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Synthesis of New DPP-4 Inhibitors Based on a Novel Tricyclic Scaffold

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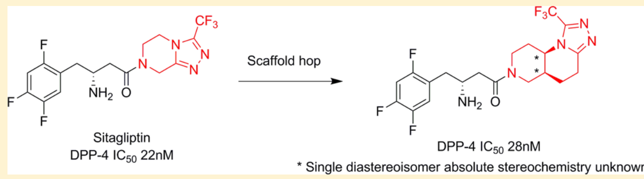
Supporting Information

ABSTRACT: A novel molecular scaffold has been synthesized, and its synthesis and incorporation into new analogues of biologically active molecules will be discussed. A comparison of the inhibitory activity of these compounds to the known type-2 diabetes compound (sitagliptin) against dipeptidyl peptidase-4 (DPP-4) will be shown.



KEYWORDS: *Diabetes, scaffold, crystal structure, dipeptyl peptidase VI (DPP-4) inhibitor, 1,2,4-triazole*

Diabetes remains one of the world's largest health problems with numerous different factors contributing to its pathogenesis. According to the WHO in 2013,[1] 347 million people were diagnosed with type-2 diabetes mellitus, with an alarming growth predicted over the next decade.

Type-2 diabetes is a chronic disease, characterized by elevated blood sugar levels, leading to severe vascular complications and an increased mortality risk. Dipeptidyl peptidase-4 (DPP-4), a widely distributed serine protease found solubilized in blood or anchored into tissue membranes, is involved in glucose metabolism and is now a validated target for antidiabetic therapy. Inhibition of DPP-4 has been shown to result in indirect stimulation of insulin secretion.[2,3] The mechanism of inhibition[4,5] is through an increase in the release of incretin (GLP-1 and GIP) following food intake, therefore inhibiting glucagon release, which in turn increases insulin secretion and decreases blood glucose levels.[6]

Sitagliptin (Januvia) was the first approved DPP-4 inhibitor launched by Merck in 2006.[7] It was followed by several, structurally diverse DPP-4 inhibitors, namely, vildagliptin, saxagliptin, alogliptin, linagliptin, and gemigliptin, and recent communications highlighting further compounds such as omarigliptin[8] and imigliptin[9] have been recently communicated (Figure 1).

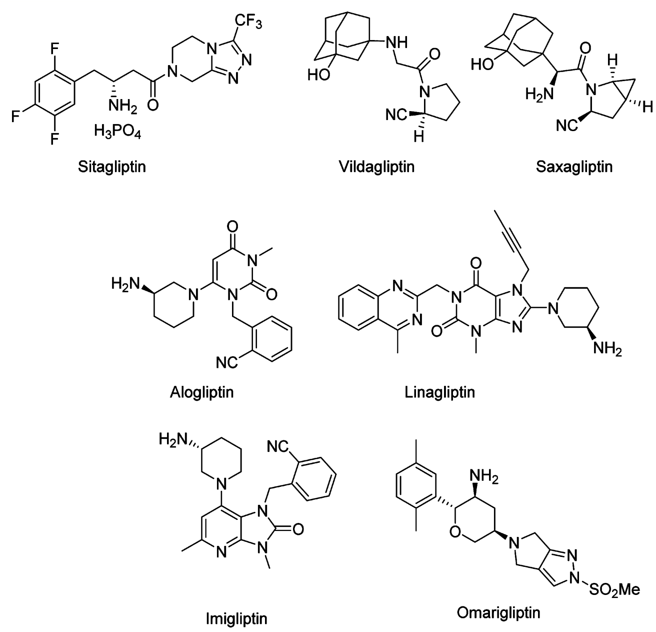


Figure 1. Approved DPP-4 inhibitors: sitagliptin, vildagliptin, saxagliptin, alogliptin, linagliptin, and the clinical candidates imigliptin and omarigliptin.

The so-called "gliptins" are under investigation for other potential therapeutic uses. For example, it was reported that potential substrates of DPP-4 could have implications in other metabolic disorders and that DPP-4 inhibitors could be utilized in the treatment of diseases associated with the immune/inflammatory response, heart failure, cancer, and neurodegenerative disorders. In addition it was stated that a positive role of DPP-4 inhibition was observed in diseases of the kidney and the cardiovascular system.[10]

In our ongoing exploration of novel constrained molecular scaffolds containing substituted ring-fused 1,2,4-triazoles, we were drawn to the possibility of substituting the piperazine-fused 1,2,4-triazolo group present in sitagliptin with a new tricyclic octahydro-[1,2,4]triazolo[4,3-*a*][1,6]naphthyridine molecular scaffold (**1a**–**b**) to generate sitagliptin hybrid structures of the type shown in compounds **2a** and **2b** (Figure 2).

To test our hypothesis, preliminary docking studies were carried out with the known crystal structure of sitagliptin in DPP-4 (pdb code 1X70).[11,12] The cis-fused diastereoisomer **2a** ($R_2 = CF_3$) showed a good overlay with sitagliptin as well as a good topographical fit into the enzyme pocket when compared to the trans-fused diastereoisomer **2b**. As expected, the 2,4,5-trifluorophenyl group fully occupied the S1 pocket, which was

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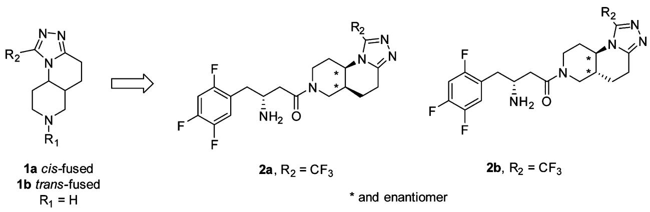


Figure 2. Novel tricyclic scaffold **1a**–**b** and proposed DPP-4 inhibitors **2a** and **2b**. Compounds **2a** and **2b** would exist as a 1:1 mixture of either cis- or trans-diastereoisomers.

previously reported by the Merck group.[13] From the docking studies it was noted that the key interactions observed in sitagliptin; namely, the four hydrogen bond interactions with Tyr662, Glu205, and Glu206 resulting from the (*R*)-amino group were still preserved along with the water molecule bridge present between the amide carbonyl of **2a** with Tyr547. Phe357 provides a $\pi-\pi$ interaction with the triazole core of compound **2a**. However, it was noticed that the inclusion of the sterically demanding tricyclic portion of **2a** led to a change in the orientation of the CF_3 group, losing the known interaction of this moiety in sitagliptin with Arg358 and Ser209. However, we felt that this potential loss in activity would be compensated by allowing us the exciting potential for discovering new interactions within the DPP-4 protease backbone (Figure 3).

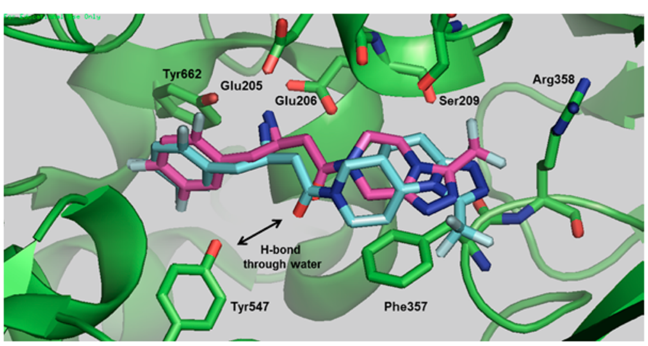
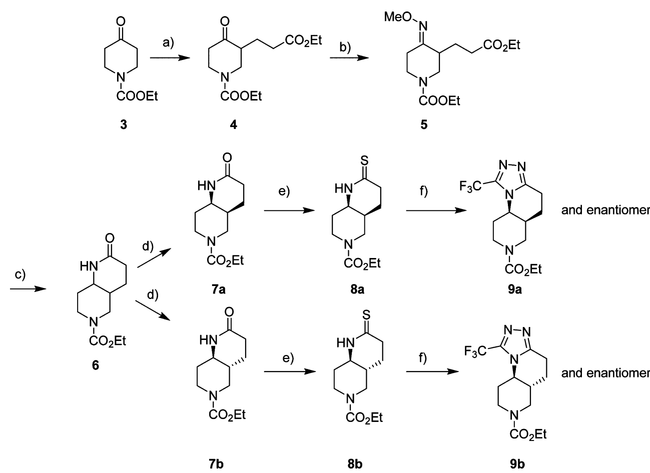


Figure 3. Compound **2a** (blue) docked into the DPP-4 active site (pdb code 1X70) overlaid with sitagliptin (magenta). Only one of the two possible diastereoisomers of **2a** with the optimal docking pose is shown. The image was generated using PyMol.

The novel scaffold **1** (represented as examples **9a** and **9b**) was synthesized in a robust six step racemic sequence starting from commercially available ethyl 4-oxopiperidine-1-carboxylate **3**. The first step in the sequence was the enamine alkylation with ethyl acrylate under Dean–Stark conditions that resulted in a high yield of the δ-keto ester **4**.[14] Conversion of **4** into the *O*-methyl oxime **5** using methoxyamine hydrochloride in pyridine, delivered the desired compound in a 1:1.5 mixture of imine isomers. These were converted to the bicyclic lactam **6** using Raney Nickel in 7 N ammonia in methanol under an atmosphere of hydrogen. At this stage the isomers generated in the ring closure were separated by flash chromatography on silica gel. Unfortunately, no assignment of the ring geometry was possible for the two separated lactam diastereoisomers (**7a** and **7b**) from NMR spectroscopy. Therefore, single crystal X-ray crystallography was needed to determine the relative configuration of the two isomers.[15] Each of the isomers (**7a** and **7b**) was converted into their corresponding thiolactams (**8a** and **8b**) using Lawessons' reagent.[16,17] These thiolactams were converted into the corresponding tricyclic triazoles (**9a** and **9b**) by refluxing in toluene with 2,2,2-trifluoroacetohydrazide (Scheme 1).

Scheme 1. Synthetic Route to Compounds 9a and 9b[a]

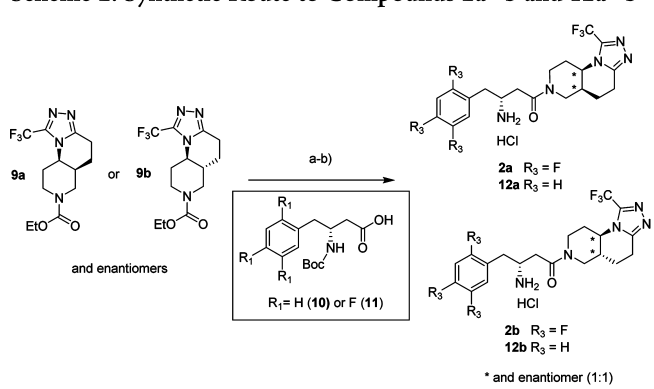


[a]Reagents and conditions: a) 1. pyrrolidine, benzene, rf, Dean–Stark; 2. ethyl acrylate, benzene, rf, o.n., 3. water, 2 h, rf, 69% (2 steps); b) $MeONH_2\cdot HCl$, pyridine, 84%; c) Raney–Nickel, 7 N ammonia in MeOH, H_2, o.n., 86%; d) flash chromatography ethyl acetate/hexane/methanol (10:1:1), ratio 1:4; e) Lawessons' reagent, toluene, rf, o.n., 90–94%; f) CF_3-hydrazide, toluene, 1–3 d, 120 °C, 40–84%. Compounds **9a** and **9b** exist as a 1:1 mixture of enantiomers.

The molecular scaffolds (**9a** and **9b**) were inserted in the sitagliptin structural motif by deprotection of the carbamates (**9a** and **9b**) in ethanol/water using potassium hydroxide under refluxing conditions to afford the key molecular scaffolds (Figure 2: **1a** and **1b**, R_1 = H, R_2 = CF_3) ready for reaction with the commercially available acids **10** and **11**. This coupling was high yielding and the final deprotection to the HCl salts (**2a**–**b** and **12a**–**b**) was carried out with 4 N HCl in dioxane (Scheme 2).

As the synthesis of the scaffolds **9a** and **9b** was in racemic form, the separation of the diastereoisomers of compounds **2a** and **2b** was required. Rewardingly, both diastereomers proved separable under chiral HPLC conditions with purity of at least

Scheme 2. Synthetic Route to Compounds 2a–b and 12a–b[a]



[a]Reagents and conditions: a) 1. KOH, water/ethanol, rf, o.n.; 2. **10** or **11** + EDCI, HOBt, DMF, rt, o.n., 77–91%; b) 4 N HCl in dioxane. Compounds **2a**–**b** and **12a**–**b** exist as a 1:1 mixture of either cis- or trans-diastereoisomers.

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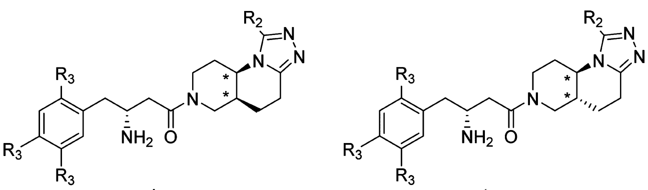
99% *de*. Therefore, **2a** was separated into the single diastereomers (**13** and **14**), and **2b** was separated as single diastereomers (**15** and **16**). Because of the robust nature of the synthetic procedure, a small series of further analogues was also produced (examples **17**, **18**, **19**, and **20**; see Supporting Information for the synthesis and yields). Inhibitory activity against DPP-4 was then determined (Table 1).[18]

Table 1. DPP-4 Inhibitory Activity



cis- *trans-*

compd	cis or trans	R3	R2	DPP-4 IC_{50}(nM)[a]
12a	cis	H	CF_3	695 ± 31[b]
12b	trans	H	CF_3	2801 ± 86[b]
13	cis	F	CF_3	28 ± 1[c]
14	cis	F	CF_3	70 ± 2[c]
15	trans	F	CF_3	145 ± 5[c]
16	trans	F	CF_3	537 ± 23[c]
17	cis	F	ethyl	85 ± 3[b]
18	trans	F	ethyl	108 ± 6[b]
19	cis	F	CF_2CF_3	67 ± 2[b]
20	trans	F	CF_2CF_3	258 ± 11[b]

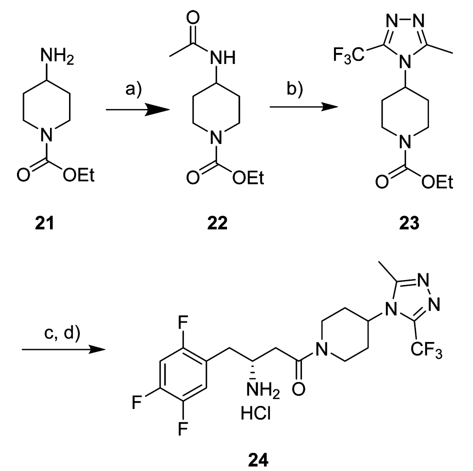
[a]Sitagliptin IC_{50} 22 ± 2 nM (n = 20). [b]One to one mixture of diastereoisomer. [c]Single diastereoisomers with unknown absolute configuration.

Gratifyingly, the compounds demonstrated very good levels of DPP-4 activity when compared to sitagliptin (IC_{50} 22 ± 2 nM). Notably, in all the tested analogues the cis-isomer reproducibly showed a better level of biological activity when compared to the trans-isomer (for example, compare **12a** and **12b**, and **17** and **18**). As this is an observation throughout all the synthesized derivatives, it demonstrates the effect of the connection of the rings to be important to allow the cis-fused compounds to establish optimal interactions within the active site of DPP-4. It was also encouraging to see that the separated diastereoisomers of **2a** and **2b** were shown to have either a 2.5-fold (cis-isomers, compare compounds **13** and **14**) or 3.6-fold (trans-isomers, compare compounds **15** and **16**) difference in activity, demonstrating further molecular recognition for either the novel cis-fused or trans-fused tricyclic scaffolds within the DPP-4 active site. This was in agreement with our original docking studies (see Figure S1, Supporting Information) where the **2a** cis-isomers (**13**–**14**) were observed to have a better topographical fit into the DPP-4 active site, preserving the water molecule bridge present between the amide carbonyl of **2a** with Tyr547. However, from the docking work the **2b** trans-isomers (**15**–**16**) were shown to lack this key interaction, and as a consequence, we proposed they would possess lower DPP-4 inhibitory activity.

The enlargement of the CF_3 group in **2a** to both the ethyl-substituted analogue **17** and the CF_2CF_3-substituted analogue **19** led to a decrease in inhibitory activity. The lack of the three fluorine atoms in the aromatic region (**12a** and **12b**) substantially decreased the activity, which concurred with similar observations previously reported by the Merck group.

In order to show the influence of the rigid tricyclic ring system toward DPP-4 inhibitory activity, the bicyclic compound **24** was synthesized. The commercially available amine **21** was converted to the substituted 1,2,4-triazole **23** in 15% yield.[19] Final deprotection of the carbamate group in **23** was carried out under established standard conditions using KOH in water/ethanol, and the coupling to the final bicyclic compound **24** was achieved using acid **11**, followed by deprotection using 4 N HCl in dioxane (Scheme 3).

Scheme 3. Synthesis of the Open Chain Compound 24[a]



[a]Reagents and conditions: a) Ac_2O, CH_2Cl_2, NEt_3, rt, o.n. 84%; b) 1. $POCl_3$, $CHCl_3$, pyridine; 2. $CF_3CONHNH_2$, $CHCl_3$; 3. 2 M HCl, 15% (3 steps); c) 1. KOH, water/EtOH; 2. **11**, EDCI, HOBt, DMF, rt, o.n., 93%; d) 4 N HCl in dioxane.

In order to gain insight into the impact of ring size for DPP-4 inhibitory activity, the synthesis of the novel cis-fused hexahydro-6*H*-[1,2,4]triazolo[4′,3′:1,5]pyrrolo[3,2-*c*]pyridine analogue **31** was carried out. The synthesis to the 5-membered lactams (**28** and **29**) followed a slightly different approach than for the 6,6-membered lactams **7a**–**b**. Commercially available ethyl 4-oxopiperidine-1-carboxylate **3** was converted into *tert*-butylester **25** using LDA and *tert*-butyl 2-bromoacetate. The *tert*-butylester **25** was converted through to the substituted benzylamine **26** via a reductive amination reaction using benzylamine and sodium triacetoxyborohydride in 1,2-dichloroethane. Compound **26** was trans-esterified with 0.6 M HCl in methanol to yield the methyl ester **27**, which was catalytically hydrogenated using Pd/C in MeOH under an atmosphere of hydrogen. The final ring closure to the key bicyclic lactams **28** and **29** was carried out with potassium carbonate in methanol and the diastereomers were separated by flash column chromatography. Once more, structural determination of the separated diastereomers (**28** and **29**) was performed through X-ray crystallography (see ref 15) (Scheme 4).

The separated cis-fused isomer **29** was converted to the final product through the standard synthetic procedure (Scheme 5).

The DPP-4 inhibitory activity for compounds **24** (IC_{50} 100 ± 4 nM) and **31** (IC_{50} 94 ± 4 nM) was established showing a reduced level of activity compared to **13** (separated most active isomer of compound **2a**, IC_{50} 28 ± 1 nM). In order to understand the DPP-4 inhibitory activity of compounds **2a**, **24**, and **31**, the compounds were energy minimized and docked into the known crystal structure of sitagliptin in DPP-4. As previously mentioned, compound **2a** displayed a good

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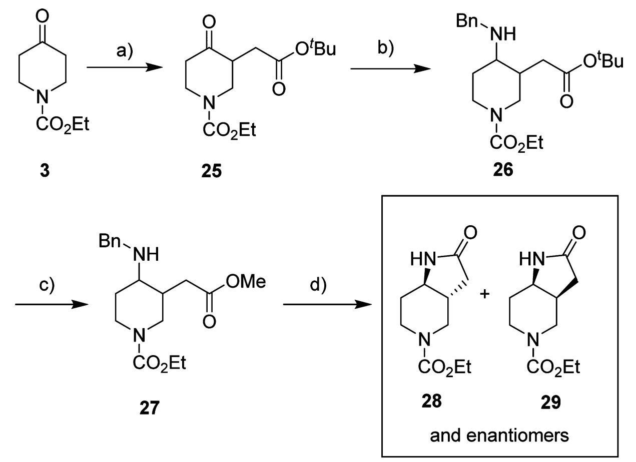
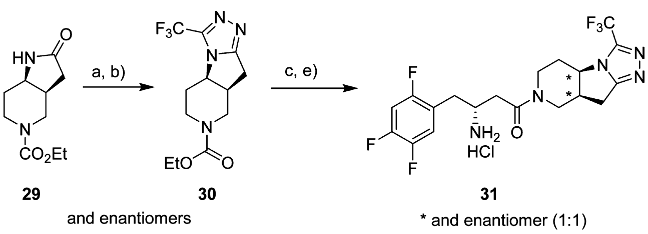
Scheme 4. Synthesis of the 6,5-Bicyclic Intermediates 28 and 29[a]



[a]Reagents and conditions: a) 1. LDA, THF, −78 °C, 30 min; 2. *tert*-butyl 2-bromoacetate, −78 °C to rt, 50%; b) benzylamine, $NaBH(OAc)_3$, 1,2-dichloroethane, 94%; c) 0.6 M HCl in MeOH; d) 1. Pd/C, MeOH; 2. K_2CO_3, MeOH, 45% (3 steps).

Scheme 5. Synthesis of the 6,5,5-Tricyclic Compound 33[a]



[a]Reagents and conditions: a) Lawessons' reagent, toluene, rf, o.n., 66%; b) CF_3-hydrazide, toluene, 1−3 d, rf, 70%; c) KOH, water/ethanol, rf, o.n., 50%; d) **11** + EDCI, HOBt, DMF, rt, o.n., 37%; e) 4 N HCl in dioxane. Compound **31** exists as a 1:1 mixture of cis-diastereoisomers.

topographical fit into the enzyme pocket, whereas compound **31** docked to allow overlay of the trifluoromethyl group of **31** with that of sitagliptin. However, in achieving this topographical fit, the Phe357 $\pi-\pi$ interaction with the triazole core of compound **31** is lost due to a steric clash imposed by the rigid tricyclic ring system and this could be an explanation for the observed reduction in DPP-4 inhibitory potency of **31** when compared to **2a**. For the bicyclic compound **24**, once more there is a good topographical fit into the enzyme pocket; however, the Phe357 $\pi-\pi$ interaction with the triazole core of compound **24** is not present, and this could again be an explanation for the observed loss in DPP-4 inhibitory potency (Figure 4).

In summary, we have shown the successful synthesis of promising new inhibitors for DPP-4 along with preliminary docking studies of the active compounds into the active site of the protease. The compounds demonstrated a range of activities with compound **13** (unknown absolute conformation) possessing similar levels of DPP-4 inhibitory activity to that of sitagliptin. We have shown the stereochemical preference for the cis-diastereomer of the novel octahydro-[1,2,4]triazolo[4,3-*a*][1,6]naphthyridine tricyclic ring system, and we are currently investigating a chiral synthesis of the key intermediates along with further structure design-based synthesis of analogues to interrogate the SAR within this interesting new tricyclic scaffold, which we will report in due course.

■ ASSOCIATED CONTENT

Supporting Information

Preparation and full characterization of the compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors.

Notes

The authors declare no competing financial interest.

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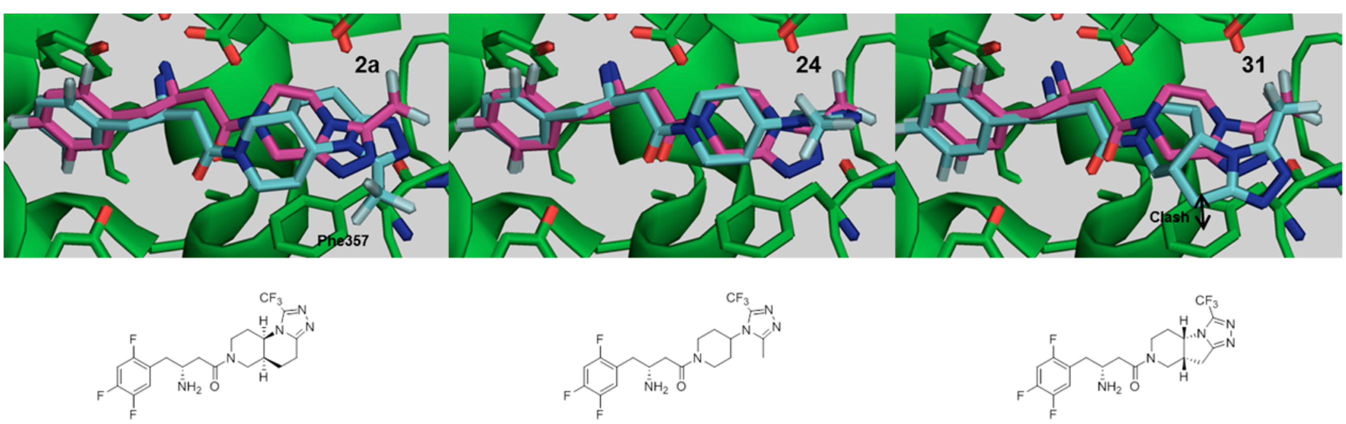

Figure 4. Compounds **2a**, **24**, and **31** (blue) docked into the DPP-4 active site (pdb code 1X70) shown overlaid with sitagliptin (magenta). For compounds **2a** and **31** only the single diastereoisomer with the optimal docking pose is shown. The image was generated using PyMol.

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■ ABBREVIATIONS

DPP-4, dipeptidyl peptidase 4; LDA, lithium diisopropylamide; SAR, structure−activity relationship; WHO, World Health Organization

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(18) The compounds were assayed for their DPP-4 inhibitory activity at HitGen using the following method: DPP-4 recombinant human protein (purchased from Sino Biological Inc.) cleaves a nonfluorescent substrate, H-Gly-Pro-AMC (purchased from Bachem Americas, Inc.), to release fluorescent, 7-amino-4-methyl coumarin (AMC) (ex/em = 360/460 nm). The initial rate of DPP-4 activity is measured over 15 min by following the fluorescent change at ex/em = 360/460 nm, and the fits are inspected to ensure that the reactions are linear to a correlation coefficient of 0.9. The resulting IC_{50} values are obtained by fitting log(inhibitor concentration) vs percentage of remaining activity using four-parameter dose−response model (Sigmaplot Version 11.0).

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