
Access from the University of Nottingham repository: http://eprints.nottingham.ac.uk/28517/1/M%20Stocks_ACS%20med%20chem%20letters_2015.pdf

Copyright and reuse:

The Nottingham ePrints service makes this work by researchers of the University of Nottingham available open access under the following conditions.

This article is made available under the University of Nottingham End User licence and may be reused according to the conditions of the licence. For more details see: http://eprints.nottingham.ac.uk/end_user_agreement.pdf

A note on versions:

The version presented here may differ from the published version or from the version of record. If you wish to cite this item you are advised to consult the publisher’s version. Please see the repository url above for details on accessing the published version and note that access may require a subscription.

For more information, please contact eprints@nottingham.ac.uk
Synthesis of new DPP-4 inhibitors based on a novel tricyclic scaffold.

Carolin Schwehm¹, Jin Li², Hongmei Song², Xiao Hu², Barrie Kellam¹, Michael J.Stocks¹*
¹ Centre for Biomolecular Sciences, University Park Nottingham, Nottingham, NG7 2RD, UK,
² Hitgen Ltd., F7-10, Building B3, Tianfu Life Science Park, 88 South Kayuan Road, Chengdu, Sichuan, China 610041

KEYWORDS Diabetes, Scaffold, Crystal Structure, dipeptidyl peptidase VI (DPP-4) inhibitor, 1,2,4-triazole

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.

Diabetes remains one of the world’s largest health problems with numerous different factors contributing to its pathogenesis. According to the WHO in 2013, 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 anti-diabetic therapy. Inhibition of DPP-4 has been shown to result in indirect stimulation of insulin secretion. The mechanism of inhibition 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.

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

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

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 utilised 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
In our on-going 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-triazole group present in sitagliptin with a new tricyclic octahetero-
\[1,2,4\text{-triazolo}[4,3-a][1,6]naphthyridine molecular scaffold (a1-b) to generate sitagliptin hybrid structures of the type shown in compounds a2 and b2 (Figure 2).

Figure 2. Novel tricyclic scaffold a1-b and proposed DPP-4 inhibitors a2 and b2. Compounds a2 and b2 would exist as a 1:1 mixture of either cis- or trans-diastereoisomers.

Reagents and conditions: a) 1. Pyrrolidine, benzene, rf, Dean Stark; 2. Ethyl acrylate, benzene, rf, o.n., 3. water, 2h, rf, 69% (2 steps); b) MeONH·HCl, pyridine, 84%; c) Raney-Nickel, 7N ammonia in MeOH, H2, 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) CF3- 

Scheme 1. Synthetic route to compounds a9 and b9.

The novel scaffold (represented as examples a9 and b9) was synthesized in a robust six step racemic sequence starting from commercially available ethyl 4-oxopiperidine-1-carboxylate. 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. Conversion of 4 into the O-methyl oxime 5 using methoxamine 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 7N 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. Each of the isomers (7a and 7b) was converted into their corresponding thiolactams (8a and 8b) using Lawessons’ reagent. These thiolactams were converted into the corresponding tricyclic triazoles (9a and 9b) by refluxing in toluene with 2,2,2-trifluoroacetohydrazide (Scheme 1).

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 (Scheme 1: 1a and 1b, R1 = H, R2 = CF3) ready for reaction with the commercially available acids 10 and 11. This coupling was high yielding and the final

Figure 3. Compound a2 (blue) docked into the DPP-4 active site (pdb code 1X70) overlaid with sitagliptin (magenta). Only one of the two possible diastereoisomers of a2 with the optimal docking pose is shown. The image was generated using PyMol.
deprotection to the HCl salts (2a-b and 12a-b) was carried out with 4N HCl in dioxane (Scheme 2).

Scheme 2. Synthetic route to compounds 2a-b and 12a-b.

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

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

Table 1. DPP-4 inhibitory activity

<table>
<thead>
<tr>
<th>Compound</th>
<th>cis- or trans-</th>
<th>R3</th>
<th>R2</th>
<th>DPP-4 IC50 [nM]a</th>
</tr>
</thead>
<tbody>
<tr>
<td>12a</td>
<td>cis-</td>
<td>H</td>
<td>CF3</td>
<td>695±31b</td>
</tr>
<tr>
<td>12b</td>
<td>trans-</td>
<td>H</td>
<td>CF3</td>
<td>280±86b</td>
</tr>
<tr>
<td>13</td>
<td>cis-</td>
<td>F</td>
<td>CF3</td>
<td>28±1e</td>
</tr>
<tr>
<td>14</td>
<td>cis-</td>
<td>F</td>
<td>CF3</td>
<td>70±2e</td>
</tr>
<tr>
<td>15</td>
<td>trans-</td>
<td>F</td>
<td>CF3</td>
<td>145±25e</td>
</tr>
<tr>
<td>16</td>
<td>trans-</td>
<td>F</td>
<td>CF3</td>
<td>537±23e</td>
</tr>
<tr>
<td>17</td>
<td>cis-</td>
<td>F</td>
<td>Ethyl</td>
<td>85±3b</td>
</tr>
<tr>
<td>18</td>
<td>trans-</td>
<td>F</td>
<td>Ethyl</td>
<td>108±6b</td>
</tr>
<tr>
<td>19</td>
<td>cis-</td>
<td>F</td>
<td>CF2CF3</td>
<td>67±2b</td>
</tr>
<tr>
<td>20</td>
<td>trans-</td>
<td>F</td>
<td>CF2CF3</td>
<td>258±11b</td>
</tr>
</tbody>
</table>

“Sitagliptin IC50 22±2 nM (n=20); |1:1 mixture of diastereomers; single diastereomer with unknown absolute configuration.

Gratifyingly, the compounds demonstrated very good levels of DPP-4 activity when compared to sitagliptin (IC50 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 supplementary material) 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 CF3 group in 2a to both the ethyl-substituted analogue 17 and the CF2CF3-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 towards 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 4N HCl in dioxane (Scheme 3).

In order to gain insight into the impact of ring size for DPP-4 inhibitory activity, the synthesis of the novel cis-fused hexahydro-6H-[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 0.6M 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 reference 15] (Scheme 4).

**Scheme 4. Synthesis of the 6,5-bicyclic intermediates 28 and 29.**

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

**Scheme 5. Synthesis of the 6,5,5-tricyclic compound 33**.

The DPP-4 inhibitory activity for compounds 24 (IC50 100±4 nM) and 31 (IC50 94±4 nM) was established showing a reduced level of activity compared to 13 (separated most active isomer of compound 2a, IC50 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 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 π-π 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 loss 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 π-π 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).

**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 enantiomer with the optimal docking pose is shown. The image was generated using PyMol.

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 characterisation of the compounds is given “This material is available free of charge via the Internet at http://pubs.acs.org.”

**AUTHOR INFORMATION**

**Corresponding Author**
* michael.stocks@nottingham.ac.uk

**Author Contributions**
The manuscript was written through contributions of all authors.

**ACKNOWLEDGMENT**
We acknowledge Dr Jinqiao Wan from HitGen for his help in arranging the assaying of the compounds. Dr Simon Hirst and Stephanie Barlow (Sygnature Discovery) for their assistance in the separation of the diastereomers using chiral HPLC.

**ABBREVIATIONS**

DPP-4 (Dipeptidyl peptidase 4); LDA (lithium diisopropylamide); SAR (structure Activity Relationship); WHO (World Health Organisation)

**REFERENCES**
Authors are required to submit a graphic entry for the Table of Contents (TOC)


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 non-fluorescent 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 insure that the reactions are linear to a correlation coefficient of 0.9. The resulting IC50 values are obtained by fitting log (inhibitor concentration) vs. the percentage of remaining activity using four-parameter dose-response model (SigmaPlot Version 11.0).