RESEARCH ARTICLE

In-Silico ADME, Toxicity and Molecular Docking Studies of Dihydropyrimidine Derivatives as Dual Dihydrofolate Reductase and DPP-4 Inhibitors

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ABSTRACT

Objectives: The present study aimed to design, synthesize, and evaluate novel compounds targeting dihydrofolate reductase (DHFR) and dipeptidyl peptidase-4 (DPP-4) for potential dual therapeutic benefits in cancer and type 2 diabetes management.

Methods: A library of designed compounds was subjected to molecular docking against DHFR (PDB ID: 1W78) and DPP-4 (PDB ID: 2ONC) using AutoDock Vina. The optimization and screening of lead molecules were guided by a structured computational workflow, including ADME profiling, toxicological assessment, and binding interaction analysis. A factorial design approach was employed to systematically assess structural features influencing binding affinity.

Result: Docking studies (Table 1) revealed high binding affinities, with 1W (-13.6 kcal/mol) for /1[-1DHFR and 1G (-12.4 kcal/mol) for DPP-4 as top performers. ADME analysis (Table 3) indicated low GI absorption, no BBB penetration, and uniform bioavailability (0.17) for most compounds, alongside acceptable skin permeation values. Toxicity assessment (Table 4) identified the majority as non-mutagenic and non-reproductive toxicants, though some displayed hepatotoxicity. The optimized leads were selected based on superior docking scores, favorable pharmacokinetics, and minimal predicted toxicity.

Conclusion: The optimized compounds demonstrated strong binding affinity towards both DHFR and DPP-4, along with favorable ADME profiles and low predicted toxicity, highlighting their potential as dual inhibitors. These findings suggest promising candidates for future therapeutic development. Further in vitro and in vivo validation studies are warranted to confirm their efficacy and clinical applicability.

Keywords: DHFR; DPP-4; Molecular docking; ADME profiling; Toxicity assessment; Dual inhibitor; Factorial design.

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INTRODUCTION

Prevalence of type 2 diabetes mellitus (T2DM) and antimicrobial resistance is also rapidly rising across the world, which essentially becomes a triple crisis to health systems globally[1]. Hundreds of millions of people are affected by and T2DM is a major cause of morbidity and mortality, as well as diminished quality of life[2]. Concurrently, microbial infections especially those caused by resistant bacterial strains have also emerged as a big threat to the health of the people necessitating long periods of illness, rising medical expenses, and enhanced rates of mortality[3]. Most patients, more so diabetics, become easily vulnerable to infections because of the compromised immune responses and damage caused by hyperglycemia on tissues. Numerous therapeutic agents are frequently required because of this clinical overlap[4]. Nonetheless, monotherapies, especially the current ones, have low efficacy, experience adverse effects, and have shown development of resistance, whereas polypharmacy enhances drug-drug interactions building obstacles to patient compliance[5]. The emergence of these challenges highlights immense necessity in new therapeutic plans that can address both microbial pathogens and the targets of diabetic metabolic pathways[6]. Dual-targeting is an emerging approach with potential to increase efficacy, minimize side effect, and streamline disease management and are likely to be an avenue of future therapeutics[7].

Dihydropyrimidine derivatives have demonstrated a privileged heterocyclic scaffold with many biological activities, and many researchers have focused on these compounds and condensed them in medicinal chemistry during the recent past[8]. The high affinity and selectivity affinity that they exert on a variety of biological targets is

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as a result of their structural similarity to naturally occurring pyrimidines[9].

They have the potential to act as inhibitors of important enzymes in the context of antimicrobial action as well as glucose regulation, including dihydrofolate reductase (DHFR) and dipeptidyl peptidase-4 (DPP-4), which has

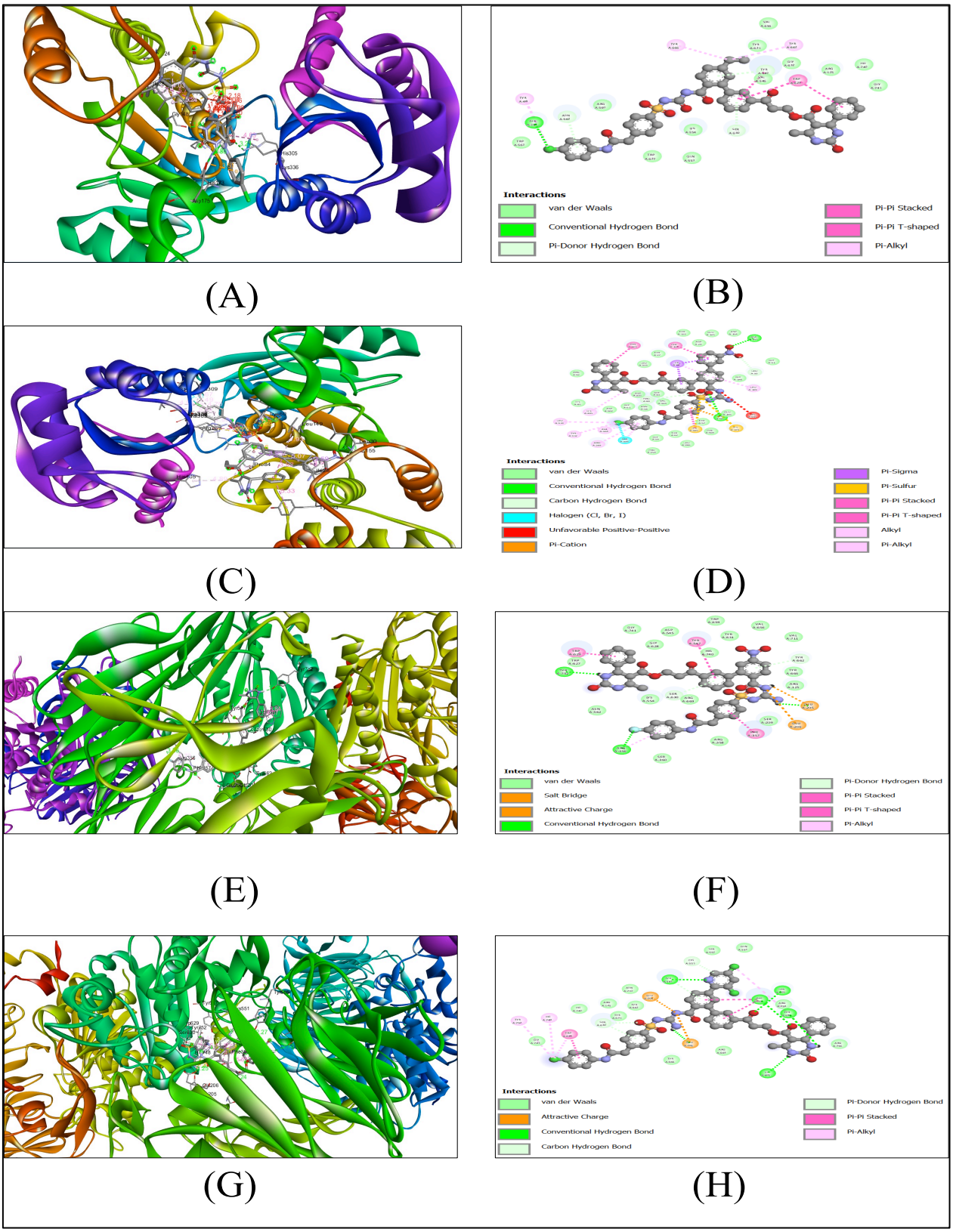

Figure 1: Binding interaction diagrams where A–B show Compound 1W (A: 3D docking pose; B: 2D interaction map) and C–D show Compound 1K (C: 3D; D: 2D) against DHFR (PDB: 1W78), while E–F show Compound 1G (E: 3D; F: 2D) and G–H show Compound 1L (G: 3D; H: 2D) against DPP-4 (PDB: 2ONC), highlighting key H-bonds, hydrophobic contacts, and π-interactions in the active sites.

been shown in previous studies[10]. The inhibition of both DHFR and DPP-4 generates undesirable effects that affect the microorganism metabolism of folate by disrupting DNA synthesis and cell replication in microorganisms and increasing the increment activity of glycemic control, respectively, in T2DM. Rationalising a molecule that will halt the activities of both targets to help reduce infection risks in diabetic patients, in addition to confronting hyperglycemia, is worth a designing strategy[11].

Dihydropyrimidine derivatives have good physicochemical properties and synthetic flexibility and can tolerate a wide range of substitutions, which underlines their potential to become a promising candidate to be screened computationally. Application of in-silico ADME screening, prediction of toxicity, and molecular docking studies as a part of their design speed up in finding strong, selective, and afterward, drug-like molecules to be studied[12].

The basic research on structure activity relationship (SAR) investigations have indicated that the modification at certain sites on the dihydropyrimidine ring has a profound role in the antimicrobial and anti-diabetic action[13]. The C4, C5, and C6 locations could be modified to regulate the hydrogen bonding, the hydrophobic interaction and the pi-pi stacking with the active site residues of DHFR and DPP-4. Aromatic exchanges can increase 1-interactions within hydrophobic crevices and large or lipophilic branches can contribute to a higher fitting and stability in enzyme binding pores[14]. Substituents that are electron-withdrawing or halogen can increase metabolic stability, electronic distribution, and the orientation of binding to increase potency. Computational analysis can be performed to screen these types of alterations in a predictive manner so that primary selection of the derivatives can be rationalized to have balanced affinity to each of DHFR and DPP-4. SAR knowledge can be combined with in-silico pharmacokinetics and toxicity predictions in a way that will allow researchers to identify the profiles with the best pharmacodynamic/safety properties in a systematic fashion[15].

This work endeavors to conduct in-silico ADME, toxicity profile, and molecular docking of new dihydropyrimidine derivatives to determine their potentially dual effect as the DHFR and DPP-4 inhibitor. The main aims are to evaluate drug likeness, anticipate safety, and interpret molecular interaction trends, so that promising lead compounds can be selected to prepare and then test biologically in both antimicrobial and antidiabetic applications.

MATERIALS AND METHODS

Materials

Dihydropyrimidine derivatives (analytical grade, synthesized as per standard protocols) were obtained from Sciquaint Innovations Pvt. Ltd. (Pune, India). Methanol (HPLC grade, ≥99.9% purity) and ethanol (analytical grade, ≥99.8% purity) were procured from Sciquaint Chemicals (Pune, India). Dimethyl sulfoxide (analytical grade, ≥99.5% purity) and phosphate buffer salts (analytical grade) were purchased from Neeta Chemicals (Pune, India). All other chemicals and reagents used in the study were of analytical grade and supplied by Research Lab Fine Chem Industries (Pune, India).

Table 1: Molecular Docking Scores of Designed Compounds Against DHFR (PDB ID: 1W78) and DPP-4 (PDB ID: 2ONC)

S.N.	Molecule	Binding affinity
DHFR (PDB ID: 1W78)		
1	1A	-10.4
2	1AA	-12.2
3	1B	-11.3
4	1BB	-12.9
5	1C	-12.3
6	1CC	-10.7
7	1D	-11.0
8	1E	-12.0
9	1F	-10.3
10	1H	-12.0
11	1I	-12.7
12	1J	-12.2
13	1K	-13.2
14	1L	-12.1
15	1M	-11.4
16	1N	-12.8
17	1O	-10.5
18	1P	-11.6
19	1Q	-11.8
20	1R	-11.8
21	1S	-12.9
22	1T	-12.6
23	1U	-10.1
24	1V	-11.5
25	1W	-13.6
26	1X	-12.3
27	1Y	-11.6
28	1Z	-13.0
DPP-4 (PDB ID: 2ONC)		
29	1A	-10.5
30	1AA	-11.4
31	1B	-10.5
32	1BB	-10.9
33	1C	-9.6
34	1CC	-10.3
35	1D	-9.8
36	1DD	-10.1
37	1E	-10.8
38	1F	-9.8
39	1G	-12.4
40	1H	-10.4
41	1I	-9.9
42	1J	-11.3
43	1K	-10.9
44	1L	-11.6
45	1M	-11.1
46	1N	-11.2
47	1O	-10.7
48	1P	-11.2
49	1Q	-10.4
50	1R	-11.1
51	1S	-10.7
52	1T	-10.3
53	1U	-10.4
54	1V	-11.8
55	1W	-9.0
56	1X	-11.1
57	1Y	-10.7

Table 2: Selected Compounds for Synthesis Based on Molecular Docking Results, Showing Key Amino Acid Interactions, Bond Lengths, and Docking Scores

Compound Name	Amino Acid	Bond Length (Å)	Docking Score
Compound 1W	LYS336, HIS173, GLY148, ASP175, LYS60, ASP175	3.21437, 2.7259, 1.9449, 2.80194, 4.04789, 3.51102	-13.6
Compound 1K	LYS60, ALA155, PRO84, HIS405, LIG1, LIG1	3.29747, 3.13018, 3.47497, 3.71091, 2.66176, 3.59429	-13.2
Compound 1Z	LIG1, GLY148, LIG1, LIG1, ILE28, ILE28	2.9668, 1.86674, 3.51129, 2.82179, 3.6476, 3.65757	-13.0
Compound 1BB	LYS60, ARG92, LIG1, LIG1, HIS305, LYS336	3.36681, 3.38514, 2.58535, 2.94848, 3.00964, 2.98865	-12.9
Compound 1S	THR122, TYR123, ARG289, HIS85, HIS305, ASP302	3.20848, 3.22325, 3.39174, 3.69593, 3.59686, 3.23106	-12.9
Compound 1G	GLU205, GLU206, ARG356, TYR752, SER630, TYR662, PHE357, TRP629, TYR547	2.64482, 3.87424, 3.36381, 1.93549, 1.89875, 3.56193, 3.81148, 4.43009, 4.06968, 3.84274, 5.47008, 4.73446	-12.4
Compound 1L	GLU205, GLU206, SER209, PHE357, TYR547, ILE405, TYR585, SER630, CYS551, TRP629, HIS748, TYR752	4.29185, 4.32689, 3.24305, 3.31298, 3.29199, 2.24512, 1.94463, 2.73144, 2.26714, 3.2437, 3.45765, 3.98815, 3.48179, 5.2186, 4.15922, 4.42525, 4.13287, 5.11052, 5.50726, 4.77797, 3.97704, 5.29915, 5.44024	-11.6
Compound 1V	TYR585, TYR547, TYR752, GLU361, SER360, TYR662, PHE357, TRP629, TYR666, ARG356, ILE374, VAL656	2.87608, 2.16216, 2.11576, 4.83857, 4.18043, 3.99278, 3.86263, 4.27398, 5.4088, 4.18991, 3.6965, 4.46126, 4.99819, 4.83652, 3.74175, 4.76532, 4.63825, 5.41134	-11.8
Compound 1AA	ARG125, LYS554, TYR662, TYR547, VAL546, SER630, PHE357, TRP627, TRP629, TYR585	3.14545, 3.31869, 3.00868, 2.77587, 2.36178, 2.28598, 3.4837, 3.89558, 3.51383, 3.73315, 4.50276, 3.95953, 5.37407, 5.34671, 4.19889	-11.4
Compound 1G	TYR547, TYR585, TYR752, PHE357, TYR666, TRP629, GLU361, ARG356, ILE374, VAL656	2.87214, 2.18963, 2.09877, 4.81524, 4.17233, 3.98721, 3.85432, 4.26895, 5.40211, 4.18027, 3.68892, 4.45218, 4.99112, 4.82817, 3.73428, 4.75892, 4.62849, 5.40115	-11.2

Methods

Ligand Preparation

Ligand preparation was intended to produce optimized 2-dimensional (2D) molecular structures of the chosen dihydropyrimidine derivatives to be used in a molecular docking study. Chemical structures have been modelled and depicted by the means of ChemDraw software (PerkinElmer Informatics, USA) that provides the appropriate valency and stereochemistry. Every structure made was saved as MOL file to preserve structure and dock compatibility. The resultant MOL files were then reconstructed as input ligand molecules into molecular docking studies. Further energy minimization was not conducted during this phase since optimization was done during docking workflow[16,17].

Selection of Target Receptor

The study constituted the dual-target docking against DHFR (Dihydrofolate Reductase; PDB ID: 1W78) and DPP-4 (Dipeptidyl Peptidase-4; PDB ID: 2ONC) which were chosen on the basis of their known functions in the microbial folate metabolism and the glucose regulation respectively. DHFR plays a critical role in the folate pathway of microorganisms and prevention through inhibition of the enzyme has been proven successful in breaking down nucleic acid production, hence a validated target in antimicrobial action. DPP-4 is highly involved in the clearance of incretin hormones and its inhibitor on type 2 diabetes mellitus improves their glycemic control. Targets were specific and were chosen based on the capacity to provide two therapeutic effects in both treatment of microbial infections and diabetes. Protein Data Bank (https://www.rcsb.org/) was used as the source of target structures that were downloaded in PDB format, and then prepared[18,19].

Protein Preparation

Protein preparation was aimed to optimize the receptor structures that have been retrieved in order to perform docking simulations. The structures of DHFR (PDB ID: 1W78) and DPP-4 (PDB ID: 2ONC) protein were downloaded in PDB format in Protein Data Bank (https://www.rcsb.org/). Discovery Studio Visualizer (version 4.5, BIOVIA, USA) allowed water molecules, heteroatoms, and any unnecessary ligands to be removed so that they do not interfere with a ligand binding. These processed structures were then written to PDB format that would later be fed into the molecular docking work process. The active site residues were not altered structurally so as to conserve the native binding conformation[20,21].

Molecular Docking

Molecular docking was performed to predict and analyse the binding affinities and patterns of the interaction of the dihydropyrimidine derivatives with the admired target proteins.

AutoDock Vina (version 1.1.2, The Scripps Research Institute, USA), together with Discovery Studio Visualizer (version 4.5, BIOVIA, USA) was used in docking simulations and visualization as well as analysis.

Ligand MOL files were transformed to PDBQT format whereas prepared protein structures, too, were transformed into PDBQT format after the addition of polar hydrogens with the help of AutoDock Tools, and the assignment of Gasteiger charges. A grid box around the active site residues was setup with the size, size_x=30.0A, size_y=30.0A, sizez=30.0A, and center, center_x= 40.105821, center_y= 18.822649, center_z= 22.698874.

Docking was used with a default exhaustiveness and the binding results poses and scores were examined in the Discovery Studio Visualizer to capture the hydrogen bonds and other important molecular contacts involving hydrophobic bonds[22,23].

Drug-Likeness and In-Silico ADME Prediction

Pharmacological characterization was assessed based on drug-likeness features and in-silico ADME (Absorption, Distribution, Metabolism and Excretion) of the chosen dihydropyrimidine derivatives through SwissADME (http://www.swissadme.ch/). Each compound was uploaded as MOL files, and it calculated parameters such as molecular mass, lipophilicity (LogP), number of hydrogen atom donors and acceptors, topological polar surface area (TPSA) and predicted gastrointestinal absorption, blood-brain barrier penetrability and bioavailability score. Based on the established pharmacokinetic principles, any prediction was done to examine the aspect of drug-likeness under Lipinski Rule of Five and the associated filters of medicinal chemistry[24,25].

Toxicity Analysis

Toxicities of all the dihydropyrimidine derivatives were also predicted via ProTox-II web-based tool on toxicity prediction (https://tox-new.charite.de/protox_II/). MOL files of the ligands were uploaded to the tool and yielded computational predictions in the three categories as outlined by mutagenicity, carcinogenicity, hepatotoxicity, skin sensitization and acute oral toxicity. Advanced QSAR-based algorithms and toxicophore mapping have been used to analyze the possible adverse effects probability. The efficacy was predicted and the toxicity end points were used in prioritizing the compounds that would be further developed[26].

Table 3: ADME Profiling of Designed Compounds Including Absorption, Distribution, Metabolism, Excretion, and Bioavailability Parameters

compound	Absorption		Distribution		Metabolism	Excretion	Bioavailability
	GI absorption	BBB permeation	skin permeation	Volume of distribution		Total Clearance	
Compd 1	Low	No	-8.23 cm/s	-1.001	P-gp substrate	-0.006	0.17
Compd 2	Low	No	-7.42 cm/s	-1.064	CYP2C9 inhibitor and P-gp substrate	-0.575	0.17
compd 3	Low	No	-7.22 cm/s	-0.973	P-gp substrate	-0.717	0.17
compd 4	Low	No	-7.12 cm/s	-0.669	P-gp substrate	0.095	0.17
compd 5	Low	No	-7.35 cm/s	-0.653	P-gp substrate	0.154	0.17
compd 6	Low	No	-8.20 cm/s	-1.102	P-gp substrate	0.039	0.17
compd 7	Low	No	-8.16 cm/s	-0.61	P-gp substrate	0.007	0.17
compd 8	Low	No	-7.97 cm/s	-1.121	P-gp substrate	0.031	0.17
compd 9	Low	No	-7.90 cm/s	-0.654	P-gp substrate	0.044	0.17
compd 10	Low	No	-7.35 cm/s	-0.71	P-gp substrate	0.191	0.17
compd 11	Low	No	-7.20 cm/s	-0.597	P-gp substrate	-0.169	0.17
compd 12	Low	No	-6.70 cm/s	-0.712	P-gp substrate	-0.13	0.17
compd 13	Low	No	-6.73 cm/s	-0.771	P-gp substrate	0.302	0.17
compd 14	Low	No	-6.43 cm/s	-0.635	P-gp substrate	-0.056	0.17
compd 15	Low	No	-6.78 cm/s	-0.517	P-gp substrate	-0.118	0.17
compd 16	Low	No	-7.81 cm/s	-0.617	P-gp substrate	-0.321	0.17
compd 17	Low	No	-7.07 cm/s	-0.754	P-gp substrate	-0.877	0.17
compd 18	Low	No	-7.05 cm/s	-0.792	P-gp substrate	-0.706	0.17
compd 19	Low	No	-6.26 cm/s	-0.772	P-gp substrate	-0.778	0.17
compd 20	Low	No	7.10 cm/s	-0.787	P-gp substrate	-0.714	0.11
compd 21	Low	No	-6.32 cm/s	-0.777	P-gp substrate	-0.703	0.17
compd 22	Low	No	-7.24 cm/s	-0.822	P-gp substrate	-0.605	0.17
compd 23	Low	No	-6.80 cm/s	-1.131	P-gp substrate	-0.658	0.17
compd 24	Low	No	-6.95 cm/s	-0.722	P-gp substrate	0.154	0.17
compd 25	Low	No	-6.63 cm/s	-0.586	P-gp substrate	-0.046	0.17
compd 26	Low	No	-6.19 cm/s	-0.662	P-gp substrate	-0.107	0.17
compd 27	Low	No	-7.02 cm/s	-1.1	P-gp substrate	-0.628	0.17
compd 28	Low	No	-7.00 cm/s	-0.62	P-gp substrate	-0.007	0.17
compd 29	Low	No	-6.25 cm/s	-0.635	P-gp substrate	-0.056	0.17
compd 30	Low	No	-7.17 cm/s	-0.634	P-gp substrate	0.099	0.17

Table 4: Toxicological Risk Assessment of Designed Compounds Including Hepatotoxicity, Skin Sensitization, Mutagenicity, Tumorigenicity, and Reproductive Effects

compounds	Hepatotoxicity	Skin Sensitization	MUTAGENIC	Tumorigenic	Reproductive effect
Compounds 1	Yes	No	No	No	No
Compounds 2	Yes	No	No	No	No
Compound 3	Yes	No	No	No	No
Compound 4	YES	NO	NO	No	No
Compound 5	YES	NO	NO	No	No
Compound 6	YES	NO	NO	No	No
Compound 7	YES	NO	NO	No	No
Compound 8	YES	NO	NO	No	No
Compound 9	YES	NO	NO	No	No
Compound 10	YES	NO	NO	No	No
Compound 11	YES	NO	NO	No	No
Compound 12	YES	NO	NO	No	No
Compound 13	YES	NO	NO	No	No
Compound 14	YES	NO	NO	No	No
Compound 15	YES	NO	NO	No	No
Compound 16	NO	NO	NO	No	No
Compound 17	YES	NO	NO	Yes	No
Compound 18	YES	NO	Yes	Yes	No
Compound 19	YES	NO	NO	Yes	No
Compound 20	YES	NO	NO	Yes	No
Compound 21	YES	NO	NO	Yes	No
Compound 22	YES	NO	NO	Yes	No
Compound 23	YES	NO	NO	Yes	No
Compound 24	YES	NO	NO	No	No
Compound 25	YES	NO	NO	No	No
Compound 26	YES	NO	NO	No	No
Compound 27	YES	NO	NO	Yes	No
Compound 28	YES	NO	NO	No	No
Compound 29	YES	NO	NO	No	No
Compound 30	YES	NO	NO	No	No

RESULTS

Results of Molecular Docking

As shown in Table 1 and visualised with the computational tool Maestro, molecular docking of the designed compounds against DHFR (PDB ID: 1W78) and DPP-4 (PDB ID: 2ONC) exhibited different binding affinity patterns. In case of DHFR binding affinities of the compounds varied between -10.1kcal/mol and -13.6kcal/mol, 1W giving the strongest binding capability (13.6kcal/mol), followed by 1K (13.2kcal/mol), 1Z (13.0 kcal/mol), 1BB (12.9 kcal/mol), and 1N (12.8 kcal/mol). It reflective of high inhibition by such molecules against DHFR. The binding affinities of the compounds ranged between -9.0, kcal/mol,-12.4 kcal/mol, with the best docking measured in 1G- -12.4 kcal/mol followed by 1V- -11.8 kcal/mol, 1L- -11.6 kcal/mol and 1AA- -11.4 kcal/mol in case of DPP-4. Interestingly, there were molecules which consistently had high affinities to both targets including 1AA, 1L and 1V that may be dual-inhibitory in nature. The general pattern in the docking results shows that the derivatives chosen display substantial affinity toward DHFR and DPP-4, which make them lucrative molecules to be subjected to further processing and profiling in the laboratory.

Results of ADME Analysis

The designed series is in silico ADME profiled and the absorption, distribution, metabolism, excretion, and bioavailability properties of the developed system are consistent among all the tested molecules (Table 3). There was minimal gastrointestinal (GI) uptake of all compounds and none permeated the blood-brain barrier (BBB) signaling that they have good potential in peripheral targeting and minimizing any potential CNS implications. The values of skin permeation (log Kp) were -8.23 to -6.19 cm/s with the least permeable compounds, i.e., 1W and 1G, being -8.23 and -8.20 cm/s respectively, indicating little transdermal absorption potential. The forecasted volume of distribution (VDss) was mostly low to moderate (-1.131 to -0.517 log L/kg) which reflected limited systemic distribution. The majority of compounds have been discovered as P-glycoprotein (P-gp) substrate providing the fact that they may be effluxed through transporters-mediated mechanisms, however, only compound 2 was shown to be P-gp substrate and CYP2C9 inhibitor simultaneously. Looking at total clearance values of between -0.877 to 0.302 mL/min/kg, the elimination of all the compounds was moderate with the exception of compound 20 that had a predicted value of 0.17. Compound 20 was predicted to have a lower (but by a

small amount) bioavailability (0.11), and this could affect its systemic bioexposure as compared to the other compounds in the series. Such similar ADME trends indicate the uniformity of pharmacokinetic properties of the designed compounds with little differences in the properties of absorption and distribution parameters, which makes it possible to consider them as suitable in the framework of further optimization in preclinical practice.

The toxicological profile of the designed compounds (Table 4) carried out in silico revealed a rather positive perspective on safety with some warning notes. Most of the compounds were predicted to have hepatotoxicity with compound 16 being the only one that could not cause hepatotoxicity. All of the substances were not of skin sensitization risk and thus could be compatible with non-irritant topical or systemic product use. All molecules except compound 18 did not possess mutagenicity which was marked mutagenic. Compounds 17 23, 27, and 18 20 had shown tumorigenic potential especially compounds 18 and 18 as they were found with tumorigenicity and mutagenicity so more structural optimization would be required to reduce these risks. More to the point, none of the compounds is expected to cause any reproductive toxicity, implying that there is a low chance of developing or reproductive system damage. In total, the series is devoid of skin sensitization or reproductive toxicity risks, however, the prevalent hepatotoxicity of most molecules and carcinogenicity warnings of several derivatives highlight the relevance of performing safety-oriented lead optimization as early as possible in developing molecules prior to in vivo testing.

DISCUSSION

The designed compounds are thoroughly computationally screened and provide the structure with significant information on their potential use as dual inhibitors of the dihydrofolate reductase (DHFR; PDB ID: 1W78) and dipeptidyl peptidase-4 (DPP-4; PDB ID: 2ONC). Molecular docking findings (Table 1) showed that most of the molecules had high scores at the binding stage, which implied good ligand-target complementarity[27]. There were several compounds that showed good docking energies of above -13.0 kcal/mol in the DHFR set of 1W, 1K, 1Z, and 1BB[28]. The mechanism behind such a tight binding in these high-affinity ligands may be attributed to 3-5 hydrogen bonds, 1 1 stacking and hydrophobic interaction with catalytically essential residues in the DHFR active locus helping to identify the ligand within the ligand binding area[29].

In the case of DPP-4, top-scoring ligands (1G, -12.4 kcal/mol; 1L, -11.6 kcal/mol; 1V, -11.8 kcal/mol) exhibited a strong interaction pattern that occupied sub-pockets in the catalytic pocket and displayed both polar and nonpolar interactions[30]. Possibility of dual inhibition by combined actions on both targets based on the capacity of certain compounds (e.g., 1K, 1L, and 1V) to achieve high binding scores on both targets could be beneficial in therapeutic contexts where comorbidities, like type 2 diabetes mellitus and cancer, may be mediated by one compound that simultaneously works through two mechanisms, one that inhibits folate metabolism and one that inhibits the degradation of incretins. Docking results (Figures 1-4) substantiated the results as it demonstrated the adequate fit of the orientations and the steady interaction networks found in both representations of the enzyme targets[31].

ADME profiling (Table 3) showed unified trends in the library. Each of the compounds had low gastrointestinal absorption predicted, which can be associated with large molecular weight or greater polarity, properties that restrict passive intestinal absorption[32]. Such decreased uptake of GI may be circumvented during optimization by incorporating prodrugs or formulations like nanoparticle encapsulation in the future[33]. All the compounds lacked blood brain barrier (BBB) penetration, which is a positive sign in terms of a non-CNS-acting drug since it decreases the likelihood of developing a drug due to neurotoxicity. There were low values of skin permeability (log Kp) indicating low risks of exposure through the transdermal route[34]. The largest proportion was found to be P-glycoprotein (P-gp) substrates, which implies that it could be prone to this clearance mechanism analogous to efflux that can be used to describe systemic bioavailability. Interestingly, the calculated bioavailability of the molecules was constant at 0.17 for almost all molecules except compound 20, which had a low bioavailability value possibly because of the structural or polar differences in absorption or distribution[35].

Favorable safety aspects with respect to absence of skin sensitization and reproductive toxicity of the compound set were highlighted in toxicological risk assessment (Table 4). Nevertheless, hepatotoxicity warnings were prevalent with the exception of the compound 16, denoting that acumen should be issued with relevance to hepatic safety during the refinement of a lead[36]. Also, there was the tumorigenic potential in specific molecules, especially in the case of certain compounds 17-23 and 27, which might be explained by structural fragments that are related with carcinogenic potentials in predicting models. Compound 18 elicited the greatest concern, given contradicting mutagenicity and tumorigenicity signals as it represented a lower-potential lead that has serious structural adjustments[37].

Combing these findings, one can conclude that some of the examined compounds can be characterized by favorable multi-target binding properties along with ADME properties suitability and a tolerable toxicity level[38]. The large docking scores indicate strong predicted binding potential, and the overall absence of BBB penetration persists with a target profile that was desired to mean the peripheral phenotype. Nonetheless, various hepatotoxicity risks and P-gp efflux liabilities as well as tumorigenicity warnings in some candidates show that selective structural optimization is necessary to advance the safety and pharmacokinetic profile[39]. Further experiments should be aimed at structure activity relationship (SAR) studies, in vitro assays of enzyme inhibition and metabolic stability to confirm such in silico predictions. This stepwise methodology will be critical to progressing the most compelling of them to preclinical development as dual DHFR/DPP-4 inhibitors and

ultimately into translation as potential components of multi-dimensional metabolism-oncology approaches to therapy[40].

CONCLUSION

The in silico screening of the developed compounds against DHFR (1W78) and DPP-4 (2ONC) showed a few candidates that showed good two-side attachment strength, accompanied by good interaction and docking images. ADME profiling suggested that GI absorption is low, with no BBB penetration, and predicted uniform bioavailability of most of the compounds compatible with a peripheral therapeutic target profile. No skin sensitization or reproductive toxicity was reported but toxicological evaluation revealed alerts of hepatotoxicity and tumorigenic impact on certain molecules.

These results give potential opportunities, which, with structural refinements, can potentially exhibit effective multi-target treatment of diseases such as type 2 diabetes mellitus and its oncologic risk. The prospective clinical advantage is that simultaneous metabolic and proliferative pathways are modulated. In vivo studies and efficacy and safety should be performed in future.

Abbreviations

DHFR: Dihydrofolate reductase; DPP-4: Dipeptidyl peptidase-4; ADME: Absorption, Distribution, Metabolism, and Excretion; BBB: Blood–brain barrier; P-gp: P-glycoprotein; CYP: Cytochrome P450; GI: Gastrointestinal; LIG: Ligand; RMSD: Root mean square deviation; H-bond: Hydrogen bond; MW: Molecular weight; TPSA: Topological polar surface area; LogP: Partition coefficient; CYP2C9: Cytochrome P450 2C9; CNS: Central nervous system.

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