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Bioorganic & Medicinal Chemistry Letters 17 (2007) 3877–3879
Bioorganic & Medicinal Chemistry Letters

Modeling assisted rational design of novel, potent, and selective pyrrolopyrimidine DPP-4 inhibitors

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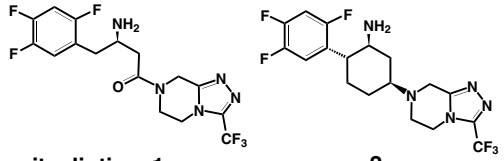
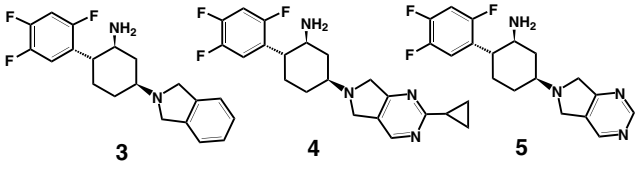
Received 30 March 2007; revised 27 April 2007; accepted 30 April 2007
Available online 3 May 2007

Abstract—Molecular modeling was used to improve potency of the cyclohexylamine series. In addition, a 3-D QSAR method was used to gain insight for reducing off-target DPP-8/9 activities. Compounds **3**, **4**, and **5** were synthesized and found to be potent DPP-4 inhibitors, in particular **4** and **5** are designed to be highly selective against off-target DASH enzymes while maintaining potency on DPP-4.

Inhibition of dipeptidyl peptidase IV (DPP-4) is a promising new approach for the treatment of type 2 diabetes.[1] DPP-4 is the enzyme responsible for inactivating the incretin hormones glucagon-like peptide 1 (GLP-1) and glucose dependent insulinotropic polypeptide (GIP). These two hormones play important roles in glucose homeostasis[2,3] and have been evaluated as potential antidiabetic agents.[4,5] However, DPP-4 rapidly cleaves the N-terminus of GLP-1 and GIP in vivo which makes the half-lives of the active peptides extremely short.[6,7] Small-molecule inhibitors of DPP-4 which prolong the beneficial effects of endogenous GLP-1[8,9] as well as stabilize GIP[10] have been pursued as a new drug class by many pharmaceutical companies. The potent, orally bioavailable and highly selective small molecule DPP-4 inhibitor sitagliptin (**1**) has obtained approval from the FDA and has been advanced to market.[12]

In a previous report, we described our effort in designing and synthesizing a rigid analog (**2**) with potency comparable to that of sitagliptin (IC_{50}s of 21 and 18 nM, respectively).[11] In this paper, we will discuss the use of docking studies to further improve potency of the cyclohexylamine series and a 3-D QSAR modeling to gain insight for reducing off-target DPP-8/9 activities.





Before the crystal structure (PDB entry 2P8S)[11] of compound **2** in complex with DPP-4 was solved, our docking study revealed that the cyclohexylamine group may be a good constraint for the middle β-amino butanoyl portion of the molecule; however, the right hand side triazolopyrazine group might be distorted in order to fit into the right hand side pocket, otherwise it would

Keywords: DPP-4; Molecular modeling; Potency; Selectivity against DPP-8/9.
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doi:10.1016/j.bmcl.2007.04.106

have close contact with the enzyme wall as depicted in Figure 1. In the crystal structure of **2** and DPP-4 complex,[11] multiple conformations are observed in the active site, and in the conformation where the triazolopyrazine group stacks face to face with the F357 side chain, it is indeed disordered as modeling predicted. The fact that multiple conformations are present in the active site also indicates that the triazolopyrazine piece is uncomfortable staying inside the right hand side pocket, it rotates itself while the enzyme takes the so-called 'breathing' motion. Taken together, a piece that better fits into the right hand pocket would improve potency on DPP-4.

To optimize the right hand group, modeling suggested that an isoindane type of 5–6 fused ring, instead of the triazolopyrazine (6–5 fused ring), may better fit into the pocket. As shown in Figure 2, the isoindane fused ring of the molecule (**3**) appears to fit into the pocket without distortion or bumping. In addition, the isoindane ring seems to π-stack with the side chain of F357 much better than the triazolopyrazine group does. This compound (**3**) was synthesized and evaluated in vitro for the inhibition of DPP-4 activity[13] and selectivity against other members in the DPP-4 activity and/or structure homologue family (DASH).[14] The data for DPP-4, DPP-8, DPP-9, FAP, and quiescent cell proline dipeptidase (QPP, also known as DPP-7)[13,15] inhibition are summarized in Table 1. On DPP-4, compound **3** gives 3-fold boost in potency compared with **2**. However, it also picked up DPP-8, DPP-9, and FAP activities. Selectivity against the off-target enzymes, in particular DPP-8 and DPP-9, was of great concern since safety studies using a selective DPP-8/9 dual inhibitor suggested that inhibition of DPP-8 and/or DPP-9 is associated with profound toxicity in preclinical species.[16]

Lacking structural information for DPP-8/9, we took a QSAR approach to understand what may contribute to high DPP-8 activity. We examined 2970 compounds that have IC_{50}s less than 500 nM on DPP-4 and have IC_{50} data

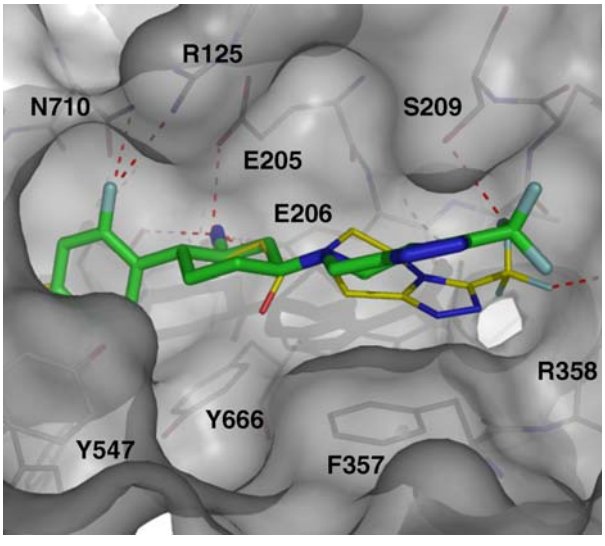

Figure 1. Compound **2** was modeled into the DPP-4 active site using the crystal structure of **1** and DPP-4 (PDB entry 1X70). Compound **1** is shown in yellow line.

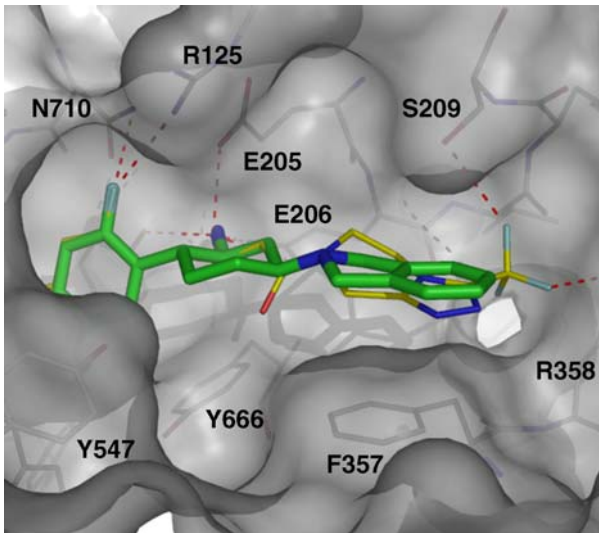

Figure 2. Compound **3** was modeled into the DPP-4 active site using the crystal structure of **1** and DPP-4 (PDB entry 1X70). Compound **1** is shown in yellow line.

available on DPP-8 as well. Trend vector analysis[17] with these data showed that DPP-4 and DPP-8 activities share very similar activity trends, therefore the trendvector models could not provide insight and ideas for reducing DPP-8 activity while maintaining the potency on DPP-4. We then used the ratio of DPP-4 IC_{50}/DPP-8 IC_{50} as input data for a 3-D QSAR approach.[18]

Structure alignment is a critical step for 3-D QSAR approaches. To ensure we were using and superimposing the biological relevant conformations for each compound in the data set, we first overlaid 25 diverse DPP-4 inhibitors using the conformations found in their (in-house) crystal structures in complex with DPP-4. Next we superimposed each of the 2970 compound structures with their most similar representative among the 25 crystal structures. This large structural set and log(DPP-4 IC_{50}/DPP-8 IC_{50}) data were then used for the 3-D QSAR analysis. Figure 3 presents the resulting contour plots from the analysis. It suggests that heteroatoms or polar substituents on the right hand group would help to increase the DPP-4 IC_{50}/DPP-8 IC_{50} ratio, namely, to provide more selective DPP-4 inhibitors. These results are in line with our chemical intuition that had been gained from known SAR of both DPP-4 and DPP-8.

To increase the polarity of the right hand group, an analog with a pyrimidine moiety, compound **4**, was synthesized and tested. While its DPP-4 activity remains the same, it is much more selective than **3**. The DPP-4 IC_{50}/DPP-8 IC_{50} ratio is 8600-fold compared with compound **3** which ratio is 258-fold. At the same time, selectivity against DPP-9 also increased significantly.

However, the SAR of compounds **3** and **4** made us wondering whether the increased selectivity was from the replacement of isoindane with heterocycle pyrimidine, or the introduction of the substitution group cyclo-propyl. To answer this question compound **5** was made and shown that the non-substituted pyrimidine **5** has about

Table 1. Activities of cyclohexylamine DPP-4 inhibitors

Compound	DPP-4 IC_{50} (nM)	DPP-8 IC_{50} (μM)	DPP-9 IC_{50} (μM)	QPP IC_{50} (μM)	FAP IC_{50} (μM)
1	18	48	>100	>100	>100
2	21	>100	>100	>100	60
3	6.6	1.7	5.5	68	7.0
4	5.3	45.6	72.3	61.0	10.6
5	0.67	5.0	45.4	>100	8.8

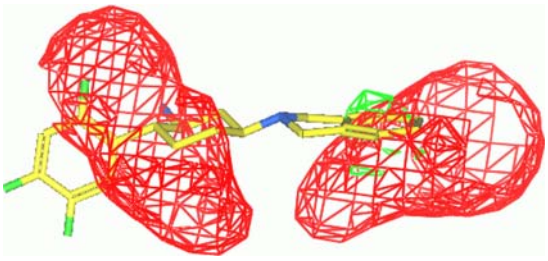

Figure 3. Contour plots for H-bond acceptor (red) and H-bond donor (green) interaction areas for increasing DPP-4 selectivity against DPP-8. Compound **3** is shown in yellow stick model.

7400-fold selectivity between DPP-4 and DPP-8. This implies that it is the replacement with pyrimidine that contributed significantly to the highly selective compounds **4** and **5**.

Rummey and Metz have recently published homology models of DPP-8 and DPP-9 based on the crystal structure of DPP-4.[19] They hypothesized that the highly selective profile of sitagliptin is a result of losing key contacts in the right hand pocket in DPP-8/9, instead of steric clashes with any of the ligand binding subsites. These missing contacts correspond to the interactions of sitagliptin with F357, R358, and S209 in DPP-4. Along these lines, the right hand groups of **3** and **4** may not have the key interactions with DPP-8/9 as well. The reduced DPP-8/9 activity for **4** could simply come from increased desolvation costs compared to **3**, while on DPP-4, the pyrimidine piece may better π-stack with the F357 side chain therefore gaining more binding energy on top of the desolvation factor. Although it would require crystal structures of DPP-8/9 to confirm these rationales, they are in line with our findings from the 3-D QSAR analysis and shed some light on designing highly selective DPP-4 inhibitors.

In summary, using docking studies based on the crystal structure of sitagliptin in complex with DPP4, and combining a 3-D QSAR method, we were able to improve the potency of the cyclohexylamine series, and gain structural insight for reducing DPP-8/9 activities. As a result, we have designed and synthesized pyrrolopyrimidine compounds as novel, potent, and selective DPP-4 inhibitors.

Acknowledgments

We thank Dr. Wendy Cornell in Molecular Systems of Merck Research Laboratories for her helpful comments on the manuscript. We are also grateful to Drs. Christian Rummey and Gunther Metz at Santhera Pharmaceuticals Ltd. for sharing their DPP-8 and DPP-9 models with us.

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