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Innovative Design, Synthesis, and *In Silico* Evaluation of Bis-Ureido Substituted Antipyrine Derivatives: Molecular Modeling and ADME Insights

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Highlights:

- Antipyrine
- In silico
- ADME
- Bis-ureido
- Anticholinesterase

Keywords:

- Antipyrine
- In silico
- ADME
- Bis-ureido
- Anticholinesterase

ABSTRACT:

Molecular docking is a computational modeling technique that predicts the interactions between molecules of interest and certain protein structures. This approach estimates binding affinities and visualizes bond interactions, making it a useful tool for drug discovery. Molecular docking helps to rationally design new therapeutic medicines by offering insight into potential binding connections between molecular structures prior to laboratory testing. ADME investigations supplement molecular docking by assessing the pharmacokinetic features of the examined compounds, consequently determining their eligibility as possible therapeutic candidates. In this study, we show the creative design, synthesis, and *In silico* evaluation of a novel series of bis-ureido substituted antipyrine derivatives, with a focus on their potential as cholinesterase inhibitors. Using molecular modeling tools, we combined the bis-ureido group with the antipyrine drug to improve the pharmacological properties of these molecules. The newly synthesized compounds were comprehensively characterized by spectroscopic approaches, including FT-IR, ^{1}H-NMR, and ^{13}C-NMR, followed by molecular docking experiments to analyze their interactions with acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Additionally, *In silico* ADME assessments were performed to determine the compounds' pharmacokinetic characteristics and drug-likeness properties. Notably, compound **10** showed strong binding affinities against AChE and BChE, with binding energies of -14.47 and -11.75 kcal/mol, respectively. The docking data revealed high binding affinities, indicating a significant inhibitory potential for both AChE and BChE. This study points out the need of combining molecular docking and ADME investigations in contemporary pharmaceutical design and development.

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INTRODUCTION

Alzheimer's disease (AD) is a progressive neurological disorder that causes cognitive decline, loss of memory, and dementia (Reale et al. 2018; Türkeş et al. 2022a). According to the World Alzheimer Report, around 50 million individuals worldwide currently have been impacted by Alzheimer's disease, with that number expected to triple to 152 million by 2050 (2024; Breijyeh et al. 2020). Despite its growing prevalence, there is no conclusive cure or treatment for Alzheimer's disease (Deture and Dickson 2019; Lane, Hardy, and Schott 2018).

The natural history of Alzheimer's disease is complicated and multifaceted, with numerous pathways contributing to its origin and progression. These include beta-amyloid plaque buildup, chronic inflammation, oxidative stress, disruption of metal ion homeostasis, and tau protein aggregation (Kumar et al., 2018). Deficits in neurotransmitters acetylcholine (ACh) and butyrylcholine (BCh) may contribute to cognitive deterioration in AD patients (Işık and Beydemir 2022). Inhibiting acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), enzymes responsible for the breakdown of ACh and BCh, is a well acknowledged therapeutic method for treating these neurotransmitter difficulties (Lolak et al. 2020; Taslimi et al. 2018; Budak et al. 2017). Cholinesterase inhibitors may improve the concentration of these neurotransmitters at synapses by blocking these enzymes, thereby contributing in the restoration of cholinergic neurotransmission and cognitive function (Walczak-Nowicka and Herbet 2021; Yang, Zou, and Wang 2023). This idea underlies the current first-line treatments for Alzheimer's disease symptoms, including rivastigmine, galantamine, and donepezil. While these drugs can be beneficial in some cases, they are frequently linked with negative side effects such as nausea, gastrointestinal disturbances, diarrhea, muscle weakness, syncope, and weight loss. Given these limitations, there is an urgent need to develop novel cholinesterase inhibitors that are not only more effective but also have fewer adverse effects (Kandimalla and Reddy 2017; Walczak-Nowicka and Herbet 2021; Yang et al. 2023).

Multifunctional molecules with notable pharmacological activity have been produced by functionalizing the pyrazole core with amino substituents at different locations (Ansari et al. 2016; Pérez-Fernández, Goya, and Elguero 2013; Akocak et al. 2023). In drug discovery, aminopyrazoles in particular have become a very useful and adaptable scaffold (Lusardi, Spallarossa, and Brullo 2023). Some derivatives incorporate the amino function into other heterocyclic systems, and these compounds can have a range of amino group configurations, from substituted amino groups to free amino groups ($-NH_2$).

Antipyrine (AP), first synthesized by Knorr in 1883 (Knorr 1883), has caused significant and long-term interest in the scientific community, particularly in studies involving its derivatives, known as antipyrine derivatives (APDs) (Nishio et al. 2005; Rostom et al. 2009). These compounds have attracted interest due to their wide range of biological actions, making them extremely valuable in medicinal chemistry.

Extensive research has been conducted to investigate the diverse therapeutic potentials of APDs, including their antitumor properties (Marengo et al. 2020), antimicrobial effects (Mamaghani et al. 2015), antiviral capabilities (Youssef et al. 2023), and role as analgesic and anti-inflammatory agents (El-Feky et al. 2015). The diversity of APDs in regulating numerous biological pathways highlights their significance in therapeutic development. Their ability to modulate cellular responses across various biological systems has led to their acceptance as model compounds for studying drug

interactions and biological mechanisms (Ansari et al. 2016; Antunes-Ricardo et al. 2022; Lusardi et al. 2023).

Beyond their traditional applications, antipyrine derivatives continue to be critical molecular frameworks for the development of new drugs. Their diverse bioactivity not only has a chance to treat chronic diseases such as cancer, infections, and inflammatory disorders, but it also sets the basis for the development of more targeted and efficient drugs (Abbas et al. 2023; Akhmadiev et al. 2021; Fatima et al. 2024). This ongoing research focus on APDs reflects their importance of enhancing our understanding of how drugs behave and processing pharmacological profiles for a wide range of clinical applications.

Our research group recently showed the powerful cholinesterase inhibition displayed by a class of aromatic and heterocyclic mono- and bis-ureido-substituted benzenesulfonamide derivatives (Akocak et al. 2021; Lolak et al. 2023; Tekeli et al. 2022; Tekeli et al. 2024). Building on these compounds' significant inhibitory potential, our current study aims to further innovate by introducing bis-ureido substitutions into the antipyrine framework. With an emphasis on their effectiveness as cholinesterase inhibitors, this method seeks to synthesize new antipyrine compounds and extensively assess their biological functions. Additionally, *In silico* methods like molecular docking studies will be used to thoroughly assess their ADME (absorption, distribution, metabolism, and excretion) profiles. By predicting and optimizing the compounds' interactions with important biological targets, these computational techniques will help us better understand the compounds' pharmacokinetic characteristics and possible therapeutic uses. The goal of this research is to help create more effective and selective treatments for neurodegenerative illnesses like Alzheimer's by combining computer analysis and rational medication design.

MATERYAL VE METOT

Chemistry

4-aminoantipyrine (2 mmol) was initially dissolved in 10–15 mL of acetonitrile, after which it was sequentially reacted with various bis-isocyanates (1 mmol). The specific bis-isocyanates used were 1,4-phenylene diisocyanide for **A1**, 4,4′-methylenebis(phenyl isocyanate) for **A2**, 3,3'-dimethyl-4,4'-biphenylene diisocyanate for **A3**, 4-methyl-1,3-phenylene diisocyanate for **A4**, and 1,5-Diisocyanatonaphthalene for **A5**. The resultant mixture was stirred at room temperature for around 6 hours before being gently heated to 50 °C to complete the reaction, which was confirmed by thin-layer chromatography (TLC). After completion, the precipitated product was carefully filtered, washed with 50 mL of diethyl ether to eliminate impurities, and then dried under vacuum conditions. The final purified products were thoroughly characterized using various spectroscopic and analytical techniques. Scheme 1 illustrates the total synthetic pathway for these reactions.

1,1'-(1,4-phenylene)bis(3-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)urea) (**6**)

Yield: 78.35 %; Color: white solid; Melting Point: >300 ^{o}C; FT-IR (cm^{-1}): 3327, 3306 (NH), 1650 (C=O); ^{1}H-NMR (DMSO-d$_6$, 500 MHz, δ ppm): 8.45 (s, 2H, -NH-), 8.32 (s, 2H, -NH-), 7.50-7.45 (m, 5H, Ar-H), 7.35-7.29 (m, 5H, Ar-H), 7.27-7.23 (m, 4H, Ar-H), 3.05 (s, 6H, -CH$_3$), 2.14 (s, 6H, -CH$_3$); ^{13}C-NMR (DMSO-d$_6$, 125 MHz, δ ppm): 162.45, 152.24, 138.37, 134.21, 133.42, 129.15, 123.35, 121.53, 118.42, 114.26, 35.40, 17.68.

1,1'-(methylenebis(4,1-phenylene))bis(3-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)urea) (**7**)

Yield: 72.18 %; Color: white solid; Melting Point: 245-246 ^{o}C; FT-IR (cm^{-1}): 3335, 3298 (NH), 1640 (C=O); ^{1}H-NMR (DMSO-d$_6$, 500 MHz, δ ppm): 8.20 (s, 2H, -NH-), 8.00 (s, 2H, -NH-), 7.90 (d, *J* = 5.6 Hz, 2H, Ar-H), 7.81 (s, 2H, Ar-H), 7.49-7.45 (m, 4H, Ar-H), 7.38-7.34 (m, 4H, Ar-H), 7.31-7.29 (m, 4H, Ar-H), 7.15 (d, *J* = 4.6 Hz, 2H, Ar-H), 7.02 (t, 2H, Ar-H), 3.02 (s, 6H, -CH$_3$), 2.21 (s, 2H, -CH$_2$-), 2.19 (s, 6H, -CH$_3$); ^{13}C-NMR (DMSO-d$_6$, 125 MHz, δ ppm): 162.63, 152.46, 138.44, 138.20, 135.50, 130.58, 129.52, 126.62, 123.82, 121.14, 113.00, 36.70, 17.69, 11.71.

1,1'-(3,3'-dimethyl-[1,1'-biphenyl]-4,4'-diyl)bis(3-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)urea) (**8**)

Yield: 69.35 %; Color: white solid; Melting Point: 276-277 ^{o}C; FT-IR (cm^{-1}): 3326, 3292 (NH), 1645 (C=O); ^{1}H-NMR (DMSO-d$_6$, 500 MHz, δ ppm): 8.36 (s, 2H, -NH-), 8.04 (s, 2H, -NH-), 7.66 (s, 2H, Ar-H), 7.62 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.49 (t, 3H, Ar-H), 7.35-7.31 (m, 3H, Ar-H), 7.25 (d, *J* = 7.5 Hz, 2H, Ar-H), 7.21 (s, 2H, Ar-H), 7.17 (d, *J* = 8.0 Hz, 2H, Ar-H), 6.64 (d, *J* = 8.4 Hz, 2H, Ar-H), 3.04 (s, 6H, -CH$_3$), 2.23 (s, 6H, -CH$_3$), 2.01 (s, 6H, -CH$_3$); ^{13}C-NMR (DMSO-d$_6$, 125 MHz, δ ppm): 162.69, 152.44, 145.97, 135.71, 129.65, 128.43, 128.25, 127.60, 127.18, 124.79, 124.46, 123.54, 122.16, 115.02, 36.08, 17.88, 11.76.

1,1'-(4-methyl-1,3-phenylene)bis(3-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)urea) (**9**)

Yield: 62.48 %; Color: white solid; Melting Point: >300 ^{o}C; FT-IR (cm^{-1}): 3308, 3287 (NH), 1640 (C=O); ^{1}H-NMR (DMSO-d$_6$, 500 MHz, δ ppm): 8.33 (s, 2H, -NH-), 8.08 (s, 2H, -NH-), 7.89 (s, 1H, Ar-H), 7.68 (d, *J* = 7.6 Hz, 2H, Ar-H), 7.55-7.51 (m, 3H, Ar-H), 7.45-7.38 (m, 3H, Ar-H), 7.21 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.08-7.02 (m, 2H, Ar-H), 3.01 (s, 6H, -CH$_3$), 2.24 (s, 6H, -CH$_3$), 2.03 (s, 3H, -CH$_3$); ^{13}C-NMR (DMSO-d$_6$, 125 MHz, δ ppm): 162.11, 152.33, 138.36, 133.15, 132.68, 129.73, 129.42, 123.54, 123.12, 121.64, 120.53, 119.32, 118.15, 117.52, 113.42, 35.63, 17.32, 11.66.

1,1'-(naphthalene-1,5-diyl)bis(3-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)urea) (**10**)

Yield: 74.54 %; Color: white solid; Melting Point: 296-297 ^{o}C; FT-IR (cm^{-1}): 3291, 3264 (NH), 1643 (C=O); ^{1}H-NMR (DMSO-d$_6$, 500 MHz, δ ppm): 8.28 (s, 2H, -NH-), 8.02 (s, 2H, -NH-), 7.86 (d, *J* = 7.5 Hz, 2H, Ar-H), 7.62-7.58 (m, 3H, Ar-H), 7.47 (d, *J* = 7.8 Hz, 2H, Ar-H), 7.39-7.35 (m, 3H, Ar-H), 7.23 (d, *J* = 8.2 Hz, 4H, Ar-H), 7.01-6.98 (m, 2H, Ar-H), 3.02 (s, 6H, -CH$_3$), 2.21 (s, 6H, -CH$_3$), 2.01 (s, 3H, -CH$_3$); ^{13}C-NMR (DMSO-d$_6$, 125 MHz, δ ppm): 162.41, 152.65, 139.43, 133.27, 130.19, 129.33, 128.62, 123.17, 121.58, 120.29, 119.49, 118.23, 117.15, 113.67, 35.42, 17.55, 11.40.

Molecular modelling and ADME(T) predictions

The AutoDock 4.2 molecular docking software was used to investigate the best binding interactions of the newly synthesized bis-ureido substituted antipyrine derivatives (**6-10**) against AChE and BChE. The three-dimensional (3D) structures of AChE (PDB ID: 4EY7) (Cheung et al. 2012) and BChE (PDB ID: 6QAA) (Meden et al. 2019) were obtained from the Protein Data Bank (PDB) and used as the target enzymes in docking examinations.

The molecular structures of the antipyrine derivatives were first created with ChemDraw and then exported to sdf format. These structures were subsequently transformed to PDB format using the Avogadro 1.2 program (Muglu et al. 2024). During this stage, energy minimization was performed, torsional flexibility of the ligands was investigated, and the files were then transformed into PDBQT format using AutoDock tools to prepare them for the docking simulation (Durgun et al. 2024; Durmaz et al. 2023; Lolak et al. 2024).

In order to identify the most advantageous binding modes between the ligands and the target enzymes, the molecular docking procedure was carried out using AutoDock 4.2. In order to explore possible binding sites over the target proteins' whole surface, a blind docking technique was used during the grid creation step (Durmaz et al. 2023). The optimization examine was the Lamarckian Genetic approach (LGA), which produced 100 docking runs for each ligand with a population size of 300 individuals, a maximum of 2,500,000 energy evaluations, and up to 54,000 generations (Yilmaz et al. 2023; Yasar et al.2021).

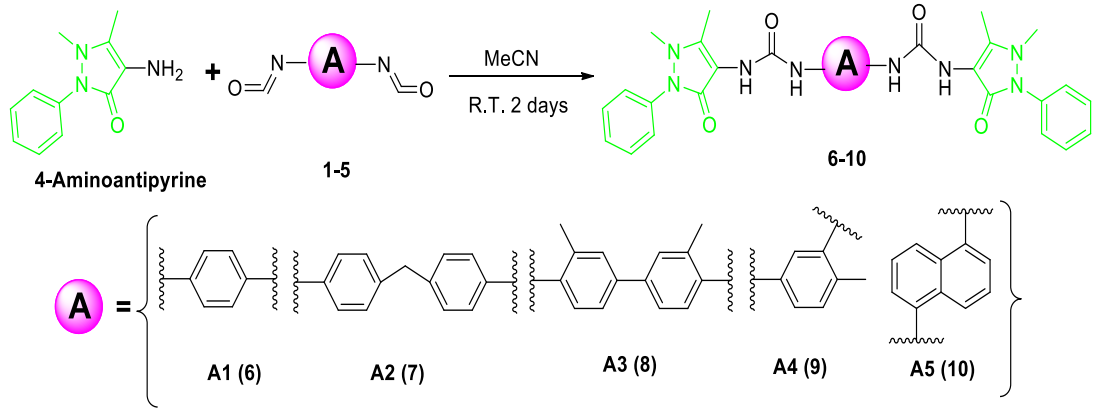
In the best docking conformations, the lowest docked binding free energy was accepted as the preferred binding pose of each compound. Inference and visualization of docking outcomes were carried out by BIOVIA Discovery Studio Visualizer 2020. The aforementioned tools were key in generating quality visual representations of the docked structures, allowing for a deeper understanding of the bis-ureido substituted antipyrine derivatives binding interactions with the target enzymes. SwissADME web tool was used for further evaluation of the physicochemical, pharmacokinetic, and solubility (ADME) properties of the synthesized compounds. The platform generates an assessment of the bioavailability, metabolic stability and solubility potential of each compound, which are necessary characteristics to assess drug candidates. Additionally, in silico toxicity prediction tools, including ProTox-II server are also highly used for preliminary toxicity estimates due to their easy accessibility and higher performance. ProTox-II enables the prediction of key toxicological profiles, including hepatotoxicity, neurotoxicity, neprotoxicity, carcinogenicity, immunetoxicity, and clinical toxicity. By evaluating these multiple toxicological attributes, the overall drug toxicity profile can be more thoroughly understood. Table 2 presents a detailed interpretation of the compounds, summarizing their predicted toxicological profiles for a more informed evaluation.

RESULTS AND DISCUSSION

Chemistry

Scheme 1 depicts the general synthesis process for producing bis-ureido substituted antipyrine derivatives. These chemicals were synthesized using a previously disclosed process from our research group (Akocak et al. 2021; Lolak et al. 2023; Tekeli et al. 2022; Tekeli et al. 2024). In summary, the procedure comprises the conjugation of 4-aminoantipyrine with bis-isocyanates, which yields bis-ureido-substituted antipyrine derivatives. Chemical diversity was achieved by using various aromatic bis-isocyanates, including 1,4-phenylene diisocyanide for **A1**, 4,4′-methylenebis(phenyl isocyanate) for **A2**, 3,3'-dimethyl-4,4'-biphenylene diisocyanate for **A3**, 4-methyl-1,3-phenylene diisocyanate for **A4**, and 1,5-Diisocyanatonaphthalene for **A5**. The reaction was carried out in acetonitrile as the solvent under mild conditions ranging from room temperature (RT) to 50 °C. After completion, the white precipitate was filtered and carefully washed with diethyl ether to assure purity. The final products were subjected to comprehensive characterization using a variety of spectroscopic and analytical techniques, including FT-IR, ^{1}H-NMR, ^{13}C-NMR, and melting point methods. This multi-technique approach provided precise structural verification and confirmed the successful synthesis of the target compounds.

Furthermore, the reaction conditions were modified to maximize yield and purity, resulting in highly reproducible bis-ureido derivatives. The mild temperature range used during the reaction emphasizes the synthetic process's efficiency and scalability. These characteristics make the synthetic route a reliable and efficient method for producing new compounds with potential biological applications.



Scheme 1. General Synthetic Route for the Synthesis of Novel Bis-ureido Substituted Antipyrine Derivatives (**6-10**)

In order to offer comprehensive information into the spectroscopic characterisation of the synthesized compounds, FT-IR spectra of the starting materials and bis-ureido derivatives were collected using U-ATR in the region of 4000-400 cm^{-1}. Sharp C=O vibrations were recorded between 1650-1640 cm^{-1}, consistent with previous findings. In the ^{1}H-NMR spectra, individual -NH- peaks of the ureido group appeared as singlets at 8.20-8.45 and 8.00-8.32 ppm, whereas aromatic protons were detected as singlet, doublet, triplet, or multiplet signals at 7.90-6.64 ppm. Antipyrine's -NCH_3 and -CH_3 signals were detected as singlets at 3.05-3.01 ppm and 2.24-2.14 ppm. The ^{13}C-NMR spectra revealed a constant carbonyl (C=O) peak around 162 ppm, indicating effective synthesis. These results are consistent with our previous studies and validate the structural strength of the synthesized compounds (Akocak et al. 2021; Lolak et al. 2023; Tekeli et al. 2022; Tekeli et al. 2024).

Ligand optimization

The molecular structures of the newly synthesized bis-ureido substituted antipyrine derivatives were first drawn with ChemDraw software. These structures were optimized for molecular modeling using the Avogadro 1.2 software, which transformed them to PDB format. During this conversion, energy minimization was used to achieve the molecules' most stable conformations (Figure 1). Furthermore, the ligands' torsional flexibility was carefully investigated to ensure an accurate description of their dynamic behavior. This stage was critical for determining the most effective binding interactions with biological targets in future molecular docking experiments. The rigorous energy minimization and flexibility analysis provided the basis for reputable *In silico* evaluation, offering that the produced structures were appropriate for comprehensive docking simulations and additional ADME characterization. These processes provided a robust foundation for the computational assessment of the bis-ureido substituted antipyrine derivatives' biological activity, particularly in their interaction with target enzymes such as AChE and BChE.

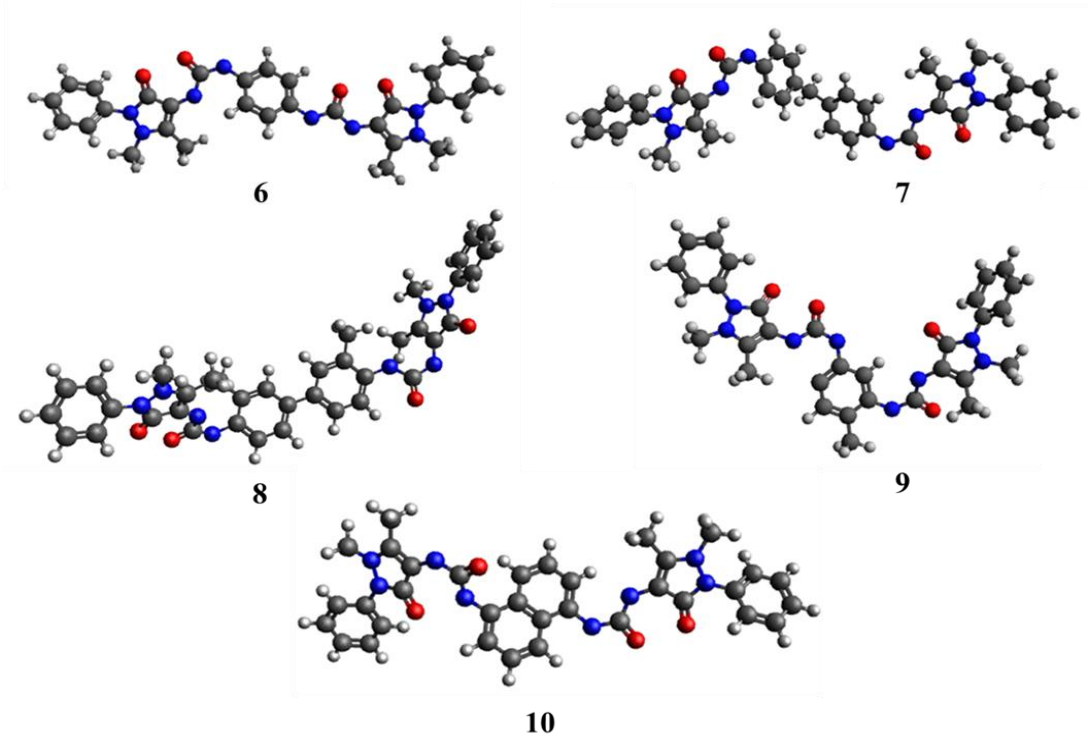


Figure 1. 3D Structures of Optimized Ligands Novel Bis-ureido Substituted Antipyrine Derivatives (**6-10**) by Avogadro 1.2 Software

Molecular docking

The AutoDock Vina software was used to investigate the binding interactions and affinities of the new bis-ureido substituted antipyrine derivatives (**6-10**) with acetylcholinesterase (AChE, PDB ID: 4EY7) and butyrylcholinesterase (BChE, PDB ID: 6QAA). The docking simulations revealed that compound **10** had the highest binding affinity for AChE, with a predicted binding energy of -14.47 kcal/mol, whereas compound **9** had the strongest binding to BChE, with a binding energy of -11.78 kcal/mol (Table 1). Docking studies revealed that hydrogen bonds, π-interactions, and van der Waals forces stabilized these ligand-enzyme complexes (Figures 2 and 3).

Table 1. The Docking Scores of Novel Bis-ureido Substituted Antipyrine Derivatives (**6-10**)

Compound ID	Binding Energy (kcal/mol) AChE (PDB ID: 4EY7)	Inhibition Constant	Binding Energy (kcal/mol) BChE (PDB ID: 6QAA)	Inhibition Constant
6	-12.95	320.56 pM	-11.46	3.96 nM
7	-12.75	448.35 pM	-11.10	7.26 nM
8	-14.12	44.62 pM	-11.56	3.34 nM
9	-12.88	359.79 pM	-11.78	2.30 nM
10	-14.47	24.69 pM	-11.75	2.42 nM
Donepezil	-11.88	1.95 nM	-9.77	69.16 nM

In the present research, all nwly synthesized compounds (**6-10**) were docked into AChE's active site in order to identify the most promising candidates. As demonstrated in Table 1, all compounds exhibited better inhibition properties as compared with standard drug donepezil (binding energy of -11.88 kcal/mol). More specifically, compound **10** has the highest binding energy of -14.47 kcal/mol, indicating a strong inhibitory activity. Compound **8** additionally showed a high binding affinity, with a binding energy of -14.12 kcal/mol. Figure 2 shows an in-depth evaluation of the docking results, highlighting crucial interactions between the synthesized molecules and essential amino acid residues

in the AChE active region. Trp86, Tyr124, Ser125, Trp286, Leu289, Ser293, Val294, Arg296, Phe338, and Tyr341 were discovered to play important roles in the stability of ligand-enzyme complexes. In the case of AChE, the pyrazole moiety of the antipyrine scaffold exhibited strong hydrogen bonding interactions with Tyr124, Ser125, and Ser293, contributing to the high binding affinity observed for compound **10** (Figure 2).

In this investigation, all produced compounds **(6-10**) were subjected to molecular docking studies to identify their binding interactions with BChE, with the goal of selecting the most promising inhibitors. The results showed that all compounds had similar inhibitory action, with binding affinities ranging from -11.10 to -11.78 kcal/mol, indicating promise as BChE inhibitors. All produced compounds had higher binding affinities than donepezil, the reference drug. Compound **9** had the most significant action, with a binding energy of -11.78 kcal/mol, as shown in Table 1. This finding identifies compound **9** as the most potent molecule in the series, indicating a strong contact with the target site and potential as a possible lead molecule for future development as a cholinesterase inhibitor. The docking studies revealed that compound **9** built several strong π-interactions, such as π-σ, π-π stacking, and π-π T-shaped interactions, which contributed significantly to its high binding affinity. These interactions were predominantly stabilized by critical amino acid residues in the BChE active site, including Trp82, Phe229, Pro285, Ala328, Tyr332, and His438. These residues were crucial in stabilizing the compound within the enzyme's binding pocket, notably through aromatic interactions, which increased compound **9**'s overall inhibitory potency.

Comparable docking studies have been reported in the literature for synthesized compounds targeting cholinesterase enzymes. For instance, novel β-lactam derivatives were synthesized and evaluated as acetylcholinesterase (AChE) inhibitors using PDB ID: 4EY7, yielding docking scores of -9.0234 and -9.8652 kcal/mol for compounds **5a** and **5b**, respectively (Fahim et al. 2021). In another study by Medetalibeyoğlu et al., novel 4,5-dihydro-1H-1,2,4-triazol-5-one derivatives demonstrated potent AChE inhibition, with compounds **4e** and **4g** exhibiting exceptional binding energies of -12.0 kcal/mol (Medetalibeyoğlu et al. 2023) Additionally, butyrylcholinesterase (BuChE, PDB ID: 6QAA) was docked with **SPH4**, showing a binding energy of -9.56 kcal/mol (Lolak et al. 2022). These findings highlight the relevance of molecular docking in confirming inhibitory activity and binding affinities for cholinesterase inhibitors.

The constant binding affinities reported across the series of bis-ureido substituted antipyrine derivatives indicate that these compounds are well-optimized for efficient inhibition of AChE and BChE. Among the investigated chemicals, **9** and **10** demonstrated superior binding interactions, making them especially appealing candidates for further research. Their strong π-interactions and favorable binding energies highlight their potential as therapeutic agents for cholinesterase-related illnesses namely Alzheimer's disease.

Our molecular docking data show that the structural design of these molecules promotes stable and efficient interactions with enzymes, making them attractive lead molecules in drug design efforts. The favorable interactions with essential amino acids in enzyme active areas emphasize their importance in therapeutic applications. Moving ahead, detailed structure-activity relationship (SAR) studies will be required to optimize the inhibitory characteristics of these derivatives, which will improve their efficacy and selectivity. Future biochemical investigations should include pharmacokinetics and toxicity profiling to optimize these newly synthesized compounds for possible therapeutic applications. This study offers the framework for further investigation of bis-ureido substituted antipyrine derivatives in the treatment of neurodegenerative diseases.

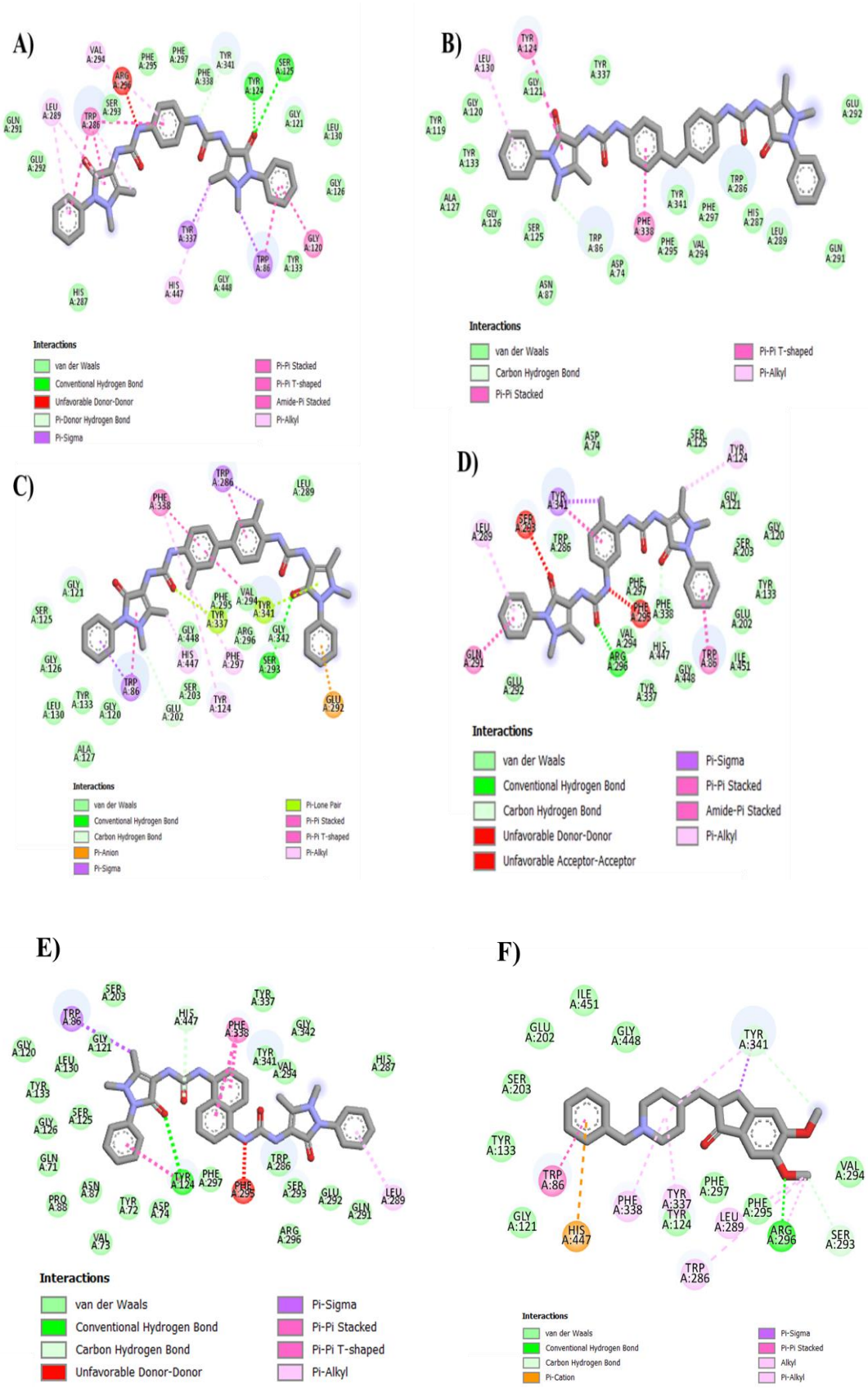


Figure 2. 2D Interactions of Novel Bis-ureido Substituted Antipyrine Derivatives (**6-10**) and Donepezil (**F**) with Amino Acids of the AChE Enzyme (PDB ID:4EY7) Binding Site

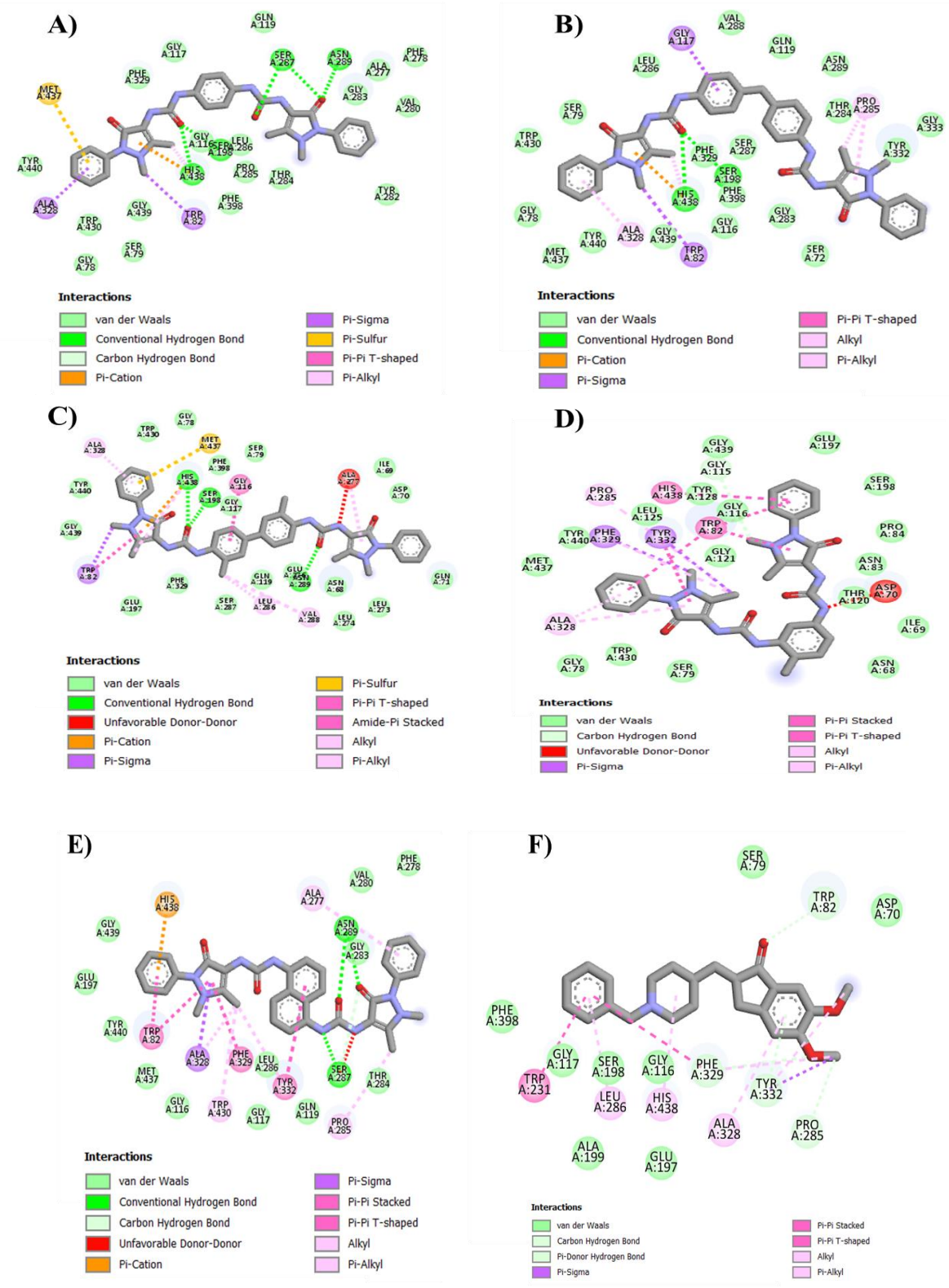


Figure 3. 2D Interactions of Novel Bis-ureido Substituted Antipyrine Derivatives (**6-10**) and Donepezil (**F**) with Amino Acids of the BChE Enzyme (PDB ID:6QAA) Binding Site

Table 2. *In silico* pharmacokinetic properties and toxicity prediction of novel bis-ureido substituted antipyrine derivatives (**6-10**).

Properties	Parameters	Compound 6	Compound 7	Compound 8	Compound 9	Compound 10
Physicochemical properties	MW (g/mol)	566.61	656.73	670.76	580.64	616.67
	Heavy atoms	42	49	50	43	46
	Aromatic heavy atoms	28	34	34	28	32
	Rotatable atoms	10	12	11	10	10
	H-bond acceptors	4	4	4	4	4
	H-bond donars	4	4	4	4	4
	Molar refractivity	163.51	192.97	198.88	168.48	181.02
Lipophilicity	M Log P	3.94	5.04	5.21	4.13	4.53
Water solubility	LogS (ESOL)	-5.45	-7.11	-7.52	-5.76	-6.57
	Solubility	Moderately soluble	Poorly soluble	Poorly soluble	Moderately soluble	Poorly soluble
Pharmacokinetics	BBB permeant	No	No	No	No	No
	CYP1A2 inhibitor	No	Yes	No	No	Yes
	CYP2C19 inhibitor	Yes	Yes	Yes	Yes	Yes
	CYP2C9 inhibitor	Yes	No	No	Yes	No
	CYP3A4 inhibitor	No	No	No	Yes	No
Druglikeness	Lipinski violation	2	3	3	2	3
	Ghose violation	3	3	4	3	3
	Veber violation	Yes	No	No	Yes	Yes
	Bioavailability score	0.17	0.17	0.17	0.17	0.17
Medicinal chemistry	Synthetic accessibility	4.00	4.55	4.69	4.24	4.24
Organ toxicity	Hepatotoxicity	Active	Active	Active	Active	Active
	Neurotoxicity	Active	Active	Active	Active	Active
	Nephrotoxicity	Inactive	Inactive	Inactive	Inactive	Inactive
Toxicity end points	Carcinogenicity	Inactive	Inactive	Inactive	Inactive	Inactive
	Immunotoxicity	Inactive	Inactive	Inactive	Inactive	Inactive
	BBB-barrier	Active	Active	Active	Active	Active
	Clinical toxicity	Active	Active	Active	Active	Active
	LD_{50} (mg/kg)	2300	1556	2300	2300	2300

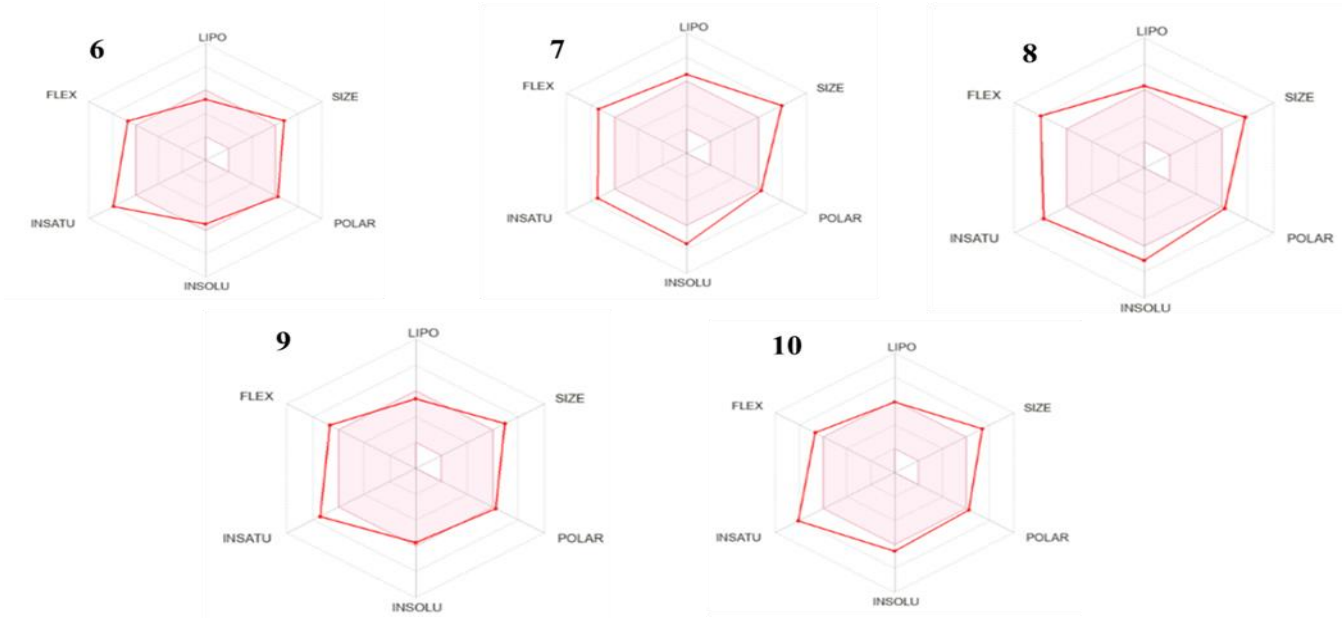


Figure 4. Bioavailability Radar Plot İllustrating the Optimal Physicochemical Parameters for Synthesized Compounds (**6-10**), Providing a Visual Representation of Key Factors That İnfluence Drug Absorption and Efficacy

To acquire a better understanding of the ADME properties of our synthesized compounds, we performed a thorough investigation using the SWISSADME website. This extensive investigation, shown in Table 2, provides essential information about the drug-likeness and pharmacokinetic characteristics of the newly synthesized bis-ureido substituted antipyrine derivatives (**6-10**). In addition to pharmacokinetic evaluation, determining possible toxicity is an important parameter in the drug discovery process. For predicting toxicity profiles, we used the ProTox-II server, a dependable and easy-to-use *In silico* tool. ProTox-II allowed us to estimate a variety of preliminary toxicity metrics, such as hepatotoxicity, neurotoxicity, neprotoxicity, carcinogenesis, immunotoxicity, and clinical toxicity. By taking into account different toxicological factors, we were able to create a thorough toxicity profile for each compound. The results, summarized in Table 2, provide a comparative interpretation of the safety profiles for these synthesized compounds, highlighting their potential suitability as therapeutic agents.

CONCLUSION

In summary, this study emphasizes the successful design and synthesis of a novel class of bis-ureido substituted antipyrine derivatives, indicating their potential as cholinesterase inhibitors. Using molecular docking and *In silico* ADME evaluations, we were able to anticipate substantial binding affinities between these compounds and important enzymes involved in neurodegenerative disorders. The strong inhibitory efficacy discovered, particularly with compound **10**, highlights the compounds' potential as new Alzheimer's disease therapeutics. This study stresses the importance of incorporating computational methods such as molecular docking and ADME analysis into the drug discovery process, providing a more efficient approach to identifying potent drug candidates with good pharmacokinetic properties.

Conflict of Interest

The article authors declare that there is no conflict of interest between them.

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