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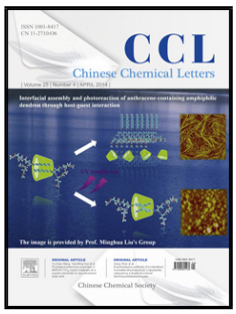

Title: Discovery of dipeptidyl peptidase IV (DPP4) inhibitors based on a novel indole scaffold

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Graphical Abstract

Discovery of dipeptidyl peptidase IV (DPP4) inhibitors based on a novel indole scaffold

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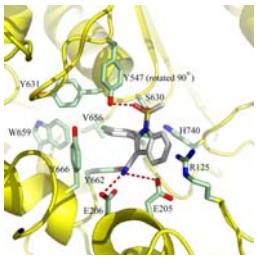

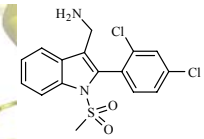

Indole sulfonamide **1**

DPP4 inhibitor IC_{50} = 232.8 nm/L

The story of our effort in the *de novo* design and development of a series of potent and selective DPP4 inhibitors based on an indole scaffold utilizing structure-based drug design (SBDD) technology.

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Original article

Discovery of dipeptidyl peptidase IV (DPP4) inhibitors based on a novel indole scaffold

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ABSTRACT

Dipeptidyl peptidase IV (DPP4) inhibitors are proven in the treatment of type 2 diabetes. We designed and synthesized a series of novel indole compounds that selectively inhibit the activity of DPP4 over dipeptidyl peptidase 9 (DPP9) (>200 fold). We further co-crystallized DPP4 with indole sulfonamide (compound **1)** to confirm a proposed binding mode. Good metabolic stability of the indole compounds represents another positive attribute for further development.

1. Introduction

Incretin hormones, including glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like-peptide-1 (GLP-1), stimulate insulin secretion, inhibit glucagon secretion, delay gastric emptying, and reduce food intake [1]. The rapid increasing plasma levels of both hormones in response to elevated serum glucose levels are negatively regulated by dipeptidyl peptidase IV (DPP4) [2], a serine protease expressed in most tissues [3]. DPP4 regulates insulin secretion by the inactivation of GIP and GLP-1 through removing two amino acids from the N terminus of both hormones [4]. Enhancing the duration of endogenous incretin hormone by inhibiting DPP4 function is now a validated approach in treatment of type 2 diabetes [1]. Several DPP4 inhibitors have been approved by the FDA, such as sitagliptin [5], saxagliptin [6], linagliptin [7] and alogliptin [8a] while several others are in late stage clinical trials [9, 10].

Structure-based drug design (SBDD) has been extensively used in modern medicinal chemistry for the discovery of many drugs. In this paper we describe our effort in the *de novo* design and development of a series of potent and selective DPP4 inhibitors based on an indole scaffold utilizing this technology.

2. Experimental

The synthesis of compound **1**, **2**, **8a-8f** and **11a-f** (Scheme 1) began with 1-(1*H*-indol-3-yl)-*N*,*N*-dimethyl methylamine (**3**) [11]. Compound **3** was treated with MeI in THF, followed by heating with potassium phthalimide in anhydrous DMF at 150 ^{o}C to generate **4** [12]. Compound **5** were prepared from **4** using pyridinium tribromide at -10 ^{o}C. The intermediate compound **6** was synthesized through Suzuki coupling reaction of compound **5** with 2,4-dichlorophenyl boronic acid in a mixture of toluene and EtOH at 105 ^{o}C. Compounds **8a-8f** and **1** were obtained by reacting **5** with R^1-sulfonyl or acyl chloride, followed by the removal of the phthalimide group using NH_2NH_2 in EtOH at room temperature. Similarly, **11a-11f** were readily synthesized in three steps including Suzuki coupling reaction between compound **5** and different phenyl boronic acids, sulfonamide formation using different alkyl sulfonyl chloride, and the removal of the phthalimide group.

Detailed synthesis procedure and characterization data of the synthesized compounds can be found in Supporting information.

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3. Results and discussion

Before the initiation of the *de novo* design, we first studied the structure of several known DPP4-inhibitor complexes [8a-d] and identified several key interactions between the protein and the inhibitors. As shown in Fig. 1, alogliptin tightly binds in the active site of DPP4 through several important interactions. The cyanobenzyl group fits in the P1 hydrophobic pocket formed by Val656, Tyr631, Try662, Trp659, Tyr666 and Val711. The cyano group also forms a hydrogen bond with Arg125. Another key interaction is a salt bridge interaction between the aminopiperidine in alogliptin and residues Glu205/Glu206 in DPP4. The 4-carbonyl oxygen in the primidindione interacts with the backbone NH of Tyr631 through a hydrogen bond, while the pyrimidindione ring itself forms π-stack interaction with the phenyl ring on the Tyr547 residue. From DPP4-alogliptin and other co-crystal structures, we recapitulated the following important interactions: (a) hydrophobic filling in the P1 pocket, (b) salt bridge to Glu205/Glu206, (c) π-stacking and hydrogen bond with NH of Tyr631or OH of Ser630. Rather than starting from a screening hit, we decided to use the information described above to *de novo* design new chemical structures that could capture all or most important interactions with DPP4. An indole scaffold having certain functional groups was one of our designs. As schematically shown in Fig. 2, a methylamine motif at C-3 provides a salt bridge to E205/E206, the phenyl group fills the P1 pocket, while an acyl group or alkyl sulfonyl group at *N*-1 could form one or two potential hydrogen bonds to Ser630, Tyr547 and Tyr361.

To evaluate the design, we first synthesized methylsulfonyl derivative **1** and acetyl derivative **2**, which showed inhibitory activity against DPP4 at an IC_{50} concentration of 232 nmol/L and 38% inhibition at 10 umol/L, respectively (Table 1). Furthermore, compound **1** also showed more than 200 fold of selectivity over DPP9. According to our modeling results, the sulfonyl group in compound **1** could form more favorable hydrogen bonds with Ser630 and Tyr547 residues of DPP4, thus explained the better activity of compound **1**. To verify our modeling results and our hypotheses, we co-crystallized compound **1** and DPP4 (Table 2). The detail structural information (Fig. 3) revealed the mode of binding of compound **1** with DPP4 in the active site. The indole ring forms a cation-π interaction with the guanidinium moiety of Arg125, the methylamine motif forms salt bridges to Glu205/Glu206, and the 2,4-dichlorobenzene group effectively fits in the P1 pocket. Comparing the proposed binding mode and the real crystal structure, the inhibitor conformation was as predicted except the amine orientation. The proposed hydrogen bond between the sulfonyl group and Tyr 547 and the orientation of the methylsulfonyl group are in good agreement with the crystal structure. However, the residue Tyr547 of DPP4 rotates 90 degrees in the co-crystal structure thus differed from the structure of DPP4-Alogliptin and other DPP4 complexes, which causes the loss of the hydrophobic interactions.

The encouraging results motivated us to develop structure activity relationship (SAR) both on the alkyl sulfonyl and Ar groups. Based on the analysis of our crystal structure, we decided to modify the R1 group on the sulfonyl group to generate more interactions with the phenyl ring of Tyr547 in order to improve the potency. According to the SAR shown in Table 1, similar to compound **1** ethylsulfonyl (**8a**), isopropylsulfonyl (**8c**) and isobutylsulfonyl (**8d**) analogs demonstrate good potency against DPP4, while the propylsulfonyl (**8c**) and cyclopropylsulfonyl (**8d**) analogs are relatively weak. Our interpretation for the SAR is that the hydrogen bond between the indole compound and the Tyr547 is not as strong comparing with the hydrogen bond between Alogliptin and Tyr631, while the phenol group in Tyr547 is more flexible compared with the backbone NH in the Tyr631. Therefore increasing the □π-stack interactions with the Tyr547 might not be beneficial.

The P1 pocket of DPP4 is the most important area utilized by others in their inhibitor design; therefore our second optimization study was focused on modifying the Ar group on the indole core. With knowledge learned from others [8a-d], the 2,4-di-Cl phenyl group was used in our first inhibitor design (compound **1**). To understand SAR at this position, compounds with mono or di-substituted phenyl group (**11a-11f**) were synthesized. The inhibition testing results (Table 3) showed that only 2,4-disubstituted phenyl compounds (**8c** and **11d**) were tolerated and all other modifications caused significant activity loss, which indicated that the P1 pocket might be rigid and sensitive to the substituents on the phenyl ring.

In general, the indole sulfonamide analogues are stable in the liver microsome metabolic stability test (see Table 1), indicating that indole sulfonamide is a suitable scaffold for further development. DPP9 belongs to the same family of DPP4 and blocking its function could cause some undesired side effects [13]. Therefore the most potent compounds (**8a-8e** and **1**) were also tested against DPP9 enzymatic activity, and more than 200 fold selectivity has been achieved for DPP4 over DPP9.

4. Conclusion

In conclusion, we have *de novo* designed and developed a series of indole DPP4 inhibitors using computer modeling and known DPP4-inhibitor complex structures. The proposed binding mode of the indole compound in the active pocket overlapped well with that observed from the co-crystal structure of DPP4 and compound **1**. Preliminary SAR around indole core has been developed and a series of potent and selective DPP4 inhibitors with favorable physical properties were obtained. Further optimization around the core is in progress.

Acknowledgments

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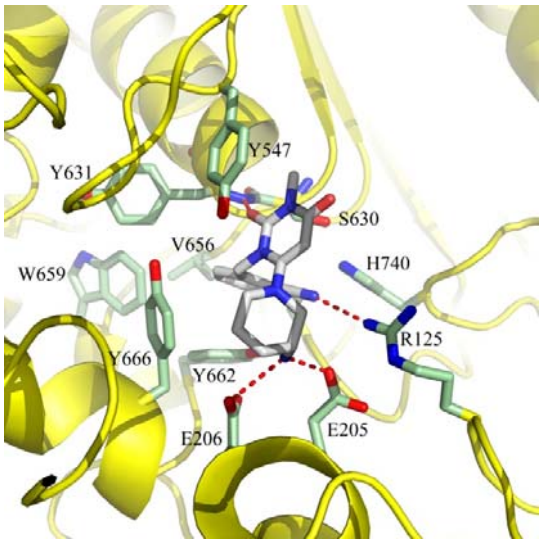


Fig. 1. Cocrystal structure of Alogliptin in the DPP4 active site.

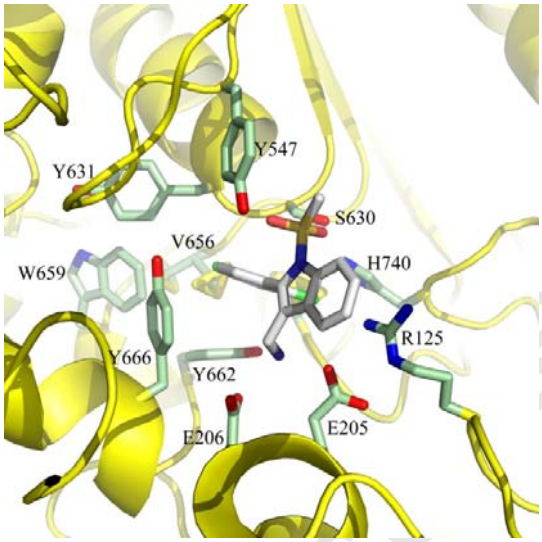


Fig. 2. A model of compound **1** in the DPP4 active site showing structure based design.

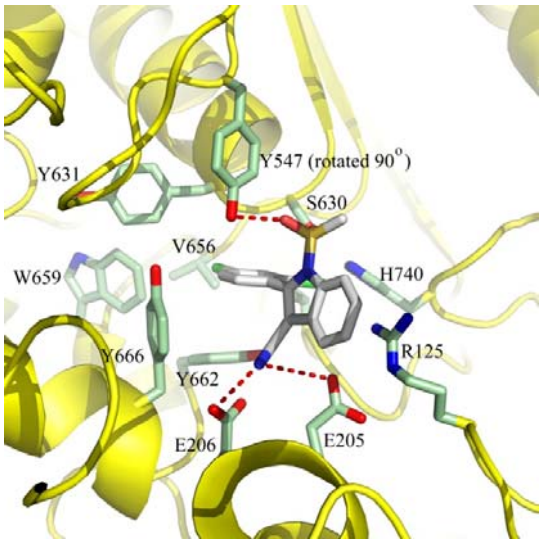
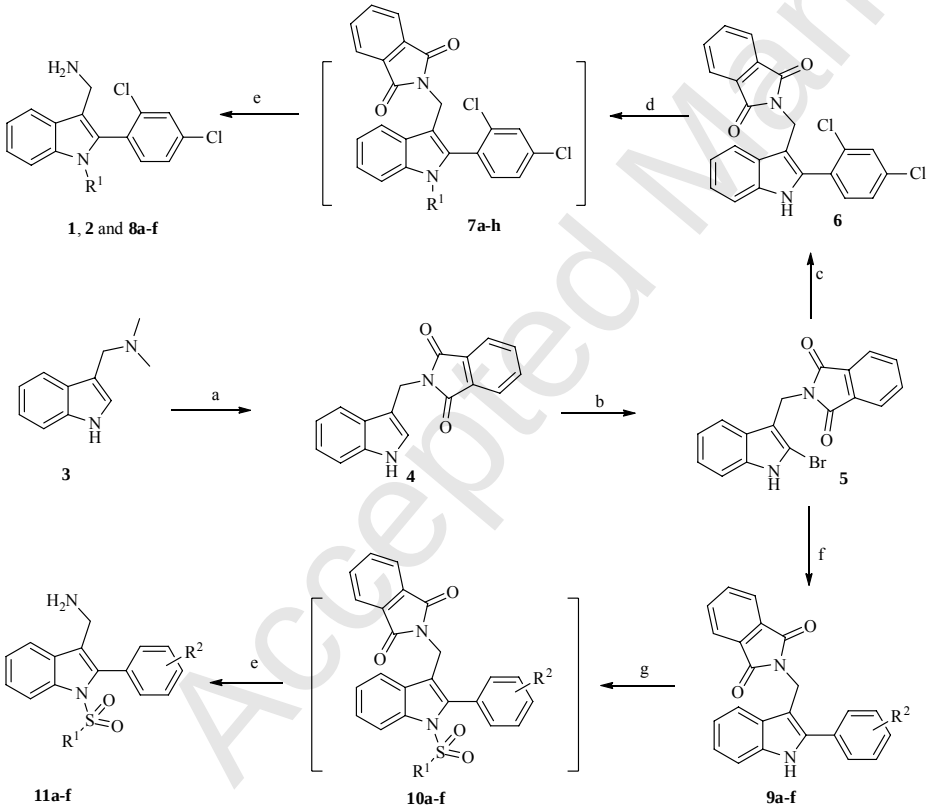
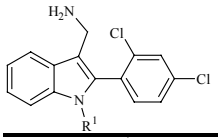


Fig. 3. Cocrystal structure of 1 in the DPP4 active site.



Scheme 1. Synthesis of compounds **1**, **2** and **8a-f** and **11a-f**. Reagents and conditions: (a) MeI, THF, potassium pthalimide, DMF, 150 ºC for 5 h, 64%; (b) THF, $CHCl_3$, pyridinium tribromide, -10 ºC for 3 h, 56%; (c) 2,4-dichlorophenylboronic acid, $Pd(PPh_3)_4$, LiCl, Na_2CO_3, PhMe, EtOH, 105 ºC for 4 h, 30%; (d) R^1-sulfonyl or acyl chloride, NaH, DMF, 0 ºC for 16 h, 31%-50%; (e) NH_2-NH_2, EtOH r.t. for 1 h, 84%-91%; (f) R^2-phenyl boronic acid, $Pd(PPh_3)_4$, LiCl, Na_2CO_3, PhMe, EtOH,105 ºC for 4 h, 31%-57%; (g) R^1-sulfonyl chloride, NaH, DMF, 0 ºC for 16 h, 31%-50%.

Table 1 Selected data for indole scaffold analogues (R^1 substitution).



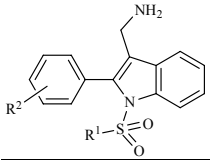
Compd.	R^1	DPP4 IC_{50} (nmol/L)	DPP9 IC_{50} (umol/L)	RLM [a] $t_{1/2}$ (min)	HLM [b] $t_{1/2}$ (min)	MLM [c] $t_{1/2}$ (min)
1	methylsulfonyl	232.8	53	99	182	84.5
2	acetyl	38%@10 umol/L	NT	NT	NT	NT
8a	ethylsulfonyl	219.63	73	73.0	347	46.8
8b	propylsulfonyl	375.93	104	70.7	99.0	26.5
8c	isopropylsulfonyl	216.39	85	85.6	693	36.9
8d	cyclopropylsulfonyl	374.96	86	66.6	347	50.2
8e	isobutylsulfonyl	214.97	110	35.5	24.2	9.33
8f	cyclohexylsulfonyl	66%@10 umol/L	NT	NT	NT	NT

[a] RLM: incubation with rat liver microsomes.
[b] HLM: incubation with human liver microsomes.
[c] MLM: incubation with mouse liver microsomes.
NT: not tested.

Table 2 X-ray data and refinement statistics.

Data set		Refinement	
Space Group	$P3_2$	Resolution (Å)	3.25
Unit Cell (a,b) (Å)	79.8, 286.8	No. of reflections |F|>0 σF	31,241
Resolution (Å)	3.25	R-factor/R-free (%)	22.7/26.6
Measured reflections	167,140	No. of protein atoms	11,914
Unique reflections	32,631	No. of compound molecules	2
Redundancy	5.1	No. of water molecules	0
Completeness (%, highest shell)	99.9 (100.0)	rmsd bond lengths (Å)	0.008
Mean I/σI (highest shell)	29.0 (2.0)	rmsd bond angles (°)	1.17
Rsym (%, highest shell)	11.0 (100)		

Table 3 Selected data for indole scaffold analogues (R^2 substitution).



Compd.	R^1	R^2	DPP4 IC_{50} (nmol/L)	DPP9 IC_{50} (umol/L)
8c	isopropyl	2,4-diCl	216.39	85
11a	isopropyl	2,3-diCl	27%@10umol/L	NT
11b	isopropyl	2,5-diCl	45%@10umol/L	NT
11c	isopropyl	2-Cl,4-F	70%@10umol/L	NT
11d	Me	2-Cl,4-Me	284.58	75
11e	isopropyl	2-Cl	34%@10umol/L	NT
11f	isopropyl	4-Cl	>10000	NT

NT: not tested.