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Designing Novel Lenalidomide Derivatives as Inhibitors of IKZF1-3 Transcription Factors Targeting CRL4CRBN E3 Ubiquitin Ligase Complex

Abstract

Cancer continues to pose a significant threat to global health despite extensive research, clinical trials, and therapeutic interventions. The emergence of targeted therapies has brought hope to the field, with lenalidomide standing out as a promising treatment option for hematological malignancies. Lenalidomide, an immune-modulating drug, exhibits potent anti-cancer, anti-angiogenic, and anti-inflammatory properties. As research advances, there is a growing focus on designing novel drug compounds to combat cancer effectively. Immunomodulatory drugs (IMiDs), including lenalidomide, target the CRL4CRBN E3 ubiquitin ligase complex and facilitate the ubiquitination of transcription factors Ikaros and Aiolos (IKZF1 and IKZF3). This article aimed to compare lenalidomide's performance with the newly designed analog LENO54 in terms of its interactions with the CRBN protein and its ability to bind to Ikaros and Aiolos transcription factors through molecular docking. Molecular docking analysis revealed that the novel analog LENO54 demonstrated significantly lower binding energy and higher inhibitory capacity compared to lenalidomide. These findings suggest that LENO54 may hold greater promise as an anti-tumor agent, particularly for patients diagnosed with multiple myeloma. In conclusion, this study highlights the potential of LENO54 as a superior therapeutic candidate, shedding light on the importance of continued research in developing targeted therapies for cancer treatment.

Keywords: *Cancer, lenalidomide, molecular docking, CRL4CRBN, IKZF1-3.*

Arefeh Esmaeili[a], Mehdi Yoosefian[*a], Mohamad Mahani[a]

[a] *Department of Chemistry, Graduate University of Advanced Technology, Kerman, Iran*

Emails of the corresponding author:
myoosefian7@gmail.com

Introduction

Cancer [1] continues to be a significant global health concern, accounting for approximately 10 million deaths in 2020 [2]. Multiple myeloma (MM) [3], comprising 2% of cancer-related deaths, has been an area of intense research for improved therapies and stem cell transplantation over the past few decades [4]. MM, a cancer originating from plasma cells within B cells, presents a subset of long-lived plasma cells. Among the various treatment options, lenalidomide, a derivative of thalidomide [5], has emerged as a potent therapeutic agent with remarkable immunomodulatory effects [6]. Its clinical success has been evident in hematological malignancies like multiple myeloma and myelodysplastic syndromes [7]. However, the precise mechanism of action for lenalidomide is still not completely understood.

Recent studies indicate that lenalidomide's immunomodulatory activities [8] are mediated through interactions with E3 ubiquitin ligases, notably the Cereblon protein [9]. Cereblon plays a pivotal role in cellular proteostasis by regulating protein degradation and turnover [10]. The interactions with lenalidomide are crucial for inducing immunomodulatory effects. To maximize its therapeutic potential, enhancing lenalidomide's binding affinity with Cereblon and expanding its molecular targets becomes imperative [11].

Lenalidomide's mechanism of action in myeloma cells is multifaceted, and it varies based on cell type and tissue [10]. It involves arresting the G0/G1 phase through the positive regulation of cyclin-dependent kinase inhibitor p21WAF-1 and reducing the expression of the regulatory factor Interferon 4 (IRF4). This leads to the selective ubiquitination and degradation of two lymphoid transcription factors, Ikaros (IKZF1), and Aiolos (IKZF3), by the CRL4-CRBN ubiquitin ligase. IKZF1 and IKZF3 are critical transcription factors in multiple myeloma, and their degradation results in clinical efficacy in treating the disease [12]. Additionally, lenalidomide induces increased release of Interleukin 2 from T2-4 cells and inhibits angiogenesis and growth factor production from bone marrow stromal cells [11].

This article focuses on designing a novel drug based on lenalidomide, specifically targeting initial binding affinity with the Cereblon protein. The primary goal of this drug design is to inhibit IKZF1-3 transcription factors, which play vital roles in various cellular processes, including immune response and oncogenesis. The paper highlights the rational drug design process, molecular interactions, ADMET screening, and potential therapeutic applications of this lenalidomide-based compound for treating various diseases, with particular emphasis on immune-related disorders and malignancies.

Method

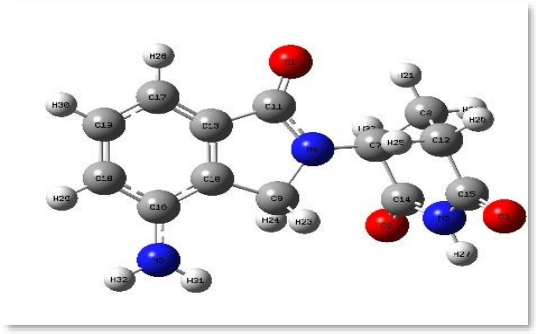
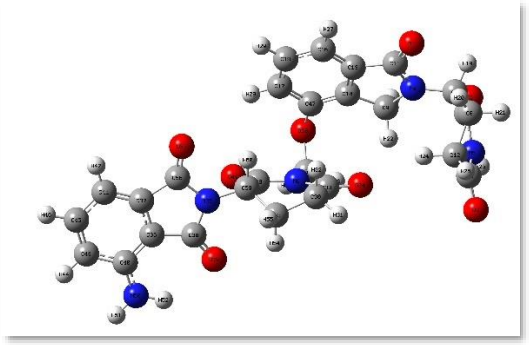
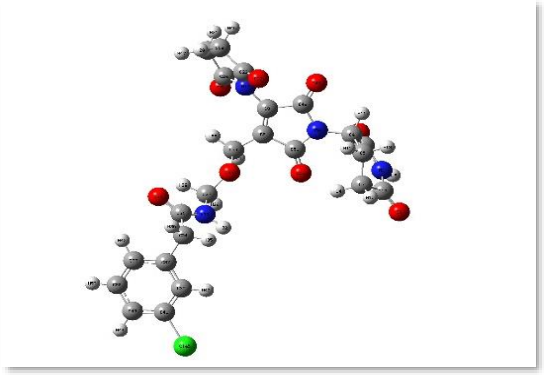
Rational drug design techniques were utilized to modify the structure of the lenalidomide molecule to enhance its binding interactions with the Cereblon protein. In addition to rational drug design, ADMET [13] (Absorption, Distribution, Metabolism, Excretion, and Toxicity) screening was carried out to investigate the physicochemical and pharmacodynamic properties of the modified compounds. Subsequently, molecular docking simulations were performed to assess the

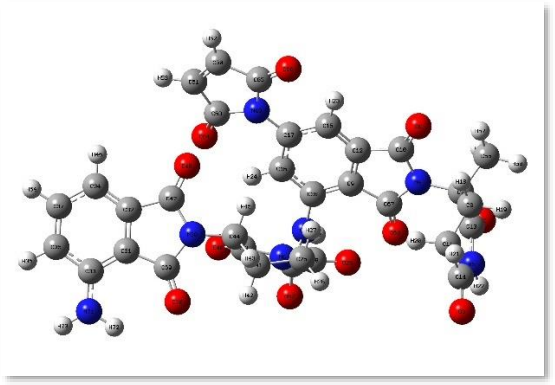
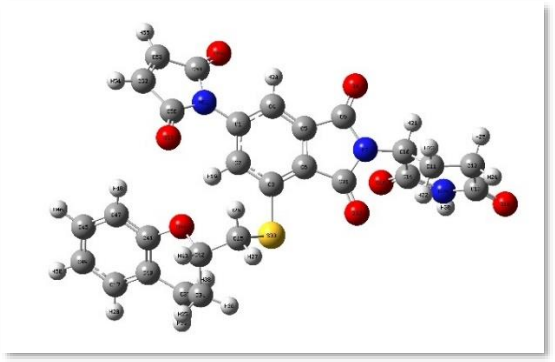
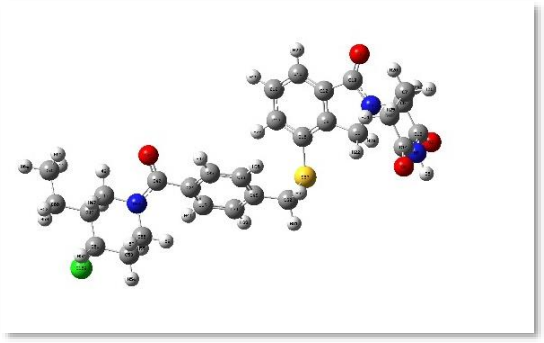
binding affinities of various lenalidomide derivatives with the Cereblon protein. Based on the docking results, the derivatives exhibiting superior binding interactions were selected for further evaluation and in-depth analysis.

Computational Design of Novel Analogs using Gaussian Software

Five novel analogs based on the drug lenalidomide (LENO) were developed using Gaussian software [14]. To ensure their stability and accuracy, all designed structures underwent optimization with the Gaussian 09 software, utilizing the Hartree-Fock level of calculations [15] and the 3-21G* basis set [16]. In Table 1, the structures of the designed lenalidomide analogs are presented, alongside their respective optimized energies for each structure. This comprehensive analysis of the analogs' structural features and energetic properties lays the foundation for further exploration of their potential pharmaceutical applications.

Table 1: Structure of LENO Drug and Designed Analogues Using Gaussian Software

Drug	**E Hartree-Fock**	**Structure**
LENO	**882.90-**	
Designed Analogues		
LENO54	**-1918.27**	
LENO8	**-2147.81**	

LENO60	**-2416.45**	
LENO38	**-2108.12**	
LENO7	**-2384.97**	

ADMET screening

The pharmacokinetic and toxicity data of the compounds were analyzed and supported by the ADMETlab 2.0 server from the website [17] (https://admetmesh.scbdd.com/) and (https://tox-new.charite.de/protox_II). The ADMET analysis plays a crucial role in providing essential insights into the drug's behavior within the body, including its absorption, distribution, metabolism, excretion, and potential toxicity. This information is invaluable for gaining a comprehensive understanding of the compounds' pharmacological properties and safety profiles, which is crucial for efficient drug design and effective management of treatments.

Molecular docking

In this study, we used AutoDock version 1.5.2.4 and AutoDock Tools version 1.5.6 for docking simulations to identify potential ligand-binding sites [18]. The Lamarckian Genetic Algorithm (LGA) 1 in AutoDock was utilized to explore various conformational arrangements of protein-ligand complexes. The protein's crystal structure with the code 8D80 was retrieved from the protein database. Protein preparation involved the removal of water molecules and ions, the addition of hydrogen atoms, and the assignment of Kollman atomic partial charges using ADT. For ligand preparation, hydrogen atoms were added to the optimized structure under neutral pH conditions. Ligand rotations and torsions were automatically determined using ADT, with all other parameters set to their default values. To identify the amino acids involved in the protein's binding site, we performed docking simulations with a box size of 50 Å × 50 Å × 44 Å along the x, y, and z axes. Flexible ligand docking simulations were conducted with 250 runs and the Genetic Algorithm to evaluate the binding energies. To visualize hydrogen bonds and hydrophobic interactions between Lenalidomide and its analogs with the protein, we used the molecular graphics program LigPlot^{+} [19].

This analysis provided valuable insights into the ligand-protein interactions, aiding in the identification of potential binding sites and informing the rational design of novel analogs with enhanced therapeutic potential.

Results and Discussion

ADMET Result

In this study, Table 2 and Table 3 present the ADME properties and biological activities of various drugs. The solubility of a drug is crucial for its absorption, distribution, and metabolism. Table 2 provides information on adsorption parameters, including Pgp-inhibitor and Pgp-substrate. The results suggest that the five investigated compounds do not inhibit P-glycoprotein (Pgp) [20], indicating a lack of hepatotoxicity risk. Distribution parameters involving liver enzymes, such as cytochrome P450 A2, C19, C9, D6, and A4, were also evaluated, and no inhibition of these enzymes was observed for any of the compounds. Metabolism parameters, such as blood-brain barrier (BBB) permeability and plasma protein binding (PPB), were analyzed. Among the analogs, LENO54, LENO7, and LENO8 were found to possess the ability to cross the blood-brain barrier. Additionally, all five compounds demonstrated a high percentage of plasma protein binding compared to the parent drug (Table 2). Elimination parameters, including clearance [21] (CL) and half-life (T1/2), play a crucial role in the excretion of compounds through the kidneys and liver. The results indicated that the elimination of all three compounds from the body is nearly similar to that of the parent drug. Furthermore, LENO54 showed a longer half-life compared to the other analogs (Table 2) Table 3 provides information on physicochemical properties [22], such as lipophilicity (Log P), aqueous solubility (Log S), molecular weight (MW), and molecular formula. The results indicate that all five designed analogs have high LogP values (optimal range: 0~3) and low water solubility, suggesting their propensity to be soluble in lipids and capable of crossing the blood-brain barrier successfully. Among the analogs, LENO54 displayed higher lipophilicity, making it more likely to cross the blood-brain barrier compared to the other compounds. Drug-likeness factors, including Lipinski's rule [23] and Pfizer's rule [24] of similarity, were evaluated for the analogs in Table 3. Moreover, the toxicity profiles of the compounds, including their potential to induce cellular toxicity, mutagenicity, immunotoxicity [25], carcinogenicity [26], and hepatotoxicity [27], were subject to prediction. Table 4 provides the in-silico toxicity predictions for the analogs. The average lethal dose (LD50), which signifies the amount of a substance that, on average, results in a 50% mortality rate in a population exposed to it [28], is also presented in Table 4. Higher LD50 values indicate lower toxicity levels for the analogs, as demonstrated in the table.

Table 2: ADME Analysis for Lenalidomide Analogues

	Absorption		**Distribution**		**Metabolism**				
Drugs	**Pgp Inhibitor category1: inhibitor category0: non-inhibitor**	**Pgp Substrate category1: Substrate category0: non-Substrate**	**BBB Penetration category1: BBB+ Category0: BBB-**	**PPB% Optimal: ≤ 90%. Drugs with high protein-bound may have a low therapeutic index**	**CYP1 A2 Inhibitor category1: inhibitor category0: non-inhibitor**	**CYP2 C19 Inhibitor category1: inhibitor category0: non-inhibitor**	**CYP2 C9 Inhibitor category1: inhibitor category0: non-inhibitor**	**CYP2 D6 Inhibitor category1: inhibitor category0: non-inhibitor**	**CYP3 A4 Inhibitor category1: inhibitor category0: non-inhibitor**
LENO	**0.00**	**0.02**	**YES**	**28.60%**	**0.03**	**0.14**	**0.18**	**0.03**	**0.04**
LENO54	**0.00**	**0.36**	**YES**	**80.30%**	**0.02**	**0.15**	**0.54**	**0.29**	**0.79**
LENO8	**0.01**	**0.07**	**YES**	**54.43%**	**0.01**	**0.10**	**0.05**	**0.19**	**0.06**
LENO60	**0.00**	**0.97**	**NO**	**71.25%**	**0.01**	**0.03**	**0.30**	**0.17**	**0.23**
LENO38	**0.053**	**0.01**	**NO**	**90.26%**	**0.11**	**0.71**	**0.81**	**0.62**	**0.88**
LENO7	**0.08**	**0.03**	**YES**	**95.75%**	**0.09**	**0.73**	**0.92**	**0.41**	**0.95**
Excretion									
Drugs	**Clearance**								

	High:>15ml/min/kg / Moderate:5-15 / Low:<5	$T_{1/2}$ Category1: Long half-life / Category 0: Low half-life
LENO	**1.93**	**0.72**
LENO54	**1.72**	**0.70**
LENO8	**1.36**	**0.42**
LENO60	**1.87**	**0.16**
LENO38	**1.68**	**0.18**
LENO7	**1.77**	**0.54**

Table 3: Prediction of physicochemical properties such as parameters: lipophilicity (Log P) and solubility in water (Log S), molecular weight (MW) and molecular formula (Formula), Druglikeness to Pfizer and Lipinski rules

	Physicochemical Property				Druglikeness	
Drugs	**Log S Optimal: -4~0.5**	**Log P Optimal: 0-3**	**Molecular Weight(g/mol) Optimal:100~600**	**Formula**	**Pfizer**	**Lipinski**
LENO	**-2.450**	**-0.31**	**259.1**	**C13H13N3O3**	**Accept**	**Accept**
LENO54	**-4.20**	**-0.04**	**545.15**	**C27H23N5O8**	**Accept**	**Accept**
LENO8	**-2.76**	**-0.32**	**516.1**	**C23H21ClN4O8**	**Accept**	**Reject**
LENO60	**-5.53**	**-0.04**	**681.15**	**C32H23N7O11**	**Accept**	**Reject**
LENO38	**-5.39**	**2.40**	**531.11**	**C27H21N3O7S**	**Accept**	**Accept**
LENO7	**-5.04**	**3.66**	**539.16**	**C28H30ClN3O4S**	**Accept**	**Accept**

Table 4: Prediction of Toxicity for Lenalidomide Analogues

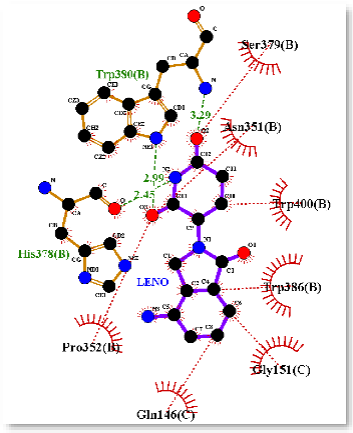
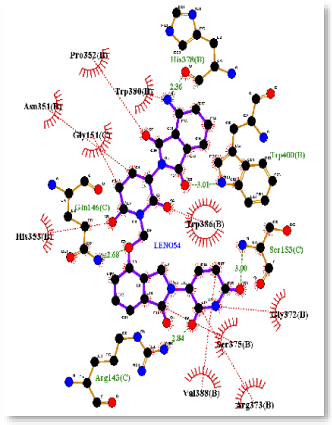
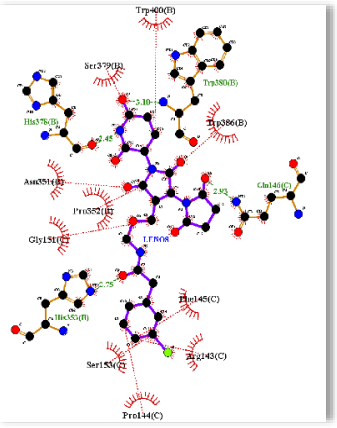
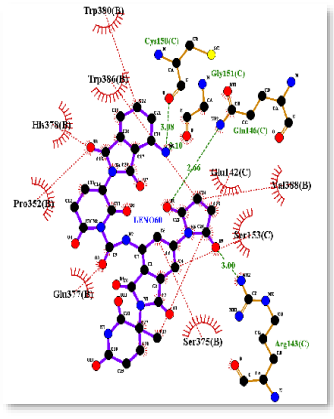
Drugs	**LD50 (mg/kg)**	**Cytotoxicity**	**Mutagenicity**	**Immunotoxicity**	**Carcinogenicity**	**Hepatotoxicity**
LENO	**700**	**Inactive**	**Inactive**	**Inactive**	**Inactive**	**Inactive**
LENO54	**700**	**Inactive**	**Inactive**	**Inactive**	**Inactive**	**Inactive**
LENO8	**1000**	**Inactive**	**Inactive**	**Inactive**	**Inactive**	**Inactive**
LENO60	**740**	**Inactive**	**Inactive**	**Active**	**Inactive**	**Inactive**
LENO38	**2232**	**Inactive**	**Inactive**	**Inactive**	**Inactive**	**Inactive**
LENO7	**700**	**Inactive**	**Inactive**	**Active**	**Inactive**	**Inactive**

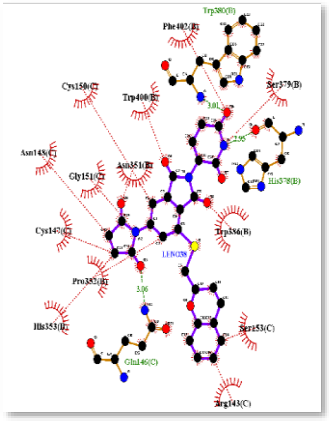
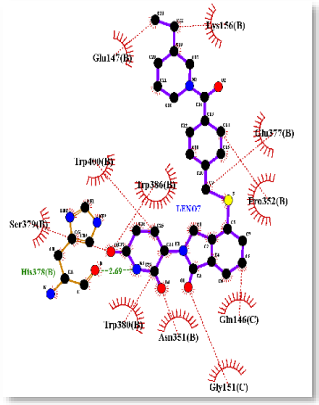
Molecular docking result

In the conducted study, each complex underwent 250 docking runs, and the conformation with the lowest binding energy was evaluated. The docking parameters, such as binding energy, inhibition constant, and intermolecular energy of Lenalidomide and the proposed analogs, are presented in Table 5.

Table 5: Binding Energy (BE in kcal/mol), Inhibition Constant [29] (Ki in nM), and Intermolecular Energy (IE in kcal/mol) of Hydrogen Bonds (green) and Hydrophobic Interactions (red) between the Parent Drug and Designed Analogues with the Protein.

Drugs	BE	Ki	IE	Hydrogen Bonds and Hydrophobic Interactions

LENO	-7.33	4240	-7.93	
LENO54	-12.38	0.85	-14.17	
LENO8	-10.03	44.10	-12.42	
LENO60	-8.83	336.51	-10.32	

LENO38	-11.04	8.03	-12.54	
LENO7	-10.59	17.23	-12.38	

Among the designed analogs, LENO54 demonstrated the lowest binding energy of -12.38 kcal/mol, indicating the strongest interaction with the Cereblon protein. This lower binding energy value suggests a higher binding affinity for LENO54 compared to other analogs. Additionally, the inhibition constant (Ki) for LENO54 was determined to be 0.85 nM, indicating its potent inhibition of Cereblon in the least concentration among all analogs. Moreover, the intermolecular energy for LENO54 was -14.17 kcal/mol, indicating robust interactions between LENO54 and the target protein.

Overall, these docking parameters highlight the superior binding capabilities of LENO54 to Cereblon, making it a promising candidate for effectively targeting and inhibiting the protein's functions.

LENO

Based on the docking results (Fig 1), Lenalidomide demonstrates several hydrophobic interactions with specific amino acids within the binding site. These interactions involve Ser379(B), Asn351(B), Trp400(B), Trp386(B), Gly151(C), Gln146(C), Pro352(B), Gly48(A), and Gly49(A). Furthermore, Lenalidomide establishes two hydrogen bonds with Trp380(B) at a distance of 2.99 and 3.29, along with another hydrogen bond with His378(B) at a distance of 2.45.

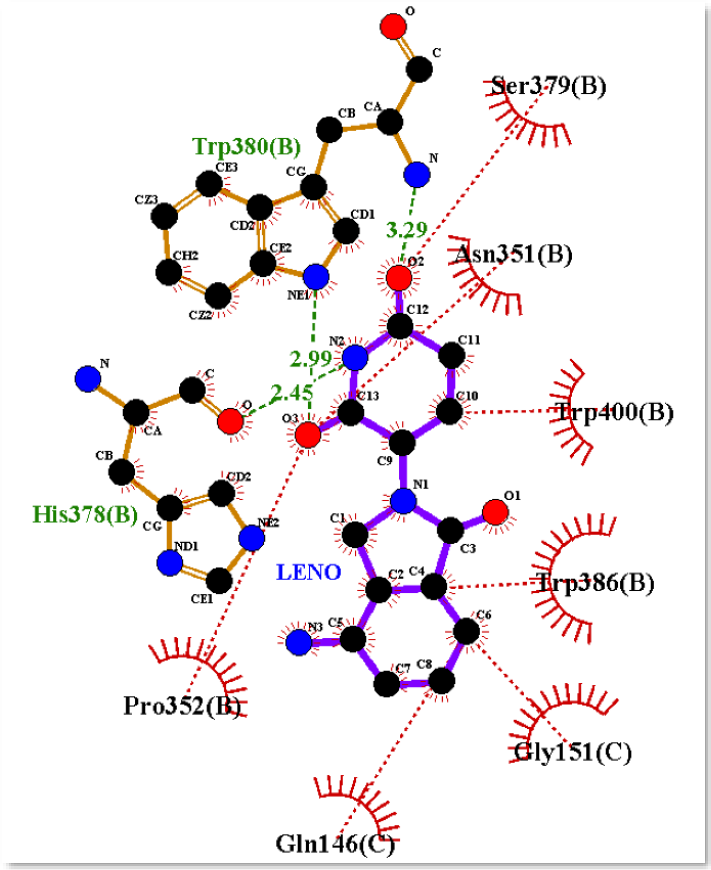


Fig 1: Hydrogen bonds and hydrophobic interactions of Lenalidomide with active site amino acids.

LENO54

In Fig 2, the lenalidomide analog LENO54 is shown to engage in significant hydrophobic interactions with specific amino acids within the binding site. These interactions involve Pro352(B), Trp380(B), Gly151(C), Asn351(B), His353(B), Trp386(B), Val388(B), Arg373(B), Ser375(B), and Gly372(B). These hydrophobic interactions contribute to the stability and binding affinity of LENO54 to the Cereblon protein. Additionally, LENO54 forms crucial hydrogen bonds with key amino acids. It establishes a hydrogen bond with His378(B) at a distance of 2.36, indicating a specific and directional attraction between the drug and the protein. Moreover, LENO54 forms two hydrogen bonds with Gln146(C) and Trp400(B) at distances of 2.68 and 3.01, respectively. Furthermore, it interacts with Ser153(C) and Arg143(C) through hydrogen bonds at distances of 2.84 and 3.00, respectively. These molecular interactions between LENO54 and the Cereblon protein suggest a strong and specific binding, potentially leading to potent inhibitory effects on the transcription factors IKZF1-3. The understanding of these interactions is crucial for optimizing drug design and developing effective therapeutic strategies for hematological malignancies and autoimmune disorders.

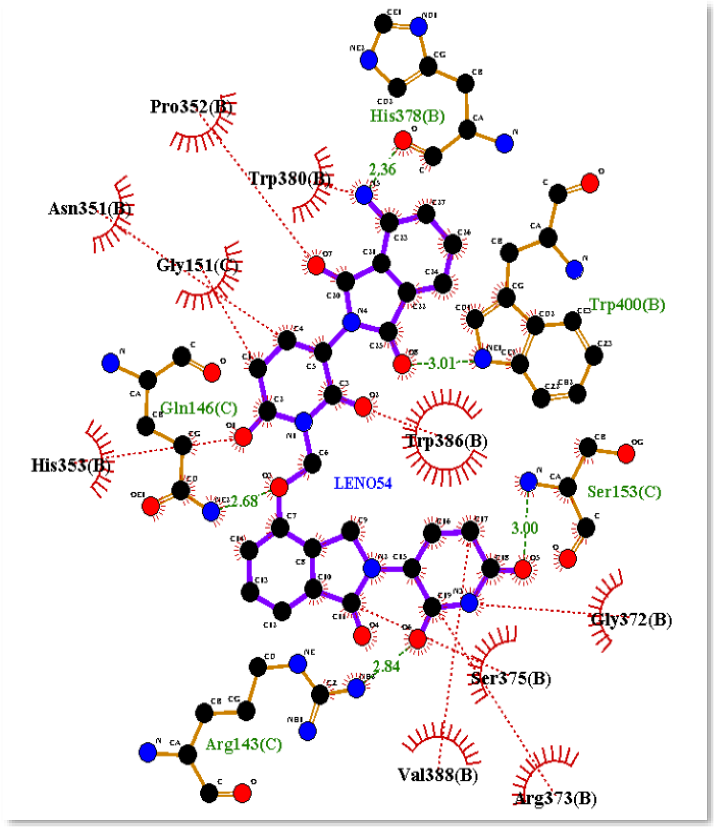


Fig 2: Hydrogen bonds and hydrophobic interactions of LENO54 with active site amino acids.

Discussion

The identified lenalidomide-based candidate, LENO54, shows promising potential as a novel drug capable of effectively binding to Cereblon and modulating its protein turnover functions. Through this mechanism, LENO54 is expected to exert a potent inhibitory effect on IKZF1-3 transcription factors [30], which are crucial players in the pathogenesis of various hematological malignancies and autoimmune disorders. Targeting these transcription factors holds great promise as a therapeutic strategy. LENO54's ability to interact with Cereblon and regulate IKZF1-3 factors makes it an attractive candidate for targeted therapy. By inhibiting these transcription factors, the drug has the potential to disrupt aberrant cellular processes responsible for disease progression in conditions such as hematological malignancies and autoimmune disorders. This targeted approach may offer improved therapeutic outcomes with fewer side effects compared to traditional therapies.

However, while the potential of LENO54 is encouraging, further preclinical studies and clinical trials are necessary to fully evaluate its therapeutic efficacy and safety profile. Rigorous testing and validation will be essential to establish the viability of LENO54 as a valuable therapeutic option for patients in need. These studies will also shed light on the drug's pharmacokinetics, pharmacodynamics, and potential interactions with other medications.

Conclusion

Finally, LENO54 exhibits promising potential as a drug candidate, opening doors to novel treatment strategies for managing hematological malignancies and autoimmune disorders. Its ability to target Cereblon and inhibit IKZF1-3 transcription factors holds great promise for therapeutic interventions in these diseases. However, further research and rigorous testing are imperative to thoroughly assess its therapeutic efficacy and safety profile. Rigorous testing and validation will be instrumental in determining the feasibility of LENO54 as a valuable therapeutic option for patients in need. The findings from this study pave the way for the development of novel treatment strategies in the management of hematological malignancies and autoimmune disorders, offering hope for improved patient outcomes and quality of life.

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Conflict of interest
None.

Financial support
None.

Ethics statement
I hereby declare that all stages of this study were conducted in accordance with the laboratory simulation protocols of the Advanced Technology Institute at Kerman Graduate University of Advanced Technology

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