In Silico Identification of Novel Dipeptidyl Peptidase 4 Inhibitors Via Pharmacophore-Guided Virtual Screening.

Khanh Huyen Thi Pham[a], Huynh Van Thi Nguyen[b], Tan Khanh Nguyen[c*]

Abstract:

Dipeptidyl peptidase 4 (DPP4) plays a crucial role in glucose metabolism and is a validated therapeutic target for type 2 diabetes mellitus. Despite the availability of several DPP4 inhibitors, the discovery of novel scaffolds with improved safety and pharmacokinetic profiles remains an unmet need. In this study, a structure-based pharmacophore model integrating key interaction features of saxagliptin and vildagliptin was constructed and applied to screen the Enamine database, aiming to identify new chemotypes distinct from existing inhibitors. The top hits were prioritized through molecular docking, drug-likeness assessment, and ADMET prediction. Among them, compound 3 emerged as a novel lead scaffold, showing strong binding affinity (-9.5 kcal/mol) and stable interactions with critical catalytic residues, including Ser630 and Tyr547. SwissADME analysis indicated favorable oral pharmacokinetics with high gastrointestinal absorption and no BBB penetration. Toxicity prediction suggested low acute toxicity (LD_{50} = 1500 mg/kg, Class 4), with minimal hepatic and cardiac risks. Overall, this work introduces a computationally validated pharmacophore-driven strategy to identify new DPP4 inhibitor scaffolds, providing a promising starting point for further experimental optimization.

Keywords: *diabetes mellitus, dipeptidyl peptidase 4 (DPP4), pharmacophore model, computer-aided drug discovery, molecular docking*

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[a] College of Pharmacy, Dongguk University; Seoul, Republic of Korea.
e-mail: phamthikhanhhuyen22299@gmail.com

[b] Scientific Management Department, Dong A University; Danang, Vietnam.
e-mail: vannth@donga.edu.vn

[c] Scientific Management Department, Dong A University; Danang, Vietnam.
e-mail: khanhnt2501@gmail.com

[*] *Corresponding author.*

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Introduction

Diabetes mellitus has become a major global public health issue, contributing significantly to illness, disability, and mortality worldwide, with both incidence and prevalence continuing to rise rapidly (Bourne, 2023). According to the International Diabetes Federation (IDF) Atlas, around 537 million adults (aged 20-79) were living with diabetes in 2021, and this number is projected to reach 783 million by 2045 (Magliano et al., 2021). The causes of this epidemic are multifactorial, overweight and obesity, unhealthy diets, and physical inactivity play crucial roles in disease development, along with genetic and epigenetic predispositions (Zheng et al., 2018). However, diabetes can be prevented and effectively managed through a balanced diet, regular physical activity, maintaining a healthy weight, avoiding tobacco use, and appropriate medication and routine screening to detect and manage complications early. In recent years, extensive research has also focused on discovering novel drugs and enzyme inhibitors, to improve glycemic control and reduce diabetes-related complications.

The Dipeptidyl peptidase (DPP) enzyme family includes Dipeptidyl peptidase 4 (DPP4), Fibroblast activation protein-α (FAP), Dipeptidyl peptidase 7 (DPP7), Dipeptidyl peptidase 8 (DPP8) Dipeptidyl peptidase 9 (DPP9), and Prolylcarboxypeptidase (PCP), among which DPP4 is a serine protease widely expressed on the surface of endothelial cells, lymphocytes, and various other tissues (Edwards et al., 2012; Gass & Khosla, 2007; Holst & Deacon, 1998; Polgar & Szeltner, 2008; Rea & Fülöp, 2006; Stonehouse et al., 2012). DPP4 catalyzes the cleavage of N-terminal dipeptides containing proline or alanine from peptide substrates, leading to the inactivation of the incretin hormones GLP-1 and GIP (Darmoul et al., 1990). These hormones stimulate insulin secretion from pancreatic β-cells, suppress glucagon release from α-cells, and reduce hepatic glucose production (Deacon, 2019; Whalley et al., 2011). Therefore, inhibition of DPP4 prolongs incretin activity, enhances glucose-dependent insulin secretion, and improves overall glycemic control (Campbell & Drucker, 2013). This inhibitory mechanism has become a well-established therapeutic strategy for developing antidiabetic drugs, particularly for patients with type 2 diabetes who do not achieve adequate glycemic control with conventional treatments such as metformin or sulfonylureas (Green et al., 2006; Yin et al., 2022).

DPP4 inhibitors, commonly known as gliptins, include several FDA-approved drugs such as sitagliptin, saxagliptin, linagliptin, and alogliptin, along with others used in Europe and Asia such as vildagliptin, gemigliptin, anagliptin, teneligliptin, trelagliptin, omarigliptin, evogliptin, and gosogliptin (Baetta & Corsini, 2011; Green et al., 2006; Yin et al., 2022). Among them, saxagliptin (SAX) and vildagliptin (VIL) act as reversible covalent inhibitors, forming an imidate adduct with the Ser630 residue at the DPP4 catalytic site, which enhances potency and minimizes side effects compared to non-covalent inhibitors

(Nabeno et al., 2013; Sutanto et al., 2020). The inhibition mechanism involves the catalytic triad Ser630-His740-Asp708, where His740 facilitates proton transfer and Tyr547 stabilizes the imidate intermediate (Berger et al., 2018; Wang et al., 2019). The distinct hydrolysis behavior between SAX (reversible reaction) and VIL (irreversible reaction) may account for the superior efficacy and safety profile of VIL, suggesting that new DPP4 inhibitors modeled on the VIL scaffold could offer improved pharmacological potential (He et al., 2009).

Research on Dipeptidyl Peptidase-4 (DPP4) inhibitors has achieved significant progress; however, there remains an urgent need to identify bioactive compounds with novel chemical scaffolds, enhanced efficacy, and improved pharmacokinetic profiles. To address this gap, the present study introduces a novel approach by employing the Pharmit platform to construct a structure-based pharmacophore model. This model was derived from the key structural and electronic features of two well-established DPP4 inhibitors, SAX) and VIL. The resulting pharmacophore was subsequently used to screen potential compounds from the Enamine commercial library, with the primary objective of discovering novel DPP4 inhibitors. The selected hits from the in silico screening were further subjected to expanded and complementary analyses compared to previous studies, including molecular docking to evaluate binding affinity, assessment based on Lipinski's rule of five, and prediction of ADMET parameters (absorption, distribution, metabolism, excretion, and toxicity). The ultimate goal of this workflow was to identify the most promising candidates exhibiting strong binding affinity, favorable pharmacokinetic properties, and potential efficacy as DPP4 inhibitors.

Materials and Methods

Pharmacophore Designing/Modeling

In this study, a structure-based pharmacophore model was constructed for the active site of DPP4 (PDB ID: 6B1E) using two active inhibitors, SAX and VIL, as reference ligands. The pharmacophore was generated through the freely accessible web server Pharmit (pharmit.csb.pitt.edu, accessed on October 20, 2025). The resulting model comprised eight key features, including three hydrogen donors, four hydrogen acceptors, and one central hydrophobic site (Table 1). These features were spatially arranged to capture the essential interactions within the DPP4 active pocket. The hydrogen bond donor and acceptor groups were primarily distributed around the coordinates (35-43 Å), corresponding to regions capable of forming strong polar interactions with catalytic residues such as Ser630 and His740. The hydrophobic feature located near (35.3, 48.4, 35.3) represented a nonpolar pocket that contributes to ligand core stabilization.

Table 1. Pharmacophore features generated from active DPP4 inhibitors (SAX and VIL).

No.	Feature Type	Coordinates (x, y, z)	Radius (r)
1	Hydrogen Donor 1	(35.9, 48.7, 38.5)	1.0
2	Hydrogen Donor 2	(39.2, 52.0, 35.0)	1.0
3	Hydrogen Donor 3	(42.5, 54.5, 37.6)	1.0
4	Hydrogen Acceptor 1	(35.9, 48.7, 38.5)	1.0
5	Hydrogen Acceptor 2	(39.2, 52.0, 35.0)	1.0
6	Hydrogen Acceptor 3	(36.8, 52.0, 36.4)	1.0
7	Hydrogen Acceptor 4	(42.5, 54.5, 37.6)	1.0
8	Hydrophobic	(35.3, 48.4, 35.3)	1.0

Pharmacophore-Based Virtual Screening

In computational drug discovery pipelines, pharmacophore-based virtual screening serves as a crucial step for identifying potential lead compounds from large chemical libraries against a specific biological target. Several tools and web servers have been developed for this purpose, among which Pharmit stands out as a freely accessible platform that enables interactive screening based on pharmacophore models or molecular shape, ranking hits according to minimized energy scores (Sunseri & Koes, 2016). Using this platform, extensive compound databases can be efficiently filtered according to their spatial and electronic features derived from known ligands. In the present study, the Enamine database (enaminestore.com, accessed on October 20, 2025) was employed for virtual screening. This commercially available library contains 60,516,302 conformers representing 4,117,328 molecules, allowing for the identification of compounds structurally compatible with the pharmacophore model generated from SAX and VIL. The top-ranked compounds obtained from this screening process are summarized in Table 2.

Table 2. Top-ranked compounds obtained from pharmacophore-based virtual screening using Pharmit

No.	Compound ID	RMSD	Molecular Mass (Da)	Number of Rotatable Bonds (RBnds)
1	Z4877467597	0.453	317	8
2	Z1552200029	0.561	765	7
3	Z4206100050	0.616	367	7
4	Z2053669773	0.654	294	3

No.	Compound ID	RMSD	Molecular Mass (Da)	Number of Rotatable Bonds (RBnds)
5	Z8333350340	0.675	373	7
6	Z4421993557	0.708	371	5
7	Z4392288899	0.758	276	4

Modecular docking

Protein and ligand preparation: The 3D structure of DPP4 (PDB ID: 6B1E) was retrieved from the Protein Data Bank. Following the established protocol in our previous study, all crystallographic water molecules, co-crystallized ligands, and heteroatoms were removed using Discovery Studio 2020 (Nguyen et al., 2025). Polar hydrogens and Kollman charges were subsequently added with Autodock Tool (version 1.5.6) (Morris et al., 2009), and the processed structure was saved in pdbqt format for docking simulations. The 3D structures of the top-ranked compounds were obtained from the PubChem database. Each ligand was energy-minimized, protonated, and converted into pdbqt format using Open Babel 3.1.1.

Molecular docking was carried out with AutoDock Vina 1.2.4 to predict the binding affinity and pose of the ligands within the DPP4 active site. The docking grid was centered on the catalytic pocket of DPP4, with the following parameters after the center position was determined: size_x: 21, size_y: 21, size_z: 21, center_x: 41 , center_y: 50, center_z: 35.. Docking scores were reported in kcal/mol to evaluate ligand binding affinity. The binding interactions and conformational poses of the protein-ligand complexes were visualized and analyzed using BIOVIA Discovery Studio Visualizer 2020.

Drug-Likeness, ADME, and Toxicity Prediction

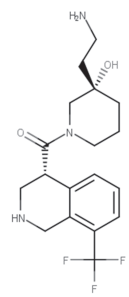
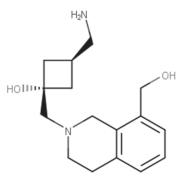
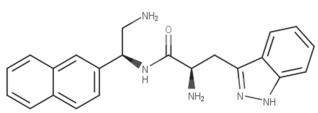
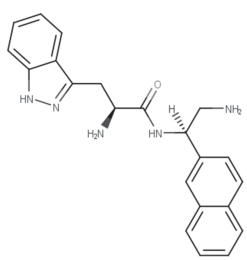
The top-ranked compound with the most favorable docking score was further evaluated for its pharmacokinetic and drug-likeness properties. Lipinski's Rule of Five was applied to assess its oral bioavailability and overall suitability as a drug-like molecule. The compound's absorption, distribution, metabolism, and excretion (ADME) characteristics were analyzed using the SwissADME web server (Daina et al., 2017). In addition, the ProTox-3.0 prediction platform was employed to estimate potential organ-specific toxicities and safety profiles (Banerjee et al., 2024). These computational evaluations provided insights into the compound's pharmacological behavior and its potential as a promising DPP4 inhibitor candidate.

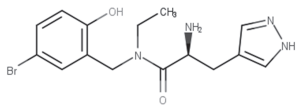
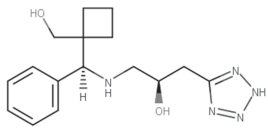
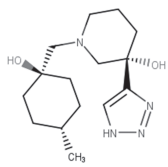
Results and Discussion

Molecular docking analysis

All seven candidate compounds were docked into the active site of DPP4, and their binding affinities and interaction profiles were analyzed in detail (Table 3). The docking scores ranged from -6.8 to -9.5 kcal/mol, suggesting moderate to strong binding affinities toward the target enzyme. Among them, compound 3 exhibited the lowest docking energy (-9.5 kcal/mol), indicating the most stable and favorable binding conformation within the catalytic pocket of DPP4.

Table 3. Molecular docking results of selected compounds with DPP4

No	Structure	Hydrogen bond	Hydrophobic interaction	Docking score (kcal/mol)
1		Glu206, Glu205, Tyr547, Ser630	Glu205, Tyr666, His740, Val711, Tyr631, Val656, Trp659	-7.9
2		Glu206, Glu205, Tyr547, Phe357	Tyr666, Arg358, Phe357	-6.8
3		Glu206, Tyr547, Ser630, Tyr662, Asn710	Tyr666, His740, Arg358, Phe357	-9.5
4		Glu206, Glu205, Tyr662, Asn710, Val711, Tyr547, Ser630,	Tyr666, His740, Phe357	-8.3

No	Structure	Hydrogen bond	Hydrophobic interaction	Docking score (kcal/mol)
5		Glu206, Glu205, Tyr662, Ser630,	His740, Phe357	-7.6
6		Glu206, Tyr547, Tyr662, Ser630, His740, Arg125	Phe357, Glu205, Arg125	-7.9
7		Tyr547, Tyr662, Ser630, His740, Glu205,	Tyr666, Phe357, Arg125	-6.9

Compound 3 formed multiple hydrogen bonds with key residues Glu206, Tyr547, Ser630, Tyr662, and Asn710, which are known to play essential roles in substrate recognition and catalytic activity. In addition, several hydrophobic interactions were observed with Tyr666, His740, Arg358, and Phe357, contributing to the stabilization of the ligand-protein complex. The presence of these interactions suggests that compound 3 effectively anchors within the active site and may interfere with the catalytic function of DPP4.

Notably, the involvement of Ser630, a core residue of the DPP4 catalytic machinery, indicates that compound 3 may act through a competitive inhibitory mechanism by directly blocking substrate access to the active site. Furthermore, interactions with aromatic residues such as Tyr662 and Tyr666, are commonly associated with potent and selective DPP4 inhibition. Compared to the other candidates, compound 3 exhibited a more comprehensive interaction network with catalytically relevant residues, supporting its superior docking performance.

Overall, the docking results highlight compound 3 as the most promising inhibitor candidate among the tested molecules, exhibiting both strong binding affinity and extensive interactions with catalytically relevant residues of DPP4. Therefore, compound 3 was selected for subsequent ADME, toxicity, and molecular docking analyses to further evaluate its pharmacological potential. These structural features provide a clear rationale for its selection for subsequent ADME and toxicity analyses.

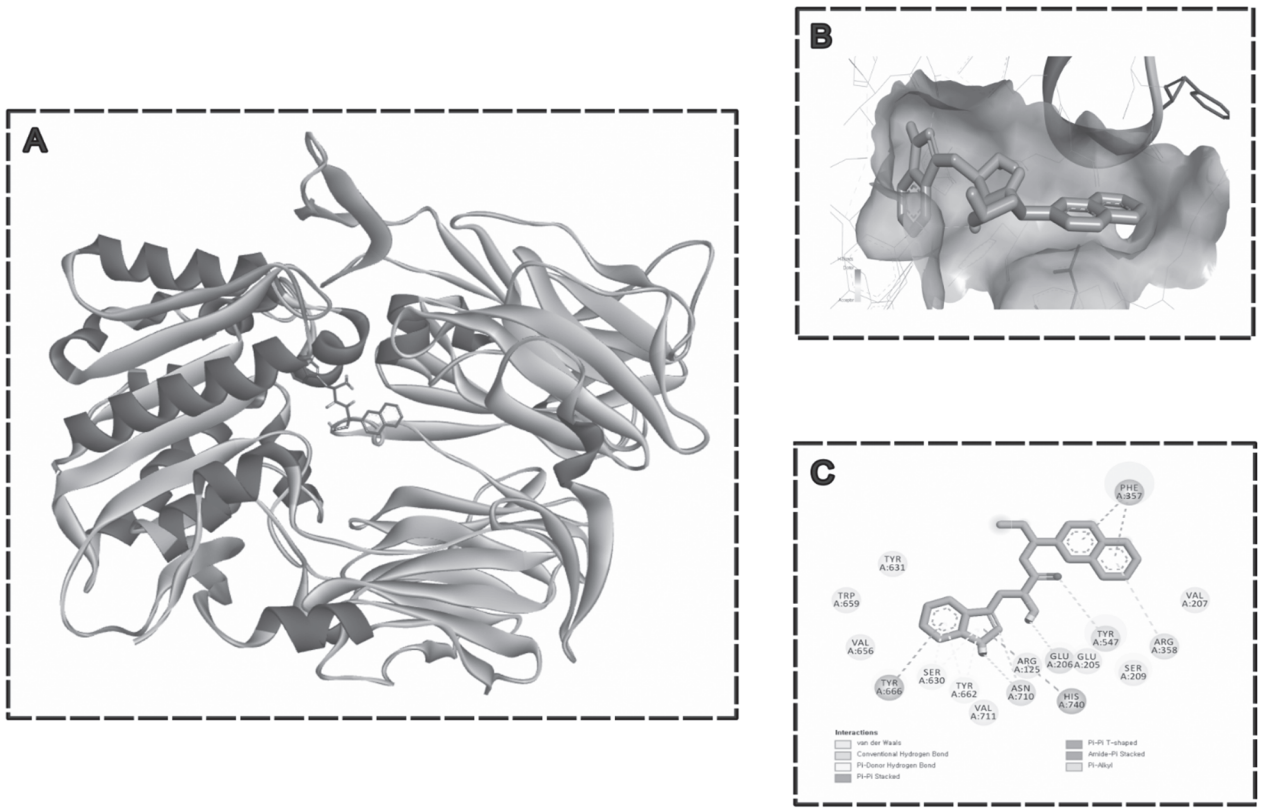


Fig 1. Molecular docking visualization of the top-ranked compound (compound 3) in the active site of DPP4.

*(**A**) Overall 3D structure of DPP4 (PDB ID: 6B1E) showing the binding position of compound 3 within the catalytic pocket. (**B**) Surface representation of the DPP4 active site illustrating the binding pose and hydrophobic environment surrounding the ligand. (**C**) 2D interaction diagram showing key hydrogen bonds and hydrophobic contacts between compound 3 and critical residues such as Glu205, Glu206, Tyr547, Ser630, Tyr662, Asn710, His740, and Tyr666.*

ADME studies

The pharmacokinetic evaluation of compound 3 using SwissADME revealed favorable physicochemical and absorption characteristics (Table 4). The compound has a molecular weight of 373.45 g/mol, Log P of 2.10, and a total polar surface area (TPSA) of 109.82 Å^2, all of which fall within the optimal range suggested by Lipinski's Rule of Five. The absence of any Lipinski violations and moderate lipophilicity indicate that compound 3 is likely to possess good oral bioavailability and membrane permeability. In terms of ADME properties, compound 3 demonstrated high gastrointestinal absorption (GI Abs) but was predicted not to cross the blood-brain barrier (BBB), suggesting limited central nervous system effects, an advantageous feature for DPP4-targeting antidiabetic agents. It was also identified as a P-gp substrate, implying potential influence on drug transport. Regarding

metabolic stability, compound 3 showed selective inhibition of CYP2C19, CYP2D6, and CYP3A4, while having no inhibitory effects on CYP1A2 and CYP2C9, which suggests a moderate risk of metabolic interactions.

From a translational perspective, the combination of high GI absorption and lack of BBB permeability is particularly desirable for antidiabetic drugs, as DPP4 inhibition is primarily required in peripheral tissues rather than the central nervous system. The moderate TPSA value further supports efficient intestinal absorption while maintaining sufficient polarity to limit CNS exposure.

Table 4. Predicted drug-likeness and ADME properties of compound 3 calculated by SwissADME.

Property	Value
Molecular weight (g/mol)	373.45
Log P	2.10
nHBD	4
nHBA	4
TPSA (Å²)	109.82
MR	110.70
Lipinski violation	0
Log Kp (cm/s)	-7.07
Log S	-3.54
nRotB	7

ADME Prediction	Result
GI absorption	High
BBB permeability	No
P-gp substrate	Yes
CYP1A2 inhibitor	No
CYP2C19 inhibitor	Yes
CYP2C9 inhibitor	No
CYP2D6 inhibitor	Yes
CYP3A4 inhibitor	Yes

LogP, Log of octanol/water partition coefficient; nHBD, Number of hydrogen bond donor(s); nHBA, Number of hydrogen bond acceptor(s); TPSA, Total polar surface area; MR, Molar refractivity; Log Kp, Log of skin permeation; Log S, log of solubility; nRotB, Number of rotatable bonds; GI Abs, Gastro-intestinal absorption; BBB Per, Blood brain barrier permeability; P-gp, P-glycoprotein; CYP, cytochrome-P

Overall, compound 3 exhibits a balanced profile of drug-likeness and ADME characteristics, aligning well with the pharmacokinetic properties expected of orally active agents. Although the predicted inhibition of certain CYP isoforms may warrant further optimization to minimize potential drug-drug interactions, these liabilities are not uncommon among clinically used DPP4 inhibitors. These results support its potential as a promising DPP4 inhibitor candidate and justify further optimization and biological validation in subsequent studies.

Toxicity prediction

The predicted LD_{50} value of compound 3 was 1500 mg/kg, placing it in toxicity class 4, which indicates a relatively low acute toxicity profile. According to ProTox 3.0 predictions, the compound was non-hepatotoxic, non-nephrotoxic, and non-cardiotoxic, suggesting a favorable safety margin for hepatic, renal, and cardiovascular systems. However, it exhibited potential respiratory toxicity (probability 0.82), implying possible adverse effects on the respiratory systems at higher concentrations. From a safety assessment standpoint, the absence of predicted hepatotoxicity and cardiotoxicity is particularly important for chronic antidiabetic therapy, as long-term DPP4 inhibition requires sustained systemic exposure. The relatively high LD_{50} value further supports a reasonable therapeutic window at the acute toxicity level. Overall, compound 3 demonstrates acceptable systemic safety, but the predicted neurological and respiratory risks warrant further *in vitro* and *in vivo* validation before clinical consideration. Notably, the respiratory toxicity prediction is based on in silico probability models and may reflect off-target or dose-dependent effects; therefore, targeted cytotoxicity and organ-specific assays will be essential to confirm these liabilities.

Table 5. Toxicity of Compound 3 Predicted by ProTox 3.0 Prediction Server

Predicted mg/kg	Predicted Toxicity Class	Organ toxicity			
		Hepatotoxicity	Nephrotoxicity	Respiratory toxicity	Cardiotoxicity
1500	4	Inactive (0.51)	Inactive (0.59)	Active (0.82)	Inactive (0.68)

Conclusion

This study employed an integrated *in silico* workflow combining structure-based pharmacophore screening, molecular docking, and ADMET-toxicity prediction to identify potential DPP4 inhibitor candidates from a large commercial chemical library. This computational strategy effectively reduced chemical space and enabled the prioritization of biologically relevant compounds with favorable drug-like properties. Among the screened hits, compound 3 exhibited the most favorable binding affinity toward the DPP4 active site, satisfactory compliance with drug-likeness criteria, and an acceptable predicted safety profile. The consistency of results across multiple computational tools supports the robustness of the proposed screening framework and highlights its utility as a rational approach for early-stage DPP4 inhibitor discovery.

Nevertheless, the findings of this study are subject to several limitations. The conclusions are based solely on computational predictions and therefore require

experimental validation, including *in vitro* enzymatic inhibition assays and subsequent *in vivo* pharmacological evaluation, to confirm biological activity and therapeutic relevance. In addition, the selectivity of compound 3 toward DPP4 relative to closely related enzymes such as DPP8 and DPP9 was not explicitly evaluated and remains an important consideration for safety assessment. Accordingly, future studies will focus on experimental validation, selectivity profiling, and further structural optimization to improve efficacy and minimize potential off-target effects. Overall, this work provides a solid computational foundation for subsequent experimental efforts toward the development of next-generation DPP4 inhibitors.

Abbreviations

DPP: Dipeptidyl peptidase DPP4: Dipeptidyl peptidase 4 DPP7: Dipeptidyl peptidase 7 DPP8: Dipeptidyl peptidase 8 DPP9: Dipeptidyl peptidase 9 BBB Per: Blood brain barrier permeability CYP, cytochrome-P FAP: Fibroblast activation protein-α GI Abs: Gastro-intestinal absorption	Log Kp: Log of skin permeation LogP: Log of octanol/water partition coefficient Log S: Log of solubility MR: Molar refractivity nHBA: Number of hydrogen bond acceptor(s) nHBD: Number of hydrogen bond donor(s) nRotB: Number of rotatable bonds P-gp: P-glycoprotein PCP: Prolylcarboxypeptidase TPSA: Total polar surface area

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