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A novel pyridine-based Schiff base with multi-target pharmacological potential: Synthesis, biological evaluation, and molecular docking analysis

Fatih Çelik[1], Aleyna Nalçaoğlu Şenocak[2], Halil İbrahim Güler[3], Kadriye İnan Bektaş[3], Yasemin Ünver[1]*

[1] Karadeniz Technical University, Faculty of Sciences, Department of Chemistry, 61080, Trabzon, Türkiye
[2] Karadeniz Technical University, Institute of Science, Gene Engineering, 61080, Trabzon, Türkiye
[3] Karadeniz Technical University, Faculty of Science, Department of Molecular Biology and Genetics, 61080, Trabzon, Türkiye

Abstract

In the present study, a novel Schiff base derived from pyridine-4-carboxaldehyde and 4-aminobutan-1-ol was synthesized via a condensation reaction and purified by recrystallization from a DMF/water solvent system. The compound was characterized by IR, ^{1}H-NMR, and ^{13}C-NMR studies. The bioactivity of the synthesized compound was examined via cytotoxicity and enzyme inhibition assays. The compound exhibited concentration-dependent cytotoxicity, with relatively greater sensitivity observed in MCF-7 breast cancer cells compared to non-tumorigenic MCF-10A cells (IC_{50} = 6.5 mM and 21.8 mM, respectively), corresponding to moderate preferential activity toward cancer cells. In enzyme inhibition studies, the compound demonstrated dose-dependent inhibitory activity against HIV-1 reverse transcriptase, acetylcholinesterase, and α-glucosidase, with IC_{50} values ranging from 11 to 14 mM. Among the tested enzymes, HIV-1 RT exhibited relatively greater sensitivity within the examined concentration range. Although the observed activities occurred at millimolar concentrations, these findings indicate measurable multi-target bioactivity and suggest that the compound may represent a preliminary lead scaffold for further optimization. Molecular docking was employed to evaluate the binding behavior of a newly synthesized Compound I against HIV-1 reverse transcriptase, epidermal growth factor receptor, and acetylcholinesterase. Docking results revealed that Compound I exhibited moderate binding affinity toward all three targets, with a relative preference for acetylcholinesterase, as evidenced by favorable binding energies and key intermolecular interactions. Although its predicted affinities were lower than those of established reference inhibitors, Compound I demonstrated stable binding orientations and conserved interaction patterns within the active sites. These findings suggest that Compound I may serve as a promising lead scaffold for further structure-based optimization.

Keywords: Schiff base, enzyme inhibition, molecular docking

1. Introduction

Schiff bases, characterized by the presence of an azomethine (–CH=N–) functional group, constitute an important class of organic compounds formed typically through the condensation of primary amines with carbonyl compounds [1,2]. Owing to their straightforward synthesis, structural versatility, and tunable electronic properties, Schiff bases have attracted sustained interest in various areas of chemistry, including coordination chemistry, materials science, catalysis, and medicinal chemistry. Their rich donor–acceptor characteristics make them suitable candidates for both fundamental structural studies and applied research [3,4].

Recent studies have increasingly focused on Schiff bases derived specifically from pyridine-4-carboxaldehyde due to their versatile coordination behavior and promising biological properties. Several reports have demonstrated that pyridine-4-carboxaldehyde-based azomethine derivatives exhibit enhanced antimicrobial, anticancer, and enzyme inhibitory activities compared to analogous benzaldehyde-derived compounds [5]. In particular, recent investigations have highlighted their significant inhibitory effects against enzymes involved in nucleic acid metabolism and oxidative stress pathways, along with favorable binding interactions revealed by

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***Corresponding author:** Yasemin Ünver
e-mail: yasemincan@ktu.edu.tr
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molecular docking studies [6]. Moreover, structural modifications at the imine nitrogen or incorporation of additional hydrogen-bond-donating groups have been shown to significantly influence their spectroscopic characteristics and biological activity profiles [7]. These findings underline the importance of systematic structural and biological evaluation of pyridine-4-carboxaldehyde-derived Schiff bases.

Another important structural feature in Schiff bases is the incorporation of hydroxyl-functionalized aliphatic chains. The presence of a hydroxyl (–OH) group can promote intra- and intermolecular hydrogen bonding, which plays a crucial role in molecular conformation, crystal packing, and solubility [8,9]. Hydroxyl-containing Schiff bases often show enhanced stability and distinct infrared and nuclear magnetic resonance spectroscopic characteristics arising from hydrogen-bonding interactions. These features are particularly relevant in the interpretation of structural data and in understanding structure–property relationships.

Schiff bases may exist as E or Z geometrical isomers around the azomethine bond. In most cases, the E-configuration is thermodynamically favored due to reduced steric hindrance and increased conjugation [10,11]. The E-isomer is therefore more commonly isolated and reported, and it is generally associated with greater structural stability. Consideration of this geometrical preference is essential for accurate structural characterization and for comparison with related compounds reported in the literature. Schiff bases have attracted considerable interest in recent years due to their ability to exhibit a broad spectrum of biological activities, including anticancer and antiviral effects [12–14]. These compounds have been reported to demonstrate strong preclinical anticancer activities through various mechanisms, primarily involving the induction of DNA damage, the generation of reactive oxygen species (ROS), and the activation of apoptotic pathways [15]. In addition, enzymatic activities associated with reverse transcriptase and nucleic acid processing pathways are known to be frequently upregulated in rapidly proliferating cancer cells [16]. This characteristic renders such enzymes attractive molecular targets for cancer therapy.

Recent studies have revealed that compounds capable of interacting with reverse transcriptase or reverse transcriptase-related enzymatic pathways can influence not only viral replication but also DNA synthesis, cell cycle progression, and survival mechanisms of cancer cells. In this context, Schiff bases exhibiting inhibitory activity against HIV-1 reverse transcriptase (HIV-1 RT) have been reported to inhibit enzymes involved in pathologies related to nucleic acid metabolism and enzymatic regulation, including HIV-associated cancers, diabetes, and Alzheimer's disease [17–19]. This multifunctional biological activity profile highlights Schiff bases as promising molecular scaffolds for the development of next-generation antiviral and anticancer therapeutic agents.

Despite numerous studies on pyridine-containing Schiff bases, compounds that combine a pyridin-4-yl azomethine unit with an aliphatic hydroxyl group remain relatively underexplored, particularly with respect to their detailed spectroscopic characterization. Systematic studies focusing on such structures are necessary to expand the existing knowledge base and to provide reliable reference data for future investigations.

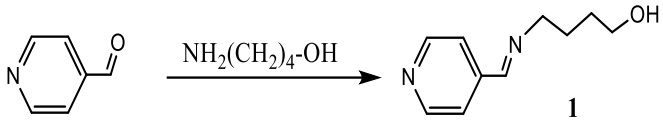
In this context, the present work reports the synthesis of (E)-4-((pyridin-4-ylmethylene)amino)butan-1-ol. The compound was characterized using FT-IR, ^{1}H NMR, and ^{13}C NMR spectroscopic techniques, and its structural features are discussed in relation to its functional groups. The novelty of the present study lies not only in the synthesis of a structurally simple pyridin-4-yl azomethine bearing a hydrogen-bond-donating hydroxyl group, but also in the integrated evaluation of its spectroscopic features, cytotoxic selectivity, multi-enzyme inhibition profile, and docking-supported interaction mechanisms within a single systematic framework. By correlating structural characteristics with biological response, this work provides new insights into structure–activity relationships of hydroxyl-substituted pyridinyl Schiff bases and proposes a multifunctional scaffold that may serve as a promising lead for further structure-based optimization in antiviral and anticancer drug development.

2. Experimental

2.1. Synthesis of (E)-4-((pyridin-4-ylmethylene)amino)-butan-1-ol (1)

Pyridine-4-carboxaldehyde and 4-aminobutan-1-ol were placed in a round-bottom flask and heated in an oil bath at 160–170 °C under continuous magnetic stirring for 2 h. The progress of the condensation reaction was monitored by Thin-Layer Chromatography (TLC). Upon completion of the reaction, as indicated by TLC, the reaction mixture was allowed to cool to room temperature. The resulting crude product was obtained as an oily residue. This material was subsequently purified by recrystallization from a DMF/water (1:3, v/v) solvent system to afford the desired Schiff base in purified form (Scheme 1). Yield: 80.78%. IR (KBr, cm^{-1}): 3249 (OH), 2987 (-CH), 1646 (C=N), 1598 (C=C); ^{1}H NMR (400 MHz, DMSO-d$_6$) δ: 1.43-1.50 (m, N-CH$_2$-C<u>H</u>$_2$, 2H), 1.62-1.69 (m, O-CH$_2$-C<u>H</u>$_2$, 2H), 3.43 (t, N-C<u>H</u>$_2$, 2H), 3.60 (t, O-C<u>H</u>$_2$, 2H), pyridine H [7.66 (d, 2H), 8.66 (d, 2H)], 8.36 (s, N=CH, 1H); ^{13}C NMR (100 Hz, DMSO-d$_6$) δ :

27.15 (N-CH_2-CH_2), 30.71 (O-CH_2-CH_2), 60.97 (N-CH_2;O-CH_2), pyridine C [122.05 (CH), 143.04 (C), 150.93 (CH)], 159.68 (N=CH).



Scheme 1. Synthetic pathway of compound 1

2.2. In silico ADME prediction

The physicochemical and drug-likeness properties of Compound I were evaluated using cheminformatics-based calculations. The MOL file of Compound I was converted into a canonical SMILES representation and analyzed using RDKit. Molecular weight (MW), lipophilicity (cLogP), topological polar surface area (TPSA), number of hydrogen bond donors (HBD), hydrogen bond acceptors (HBA), and rotatable bonds were calculated. Drug-likeness was assessed according to Lipinski's rule of five and Veber criteria to estimate the oral drug-likeness and pharmacokinetic suitability of the compound.

3. Results and discussion

3.1. Synthesis

The successful formation of imine functionality was clearly evidenced by spectroscopic analyses. In the IR spectrum, the characteristic absorption band observed at 1646 cm^{-1} corresponds to the azomethine (C=N) stretching vibration, confirming Schiff base formation. The presence of the hydroxyl group was supported by the broad absorption at 3249 cm^{-1}, while bands at 2987 cm^{-1} and 1598 cm^{-1} were assigned to aliphatic C–H and aromatic C=C stretching vibrations, respectively.

The ^1H NMR spectrum further substantiated the proposed structure. The azomethine proton appeared as a singlet at δ 8.36 ppm, a diagnostic signal for imine formation. The pyridine ring protons were observed as two doublets at δ 7.66 and 8.66 ppm, consistent with a para-substituted pyridine moiety. The aliphatic chain protons of the 4-aminobutan-1-ol fragment appeared as multiplets and triplets in the expected regions, with signals at δ 3.43 and 3.60 ppm corresponding to the N–CH_2 and O–CH_2 groups, respectively.

The ^{13}C NMR spectrum provided further confirmation of the molecular structure, displaying a distinct signal at δ 159.68 ppm attributable to the imine carbon (C=N). Signals corresponding to the pyridine carbons and aliphatic methylene carbons were observed in agreement with the proposed structure. Overall, the combined spectroscopic data unequivocally confirm the successful synthesis of the target Schiff base.

3.2. Biological activity

The present study aimed to investigate the multifunctional biological potential of the synthesized scaffold. While enzyme inhibition assays provide molecular-level evidence of target interaction, cytotoxicity assays were conducted to evaluate the broader biological impact of the compound. These experiments were designed to characterize the compound's overall bioactivity profile rather than to establish a direct mechanistic relationship between enzyme inhibition and cytotoxicity.

3.2.1. Determination of cellular viability

The effects of the tested compound on cell viability were evaluated using the MTT assay. The results demonstrated a concentration-dependent reduction in cell viability following 24 h of treatment in both cell lines. In the MCF-10A non-tumorigenic breast epithelial cell line, no significant cytotoxic effect was observed at concentrations of 10 mM and below, where cell viability remained close to that of the untreated control. However, higher concentrations resulted in a gradual decrease in viability, indicating dose-dependent cytotoxicity. In the MCF-7 breast cancer cell line, the compound exhibited a more pronounced reduction in cell viability across the tested concentration range. Treatment with 40, 20, 10, 5, 2.5, and 1.25 mM resulted in approximately 7%, 9%, 20%, 62%, 89%, and ~100% cell viability, respectively, after 24 h of exposure (Fig. 1). The calculated IC_{50} values were 21.8 mM for MCF-10A cells and 6.5 mM for MCF-7 cells, corresponding to a selectivity index of approximately 3.3. These findings indicate moderate preferential cytotoxicity toward cancer cells rather than complete selectivity.

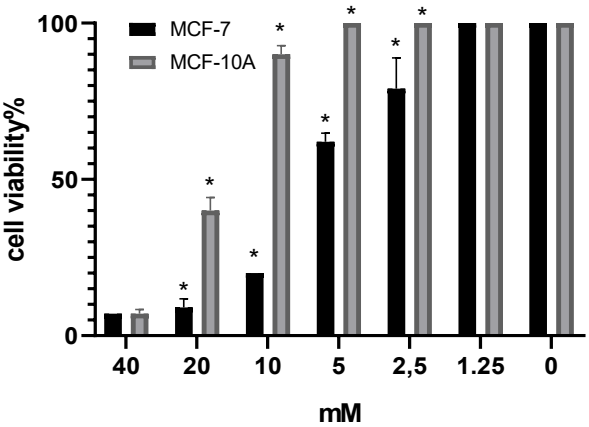


Figure 1. Effects of the synthesized compound on cell viability of MCF-7 breast cancer cells and MCF-10A non-tumorigenic breast epithelial cells following 24 h of treatment. *p<0.05 compared with MCF-10A at the same concentration.

3.2.2. Enzyme inhibition potential of compound

Based on the inhibition assay results, the compound exhibited a concentration-dependent inhibitory effect

against all enzymes tested. The inhibitory potential of the compound against HIV-1 reverse transcriptase (RT) was evaluated in vitro using a colorimetric assay. Treatment with the compound at concentrations of 20, 10, 5, 2.5, and 1.25 mM resulted in inhibition rates of 52.27%, 50.00%, 46.72%, 41.30%, and 36.10%, respectively. The IC_{50} value of the compound against HIV-1 RT was calculated as 11.09 ± 0.09 mM, whereas nevirapine, used as a positive control, exhibited a substantially lower IC_{50} value of 4.0 ± 0.05 μM (Fig. 2).

For acetylcholinesterase (AChE) inhibition, the compound demonstrated dose-dependent activity, with inhibition rates of 60.00%, 40.25%, 33.20%, 17.73%, and 0% at concentrations of 20, 10, 5, 2.5, and 1.25 mM, respectively. The IC_{50} value of the compound against AChE was determined as 13.08 ± 0.12 mM, while the reference inhibitor galantamine showed an IC_{50} value of 0.30 ± 0.05 mM (Fig. 2).

The inhibitory activity of the compound against α-glucosidase was assessed using the same concentration range to enable direct comparison of enzyme selectivity. The compound exhibited a concentration-dependent inhibitory profile, yielding inhibition rates of 57.90%, 42.30%, 29.00%, 11.90%, and 10.00% at 20, 10, 5, 2.5, and 1.25 mM, respectively. The IC_{50} value for α-glucosidase inhibition was calculated as 14.82 ± 0.09 mM, whereas acarbose, used as a standard inhibitor, displayed an IC_{50} value of 1.00 ± 0.05 mM (Fig. 2). In the present study, the pharmacological profile of the synthesized compound was evaluated using cell viability assays and enzyme inhibition studies. The compound demonstrated concentration-dependent cytotoxic effects in both cell lines, with relatively greater sensitivity observed in MCF-7 breast cancer cells compared to non-tumorigenic MCF-10A cells. The calculated IC_{50} values (6.5 mM for MCF-7 and 21.8 mM for MCF-10A) correspond to a selectivity index of approximately 3.3, indicating moderate preferential cytotoxicity toward cancer cells rather than absolute selectivity. Although the inhibitory activity was observed at millimolar concentrations and therefore reflects modest potency compared to reference compounds, the simultaneous multi-enzyme inhibition and antiproliferative effects observed at comparable dose levels suggest that the compound may serve as a preliminary lead scaffold for further structural optimization.

Furthermore, the synthesized compound exhibited IC_{50} values in the range of 11-14 mM against HIV-1 reverse transcriptase (RT), acetylcholinesterase (AChE), and α-glucosidase. Although the observed inhibitory effects occurred at millimolar concentrations, HIV-1 RT showed relatively greater sensitivity compared to the other enzymes within the tested concentration range. The ability of the compound to inhibit multiple biologically relevant enzymes at comparable dose levels highlights its broad bioactivity profile. However, given the modest potency observed, these findings should be interpreted as preliminary evidence of multi-target activity rather than definitive therapeutic efficacy. The compound may therefore be considered a preliminary lead scaffold requiring further structural optimization and mechanistic investigation.

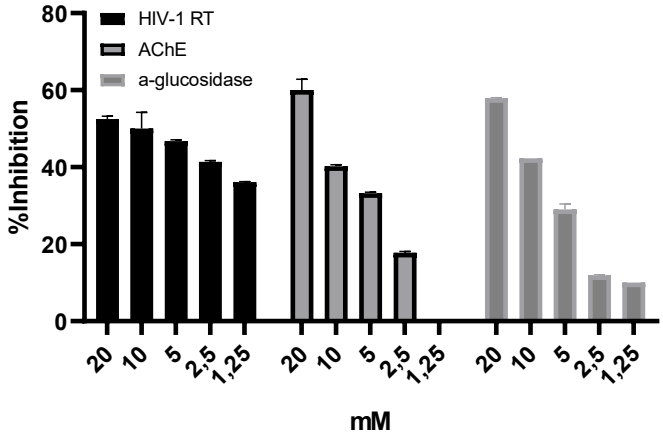


Figure 2. Concentration-dependent inhibitory effects of the synthesized compound against HIV-1 reverse transcriptase (RT), acetylcholinesterase (AChE), and α-glucosidase.

3.3. Molecular docking analysis

Molecular docking analyses were performed to investigate the binding behavior of the newly synthesized compound I against three therapeutically important targets HIV-1 reverse transcriptase (RT), epidermal growth factor receptor (EGFR), and acetylcholinesterase (AChE) and to compare its interaction profile with those of established reference inhibitors. The predicted binding energies, inhibition constants (K_i), hydrogen bond counts, and key interacting residues are summarized in Table 1, while the binding modes and interaction patterns are illustrated in Fig. 3 – Fig.5.

Docking studies of Compound I into the HIV-1 RT active site (PDB ID: 3LP1) revealed a moderate binding affinity, with a predicted binding free energy of −4.50 kcal/mol and an estimated K_i value of 501.53 μM (Table 1). The ligand established three conventional hydrogen bonds with Asn265, Asp498, and Ala538, with bond distances ranging from 1.90 to 2.04 Å, indicating geometrically favorable polar interactions. In addition, π–π stacking and T-shaped π interactions involving aromatic residues within the non-nucleoside inhibitor-binding pocket were observed (Fig. 3).

In contrast, the reference inhibitor nevirapine exhibited a substantially stronger binding affinity (−9.35 kcal/mol; K_i = 140.44 nM), consistent with its well-documented potency as a first-generation non-nucleoside reverse transcriptase inhibitor (NNRTI) [20].

Nevirapine formed multiple stabilizing hydrophobic and hydrogen-bond interactions with key residues such as Lys101, Tyr181, Tyr188, and Phe227, which are critical for NNRTI binding and allosteric inhibition of RT [21,22]. The weaker binding of Compound I relative to nevirapine suggests suboptimal occupancy of the NNRTI pocket, although the presence of conserved polar contacts indicates a binding orientation compatible with further structural optimization.

Within the EGFR kinase domain (PDB ID: 1M17), Compound I displayed a predicted binding energy of −4.67 kcal/mol and a K_i value of 378.28 μM (Table 1). The ligand formed three hydrogen bonds, notably with Lys721 and Arg752, residues located in the ATP-binding cleft that are known to participate in ligand recognition (Fig. 4). Additional van der Waals contacts with surrounding hydrophobic residues, including Met742 and Ala719, contributed to stabilization of the complex. However, the reference inhibitor erlotinib demonstrated a markedly superior binding profile (−7.13 kcal/mol; Ki = 5.94 μM), in agreement with its established role as a clinically approved ATP-competitive EGFR inhibitor [23]. Erlotinib's interaction network included multiple hydrogen bonds and extensive hydrophobic contacts within the hinge region and adjacent subpockets, a hallmark of potent EGFR inhibition [24]. Compared to erlotinib, Compound I showed reduced interaction density within the hinge region, which likely accounts for its weaker predicted affinity and suggests that structural features required for optimal hinge binding are not fully satisfied.

Among the three targets examined, Compound I exhibited its most favorable docking performance against acetylcholinesterase (PDB ID: 4EY7). The predicted binding free energy was −5.94 kcal/mol, with an estimated K_i of 44.44 μM and four hydrogen bonds formed within the active-site gorge (Table 1). Key interactions involved Ser292, Phe294, Arg295, and Phe296, residues located in the peripheral anionic site (PAS) and mid-gorge region of AChE (Fig. 5). Notably, π–π stacking interactions with aromatic residues such as Tyr340 further stabilized the ligand within the gorge, a feature frequently associated with effective AChE inhibitors [25,26]. The reference inhibitor donepezil showed significantly stronger binding (−11.55 kcal/mol; K_i = 3.42 nM), consistent with its high clinical potency and dual interaction with both the catalytic active site (CAS) and PAS of AChE [27]. Donepezil's interaction pattern included deep penetration into the catalytic gorge and extensive aromatic stacking with Trp86 and Tyr337, interactions that were only partially reproduced by Compound I. Nevertheless, the binding mode of Compound I suggests effective engagement with the PAS region, which is increasingly recognized as a promising target for multifunctional AChE inhibitors aimed at modulating both cholinergic signaling and amyloid aggregation [28].

Overall, comparative docking results indicate that Compound I exhibits moderate binding affinity toward all three targets, with a relative preference for AChE over EGFR and HIV-1 RT. While its predicted affinities are lower than those of the corresponding reference inhibitors, the observed interaction patterns, particularly hydrogen bonding and aromatic stacking within functionally relevant regions support the validity of its binding poses (Fig. 3 – Fig.5). These findings suggest that Compound I may serve as a viable lead scaffold, especially for AChE inhibition, and that rational structural modifications could enhance its target specificity and binding strength.

3.4. ADME prediction and drug-likeness evaluation

To further support the lead-like character of Compound I, in silico ADME and drug-likeness analyses were performed using cheminformatics-based calculations. The predicted physicochemical parameters are summarized in Table 2. Compound I satisfies Lipinski's rule of five without any violations, indicating favorable drug-like characteristics. The molecular weight (178.23 g/mol) and lipophilicity (cLogP = 1.27) fall well within the acceptable range for orally active small molecules.

Table 1. Predicted binding energies, inhibition constants (K_i), hydrogen bond counts, and key interacting residues of Compound I and reference inhibitors docked against HIV-1 reverse transcriptase, EGFR, and acetylcholinesterase.

Target	Ligand	Predicted Binding Energy (kcal/mol)	Predicted K_i value	Number of HBs	Interacting Key Residues
HIV-1 RT (3LP1)	Compound I	-4.50	501.53 μM	3	Asn265, Asp498, Trp535, Ala538
	*nevirapine	-9.35	140.444 nM	3	Val179, Leu100, Lys101, Lys103, Val106, Tyr181, Tyr188, Phe227, Leu234, Tyr318
EGFR (1M17)	Compound I	-4.67	378.28 μM	3	Lys721, Glu738, Met742, Arg752, Gln767, Asp831
	*erlotinib	-7.13	5.94 μM	5	Leu694, Ala719, Lys721, Leu764, Gln767, Met769, Cys773, Leu820
AChE (4EY7)	Compound I	-5.94	44.44 μM	4	Ser292, Phe294, Arg295, Phe296, Tyr340
	*donepezil	-11.55	3.42 nM	2	Trp86, Ser292, Phe294, Tyr336, Phe337, His446

*Reference molecules

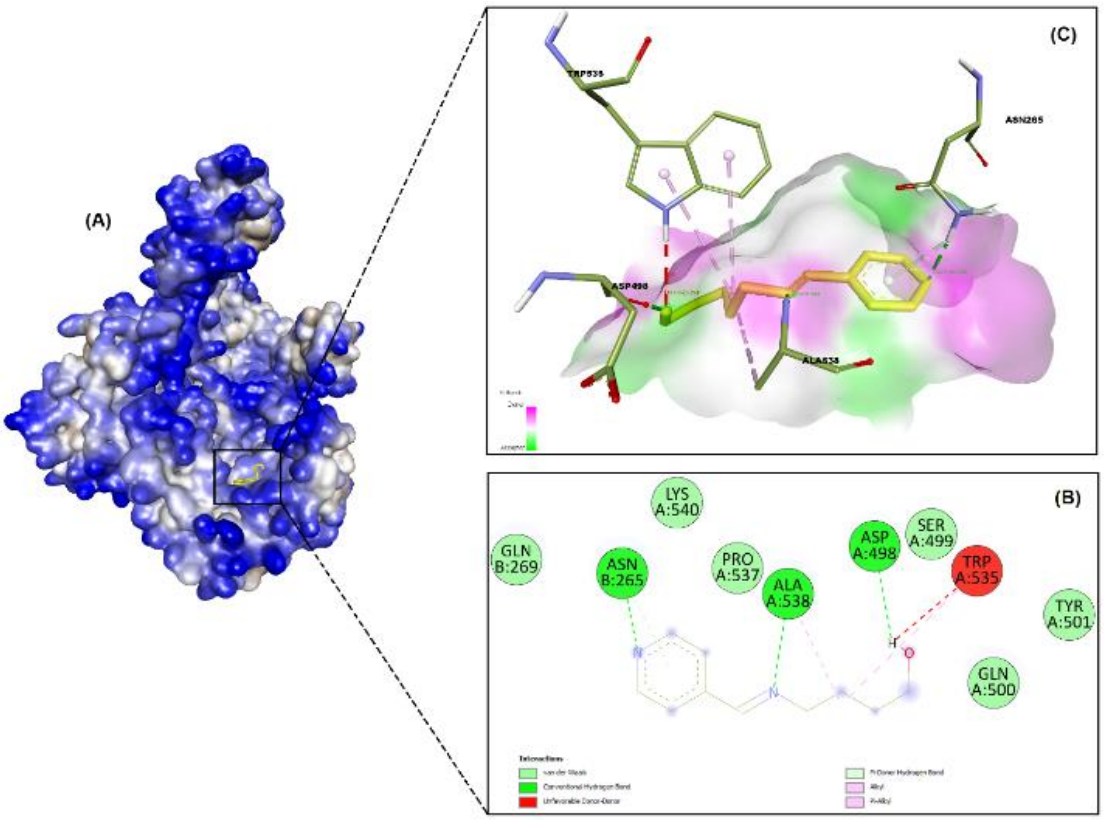


Figure 4. Binding mode of Compound I within the HIV-1 reverse transcriptase active site. (A) Surface representation showing the localization of Compound I in the NNRTI-binding pocket. (B) Two-dimensional interaction diagram illustrating hydrogen bonds and π–π interactions with key residues. (C) Three-dimensional close-up view highlighting the binding orientation and intermolecular interactions of Compound I within the active site.

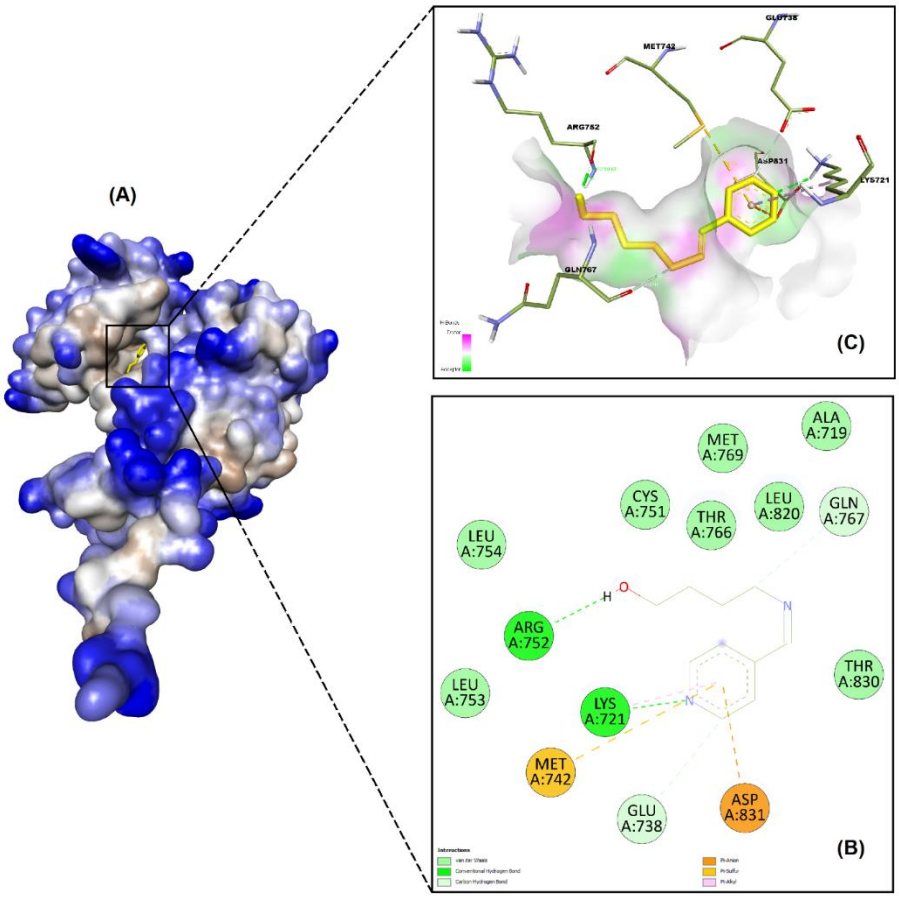


Figure 3. Docking pose of Compound I in the ATP-binding site of EGFR. (A) Surface representation of EGFR indicating the binding cavity occupied by Compound I. (B) Two-dimensional interaction map showing hydrogen bonding and hydrophobic interactions with critical kinase residues. (C) Three-dimensional representation of the ligand–protein complex emphasizing key stabilizing contacts within the ATP-binding cleft.

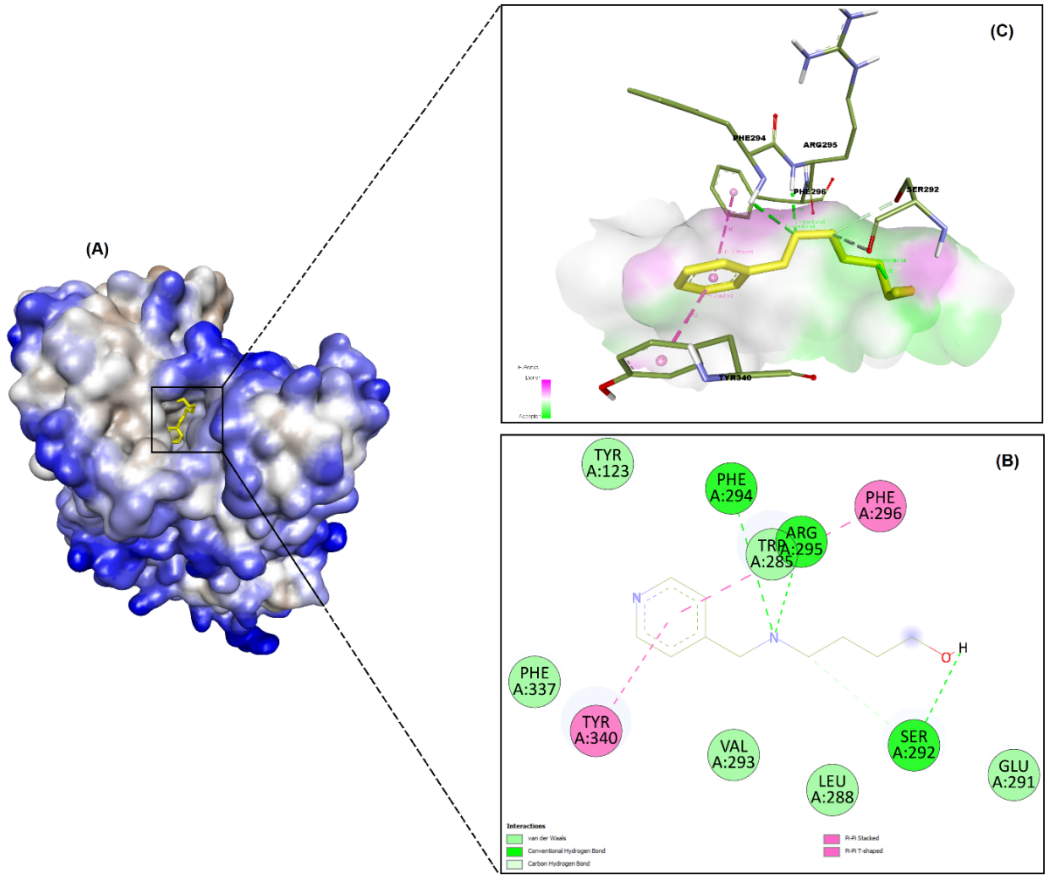


Figure 5. Predicted binding mode of Compound I in the active-site gorge of acetylcholinesterase. (A) Surface representation depicting ligand accommodation within the enzyme gorge. (B) Two-dimensional interaction diagram highlighting hydrogen bonds and aromatic π–π interactions with peripheral and mid-gorge residues. (C) Three-dimensional close-up view showing the binding orientation of Compound I and its interactions with key AChE residues.

In addition, the compound exhibits a moderate topological polar surface area (TPSA = 45.48 Å²) and a limited number of rotatable bonds (5), satisfying Veber criteria associated with good oral bioavailability. The presence of one hydrogen bond donor and three hydrogen bond acceptors further support balanced polarity and interaction potential.

Overall, these predicted ADME-related properties suggest that Compound I possesses suitable physicochemical characteristics for a lead scaffold, complementing the molecular docking results and supporting its potential for further structure-based optimization.

Table 2. Predicted physicochemical and drug-likeness properties of Compound I.

Parameter	Value
Molecular weight (g/mol)	178.23
cLogP	1.27
H-bond donors (HBD)	1
H-bond acceptors (HBA)	3
Rotatable bonds	5
TPSA (Å²)	45.48
Lipinski rule violations	0
Veber rule compliance	Yes

3.5. Structure–Activity Relationship (SAR) Analysis

From a structure–activity relationship (SAR) perspective, the biological profile of (E)-4-((pyridin-4-ylmethylene)amino)butan-1-ol can be correlated with its distinct pharmacophoric architecture. The molecule integrates a heteroaromatic pyridine ring, an azomethine (–CH=N–) linkage, and a flexible hydroxyl-terminated aliphatic chain, each of which may contribute to target recognition and binding stability. The azomethine (imine) functionality represents a well-established bioactive motif in Schiff bases. The conjugated –CH=N– group contributes to partial planarity and electron delocalization, which may facilitate stable ligand–enzyme complex formation. Additionally, the imine nitrogen can act as a coordination or hydrogen bond acceptor site, supporting molecular recognition processes. Numerous studies have associated this pharmacophore with cytotoxic and enzyme inhibitory activities, suggesting its central role in the observed biological effects.

4. Conclusion

In conclusion, a novel Schiff base derived from pyridine-4-carboxaldehyde and 4-aminobutan-1-ol was successfully synthesized and structurally confirmed by

comprehensive spectroscopic analyses (IR, ^{1}H-NMR, and ^{13}C-NMR), establishing the reliability of its molecular framework. The biological evaluation demonstrated concentration-dependent cytotoxic effects with moderate preferential activity toward MCF-7 cells compared to MCF-10A cells. The compound also exhibited dose-dependent inhibition of HIV-1 reverse transcriptase, acetylcholinesterase, and α-glucosidase at millimolar concentrations, indicating modest multi-target activity. Molecular docking studies provided mechanistic insight into the observed biological activities. The compound demonstrated stable binding conformations within the active sites of HIV-1 reverse transcriptase, EGFR, and acetylcholinesterase. Particularly in acetylcholinesterase, favorable hydrogen bonding interactions and π–π stacking within catalytically significant regions were observed, supporting its experimental inhibitory activity. Although the calculated binding affinities were lower than those of reference inhibitors, the consistent interaction patterns and stable docking poses indicate that the compound possesses a structurally competent pharmacophore suitable for further optimization.

Data availability

The data supporting the findings of this study are available within the published article and its Supplementary File.

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Disclosure of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of Funding

No funding was received for this study.

Use of Generative AI

The authors used generative artificial intelligence tools only for language editing and improving the readability of the manuscript. All scientific content, data interpretation, and conclusions were created and verified by the authors.

Ethics Statement

This study did not involve human participants or live animals; therefore, ethical approval was not required.

Author Contributions

Fatih Çelik: Investigation, methodology, synthesis, and experimental studies.

Aleyna Nalçaoğlu Şenocak: Investigation and biological activity studies.

Halil İbrahim Güler: In silico studies, data analysis, and writing—original draft preparation.

Kadriye İnan Bektaş: Biological activity studies, data curation, and writing—review and editing.

Yasemin Ünver: Conceptualization, supervision, project administration, methodology, and writing—review and editing.

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