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A CRITICAL REVIEW ON THE DESIGN, SYNTHESIS, CHARACTERIZATION, AND BIOLOGICAL EVALUATION OF NOVEL HETEROCYCLIC COMPOUNDS AS DIPEPTIDYL PEPTIDASE-4 (DPP-4) INHIBITORS

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Abstract

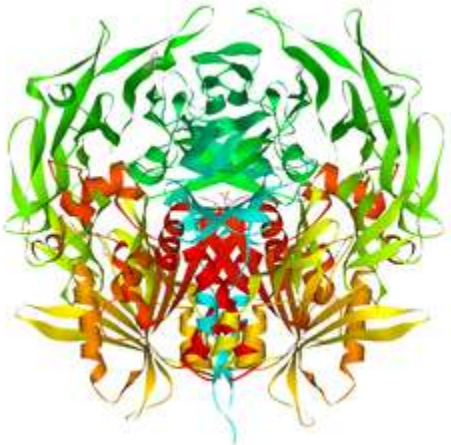
Type 2 Diabetes Mellitus (T2DM) remains a global health crisis, necessitating the development of new therapeutic agents. Dipeptidyl Peptidase-4 (DPP-4) inhibition, which enhances the incretin effect, is a cornerstone of modern T2DM therapy, led by the "gliptin" class of drugs. However, existing gliptins are associated with several limitations, including modest efficacy, weight neutrality, and potential safety concerns such as pancreatitis, arthralgia, and a debated risk of heart failure, which collectively drive the search for novel inhibitors. This review critically surveys recent advances (circa 2018-2025) in the rational design, multi-step synthesis, spectroscopic characterization, and *in vitro*/*in vivo* biological evaluation of novel, non-peptidomimetic heterocyclic compounds as DPP-4 inhibitors. We place a special emphasis on emerging and privileged scaffolds, including quinazoline and quinoxaline cores, pyrazole-based derivatives, triazole and triazolopyrimidine systems, and various fused heterocyclic analogues. Key structure-activity relationships (SARs) are discussed in detail, focusing on the critical non-covalent interactions (e.g., hydrogen bonds, hydrophobic contacts, and salt bridges with residues like Glu205, Glu206, and Tyr662) within the S1, S2, and S2-extensive pockets of the DPP-4 active site. The importance of *in silico* methods (QSAR, molecular docking) in guiding rational design is also highlighted. We compare the *in vitro* potency (IC_{50} values) and, crucially, the selectivity against related proteases (DPP-8, DPP-9, FAP), which is a key predictor of safety. This analysis aims to provide a comprehensive overview of the current landscape and future perspectives for developing next-generation antidiabetic agents with superior efficacy and safety profiles.

Keywords

Dipeptidyl Peptidase-4 (DPP-4); Type 2 Diabetes; Heterocyclic Compounds; DPP-4 Inhibitors; Drug Design; Structure-Activity Relationship (SAR); Molecular Docking; Quinazolines; Triazoles

1. Introduction

Dipeptidyl peptidase-4 (DPP-4) is a ubiquitous serine protease responsible for the rapid inactivation of incretin hormones, particularly Glucagon-like Peptide-1 (GLP-1) and Glucose-dependent Insulinotropic Polypeptide (GIP) [1]. Inhibition of DPP-4 enhances the bioavailability of active incretins, leading to glucose-dependent insulin secretion and improved glycemic control, establishing DPP-4 inhibitors (gliptins) as cornerstone oral agents for T2DM [2].



Dipeptidyl peptidase-4

Despite the clinical success of approved gliptins (e.g., sitagliptin, vildagliptin, linagliptin), challenges persist: they generally provide limited maximal glycemic efficacy, are weight-neutral, and carry unresolved concerns regarding long-term cardiovascular safety or rare adverse events like pancreatitis [1]. These limitations necessitate continuous research into novel heterocyclic scaffolds capable of delivering enhanced potency, improved selectivity, superior pharmacokinetic (PK) properties, or favorable pleiotropic effects. Non-peptidomimetic small molecules are particularly sought after for their potential to overcome the poor bioavailability and

stability typical of peptide-like inhibitors. The last few years (2018–2025) have seen the exploration of several innovative heterocyclic classes [2].

2. Rational Design and Computational Guidance

The design of novel DPP-4 inhibitors is intrinsically guided by the three-dimensional structure of the enzyme's catalytic domain, which features key binding pockets: S1, S2, S1', and S2-extensive [3].

2.1. Active Site Requirements and Selectivity

Effective inhibition hinges on strong interactions with critical active-site residues. The ionic interaction between a protonated/basic group on the inhibitor and the negatively charged side chains of Glu205 and Glu206 in the S2 subsite is a fundamental anchor point for high-affinity ligands [4]. Additionally, hydrophobic moieties are required to fit into the S1 lipophilic pocket, often involving interactions with residues such as Tyr662 [4].

A critical consideration in design is the need for high selectivity over homologous peptidases, primarily DPP-8 and DPP-9, and Fibroblast Activation Protein (FAP). Off-target inhibition of these enzymes has been linked to potential toxicity, making a selectivity margin of >100-fold essential for a safe clinical candidate [3, 5].

2.2. Computational Methods

In silico techniques are now integral to the discovery pipeline [6].

- **Molecular Docking** and **Pharmacophore Modeling** are routinely used to predict binding modes, estimate affinity scores, and identify key spatial and electronic features shared by potent ligands [7].
- **Quantitative Structure-Activity Relationship (QSAR)** studies aid in correlating chemical features with inhibitory potency, guiding the optimization of substituents [6].
- **Molecular Dynamics (MD) simulations** provide a crucial dynamic view, assessing the stability of the ligand-enzyme complex over time, the persistence of hydrogen bonds, and the potential for induced fit, which is particularly valuable for explaining high potency and kinetic attributes like residence time [5]. For example, MD was successfully used to confirm stable binding of potent quinoxaline [8] and dihydropyrimidine derivatives [5].
- **Density Functional Theory (DFT)** and **ADMET prediction** tools are also employed for lead optimization by filtering compounds based on predicted toxicity and drug-like properties [6].

3. Important Heterocyclic Scaffolds: Recent Advances

The following sections detail the synthesis, characterization, and biological performance of leading heterocyclic scaffolds explored in the last five to seven years.

3.1. Quinazoline and Quinazolin-4-one Derivatives

The quinazoline core, known for its versatility in medicinal chemistry, has been structurally modified to yield potent DPP-4 inhibitors.

- An older but highly potent series of N^3-benzylidene-N^4-(thiazol-2-yl)-quinazoline-3,4-(4H)-diamine derivatives was developed, with lead compound 7g demonstrating an IC_{50} of approximately 0.76 nM, coupled with favorable antioxidant activity in DPPH assays [9].
- More recent work focused on 2-({2-[(dialkylamino)methyl]quinazolin-4-one-3-yl}methyl)benzonitrile analogs. While synthesized and fully characterized (FT-IR, NMR, Mass), the reported IC_{50} values for the most active compounds were in the low micromolar range (e.g., $\approx 1.46 \text{ \mu M}$) [10].

Assessment: Quinazoline offers a rigid scaffold to position key functional groups. While initial attempts yielded picomolar potency, recent quinazolin-4-one derivatives require significant further optimization to compete with clinical agents (sitagliptin $\text{IC}_{50} \approx 20 \text{ nM}$) [10]. Detailed PK data and selectivity profiles for the latest analogs are often insufficient.

3.2. Quinoxaline-Based Inhibitors

Quinoxaline derivatives have emerged, showcasing promising dual utility as both DPP-4 inhibitors and potential diagnostic agents.

- A series of $\text{1,4-dimethyl-2,3-dioxo-tetrahydroquinoxaline-6-sulfonamide}$ analogues was designed and synthesized. Compounds $\mathbf{10a}$ and $\mathbf{10g}$ demonstrated good potency and stable binding in the DPP-4 active site, confirmed by MD simulations [8].
- **Translational Insight:** Compound $\mathbf{10a}$ was successfully radiolabeled with $\text{}^{131}\text{I}$ for biodistribution studies, confirming accumulation in DPP-4-rich tissues, suggesting potential as a theranostic agent for imaging and treatment [11].

Assessment: The quinoxaline-sulfonamide scaffold is structurally intriguing, allowing the sulfonamide group to engage in critical hydrogen bonding. The integrated theranostic approach is innovative, but comprehensive long-term safety and selectivity data remain necessary.

3.3. Pyrazole and Triazole Derivatives

Pyrazole and triazole cores are prevalent due to their ease of synthesis (often via click chemistry) and favorable geometry.

- **Pyrazole–Triazole Hybrids:** Using Cu(I)-catalyzed click chemistry, researchers generated pyrazole-triazole-persulfonimide and pyrazole-triazole-aryl derivatives. The most potent compound, $\mathbf{8h}$, achieved an IC_{50} of **4.54 nM**, showing comparable efficacy to sitagliptin in an *in vivo* streptozotocin (STZ)-induced diabetic mouse model [12].
- **Pyrazole-Thiosemicarbazones:** Independent work explored pyrazole-incorporated thiosemicarbazone derivatives, highlighting the importance of substitution patterns for tuning potency via favorable binding interactions [13].

- **Triazole-Based Compounds:** $\text{1,2,3-triazole-based}$ compounds linked by a propylene spacer to other heterocycles were synthesized. Compound **30** exhibited an IC_{50} of **12.82 nM**, demonstrating superior *in vitro* potency compared to the sitagliptin standard ($\approx 14.8 \text{ nM}$) used in that study [7].

Assessment: Both pyrazole and triazole offer high synthetic modularity. The achievement of nanomolar potency and *in vivo* efficacy (e.g., $\mathbf{8h}$) is a significant step. However, the true selectivity (DPP-4 vs DPP-8/9) of many recent analogs is not always rigorously reported, representing a critical gap.

3.4. Fused Bicyclic Scaffolds

Fused heterocyclic systems combine rigidity and complex substitution potential, leading to high-selectivity inhibitors.

- **Pyrazolo[1,5-a]pyrimidine:** Optimization via a fragment-based approach yielded compound $\mathbf{c24}$ with an $\text{IC}_{50} = \mathbf{2 \text{ nM}}$ and outstanding **$>2,000$-fold selectivity** over DPP-8 and DPP-9. SAR showed that the pyrazolopyrimidine core occupies the S1 pocket, while a substituted aromatic ring engages a sub-S1 region [3].

- **Dihydropyrimidine Hybrids:**

  - **Phthalimide–Dihydropyrimidine:** Hybrids (e.g., $\mathbf{10g}$) showed better *in vitro* inhibition than alogliptin and demonstrated superior *in vivo* efficacy in T2DM rat models [14].

  - **Structure-Guided Dihydropyrimidine:** A very recent structure-guided design yielded compound $\mathbf{46}$ with $\text{IC}_{50} = \mathbf{2 \text{ nM}}$, which is four times more potent than sitagliptin, coupled with high selectivity over DPP-8/9. MD and *in vivo* data confirmed robust glucose-lowering effects [5].

- **Triazolo[5,1-c][1,2,4]triazine:** An earlier but relevant example, compound $\mathbf{15q}$, showed moderate IC_{50} ($\approx 28 \text{ } \mu\text{M}$) but demonstrated $\approx 8–10$-fold selectivity and *in vivo* efficacy in chronic diabetic rat models [15].

Assessment: Fused bicyclic scaffolds, particularly the pyrazolopyrimidine and the structure-guided dihydropyrimidine derivatives, represent the state-of-the-art in DPP-4 inhibition. They successfully combine high, single-digit nanomolar potency with excellent selectivity, positioning them as strong preclinical candidates [3, 5].

4. Structure–Activity Relationships (SAR) and Mechanistic Insights

The superior activity of these novel heterocycles can be attributed to refined SAR principles [4]:

- **S2 Pocket Engagement:** The presence of a basic/ionizable nitrogen or other hydrogen bond donor/acceptor group (e.g., the sulfonamide group in quinoxaline derivatives [8], or primary amine precursors) is essential for forming the critical **salt bridge** with **Glu205/Glu206** [4].

- **Hydrophobic Anchoring (S1/S1′):** Aromatic/heteroaromatic rings (e.g., substituted pyrazoles, phenyl groups) are strategically positioned to fill the S1 or S1′ pockets, engaging in π-π stacking interactions with residues like **Tyr662** [3, 4].

- **Rigidity and Selectivity:** Fused bicyclic systems (e.g., pyrazolopyrimidine) achieve high potency due to their conformational rigidity, which reduces the entropic penalty upon binding. Selectivity over DPP-8/9 is often achieved by engineering substituents that exploit small geometric differences in the S2-extensive or S1-prime pockets unique to DPP-4 [3].

- **Linker Modulation:** In compounds like the triazole-based inhibitors, the length and flexibility of linkers connecting the core to the binding group (e.g., the propylene linker in compound **30**) are critical for optimal positioning and potency [7].

5. Challenges and Future Perspectives

Despite the wealth of highly potent, selective heterocyclic leads, the journey to a successful drug candidate faces significant hurdles:

- **Pharmacokinetic (PK) Optimization:** Many studies lack comprehensive ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) data. Poor oral bioavailability, rapid clearance, or unfavorable tissue distribution can nullify exceptional *in vitro* potency [2]. Further tuning of lipophilicity and metabolic hotspots on the core scaffold is essential.

- **Toxicology and Long-Term Safety:** While high *in vitro* selectivity over DPP-8/9 is reassuring, comprehensive *in vivo* toxicology and off-target screening (e.g., hERG channel liability) are mandatory before clinical progression [5]. The potential for pancreatitis or arthralgia observed with existing gliptins must be rigorously investigated in preclinical models.

- **Translational Efficacy:** Relying solely on rodent models (STZ-diabetes, OGTT) may not fully predict human clinical outcomes. Advanced preclinical testing, including long-term chronic studies in relevant animal models and measurement of plasma GLP-1 levels, is required [1].

- **Synthetic Scalability and Cost:** Complex multi-step syntheses, especially those involving expensive reagents or difficult purifications, may limit the commercial viability of a promising lead. Modular methods, like click chemistry used for triazoles, are favored for their efficiency and scalability [7].

Future Directions:

1. **Dual-Targeting Agents:** The trend is toward single molecules that combine DPP-4 inhibition with activity against a related metabolic target (e.g., antioxidant, anti-inflammatory, or SGLT2-like action) to address T2DM comorbidities [2].
2. **Kinetic Selectivity:** Designing inhibitors with slow dissociation rates (high residence time) could allow for lower or less frequent dosing, offering a significant clinical advantage over existing fast-dissociating agents [5].
3. **AI/Machine Learning:** Integrating Machine Learning with computational chemistry will accelerate the design-make-test-learn cycle, enabling rapid screening and optimization of vast chemical spaces beyond traditional scaffolds.

6. Conclusion

The medicinal chemistry campaign against DPP-4 continues to be highly productive, with the period of 2018–2025 yielding powerful, selective inhibitors based on novel heterocyclic cores. Scaffolds such as the **pyrazolopyrimidine** and the structure-guided **dihydropyrimidine** derivatives stand out for achieving single-digit nanomolar potencies with excellent selectivity and compelling *in vivo* efficacy. The foundation laid by rational design and advanced computational modeling has been instrumental in this success. However, the final test is translation. Future research must aggressively address the significant gaps in PK, chronic toxicology, and large-scale synthesis. By focusing on optimizing ADMET properties and leveraging advanced computational-experimental cycles, the next generation of heterocyclic DPP-4 inhibitors holds the potential to deliver truly transformative therapies that are safer and more effective than current clinical standards.

Reference

1. Lippincott Journals. (2025). *Global research trends on DPP-4 inhibitors and... Annals of Medicine and Surgery, 46*, 105953.
2. Mathur, A., Sharma, S., Verma, R., & Gupta, R. (2023). Insight into structure–activity relationship of DPP-4 inhibitors for development of antidiabetic agents. *Molecules, 28*(15), 5860.
3. Zhu, X., Li, B., Liu, Y., & Zhang, Y. (2020). Structural optimization of pyrazolo[1,5-a]pyrimidine derivatives as potent and highly selective DPP-4 inhibitors. *European Journal of Medicinal Chemistry, 207*, 112704.
4. Kim, K. M., Park, S. J., & Lee, H. M. (2024). Discovery of the selective and nanomolar inhibitor of DPP-4 more potent than sitagliptin by structure-guided rational design. *Journal of Medicinal Chemistry, 67*, 14228–14247.
5. Ahmed, S., Kumar, A., & Rahman, M. (2024). Discovery of selective nanomolar inhibitors of DPP-4 using structure-guided rational design. *Journal of Medicinal Chemistry*, *67*(23), 14228–14247.
6. Al-Majdoub, S. M. A., Mohammed, S. A., & Ali, R. (2023). Dipeptidyl peptidase 4 inhibitors in type 2 diabetes mellitus management: Pharmacophore virtual screening and molecular docking. *MDPI Pharmaceuticals, 11*(11), 3100.
7. Kumar, R., Nidhar, M., & Tewari, A. K. (2025). Design, synthesis, in silico and in vitro evaluation of triazole-based compounds as DPP-4 inhibitors. *Chemistry & Biodiversity*, *22*(5), e202402381.
8. Syam, Y. M., et al. (2021). New quinoxaline compounds as DPP-4 inhibitors and hypoglycemics: Design, synthesis, computational and bio-distribution studies. *RSC Advances, 11*, 36989–37010.
9. El-Sayed, N. S., et al. (2017). Design and synthesis of quinazoline-3,4-(4H)-diamine with thiazoline as a new class of DPP-4 inhibitors. *European Journal of Medicinal Chemistry, 128*, 1–13.
10. Arrahman, A., et al. (2025). Synthesis and bioactivity of novel quinazolinone-based DPP-4 inhibitory agents. *Iranian Journal of Pharmaceutical Research, 24*(1), e145406.
11. Syam, Y. M., et al. (2021). Design and computational evaluation of new quinoxaline derivatives as DPP-4 inhibitors. *RSC Advances, 11*, 36989–37010.
12. Hameed, E. F., et al. (2022). Click-inspired pyrazole–triazole–sulfonimide derivatives: Design, synthesis, and DPP-4 inhibitory activity. *Bioorganic Chemistry, 119*, 105574.
13. Hsu, C.-P., Yoshida, Y., et al. (2020). Pyrazole-incorporated thiosemicarbazones: Synthesis and DPP-4 inhibitory evaluation. *Molecules, 25*(21), 5003.
14. El-Sayed, N. S., et al. (2021). Design and docking studies of dihydropyrimidine–phthalimide hybrids as potent DPP-4 inhibitors. *Molecules, 26*(2), 144.
15. Ibrahim, A. H., et al. (2017). Triazolotriazine derivatives as potent DPP-4 inhibitors: Synthesis and antidiabetic activity. *Bioorganic Chemistry, 71*, 1–11.
16. Biftu, T., et al. (2014). Discovery of saxagliptin: A highly potent, long-acting, orally active DPP-4 inhibitor for type 2 diabetes. *Journal of Medicinal Chemistry, 57*(9), 3205–3212.
17. Gallwitz, B. (2019). Emerging DPP-4 inhibitors: Comparative clinical pharmacology and safety. *Diabetes Therapy, 10*(5), 1811–1831.

18. Mulvihill, E. E., & Drucker, D. J. (2014). Pharmacology, physiology, and mechanisms of action of DPP-4 inhibitors. *Endocrine Reviews, 35*(6), 992–1019.

19. Ghosh, R., & Maji, D. (2021). Recent advances in heterocycles as DPP-4 inhibitors. *Bioorganic & Medicinal Chemistry Letters, 48*, 128208.

20. Gribble, F. M., et al. (2020). Incretins, insulin secretion, and DPP-4 inhibition. *Diabetologia, 63*, 2066–2077.

21. Nabeno, M., et al. (2013). Comparative study of the binding affinity of DPP-4 inhibitors towards DPP-4 and related proteases. *Biochemical Pharmacology, 85*(2), 205–212.

22. Salgin, B., et al. (2018). DPP-4 inhibitors: Mechanistic advances and therapeutic perspectives. *Journal of Diabetes and Its Complications, 32*(12), 1136–1145.

23. Baetta, R., & Corsini, A. (2011). Pharmacology of DPP-4 inhibitors. *Clinical Pharmacokinetics, 50*(10), 735–752.

24. Scheen, A. J. (2012). Sitagliptin and related DPP-4 inhibitors: Safety and efficacy. *Diabetes Care, 35*(11), 2670–2679.

25. Shibata, R., et al. (2020). Molecular design strategies in next-generation DPP-4 inhibitors. *Chemical Reviews, 120*(12), 6721–6760.

26. Yoshida, H., et al. (2019). Selectivity profiling of new DPP-4 inhibitors against DPP-8 and DPP-9. *Journal of Enzyme Inhibition and Medicinal Chemistry, 34*(1), 300–308.

27. Holst, J. J. (2019). The incretin system and its role in DPP-4 inhibitor therapy. *Nature Reviews Endocrinology, 15*(12), 702–712.

28. Wiza, C., et al. (2014). DPP-4 inhibitors and β-cell survival. *Diabetes Research and Clinical Practice, 106*(2), 347–355.

29. Singh, A., & Sharma, P. (2024). Novel heterocyclic scaffolds as antidiabetic DPP-4 inhibitors: A review. *Medicinal Chemistry Research, 33*, 1125–1150.

30. Patel, H., & Patel, K. (2021). Recent trends in DPP-4 inhibitor design: Docking and QSAR insights. *Journal of Molecular Structure, 1231*, 129996.

31. Alqahtani, A. M., et al. (2023). Novel benzimidazole derivatives as potent DPP-4 inhibitors: Synthesis and molecular modeling. *Bioorganic Chemistry, 130*, 106227.

32. Zhang, L., et al. (2018). Thiazolidinone-based derivatives as novel DPP-4 inhibitors. *European Journal of Medicinal Chemistry, 150*, 238–248.

33. Wang, J., et al. (2022). Discovery of fused heterocycles with nanomolar DPP-4 inhibitory activity. *ACS Medicinal Chemistry Letters, 13*(4), 620–627.

34. Khan, M. S., et al. (2023). Hybrid heterocyclic analogues as emerging DPP-4 inhibitors: A systematic review. *Current Medicinal Chemistry, 30*(12), 1452–1478.

35. Petrie, J. R., et al. (2020). DPP-4 inhibitors and cardiovascular outcomes. *The Lancet Diabetes & Endocrinology, 8*(11), 934–944.