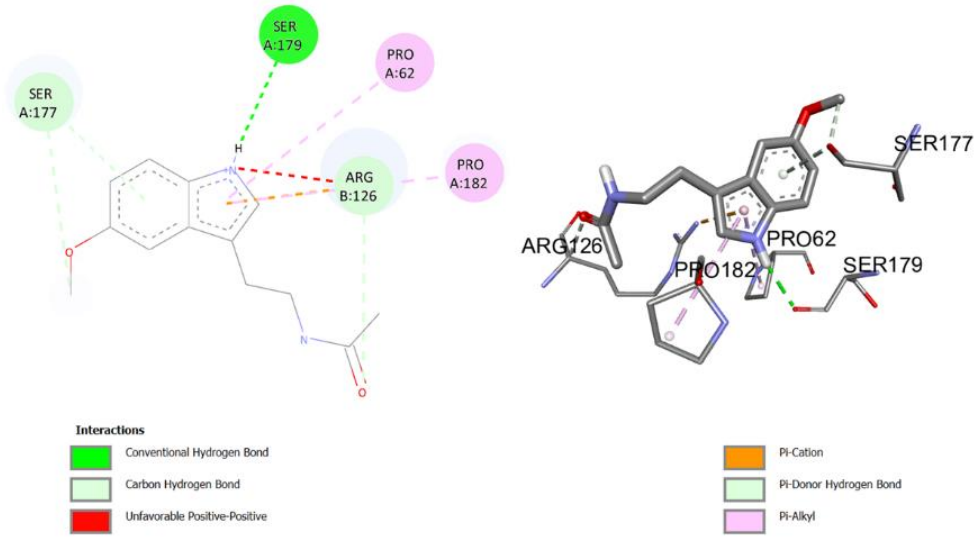


Figure 16: The interactions of melatonin in the CLOCK: BMAL1 active pocket are illustrated in both 2D (left) and 3D (right) formats. Each interaction type is color-coded for clarity.

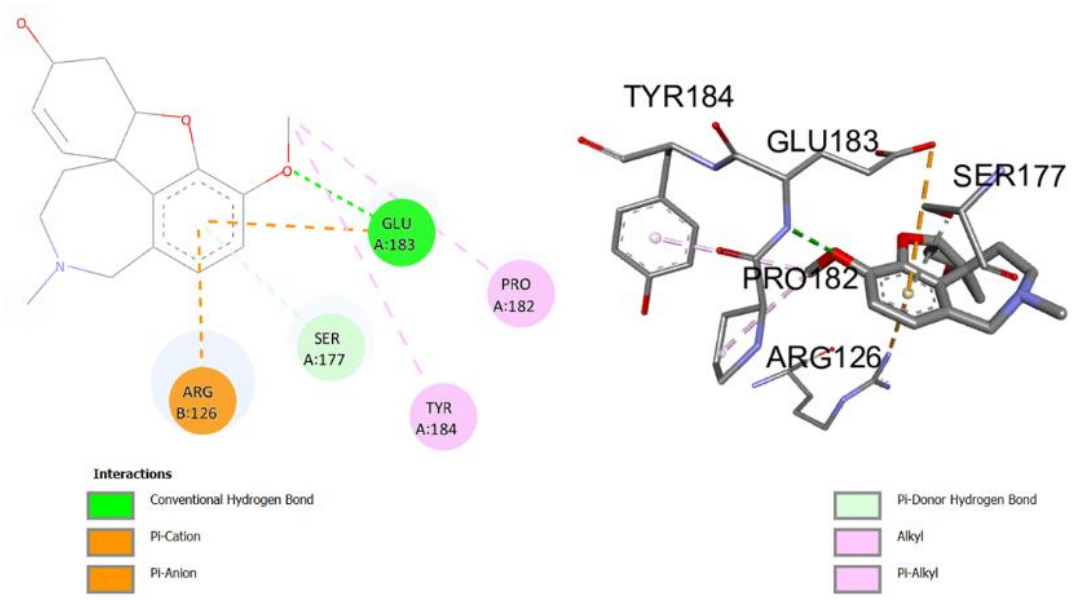


Figure 17: Visualization of bonding types for galantamine within the CLOCK: BMAL1 active pocket in 2D (left) and 3D (right) representations. Each interaction type is color-coded for clarity.

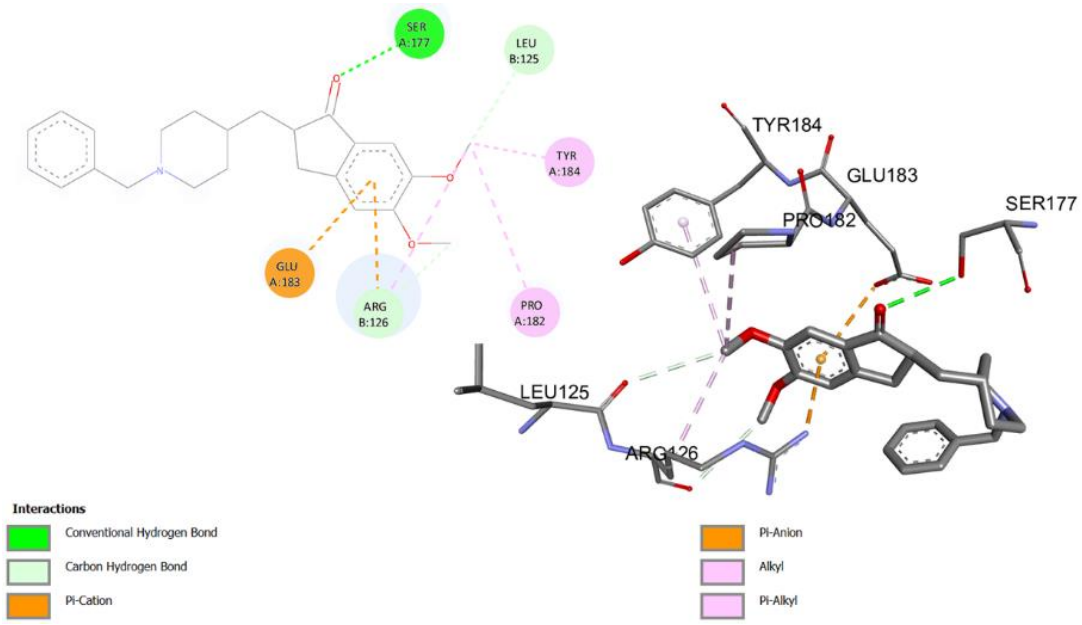


Figure 18: The 2D (left) and 3D (right) structures of donepezil depicted in the binding region of CLOCK: BMALI dimer. The interaction types are distinctly color-coded for clarity in both visualizations.

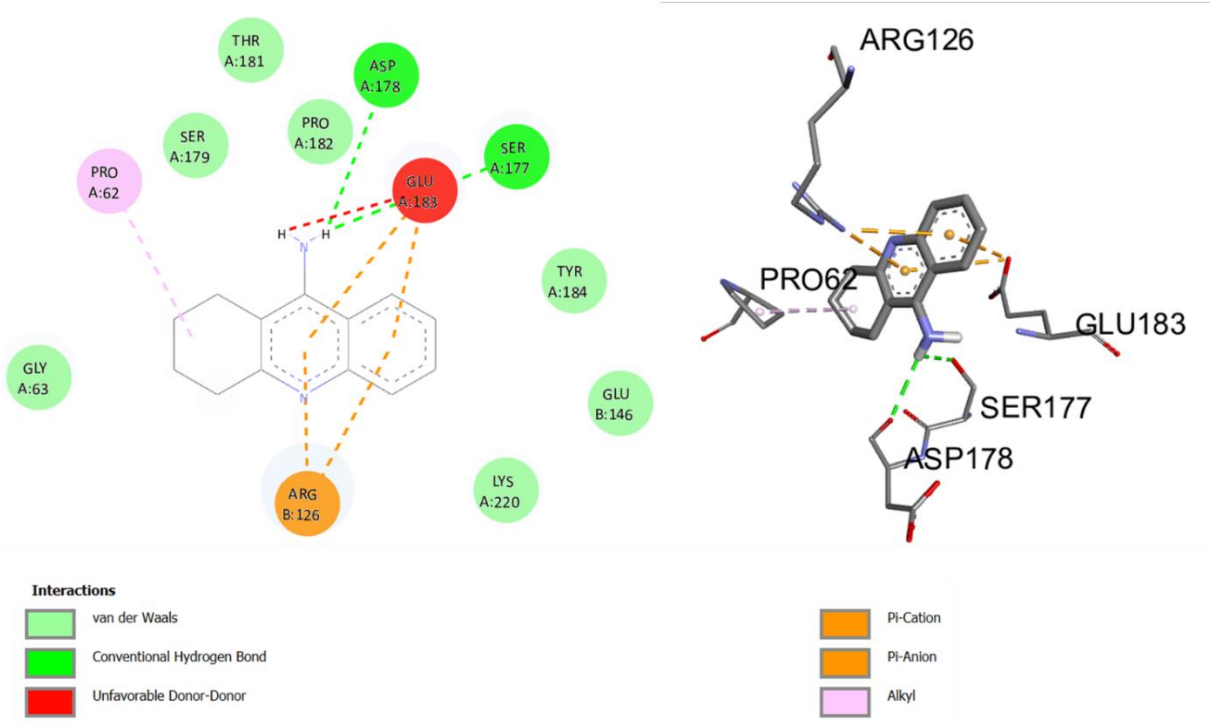


Figure 19: Interaction map and 3D visualization of the CLOCK: BMAL1 dimer with memantine. The left panel illustrates the 2D interaction diagram, highlighting key residues involved in hydrogen bonding, π-cation, π-anion, and van der Waals interactions.

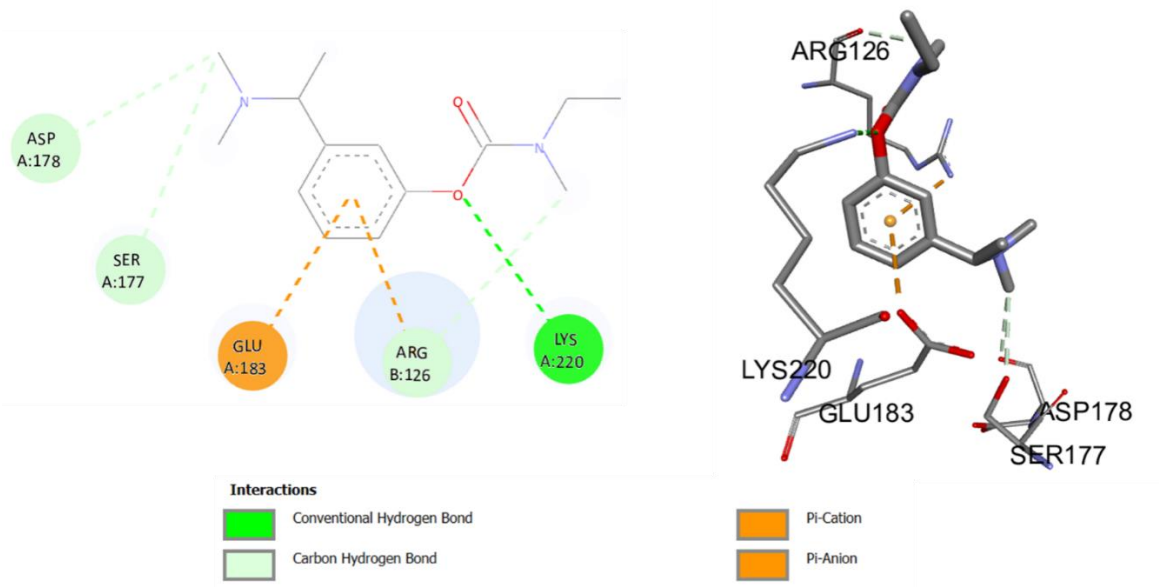


Figure 20: The interactions between rivastigmine and the CLOCK: BMAL1 complex are depicted in both 2D (left) and 3D (right) formats. In these illustrations, dark green represents conventional hydrogen bonding, whereas light green highlights C-H bonding. Additionally, orange indicates pi-anion and pi-cation interactions.

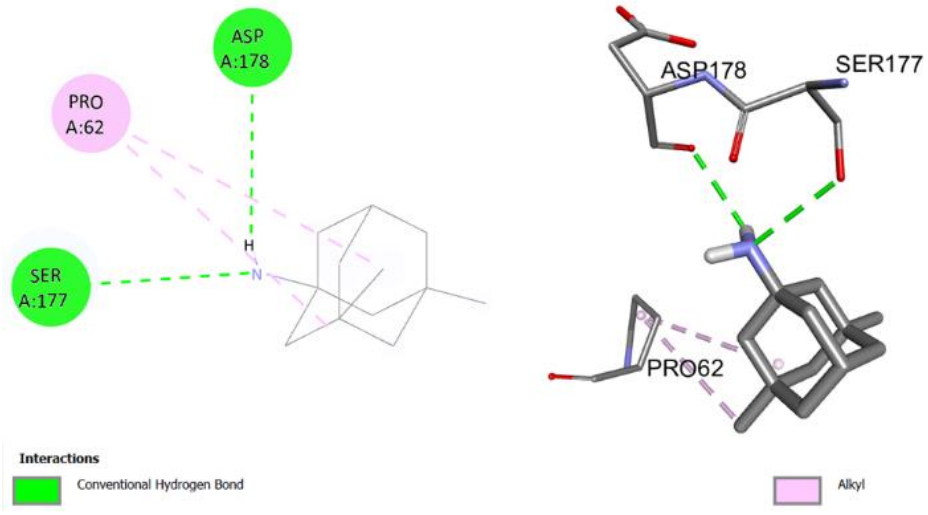


Figure 21: The bonding types in the CLOCK: BMAL1 complex with memantine are illustrated in both 2D (left) and 3D (right) formats.

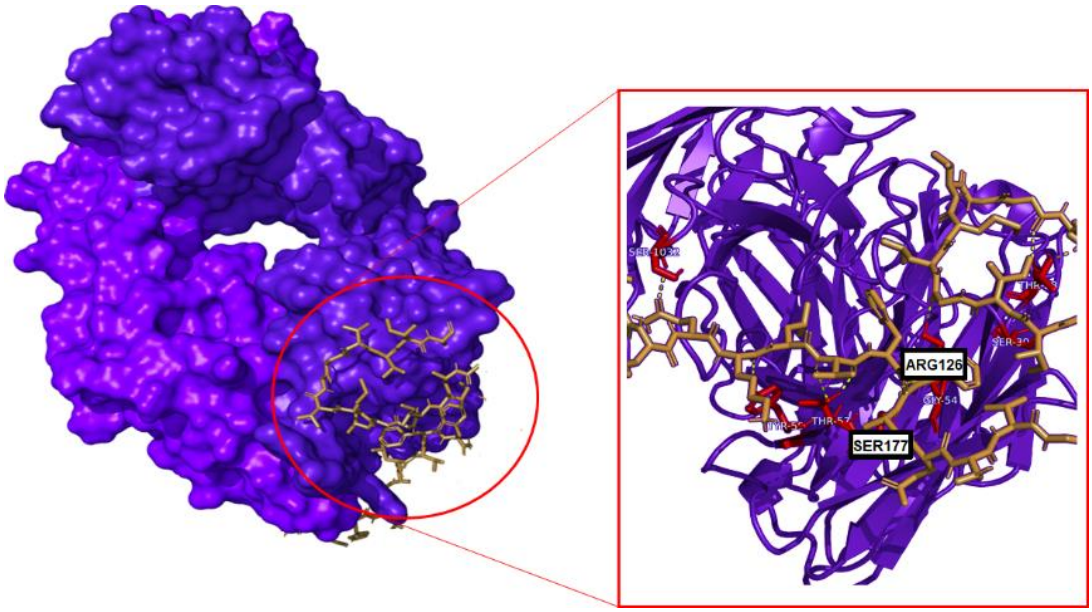


Figure 22: The three-dimensional interaction profile between amino acids in the CLOCK:BMAL1 complex and Lecanemab is illustrated. The CLOCK: BMAL1 dimer is shown in purple, with red highlighting the amino acids involved in the dimer's binding site. Brown indicates the specific residues that interact with Lecanemab.

The structural characteristics of melatonin, particularly its indole amine core, significantly contributed to its binding efficiency with active residues in the CLOCK: BMAL1 complex. Melatonin formed a range of interactions, including conventional hydrogen bonds, carbon-hydrogen bonds, and pi interactions, mediated by its aromatic rings and methoxy groups. Its amide functional group was particularly instrumental in hydrogen bonding at the target site, highlighting the crucial role of melatonin's diverse functional groups in stabilizing BMAL1 interactions.

A thorough examination of the interactions showed that melatonin's indole amine structure caused it to create hydrogen bonds with SER179 in the BMAL1 active site. Moreover, the compound's aromatic and amide functional groups facilitated binding with ARG126 in the B Chain of BMAL1 through a combination of cation-π, C-H interactions, and hydrogen bond donor capabilities. Additionally, melatonin's methoxy groups allowed for alkyl connections with PRO62—a characteristic shared

by all examined ligands in the A Chain—and carbon-hydrogen bonding with SER177.

Galantamine showed noteworthy interactions, especially with ARG126 in Chain B, and came in second in binding energy rankings following melatonin. Its methoxy groups facilitated hydrogen bonding with GLU183 and served as hydrogen bond donors for SER177, while the aromatic ring configuration enabled interactions through cation-π and anion-π bonding. Despite the formation of extra alkyl and Pi-alkyl interactions, galantamine's other structural functional groups had no discernible effect on BMAL1 binding.

Similar to melatonin and galantamine, donepezil regularly interacted with important residues like GLU183, SER177, and ARG126. A common stabilizing mechanism within the BMAL1 active site was reflected in these recurring interaction patterns across several pharmacological compounds.

In contrast, memantine demonstrated the weakest binding affinity and interaction profile among the tested ligands. Its secondary amine group formed limited hydrogen bonds with SER177, while alkyl interactions involving its cycloalkane structure and PRO62 were observed. However, these interactions were less extensive, underscoring its comparatively reduced efficacy in stabilizing BMAL1 interactions.

This comprehensive analysis highlights melatonin's superior binding efficiency, driven by its unique structural features, and positions it as a potential candidate for further investigation in BMAL1-targeted chronotherapeutic strategies.

Lecanemab’s interaction with CLOCK: BMAL1 highlighted the significance of serine and arginine residues, achieving a high binding energy. The similarity in active residue interactions across the tested ligands suggests a common binding mechanism within the CLOCK: BMAL1 dimer structure. The shared residues involved in Lecanemab’s interactions are summarized in **Table 5.**

Table 5. Analysis of the binding energy of Lecanemab and CLOCK: BMAL1 dimer, emphasizing shared interacting residues.

Name of mAb	Interacting Residues	Binding Affinity, ΔG (kcal/mol)
Lecanemab	ARG126, SER177	-869.7

Numerous studies have emphasized the reciprocal relationship between beta-amyloid (Aβ) accumulation and decreased BMAL1 function in AD. Research in mouse models has shown that Aβ disrupts BMAL1 expression in the hippocampus, while BMAL1 deficiency accelerates amyloid plaque formation (Kress et al., 2018). BMAL1 ablation, including in the SCN, has been linked to disrupted diurnal variations of Aβ, impaired clearance, and increased plaque accumulation. Enhancing BMAL1 transcription through REV-ERB inhibition has been shown to improve microglial Aβ phagocytosis in animal models of AD (Lee et al., 2020). However, BMAL1 deficiency exacerbates sleep disruptions, inflammation, and neurodegeneration (Schurhoff and Toborek, 2023).

BMAL1 dysfunction also affects tau pathology. Disruptions in circadian rhythm negatively influence tau protein phosphorylation, suggesting a strong correlation between BMAL1 expression and tau-related neurodegeneration (Hulme et al., 2020). Reduced BMAL1 levels are associated with cognitive impairments, reinforcing its critical role in AD pathology.

Astrocytic BMAL1 plays a key role in maintaining neuronal homeostasis. BMAL1-deficient astrocytes exhibit structural abnormalities, impaired synaptic coverage, and altered GABA regulation, contributing to cognitive deficits (Barca-Mayo et al., 2017). In vitro and in vivo studies have revealed that BMAL1 deficiency increases astrogliosis, promotes inflammatory gene activation, and accelerates neurodegeneration, underscoring its importance in astrocyte-neuron communication (Lananna et al., 2018).

Melatonin, a neuroprotective hormone, plays a pivotal role in maintaining circadian rhythms and exhibits anti-amyloidogenic and antioxidant properties. Its decline with aging has been linked to increased oxidative stress and AD pathology (Hossain et al., 2021). Melatonin supplementation has shown potential in mitigating amyloid plaque accumulation, tau hyperphosphorylation, and neurofibrillary tangle formation. By stabilizing circadian rhythms, melatonin alleviates cognitive decline and restlessness, common in AD patients (Musiek et al., 2015).

The interaction between melatonin and BMAL1 provides valuable insights into potential therapeutic strategies. Melatonin's structural features enable it to form strong interactions with BMAL1 binding sites, contributing to its regulatory effects on circadian rhythms. Similarities between the amino acid interactions of melatonin and FDA-approved drugs, including Lecanemab, suggest that these compounds may also modulate BMAL1 expression.

This study is the first to employ in silico techniques to evaluate the effects of Lecanemab and FDA-approved AD medications on BMAL1. The study finds a possible synergistic impact when these medications are combined with melatonin by comparing their binding affinities, providing a unique chronotherapeutic approach for AD. The results demonstrate the significance of BMAL1 in AD pathophysiology and lay the groundwork for upcoming in vivo investigations into the possible therapeutic benefits of BMAL1 expression targeting.

CHAPTER V

Conclusion and Future Directions

This thesis explores the intricate connections between circadian rhythms and various diseases, emphasizing the potential of targeting clock genes for therapeutic purposes. Through a comprehensive evaluation of benzoxazolone derivatives, melatonin, and FDA-approved drugs, the research underscores the importance of circadian rhythm modulation in managing neurodegenerative diseases and pain-related disorders.

The studies presented highlight the promising role of benzoxazolone derivatives in regulating clock proteins like CLOCK, BMAL1, PER, and CRY, opening new avenues for drug design. Similarly, melatonin's interactions with core circadian components demonstrate its dual potential in alleviating inflammation and neurodegeneration, particularly in diseases such as ALS and AD. These findings not only validate the therapeutic potential of targeting circadian genes but also pave the way for chronotherapeutic approaches that align with biological rhythms for optimized efficacy.

The computational methods employed provided significant insights into ligand-protein interactions and their implications for drug discovery. However, these findings require further validation through experimental and clinical studies to translate theoretical predictions into practical applications. Future research should focus on the synergistic effects of combining circadian modulators with conventional treatments, aiming to enhance therapeutic outcomes while minimizing side effects.

In conclusion, the modulation of circadian rhythms offers a transformative perspective in tackling complex diseases. By integrating computational techniques with pharmacological advancements, this thesis contributes to the growing body of knowledge in chronopharmacology and underscores the potential for innovation in disease treatment through the lens of biological timing.

REFERENCES

Ahmad, F., Sachdeva, P., Sarkar, J., & Izhaar, R. (2022). Circadian dysfunction and Alzheimer's disease - An updated review. *Aging Medicine (Milton)*, 6, 71-81. https://doi.org/10.1002/agm2.12221

Ahmad, S. B., Ali, A., Bilal, M., Rashid, S. M., Wani, A. B., Bhat, R. R., & Rehman, M. U. (2023). Melatonin and health: Insights of melatonin action, biological functions, and associated disorders. *Cellular and Molecular Neurobiology*, 43, 2437-2458. https://doi.org/10.1007/s10571-023-01324-w

Alexander, R. K., Liou, Y. H., Knudsen, N. H., Starost, K. A., Xu, C., Hyde, A. L., Liu, S., Jacobi, D., Liao, N. S., & Lee, C. H. (2020). Bmal1 integrates mitochondrial metabolism and macrophage activation. *eLife*, 9, e54090. https://doi.org/10.7554/eLife.54090

Alotaibi, M., Aljahany, M., Alhamdan, Z., Alsaffar, M., Almojally, A., & Alassaf, W. (2022). Differences in acute pain perception between patients and physicians in the emergency department. *Heliyon*, 8(e11462).

Al-Waeli, H., Nicolau, B., Stone, L., Abu Nada, L., Gao, Q., Abdallah, M. N., & Tamimi, F. (2020). Chronotherapy of Non-Steroidal Anti-Inflammatory Drugs May Enhance Postoperative Recovery. *Scientific Reports*, 10(468). https://doi.org/10.1038/s41598-019-57318-0

Amanpour, A., Kahraman, S., Çınar, B., & Çelik, F. (2021). Mavi Işık Maruziyetinin Sirkadiyen Ritim ve Beslenme Üzerindeki Etkisi. *Celal Bayar Üniversitesi Sağlık Bilimleri Enstitüsü Dergisi*, *8*(3), 566-573. https://doi.org/10.34087/cbusbed.891351

Andrade, M. K., Souza, L. C., Azevedo, E. M., Bail, E. L., Zanata, S. M., Andreatini, R., & Vital, M. A. B. F. (2023). Melatonin reduces β-amyloid accumulation and improves short-term memory in streptozotocin-induced sporadic Alzheimer's disease model. *IBRO Neuroscience Reports*, 14, 264-272. https://doi.org/10.1016/j.ibneur.2023.01.005

Arendt, J. (2006). Melatonin and human rhythms. *Chronobiology International*, 23(1-2), 21-37.

Artime-Naveda, F., Alves-Pérez, L., Hevia, D., Alcón-Rodríguez, S., Fernández-Vega, S., Alvarez-Artime, A., ... & Sainz, R. M. (2023). A novel study of melatonin diffusion in a 3D cell culture model. *Melatonin Research*, 6(2), 173-188. https://doi.org/10.32794/mr112500116

Ayyar, V. S., & Sukumaran, S. (2021). Circadian rhythms: Influence on physiology, pharmacology, and therapeutic interventions. *Journal of Pharmacokinetics and Pharmacodynamics*, 48, 321-338. https://doi.org/10.1007/s10928-021-09751-2

Bacchi, S., Palumbo, P., Sponta, A., & Coppolino, M. F. (2012). Clinical pharmacology of non-steroidal anti-inflammatory drugs: A review. *Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry*, 11(1), 52-64. https://doi.org/10.2174/187152312800166811

Bae, K., Jin, X., Maywood, E. S., Hastings, M. H., Reppert, S. M., & Weaver, D. R. (2001). Differential functions of mPer1, mPer2, and mPer3 in the SCN circadian clock. *Neuron*, 30(2), 525-536. https://doi.org/10.1016/S0896-6273(01)00292-4

Bali, T., & Miller, T. M. (2013). Management of amyotrophic lateral sclerosis. *Mo Med*, 110, 417-421.

Barca-Mayo, O., Pons-Espinal, M., Follert, P., Armirotti, A., Berdondini, L., & De Pietri Tonelli, D. (2017). Astrocyte deletion of Bmal1 alters daily locomotor activity and cognitive functions via GABA signalling. *Nature Communications*, 8, 14336. https://doi.org/10.1038/ncomms14336

Bass, J., & Takahashi, J. S. (2010). Circadian integration of metabolism and energetics. *Science*, 330(6009), 1349-1354.

Brenke, R., Hall, D. R., Chuang, G. Y., Comeau, S. R., Bohnuud, T., Beglov, D., Schueler-Furman, O., Vajda, S., & Kozakov, D. (2012). Application of asymmetric statistical potentials to antibody-protein docking. *Bioinformatics*, 28, 2608-2614. https://doi.org/10.1093/bioinformatics/bts493

Brzezinski, A., Rai, S., Purohit, A., & Pandi-Perumal, S. R. (2021). Melatonin, clock genes, and mammalian reproduction: what is the link? *International Journal of Molecular Sciences*, 22(24), 13240. https://doi.org/10.3390/ijms222413240

Buhr, E. D., & Takahashi, J. S. (2013). Molecular components of the mammalian circadian clock. *Cold Spring Harbor Perspectives in Biology*, 5(11), a021139.

Bumgarner, J. R., McCray, E. W., & Nelson, R. J. (2023). The disruptive relationship among circadian rhythms, pain, and opioids. *Frontiers in Neuroscience*, 17(1109480). https://doi.org/10.3389/fnins.2023.1109480

Butterfield, D. A., Drake, J., Pocernich, C., & Castegna, A. (2001). Evidence of oxidative damage in Alzheimer's disease brain: Central role for amyloid beta-peptide. *Trends in Molecular Medicine*, 7, 548-554. https://doi.org/10.1016/s1471-4914(01)02173-6

Cajochen, C., Kräuchi, K., & Wirz-Justice, A. (2003). Role of melatonin in the regulation of human circadian rhythms and sleep. *Journal of Neuroendocrinology*, 15(4), 432-437.

Canever, J. B., Queiroz, L. Y., Soares, E. S., & de Avelar, N. C. P., & Cimarosti, H. I. (2023). Circadian rhythm alterations affecting the pathology of neurodegenerative diseases. *Journal of Neurochemistry*, 00, 1-15. https://doi.org/10.1111/jnc.15883

Cao, X., Yang, Y., Selby, C. P., Liu, Z., & Sancar, A. (2021). Molecular mechanism of the repressive phase of the mammalian circadian clock. *Proceedings of the National Academy of Sciences*, 118(22), e2021174118. https://doi.org/10.1073/pnas.2021174118

Chan, M. C., Spieth, P. M., Quinn, K., Parotto, M., Zhang, H., & Slutsky, A. S. (2012). Circadian rhythms: From basic mechanisms to the intensive care unit. *Critical Care Medicine*, 40(1), 246-253. https://doi.org/10.1097/CCM.0b013e31822f0abe

Chen, L., Cruz, A., Roe, D. R., Simmonett, A. C., Wickstrom, L., Deng, N., ... & Case, D. A. (2021). Thermodynamic decomposition of solvation free energies with particle mesh Ewald and long-range Lennard-Jones interactions in grid inhomogeneous solvation theory. *Journal of Chemical Theory and Computation*, 17(5), 2714-2724. https://doi.org/10.1021/acs.jctc.0c01337

Chen, S., Sayana, P., Zhang, X., & Le, W. (2013). Genetics of amyotrophic lateral sclerosis: an update. *Molecular Neurodegeneration*, 8, 28. https://doi.org/10.1186/1750-1326-8-28

Chen, W. W., Zhang, X., & Huang, W. J. (2016). Pain control by melatonin: Physiological and pharmacological effects. *Experimental and Therapeutic Medicine*, 12(3), 1963-1968. https://doi.org/10.3892/etm.2016.3560

Chu, Y., He, H., Liu, Q., Jia, S., Fan, W., & Huang, F. (2023). The Circadian Clocks, Oscillations of Pain-Related Mediators, and Pain. *Cellular and Molecular Neurobiology*, 43(2), 511-523. https://doi.org/10.1007/s10571-022-01285-8

Chu, Y., Wu, Y., Jia, S., Xu, K., Liu, J., Mai, L., Zhao, J., Song, X., Huang, W., Li, Z., Yang, S., & Zhang, M. (2023). Single-nucleus transcriptome analysis reveals transcriptional profiles of circadian clock and pain-related genes in human and mouse trigeminal ganglion. *Frontiers in Neuroscience*, 17, 1176654. https://doi.org/10.3389/fnins.2023.1176654

Comai, S., & Gobbi, G. (2014). Unveiling the role of melatonin MT2 receptors in sleep, anxiety and neuroprotection: a novel target for the pharmacotherapy of CNS disorders. *Pharmacology & Therapeutics*, 141(2), 126-139. doi:10.1016/j.pharmthera.2013.09.002

Comai, S., & Gobbi, G. (2022). Melatonin and its analogs: From neurobiology to clinical applications. *Neuroscience & Biobehavioral Reviews*, 136, 104608. doi:10.1016/j.neubiorev.2022.104608

Cudney, L. E., Sliwinski, M. J., & Almeida, D. M. (2022). Associations of daily pain with nightly sleep in midlife adults: The role of bedtime and wake time. *Journal of Sleep Research*, 31(2), e13491. doi:10.1111/jsr.13491

Daguet, I., Raverot, V., Bouhassira, D., & Gronfier, C. (2022). Circadian rhythmicity of pain sensitivity in humans. *Brain*, 145(11), 3225-3235. https://doi.org/10.1093/brain/awac223

Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, 7(1), 42717. doi:10.1038/srep42717

Devi, L., Prabhu, B. M., Galati, D. F., Avadhani, N. G., & Anandatheerthavarada, H. K. (2006). Accumulation of amyloid precursor protein in the mitochondrial import channels of human Alzheimer's disease brain is associated with mitochondrial dysfunction. *Journal of Neuroscience*, 26, 9057-9068. https://doi.org/10.1523/JNEUROSCI.1469-06.2006

Doruk, Y. U., Yarparvar, D., Akyel, Y. K., Gul, S., Taskin, A. C., Yilmaz, F., Baris, I., Ozturk, N., Türkay, M., Ozturk, N., Okyar, A., & Kavakli, I. H. (2020). A CLOCK-binding small molecule disrupts the interaction between CLOCK and BMAL1 and enhances circadian rhythm amplitude. *Journal of Biological Chemistry*, 295, 3518-3531. https://doi.org/10.1074/jbc.RA119.011332

Dose, B., Yalçin, M., Dries, S. P. M., & Relógio, A. (2023). TimeTeller for timing health: The potential of circadian medicine to improve performance, prevent disease and optimize treatment. *Frontiers in Digital Health*, 5, 1157654. https://doi.org/10.3389/fdgth.2023.1157654

Dundas, J., Ouyang, Z., Tseng, J., Binkowski, A., Turpaz, Y., & Liang, J. (2006). CASTp: Computed atlas of surface topography of proteins with structural and topographical mapping of functionally annotated residues. *Nucleic Acids Research*, 34, W116-118. https://doi.org/10.1093/nar/gkl282

Eberhardt, J., Santos-Martins, D., Tillack, A. F., & Forli, S. (2021). AutoDock Vina 1.2.0: New docking methods, expanded force field, and Python bindings. *Journal of Chemical Information and Modeling*, 61(8), 3891-3898. https://doi.org/10.1021/acs.jcim.1c00203

Erdag, E., Haskologlu, I. C., Mercan, M., Abacioglu, N., & Sehirli, A. O. (2023). An in silico investigation: Can melatonin serve as an adjuvant in NR1D1-linked chronotherapy for amyotrophic lateral sclerosis? *Chronobiology International*, 40, 1395-1403. https://doi.org/10.1080/07420528.2023.2265476

Fagiani, F., Di Marino, D., Romagnoli, A., Travelli, C., Voltan, D., Di Cesare Mannelli, L., Racchi, M., Govoni, S., & Lanni, C. (2022). Molecular regulations of circadian rhythm and implications for physiology and diseases. *Signal Transduction and Targeted Therapy*, 7, 41. https://doi.org/10.1038/s41392-022-00899-y

Fan, R., Peng, X., Xie, L., Dong, K., Ma, D., Xu, W., Shi, X., Zhang, S., Chen, J., & Yu, X. (2022). Importance of Bmal1 in Alzheimer's disease and associated aging-related diseases: Mechanisms and interventions. *Aging Cell*, 21, e13704. https://doi.org/10.1111/acel.13704

Fei, J., Zhou, L., Liu, T., & Tang, X. Y. (2013). Pharmacophore modeling, virtual screening, and molecular docking studies for discovery of novel Akt2 inhibitors. *International Journal of Medical Sciences*, 10(3), 265-275. https://doi.org/10.7150/ijms.5294

Fifel, K., & Videnovic, A. (2021). Circadian and sleep dysfunctions in neurodegenerative disorders—An update. *Frontiers in Neuroscience*, 14, 627330. https://doi.org/10.3389/fnins.2020.627330

Forli, S., Huey, R., Pique, M. E., Sanner, M. F., Goodsell, D. S., & Olson, A. J. (2016). Computational protein-ligand docking and virtual drug screening with the AutoDock suite. *Nature Protocols*, 11(5), 905-919. https://doi.org/10.1038/nprot.2016.051

Foster, R. G., & Kreitzman, L. (2014). The rhythms of life: The biological clocks that control the daily lives of every living thing. Profile Books.

Fowler, S., Hoedt, E. C., Talley, N. J., Keely, S., & Burns, G. L. (2022). Circadian rhythms and melatonin metabolism in patients with disorders of gut-brain interactions. *Frontiers in Neuroscience*, 16, 825246. https://doi.org/10.3389/fnins.2022.825246

Fribourgh, J. L., & Partch, C. L. (2017). Assembly and function of bHLH-PAS complexes. *Proceedings of the National Academy of Sciences*, 114(20), 5330-5332. https://doi.org/10.1073/pnas.1704320114

Gao, T., Li, Y., Wang, X., & Ren, F. (2023). The Melatonin–Mitochondrial Axis: Engaging the Repercussions of Ultraviolet Radiation Photoaging on the Skin's Circadian Rhythm. *Antioxidants*, 12(5), 1000. https://doi.org/10.3390/antiox12051000

Genheden, S., & Ryde, U. (2015). The MM/PBSA and MM/GBSA methods to estimate ligand-binding affinities. *Expert Opinion on Drug Discovery*, 10(5), 449-461. doi:10.1517/17460441.2015.1032936

Gökhan-Kelekçi, N., Köksal, M., Unüvar, S., Aktay, G., & Erdoğan, H. (2009). Synthesis and characterization of some new 2(3H)-benzoxazolones with analgesic and anti-inflammatory activities. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 24(1), 29-37. https://doi.org/10.1080/14756360802020819

Gradisnik, L., & Velnar, T. (2023). Astrocytes in the central nervous system and their functions in health and disease: A review. *World Journal of Clinical Cases*, 11, 3385-3394. https://doi.org/10.12998/wjcc.v11.i15.3385

Griffin, P., Dimitry, J. M., Sheehan, P. W., Lananna, B. V., Guo, C., Robinette, M. L., Hayes, M. E., Cedeño, M. R., Nadarajah, C. J., Ezerskiy, L. A., et al. (2019). Circadian clock protein Rev-erbα regulates neuroinflammation. *Proceedings of the National Academy of Sciences*, 116, 5102-5107. https://doi.org/10.1073/pnas.1812405116

Grossberg, G. T. (2003). Cholinesterase inhibitors for the treatment of Alzheimer's disease: Getting on and staying on. *Current Therapeutic Research, Clinical and Experimental*, 64, 216-235. https://doi.org/10.1016/S0011-393X(03)00059-6

Gulino, R. (2023). Synaptic Dysfunction and Plasticity in Amyotrophic Lateral Sclerosis. *International Journal of Molecular Sciences*, 24, 4613. https://doi.org/10.3390/ijms24054613

Gunaydin, C., & Bilge, S. S. (2018). Effects of Nonsteroidal Anti-Inflammatory Drugs at the Molecular Level. *Eurasian Journal of Medicine*, 50(2), 116-121. https://doi.org/10.5152/eurasianjmed.2018.17064

Guo, D. K., Zhu, Y., Sun, H. Y., Xu, X. Y., Zhang, S., Hao, Z. B., Wang, G. H., Mu, C. C., & Ren, H. G. (2019). Pharmacological activation of REV-ERBα represses LPS-induced microglial activation through the NF-κB pathway. *Acta Pharmacologica Sinica*, 40, 26-34. https://doi.org/10.1038/s41401-018-0064-0

Güner, O. F. (2020). Pharmacophore Perception, Development, and Use in Drug Design. Springer.

Hansen, R. A., Gartlehner, G., Webb, A. P., Morgan, L. C., Moore, C. G., & Jonas, D. E. (2008). Efficacy and safety of donepezil, galantamine, and rivastigmine for the treatment of Alzheimer's disease: A systematic review and meta-analysis. *Clinical Interventions in Aging*, 3, 211-225.

Hastings, M. H., & Goedert, M. (2013). Circadian clocks and neurodegenerative diseases: Time to aggregate? *Current Opinion in Neurobiology*, 23, 880-887. https://doi.org/10.1016/j.conb.2013.05.004

Hirota, T., & Kay, S. A. (2015). Identification of small-molecule modulators of the circadian clock. *Methods in Enzymology*, 551, 131-152. doi:10.1016/bs.mie.2014.10.026

Hollingsworth, S. A., & Dror, R. O. (2018). Molecular dynamics simulation for all. *Neuron*, 99(6), 1129-1143. doi:10.1016/j.neuron.2018.08.011

Hollingworth, M., & Young, T. M. (2014). Melatonin responsive hemicrania continua in which indomethacin was associated with contralateral headache. *Headache*, 54(6), 916-919. https://doi.org/10.1111/head.12346

Homolak, J., Mudrovčić, M., Vukić, B., & Toljan, K. (2018). Circadian rhythm and Alzheimer's disease. *Medical Sciences*, 6, 52. https://doi.org/10.3390/medsci6030052

Hossain, M. F., Wang, N., Chen, R., Li, S., Roy, J., Uddin, M. G., Li, Z., Lim, L. W., & Song, Y. Q. (2021). Exploring the multifunctional role of melatonin in regulating autophagy and sleep to mitigate Alzheimer's disease neuropathology. *Ageing Research Reviews*, 67, 101304. https://doi.org/10.1016/j.arr.2021.101304

Hoyt, K. R., & Obrietan, K. (2022). Circadian clocks, cognition, and Alzheimer's disease: Synaptic mechanisms, signaling effectors, and chronotherapeutics. *Molecular Neurodegeneration*, 17, 35. https://doi.org/10.1186/s13024-022-00537-9

Huang, N., Chelliah, Y., Shan, Y., Taylor, C. A., Yoo, S. H., Partch, C., Green, C. B., Zhang, H., & Takahashi, J. S. (2012). Crystal structure of the heterodimeric CLOCK: BMAL1 transcriptional activator complex. *Science*, 337, 189-194. https://doi.org/10.1126/science.1222804

Huang, W., Lin, Z., & van Gunsteren, W. F. (2011). Validation of the GROMOS 54A7 force field with respect to β-peptide folding. *Journal of Chemical Theory and Computation*, 7(4), 1237-1243. https://doi.org/10.1021/ct100747h

Hulme, B., Didikoglu, A., Bradburn, S., Robinson, A., Canal, M., Payton, A., Pendleton, N., & Murgatroyd, C. (2020). Epigenetic regulation of BMAL1 with sleep disturbances and Alzheimer's disease. *Journal of Alzheimer's Disease*, 77, 1783-1792. https://doi.org/10.3233/JAD-200634

Iweka, C. A., Seigneur, E., Hernandez, A. L., Paredes, S. H., Cabrera, M., Blacher, E., Pasternak, C. T., Longo, F. M., de Lecea, L., & Andreasson, K. I. (2023). Myeloid deficiency of the intrinsic clock protein BMAL1 accelerates cognitive aging by disrupting microglial synaptic pruning. *Journal of Neuroinflammation*, 20, 48. https://doi.org/10.1186/s12974-023-02727-8

Jerigova, V., Zeman, M., & Okuliarova, M. (2022). Circadian Disruption and Consequences on Innate Immunity and Inflammatory Response. *International Journal of Molecular Sciences*, 23(24), 13722. https://doi.org/10.3390/ijms232413722

Juliana, N., Azmi, L., Effendy, N. M., Mohd Fahmi Teng, N. I., Abu, I. F., Abu Bakar, N. N., Azmani, S., Yazit, N. A. A., Kadiman, S., & Das, S. (2023). Effect of circadian rhythm disturbance on the human musculoskeletal system and the importance of nutritional strategies. *Nutrients*, 15, 734. https://doi.org/10.3390/nu15030734

Kaur, A., Pathak, D. P., Sharma, V., & Wakode, S. (2018). Synthesis, biological evaluation and docking study of a new series of di-substituted benzoxazole derivatives as selective COX-2 inhibitors and anti-inflammatory agents. *Bioorganic & Medicinal Chemistry*, 26(4), 891-902. https://doi.org/10.1016/j.bmc.2017.12.047

Kerstjens, A., & De Winter, H. (2022). LEADD: Lamarckian evolutionary algorithm for de novo drug design. *Journal of Cheminformatics*, 14(1), 3. https://doi.org/10.1186/s13321-022-00558-8

Killoy, K. M., Pehar, M., Harlan, B. A., & Vargas, M. R. (2021). Altered expression of clock and clock-controlled genes in a hSOD1-linked amyotrophic lateral sclerosis mouse model. *FASEB Journal*, 35, e21343. https://doi.org/10.1096/fj.202000386RR

Kim, S. M., Neuendorff, N., & Earnest, D. J. (2019). Role of Proinflammatory Cytokines in Feedback Modulation of Circadian Clock Gene Rhythms by Saturated Fatty Acids. *Scientific Reports*, 9(8909). https://doi.org/10.1038/s41598-019-45202-3

Kim, Y. H., & Lazar, M. A. (2020). Transcriptional Control of Circadian Rhythms and Metabolism: A Matter of Time and Space. *Endocrine Reviews*, 41, 707-732. https://doi.org/10.1210/endrev/bnaa014

Kou, L., Chi, X., Sun, Y., Han, C., Wan, F., Hu, J., Yin, S., Wu, J., Li, Y., Zhou, Q., et al. (2022). The circadian clock protein Rev-erbα provides neuroprotection and attenuates neuroinflammation against Parkinson's disease via the microglial NLRP3 inflammasome. *Journal of Neuroinflammation*, 19, 133. https://doi.org/10.1186/s12974-022-02494-y

Kozakov, D., Hall, D. R., Beglov, D., Brenke, R., Comeau, S. R., Shen, Y., Li, K., Zheng, J., Vakili, P., Paschalidis, I., et al. (2010). Achieving reliability and high accuracy in automated protein docking: ClusPro, PIPER, SDU, and stability analysis in CAPRI rounds 13-19. *Proteins*, 78, 3124-3130. https://doi.org/10.1002/prot.22835

Kress, G. J., Liao, F., Dimitry, J., Cedeno, M. R., FitzGerald, G. A., Holtzman, D. M., & Musiek, E. S. (2018). Regulation of amyloid-β dynamics and pathology by the circadian clock. *Journal of Experimental Medicine*, 215, 1059-1068. https://doi.org/10.1084/jem.20172347

Kucera, N., Schmalen, I., Hennig, S., Öllinger, R., Strauss, H. M., Grudziecki, A., Wieczorek, E., Kramer, A., Wolf, E., & Herzel, H. (2012). Unwinding the differences of the mammalian PERIOD clock proteins from crystal structure to cellular function. *Proceedings of the National Academy of Sciences*, 109(9), 3311-3316. https://doi.org/10.1073/pnas.1113280109

Lananna, B. V., & Musiek, E. S. (2020). The wrinkling of time: Aging, inflammation, oxidative stress, and the circadian clock in neurodegeneration. *Neurobiology of Disease*, 139, 104832. https://doi.org/10.1016/j.nbd.2020.104832

Lananna, B. V., Nadarajah, C. J., Izumo, M., Cedeño, M. R., Xiong, D. D., Dimitry, J., Tso, C. F., McKee, C. A., Griffin, P., Sheehan, P. W., et al. (2018). Cell-autonomous regulation of astrocyte activation by the circadian clock protein BMAL1. *Cell Reports*, 25, 1-9.e5. https://doi.org/10.1016/j.celrep.2018.09.015

Lavecchia, A., & Di Giovanni, C. (2013). Virtual screening strategies in drug discovery: A critical review. *Current Medicinal Chemistry,* 20, 2839-2860. https://doi.org/10.2174/09298673113209990001

Le Gall, L., Anakor, E., Connolly, O., Vijayakumar, U. G., Duddy, W. J., & Duguez, S. (2020). Molecular and Cellular Mechanisms Affected in ALS. *Journal of Personalized Medicine,* 10, 101. https://doi.org/10.3390/jpm10030101

Lee, J., Kim, D. E., Griffin, P., Sheehan, P. W., Kim, D. H., Musiek, E. S., & Yoon, S. Y. (2020). Inhibition of REV-ERBs stimulates microglial amyloid-beta clearance and reduces amyloid plaque deposition in the 5XFAD mouse model of Alzheimer's disease. *Aging Cell*, 19, e13078. https://doi.org/10.1111/acel.13078

Li, Y., Zhang, J., Wan, J., Liu, A., & Sun, J. (2020). Melatonin regulates Aβ production/clearance balance and Aβ neurotoxicity: A potential therapeutic molecule for Alzheimer's disease. *Biomedicine & Pharmacotherapy,* 132, 110887. https://doi.org/10.1016/j.biopha.2020.110887

Lima, L. M., & Barreiro, E. J. (2005). Bioisosterism: A useful strategy for molecular modification and drug design. *Current Medicinal Chemistry*, 12(1), 23-49. https://doi.org/10.2174/0929867053363268

Lin, L., Huang, Q. X., Yang, S. S., Chu, J., Wang, J. Z., & Tian, Q. (2013). Melatonin in Alzheimer's disease. *International Journal of Molecular Sciences*, 14, 14575-14593. https://doi.org/10.3390/ijms140714575

Liu, H., Zhu, Y., Gao, Y., Qi, D., Zhao, L., Zhao, L., Liu, C., Tao, T., Zhou, C., & Sun, X., et al. (2020). NR1D1 modulates synovial inflammation and bone destruction in rheumatoid arthritis. *Cell Death and Disease,* 11, 129. https://doi.org/10.1038/s41419-020-2314-6

Liu, J., Clough, S. J., Hutchinson, A. J., Adamah-Biassi, E. B., Popovska-Gorevski, M., & Dubocovich, M. L. (2022). MT1 and MT2 Melatonin receptors: A therapeutic

perspective. *Annual Review of Pharmacology and Toxicology*, 52, 23-38. doi:10.1146/annurev-pharmtox-010617-052900

Liu, X., Ouyang, S., Yu, B., Liu, Y., Huang, K., Gong, J., Zheng, S., Li, Z., & Jiang, H. (2010). PharmMapper server: A web server for potential drug target identification using pharmacophore mapping approach. *Nucleic Acids Research*, 38(Suppl 2), W609-W614. https://doi.org/10.1093/nar/gkq300

Loksha, Y. M., & Abd-Alhaseeb, M. M. (2020). Synthesis and biological screening of some novel 6-substituted 2-alkylpyridazin-3(2H)-ones as anti-inflammatory and analgesic agents. *Archiv der Pharmazie*, 353(e1900295). https://doi.org/10.1002/ardp.201900295

Lustoza Rodrigues, T. C. M., de Sousa, N. F., Dos Santos, A. M. F., Aires Guimarães, R. D., Scotti, M. T., & Scotti, L. (2023). Challenges and Discoveries in Polypharmacology of Neurodegenerative Diseases. *Current Topics in Medicinal Chemistry*, 23, 349-370. https://doi.org/10.2174/1568026623666230126112628

Manouchehri, N., Rezaie, R., & Nasiri, M. (2020). The role of melatonin in the treatment of multiple sclerosis: A review study. *Journal of Cellular Neuroscience and Oxidative Stress*, 12(1), 879-887. doi:10.1044/2019_JNE

Margaritelis, N. V., Christodoulou, P., Paizis, C., & Veskoukis, A. S. (2018). Melatonin supplementation in Parkinson's disease: A systematic review. *Critical Reviews in Clinical Laboratory Sciences*, 55(4), 274-292. doi:10.1080/10408363.2018.1456839

Martinez, V., Nielsen, S. D., & Gabay, C. (2021). Impact of circadian rhythms on the immune-inflammatory response in chronic inflammatory diseases. *Frontiers in Immunology*, 12, 676198. doi:10.3389/fimmu.2021.676198

Masters, A., Pandi-Perumal, S. R., Seixas, A., Girardin, J. L., & McFarlane, S. I. (2014). Melatonin, the Hormone of Darkness: From Sleep Promotion to Ebola Treatment. *Brain Disorders & Therapy*, 4(1000151). https://doi.org/10.4172/2168-975X.1000151

McClelland, S., Brennan, G., Collins, L. M., Devine, E., & Curtis, A. M. (2020). Circadian control of neuroinflammation and neurodegeneration: A role for the

circadian clock in the ALS/FTD spectrum. *Journal of Neuroinflammation*, 17(1), 298. doi:10.1186/s12974-020-01954-5

McKee, C. A., Lee, J., Cai, Y., Saito, T., Saido, T., & Musiek, E. S. (2022). Astrocytes deficient in circadian clock gene Bmal1 show enhanced activation responses to amyloid-beta pathology without changing plaque burden. *Scientific Reports*, 12, 1796. https://doi.org/10.1038/s41598-022-05862-z

Meléndez-Fernández, O. H., Liu, J. A., & Nelson, R. J. (2023). Circadian Rhythms Disrupted by Light at Night and Mistimed Food Intake Alter Hormonal Rhythms and Metabolism. *International Journal of Molecular Sciences*, 24, 3392. https://doi.org/10.3390/ijms24043392

Memiş, Ö. (2019). İnsan odaklı aydınlatma. *International Periodical of Recent Technologies in Applied Engineering*, *1*(1), 30-35. https://doi.org/10.35333/porta.2019.23

Mendoza-Viveros, L., Marmolejo-Gutierrez, C., Cid-Castro, C., & Escalante-Covarrubias, Q. (2023). Astrocytic circadian clock control of energy expenditure by transcriptional stress responses in the ventromedial hypothalamus. *Glia*, 71(8), 1626-1647. https://doi.org/10.1002/glia.24360

Meng, X. Y., Zhang, H. X., Mezei, M., & Cui, M. (2021). Molecular docking: A powerful approach for structure-based drug discovery. *Current Computer-Aided Drug Design*, 17(4), 365-376. doi:10.2174/1573409917666201231124530

Miller, S., Srivastava, A., Nagai, Y., Aikawa, Y., Tama, F., & Hirota, T. (2021). Structural differences in the FAD-binding pockets and lid loops of mammalian CRY1 and CRY2 for isoform-selective regulation. *Proceedings of the National Academy of Sciences*, 118(22), e2026191118. https://doi.org/10.1073/pnas.2026191118

Morton, A. J., Symonds, H. R., & Wood, N. I. (2019). Disrupted sleep and circadian rhythms in Huntington's disease. *Neurobiology of Disease*, 130, 104513. doi:10.1016/j.nbd.2019.104513

Morton, G., Elrington, A., & Payne, R. A. (2020). The role of circadian rhythms in the management of chronic pain: A review of the literature. *Pain Medicine*, 21(10), 2150-2158. doi:10.1093/pm/pnaa193

Morvaridzadeh, M., Sadeghi, E., Agah, S., Nachvak, S. M., Fazelian, S., Moradi, F., Persad, E., & Heshmati, J. (2020). Effect of melatonin supplementation on oxidative stress parameters: A systematic review and meta-analysis. *Pharmacological Research*, 161, 105210. https://doi.org/10.1016/j.phrs.2020.105210

Murray, M. H., Valfort, A. C., Koelblen, T., Ronin, C., Ciesielski, F., Chatterjee, A., Veerakanellore, G. B., Elgendy, B., Walker, J. K., Hegazy, L., et al. (2022). Structural basis of synthetic agonist activation of the nuclear receptor REV-ERB. *Nature Communications*, 13, 7131. https://doi.org/10.1038/s41467-022-34892-4

Musiek, E. S., & Holtzman, D. M. (2016). Mechanisms linking circadian clocks, sleep, and neurodegeneration. *Science*, 354(6315), 1004-1008.

Musiek, E. S., Lim, M. M., Yang, G., Bauer, A. Q., Qi, L., Lee, Y., Roh, J. H., Ortiz-Gonzalez, X., Dearborn, J. T., Culver, J. P., et al. (2013). Circadian clock proteins regulate neuronal redox homeostasis and neurodegeneration. *Journal of Clinical Investigation*, 123, 5389-5400. https://doi.org/10.1172/JCI70317

Musiek, E. S., Xiong, D. D., & Holtzman, D. M. (2015). Sleep, circadian rhythms, and the pathogenesis of Alzheimer disease. *Experimental & Molecular Medicine*, 47, e148. https://doi.org/10.1038/emm.2014.121

Nader, N., Chrousos, G. P., & Kino, T. (2021). Circadian clocks and inflammation: Molecular mechanisms and clinical implications. *Journal of Clinical Investigation*, 131(1), e140282. doi:10.1172/JCI140282

Nakazato, R., Kawabe, K., Yamada, D., Ikeno, S., Mieda, M., Shimba, S., Hinoi, E., Yoneda, Y., & Takarada, T. (2017). Disruption of Bmal1 impairs blood-brain barrier integrity via pericyte dysfunction. *Journal of Neuroscience*, 37, 10052-10062. https://doi.org/10.1523/JNEUROSCI.3639-16.2017

Neupane, P., Thada, P. K., Singh, P., Faisal, A. R., Rai, N., Poudel, P., Waleed, M. S., Quinonez, J., Ruxmohan, S., & Jain, E. (2023). Investigating Edaravone Use for

Management of Amyotrophic Lateral Sclerosis (ALS): A Narrative Review. *Cureus*, 15, e33746. https://doi.org/10.7759/cureus.33746

Nous, A., Engelborghs, S., & Smolders, I. (2021). Melatonin levels in the Alzheimer's disease continuum: A systematic review. *Alzheimer's Research & Therapy*, 13, 52. https://doi.org/10.1186/s13195-021-00788-6

Oduselu, G. O., Afolabi, R., Ademuwagun, I., Vaughan, A., & Adebiyi, E. (2023). Structure-based pharmacophore modeling, virtual screening, and molecular dynamics simulation studies for identification of Plasmodium falciparum 5-aminolevulinate synthase inhibitors. *Frontiers in Medicine*, 9, 1022429. https://doi.org/10.3389/fmed.2023.1022429

Opperhuizen, A. L., van Kerkhof, L. W. M., Proper, K. I., Rodenburg, W., & Kalsbeek, A. (2019). Rodent models to study the metabolic effects of shiftwork in humans. *Frontiers in Pharmacology*, 10, 1429. doi:10.3389/fphar.2019.01429

Ortaakarsu. (2020, August 25). *Moleküler dinamik simülasyonları ve kullanımları*. Ortaakarsu.net. Retrieved February 8, 2025, from https://www.ortaakarsu.net/?p=6696

Panda, S. (2016). Circadian physiology of metabolism. *Science*, 354(6315), 1008-1015.

Parrinello, M., & Rahman, A. (1981). Polymorphic transitions in single crystals: A new molecular dynamics method. *Journal of Applied Physics*, 52(12), 7182-7190. https://doi.org/10.1063/1.328693

Partch, C. L., Green, C. B., & Takahashi, J. S. (2014). Molecular architecture of the mammalian circadian clock. *Trends in Cell Biology*, 24(2), 90-99.

Patel, M. M., Patel, B. M., & Pandya, D. (2021). Recent updates on circadian rhythm-related small-molecule inhibitors in therapeutic strategies. *Current Topics in Medicinal Chemistry*, 21(18), 1634-1644. doi:10.2174/1568026621666210723111800

Patel, N., Huang, X. P., Grandner, J. M., Johansson, L. C., Stauch, B., McCorvy, J. D., Roth, B. L., & Stevens, R. C. (2020). Structure-based discovery of potent and

selective melatonin receptor agonists. *Elife*, 9, e53779. https://doi.org/10.7554/eLife.53779

Patke, A., Young, M. W., & Axelrod, S. (2017). Molecular mechanisms and physiological importance of circadian rhythms. *Nature Reviews Molecular Cell Biology*, 18(12), 761-774.

Peres, M. F., Stiles, M. A., Oshinsky, M., & Rozen, T. D. (2001). Remitting form of hemicrania continua with seasonal pattern. *Headache*, 41(6), 592-594. https://doi.org/10.1046/j.1526-4610.2001.041006592.x

Pilorz, V., Astiz, M., Heinen, K. O., Rawashdeh, O., & Oster, H. (2020). The Concept of Coupling in the Mammalian Circadian Clock Network. *Journal of Molecular Biology*, 432(12), 3618-3638. https://doi.org/10.1016/j.jmb.2020.01.019

Pimentel-Coelho, P. M., Almeida, A., & Newman, S. A. (2020). Therapeutic implications of circadian rhythms for amyotrophic lateral sclerosis. *Frontiers in Neuroscience*, 14, 573830. doi:10.3389/fnins.2020.573830

Poeggeler, B., Miravalle, L., Zagorski, M. G., Wisniewski, T., Chyan, Y. J., Zhang, Y., Shao, H., Bryant-Thomas, T., Vidal, R., Frangione, B., et al. (2001). Melatonin reverses the profibrillogenic activity of apolipoprotein E4 on the Alzheimer amyloid Abeta peptide. *Biochemistry*, 40, 14995-15001. https://doi.org/10.1021/bi0114269

Poupaert, J., Carato, P., Colacino, E., & Yous, S. (2005). 2(3H)-benzoxazolone and bioisosters as "privileged scaffold" in the design of pharmacological probes. *Current Medicinal Chemistry*, 12(8), 877-885. https://doi.org/10.2174/0929867053363268

Pourcet, B., Zecchin, M., Ferri, L., Beauchamp, J., Sitaula, S., Billon, C., Delhaye, S., Vanhoutte, J., Mayeuf-Louchart, A., Thorel, Q., et al. (2018). Nuclear Receptor Subfamily 1 Group D Member 1 Regulates Circadian Activity of NLRP3 Inflammasome to Reduce the Severity of Fulminant Hepatitis in Mice. *Gastroenterology*, 154, 1449-1464.e20. https://doi.org/10.1053/j.gastro.2017.12.019

Prasher, P., Mall, T., & Sharma, M. (2023). Synthesis and biological profile of benzoxazolone derivatives. *Archiv der Pharmazie*, 356(4), e2300245. https://doi.org/10.1002/ardp.202300245

Rajmohan, R., & Reddy, P. H. (2017). Amyloid-beta and phosphorylated tau accumulations cause abnormalities at synapses of Alzheimer's disease neurons. *Journal of Alzheimer's Disease*, 57, 975-999. https://doi.org/10.3233/JAD-160612

Rastelli, G., Del Rio, A., Degliesposti, G., & Sgobba, M. (2010). Fast and accurate predictions of binding free energies using MM-PBSA and MM-GBSA. *Journal of Computational Chemistry*, 31, 797-810. https://doi.org/10.1002/jcc.21372

Reiter, R. J., Sharma, R., Rosales-Corral, S., & Tan, D. X. (2022). Circadian medicine in the future: Bright times ahead for human health. *Journal of Pineal Research*, 72(2), e12792. doi:10.1111/jpi.12792

Reuter, E., Popp, R., & Munch, M. (2020). Sleep and circadian rhythm disruption in multiple sclerosis and its potential impact on pain, fatigue, and cognition: A review. *Journal of Clinical Sleep Medicine*, 16(12), 1983-1990. doi:10.5664/jcsm.8744

Ricciotti, E., & FitzGerald, G. A. (2011). Prostaglandins and inflammation. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 31(5), 986-1000. https://doi.org/10.1161/ATVBAHA.110.207449

Rodríguez-Santana, C., Florido, J., Martínez-Ruiz, L., López-Rodríguez, A., Acuña-Castroviejo, D., & Escames, G. (2023). Role of melatonin in cancer: effect on clock genes. *International Journal of Molecular Sciences*, 24(4), 1919. https://doi.org/10.3390/ijms24041919

Rosato, V., Bosetti, C., Tanda, E. T., & Polesel, J. (2019). A systematic review and meta-analysis on melatonin supplementation in amyotrophic lateral sclerosis. *Journal of Pineal Research*, 66(3), e12549. doi:10.1111/jpi.12549

Rosato, V., Bosetti, C., Tanda, E. T., & Polesel, J. (2021). Melatonin and its metabolites in amyotrophic lateral sclerosis: A systematic review and meta-analysis. *Journal of Pineal Research*, 70(1), e12685. doi:10.1111/jpi.12685

Roy, J., Tsui, K. C., Ng, J., Fung, M. L., & Lim, L. W. (2021). Regulation of melatonin and neurotransmission in Alzheimer's disease. *International Journal of Molecular Sciences*, 22, 6841. https://doi.org/10.3390/ijms22136841

Rozen, T. D. (2006). Melatonin responsive hemicrania continua. *Headache*, 46(8), 1203-1204. https://doi.org/10.1111/j.1526-4610.2006.00513_2.x

Rozen, T. D. (2015). How effective is melatonin as a preventive treatment for hemicrania continua? A clinic-based study. *Headache*, 55(4), 430-436. https://doi.org/10.1111/head.12503

Saha, S., Singh, K. M., & Gupta, B. B. P. (2019). Melatonin synthesis and clock gene regulation in the pineal organ of teleost fish compared to mammals: Similarities and differences. *General and Comparative Endocrinology*, 279, 27-34. https://doi.org/10.1016/j.ygcen.2019.02.017

Sahar, S., & Sassone-Corsi, P. (2009). Metabolism and cancer: The circadian clock connection. *Nature Reviews Cancer*, 9(12), 886-896.

Savvidis, C., & Koutsilieris, M. (2012). Circadian rhythm disruption in cancer biology. *Molecular Medicine*, 18(1), 1249-1260.

Schmidt, C., Peigneux, P., & Cajochen, C. (2012). Age-related changes in sleep and circadian rhythms: Impact on cognitive performance and underlying neuroanatomical networks. *Frontiers in Neurology*, 3, 118. https://doi.org/10.3389/fneur.2012.00118

Schurhoff, N., & Toborek, M. (2023). Circadian rhythms in the blood-brain barrier: Impact on neurological disorders and stress responses. *Molecular Brain*, 16, 5. https://doi.org/10.1186/s13041-023-00997-0

Serin, Y., & Tek, N. A. (2019). Effect of Circadian Rhythm on Metabolic Processes and the Regulation of Energy Balance. *Annals of Nutrition and Metabolism*, 74, 322-330. https://doi.org/10.1159/000500071

Shearman, L. P., Jin, X., Lee, C., Reppert, S. M., & Weaver, D. R. (2000). Targeted disruption of the mPer3 gene: Subtle effects on circadian clock function. *Molecular and Cellular Biology*, 20(17), 6269-6275. https://doi.org/10.1128/MCB.20.17.6269-6275.2000

Shukla, M., Govitrapong, P., Boontem, P., Reiter, R. J., & Satayavivad, J. (2017). Mechanisms of melatonin in alleviating Alzheimer's disease. *Current*

Neuropharmacology, 15, 1010-1031. https://doi.org/10.2174/1570159X15666170313123454

Sierk, M. L., Zhao, Q., & Rastinejad, F. (2001). DNA deformability as a recognition feature in the reverb response element. *Biochemistry*, 40, 12833-12843. https://doi.org/10.1021/bi011086r

Soares, P., Silva, C., Chavarria, D., Silva, F. S. G., Oliveira, P. J., & Borges, F. (2023). Drug discovery and amyotrophic lateral sclerosis: Emerging challenges and therapeutic opportunities. *Ageing Research Reviews*, 83, 101790. https://doi.org/10.1016/j.arr.2022.101790

Srinivasan, V., Spence, D. W., Pandi-Perumal, S. R., Brown, G. M., & Cardinali, D. P. (2020). Melatonin in mitochondrial dysfunction and related disorders. *International Journal of Alzheimer's Disease*, 2020, 8363738. doi:10.1155/2020/8363738

Stevens, R. G. (2009). Light-at-night, circadian disruption and breast cancer: Assessment of existing evidence. *International Journal of Epidemiology*, 38(4), 963-970.

Summ, O., Andreou, A. P., Akerman, S., Holland, P. R., Hoffmann, J., & Goadsby, P. J. (2021). Differential actions of indomethacin: clinical relevance in headache. *Pain*, 162(3), 591-599. https://doi.org/10.1097/j.pain.0000000000002077

Tai, F. W. D., & McAlindon, M. E. (2021). Non-steroidal anti-inflammatory drugs and the gastrointestinal tract. *Clinical Medicine*, 21(2), 131-134. https://doi.org/10.7861/clinmed.2021-0036

Takahashi, J. S. (2017). Transcriptional architecture of the mammalian circadian clock. *Nature Reviews Genetics*, 18(3), 164-179.

Takahashi, J. S., Hong, H. K., Ko, C. H., & McDearmon, E. L. (2008). The genetics of mammalian circadian order and disorder: Implications for physiology and disease. *Nature Reviews Genetics*, 9(10), 764-775. https://doi.org/10.1038/nrg2430

Tang, L., Luo, J. R., Wang, X. Y., Zhao, B., Ge, R., Liang, T. G., & Shi, Y. J. (2021). 4-Sulfonyloxy/alkoxy benzoxazolone derivatives with high anti-

inflammatory activities: Synthesis, biological evaluation, and mechanism of action via p38/ERK-NF-κB/iNOS pathway. *Chemical Biology & Drug Design*, 97(2), 200-209. https://doi.org/10.1111/cbdd.13760

Tarocco, A., Caroccia, N., Morciano, G., Wieckowski, M. R., Ancora, G., Garani, G., & Pinton, P. (2019). Melatonin as a master regulator of cell death and inflammation: molecular mechanisms and clinical implications for newborn care. *Cell Death and Disease*, 10, 317. https://doi.org/10.1038/s41419-019-1556-7

Teleanu, R. I., Niculescu, A. G., Roza, E., Vladâcenco, O., Grumezescu, A. M., & Teleanu, D. M. (2022). Neurotransmitters-Key Factors in Neurological and Neurodegenerative Disorders of the Central Nervous System. *International Journal of Molecular Sciences*, 23, 5954. https://doi.org/10.3390/ijms23115954

Tian, W., Chen, C., Lei, X., Zhao, J., & Liang, J. (2018). CASTp 3.0: Computed atlas of surface topography of proteins. *Nucleic Acids Research*, 46(W1), W363-W367. https://doi.org/10.1093/nar/gky473

Tordjman, S., Chokron, S., Delorme, R., Charrier, A., Bellissant, E., Jaafari, N., & Fougerou, C. (2017). Melatonin: Pharmacology, Functions and Therapeutic Benefits. *Current Neuropharmacology*, 15(3), 434-443. https://doi.org/10.2174/1570159X14666161202122100

Ucar, H., Cacciaguerra, S., Spampinato, S., Van derpoorten, K., Isa, M., Kanyonyo, M., & Poupaert, J. H. (1997). 2(3H)-benzoxazolone and 2(3H)-benzothiazolone derivatives: Novel, potent and selective sigma1 receptor ligands. *European Journal of Pharmacology*, 335(2-3), 267-273. https://doi.org/10.1016/S0014-2999(97)01221-5

van Dyck, C. H., Swanson, C. J., Aisen, P., Bateman, R. J., Chen, C., Gee, M., Kanekiyo, M., Li, D., Reyderman, L., Cohen, S., et al. (2023). Lecanemab in early Alzheimer's disease. *The New England Journal of Medicine*, 388, 9-21. https://doi.org/10.1056/NEJMoa2212948

Verma, M., Lizama, B. N., & Chu, C. T. (2022). Excitotoxicity, calcium and mitochondria: a triad in synaptic neurodegeneration. *Translational Neurodegeneration*, 11, 3. https://doi.org/10.1186/s40035-021-00278-7

Videnovic, A., & Golombek, D. (2013). Circadian and sleep disorders in Parkinson's disease. *Experimental Neurology*, 243, 45-56. doi:10.1016/j.expneurol.2012.07.017

Videnovic, A., Lazar, A. S., Barker, R. A., & Overeem, S. (2014). 'The clocks that time us'—circadian rhythms in neurodegenerative disorders. *Nature Reviews Neurology*, 10(12), 683-693.

Vriend, J., & Reiter, R. J. (2015). Melatonin feedback on clock genes: a theory involving the proteasome. *Journal of Pineal Research*, 58(1), 1-11. https://doi.org/10.1111/jpi.12192

Wang, H., Zheng, W., Huang, H., & Huang, Y. (2021). Molecular dynamics simulations and MM/PBSA calculations reveal the binding mechanism between flavonoid inhibitors and acetylcholinesterase. *Journal of Biomolecular Structure and Dynamics*, 39(4), 1362-1375. doi:10.1080/07391102.2020.1735138

Wang, J. Z., Wang, Z. F., & Chen, Y. S. (2020). Role of melatonin in Alzheimer's disease. *Sleep Medicine Reviews*, 52, 101317. doi:10.1016/j.smrv.2020.101317

Wang, X. L., & Li, L. (2021). Circadian Clock Regulates Inflammation and the Development of Neurodegeneration. *Frontiers in Cellular and Infection Microbiology*, 11, 696554. https://doi.org/10.3389/fcimb.2021.696554

Wang, X., Pan, C., Gong, J., Liu, X., & Li, H. (2016). Enhancing the enrichment of pharmacophore-based target prediction for the polypharmacological profiles of drugs. *Journal of Chemical Information and Modeling*, 56(6), 1175-1183. https://doi.org/10.1021/acs.jcim.6b00076

Warfield, A. E., Prather, J. F., & Todd, W. D. (2021). Systems and Circuits Linking Chronic Pain and Circadian Rhythms. *Frontiers in Neuroscience*, 15(705173). https://doi.org/10.3389/fnins.2021.705173

Wolber, G., & Langer, T. (2005). LigandScout: 3-D pharmacophores derived from protein-bound ligands and their use as virtual screening filters. *Journal of Chemical Information and Modeling*, 45(1), 160-169. https://doi.org/10.1021/ci049885e

Wolff, S. E. C., Wang, X. L., Jiao, H., Sun, J., Kalsbeek, A., Yi, C. X., & Gao, Y. (2020). The Effect of Rev-erbα Agonist SR9011 on the Immune Response and Cell

Metabolism of Microglia. *Frontiers in Immunology*, 11, 550145. https://doi.org/10.3389/fimmu.2020.550145

Wong, S. D., Wright, K. P., Jr., Spencer, R. L., Vetter, C., Hicks, L. M., Jenni, O. G., & LeBourgeois, M. K. (2022). Development of the circadian system in early life: Maternal and environmental factors. *Journal of Physiological Anthropology*, 41, 22. https://doi.org/10.1186/s40101-022-00294-0

Wu, Y. H., Feenstra, M. G., Zhou, J. N., & Van Heerikhuize, J. J. (2019). Sleep disturbances in Alzheimer's disease and circadian rhythms: A potential target for light therapy. *Journal of Biological Rhythms*, 34(3), 211-223. doi:10.1177/0748730419846891

Xia, B., Vajda, S., & Kozakov, D. (2016). Accounting for pairwise distance restraints in FFT-based protein-protein docking. *Bioinformatics*, 32, 3342-3344. https://doi.org/10.1093/bioinformatics/btw306

Ye, R., Selby, C. P., Chiou, Y. Y., Ozkan-Dagliyan, I., Gaddameedhi, S., & Sancar, A. (2014). Dual modes of CLOCK

Yıldırım, Z., Toktaş, D. N., Demir, Ö., Gül, Z., & Şen Utsukarçi, B. (2023). Amyotrofik Lateral Skleroz Patofizyolojisi ve Tedavi Yaklaşımları. *HUJPHARM*, 43, 50-69. https://doi.org/10.52794/hujpharm.1064372

Yiannopoulou, K. G., & Papageorgiou, S. G. (2020). Current and future treatments in Alzheimer disease: An update. *Journal of Central Nervous System Disease*, 12, 1179573520907397. https://doi.org/10.1177/1179573520907397

Yuet, P. K., & Blankschtein, D. (2010). Molecular dynamics simulation study of water surfaces: Comparison of flexible water models. *Journal of Physical Chemistry B*, 114(42), 13786-13795. https://doi.org/10.1021/jp1050002

Zhang, W., Xiong, Y., Tao, R., Panayi, A. C., Mi, B., & Liu, G. (2022). Emerging insight into the role of circadian clock gene BMAL1 in cellular senescence. *Frontiers in Endocrinology (Lausanne)*, 13, 915139. https://doi.org/10.3389/fendo.2022.915139

Zheng, B., Larkin, D. W., Albrecht, U., Sun, Z. S., Sage, M., Eichele, G., Lee, C. C., & Bradley, A. (1999). The mPer2 gene encodes a functional component of the mammalian circadian clock. *Nature*, 400(6740), 169-173. https://doi.org/10.1038/22118

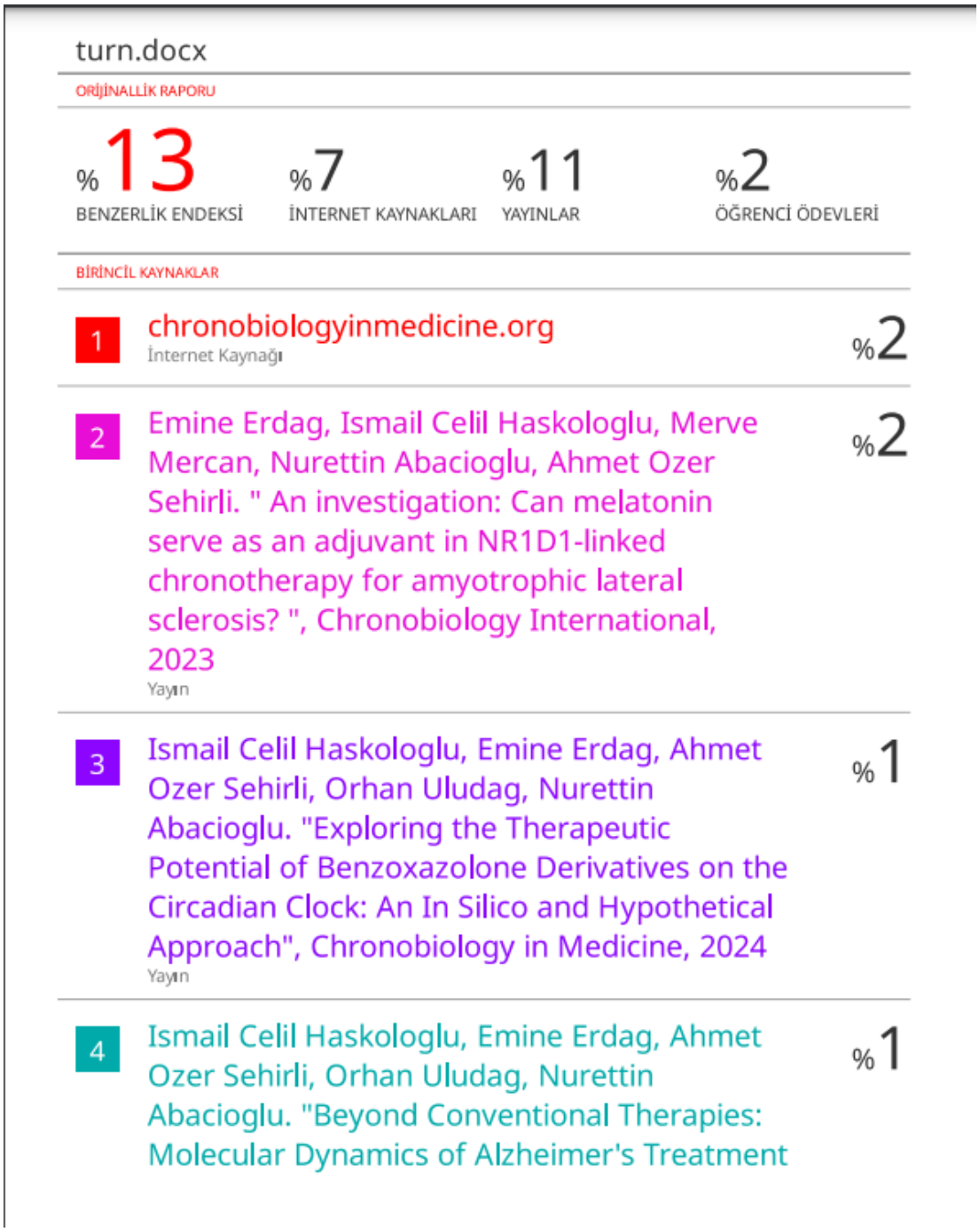
Zhu, L. Q., Wang, S. H., Ling, Z. Q., Wang, D. L., & Wang, J. Z. (2004). Effect of inhibiting melatonin biosynthesis on spatial memory retention and tau phosphorylation in rat. *Journal of Pineal Research*, 37, 71-77. https://doi.org/10.1111/j.1600-079X.2004.00136.x

Zhu, S., & McGeeney, B. (2015). When indomethacin fails: Additional treatment options for "indomethacin responsive headaches". *Current Pain and Headache Reports*, 19(1), 7. https://doi.org/10.1007/s11916-015-0494-0

Zisapel, N. (2018). New perspectives on the role of melatonin in human sleep, circadian rhythms and their regulation. *British Journal of Pharmacology*, 175, 3190-3199. https://doi.org/10.1111/bph.14116

Appendices

Similarity Report



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ORİJİNALLİK RAPORU

%13	%7	%11	%2
BENZERLİK ENDEKSİ	İNTERNET KAYNAKLARI	YAYINLAR	ÖĞRENCİ ÖDEVLERİ

BİRİNCİL KAYNAKLAR

1	chronobiologyinmedicine.org İnternet Kaynağı	%2
2	Emine Erdag, Ismail Celil Haskologlu, Merve Mercan, Nurettin Abacioglu, Ahmet Ozer Sehirli. " An investigation: Can melatonin serve as an adjuvant in NR1D1-linked chronotherapy for amyotrophic lateral sclerosis? ", Chronobiology International, 2023 Yayın	%2
3	Ismail Celil Haskologlu, Emine Erdag, Ahmet Ozer Sehirli, Orhan Uludag, Nurettin Abacioglu. "Exploring the Therapeutic Potential of Benzoxazolone Derivatives on the Circadian Clock: An In Silico and Hypothetical Approach", Chronobiology in Medicine, 2024 Yayın	%1
4	Ismail Celil Haskologlu, Emine Erdag, Ahmet Ozer Sehirli, Orhan Uludag, Nurettin Abacioglu. "Beyond Conventional Therapies: Molecular Dynamics of Alzheimer's Treatment	%1

CV

1. Name Surname **:** İSMAİL CELİL HASKOLOĞLU

2. Date of Birth **:** 17.02.1997- MALATYA/ TÜRKİYE

3. Title **:** Res. Assist.

4. State of Education **:** PhD Student

5. Current Institution **:** Near East University, Faculty of Pharmacy, Department of Pharmacology.

6. Foreign Languages **:** English (YÖKDİL: 62.50)

Degree	Department	University	Date
Bachelor's Degree	Faculty of Pharmacy	Near East University	2021
PhD	Faculty of Pharmacy, Pharmacology	Near East University	Still

7. Academic Appointments

Res. Assistant (Date) : 2021- Still

8. Publications

8.1. Articles published in internationally refereed journals (SCI, SSCI, ESCI, Arts and Humanities)

1. **Haskologlu, I. C**., Erdag, E., Sayiner, S., Abacioglu, N., & Sehirli, A. O. (2022). Melatonin and REGN-CoV2 combination as a vaccine adjuvant for Omicron variant of SARS-CoV-2. *Molecular biology reports*, *49*(5), 4061–4068. (SCI)

2. **Haskologlu, I. C**., Erdag, E., Sayiner, S., Mercan, M., Chukwunyere, U., Abacioglu, N., & Sehirli, A. O. (2023). The Concomitant Use of Melatonin and Molnupiravir in the Treatment of COVID-19: Mini Review. Bangladesh Journal of Medical Science, 22(1), 32-37. (ESCI)

3. Erdag, E., **Haskologlu, I. C**., Mercan, M., Abacioglu, N., & Sehirli, A. O. (2023). An in silico investigation: Can melatonin serve as an adjuvant in NR1D1-linked chronotherapy for amyotrophic lateral sclerosis?. Chronobiology International, 40(10), 1395-1403. (SCI)

4. **Haskologlu, I. C**., Erdag, E., Sehirli, A. O., Uludag, O., & Abacioglu, N. (2024). Exploring the Therapeutic Potential of Benzoxazolone Derivatives on the Circadian Clock: An In Silico and Hypothetical Approach.

5. **Haskologlu, I. C**., Erdag, E., Sehirli, A. O., Uludag, O., & Abacioglu, N. (2023). Beyond Conventional Therapies: Molecular Dynamics of Alzheimer's Treatment through CLOCK/BMAL1 Interactions. Current Alzheimer Research, 20(12), 862-874.

6. **Haskologlu, I. C**., Erdag, E., Sehirli, A. O., Uludag, O., & Abacioglu, N. (2024). Exploring the therapeutic potential of benzoxazolone derivatives on the circadian clock: An in silico and hypothetical approach. Chronobiology in Medicine, 6(2), 87-99.

7. **Haskologlu, I. C.,** Erdag, E., Uludag, O., & Abacioglu, N. (2024). A Chronobiological Approach: The Potential of Photoswitchable Drug Derivatives in the Treatment of Alzheimer’s Disease. Chronobiology in Medicine, 6(4):194-204.

9. Book Chapters

1. Taner, N., **Haskologlu, I. C**., Erdag, E., Mercan, M., Chuckwunyere, U., Ulker, D., ... & Abacioglu, N. (2023). Chronobiological efficacy of combined therapy of pelargonium sidoides and melatonin in acute and persistent cases of COVID-19: A

hypothetical approach. Application of Omic Techniques to Identify New Biomarkers and Drug Targets for COVID-19, 427-442.

2. **Haskologlu, I. C**., Erdag, E., Ulker, D., Uludag, M. O., Sehirli, A. O., & Abacioglu, N. (2024). Chronobiologically Targeted Anticancer Strategy: Synergistic Inhibition of CD39 and CD73 with Adenosine Receptor Agonists.

10. Certificates

CERTIFICATE OF USE OF EXPERIMENTAL ANIMALS - CATEGORY A (Mouse, Rat, Guinea Pig, and Rabbit)

11. Teaching Experience (Last 2 years)

PHA333 Pharmacology III

ECZ419 Farmakoloji IV

ECZ313 Farmakoloji II

ECZ210 Farmakoloji I

ECZ320 Farmakoloji III

ECZ418 Farmakoloji V

ECZ503 Akılcı İlaç Kullanımı

12. Short Biography

Pharm. Ismail Celil Haskologlu was born in Malatya in 1997. He completed his high school education at Malatya Cumhuriyet Anatolian High School. He completed his undergraduate education in June 2021 and started his Ph.D in the Department of Pharmacology at the Faculty of Pharmacy, Near East University in the same year.