Design and Synthesis of Novel Thiazolidine and Pyrrolidine Derivatives as DPP-IV Inhibitors

Ramesh C. Gupta, Laxmikant Chhipa, A. B. Mandhare, Sunil S. Nadkarni
Deepa Joshi, Shital Zambad, Padmaja Pathak, Vijay Chauthaiwale, C. Dutt

Torrent Research Centre, Torrent Pharmaceuticals Ltd. Village: BHAT, Tal. & Dist. Gandhinagar-382 428, Gujarat, INDIA. www.torrentpharma.com

Abstract

Various novel derivatives of substituted thiazolidine and pyrrolidine were designed and synthesized as Dipeptidyl peptidase-IV inhibitors (DPP-IV). Based on the in-vitro DPP-IV activity, selected molecules were further evaluated for their ADME and in-vivo profile in various animal models. TRC8156 (IC50= 88 nM) has shown good in-vivo profile at dose 40 and 80μM/kg b.i.d. TRC-8156 has been found potent, selective, competitive, reversible, hERG negative, non-mutagenic in AMES test and does not show micronuclei induction potential in mammalian erythrocyte micronucleus tests. It was also found safe in 30 days rodent toxicity studies. Based on its DPP-IV potency, selectivity, in vivo efficacy and pharmacokinetic profile TRC8156 has been taken for further development as DPP-IV inhibitor for the treatment of type 2 diabetes mellitus.

1. Introduction

Diabetes mellitus is a metabolic condition in which the body fails to produce enough insulin action for clearance of glucose from plasma. Primary defects in insulin secretion along with development of insulin resistance, contribute to the etiology of type 2 diabetes mellitus. Modern lifestyle and dietary factors also contribute to an alarming rise in the incidence of type 2 diabetes. Hence, search for novel mechanistic approaches to control chronic metabolic disease was intensified.

Glucagon like peptide (GLP-1), produced by L- cell in distal small bowel, stimulates glucose dependent insulin secretion. However, its effects are short-lived as a result of rapid inactivation by Dipeptidyl peptidase-IV (DPP-IV).The mechanism of insulinotropic action of GLP-1 involves interaction with a specific receptor belonging to the glucogon subfamily of G-protein coupled receptor located on the pancreatic beta-cell, with subsequent activation of adenylate cyclase, which leads to increase in c-AMP, results in increased insulin release. GLP-1 was shown to retain insulinotropic action without risk of hypoglycemia when given to insulin sensitive normal weight type-2 diabetic patient[1].

DPP-IV is an abundant and widely distributed serine protease. It is located on endothelial cells of the blood vessels throughout the body and circulates as soluble enzyme. It cleaves dipeptides containing proline or alanine at penultimate position from the amino termini of substrate proteins[2] (Fig.-1).

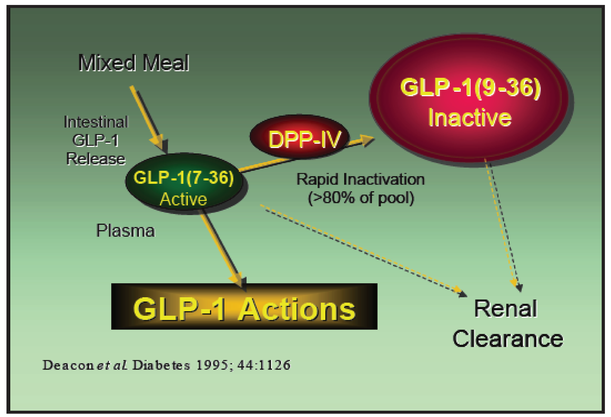


Fig.-1: Mechanism of DPP-IV inhibitors

DPP-IV inhibitors have many advantages like increased insulin release and suppressed glucagon release in a glucose-dependent manner. Hence they pose lower risk of a hypoglycemia and weight gain than that observed with other antihyperglycemic agents.

Since the knowledge of the rapid inactivation of GLP-1 by DPP-IV was established in mid 1990s, the widely accepted strategy is towards to development of small molecules as DPP-IV inhibitors. There are several other DPPs like DPP-II, DPP6, DPP7, DPP8 and DPP9 are also present, hence the high selectivity of inhibitor would be advantage to eliminate side effect associated with other dipeptidyl peptidases.

Sitagliptin (MK-0431) is now in market while vildagliptin (LAF237) and several other DPP-IV inhibitors are in the advanced developmental stage and some of them are listed in table-1 and structures are shown in Fig.-2.

Table-1: List of DPP-IV inhibitors at Advance clinical stage

Company	Drug	Status
Novartis	Vildagliptin	III
Merck & Co.	Sitagliptin	Launched
Bristol-Myers	Sexagliptin	III
GlaxoSmithKline	Denagliptin	III
Takeda	Alogliptin	III
Roche	R1438	II
OSI	PSN9301	II
Glenmark	GRC-8200	II
Taisho Pharma	TS-021	I

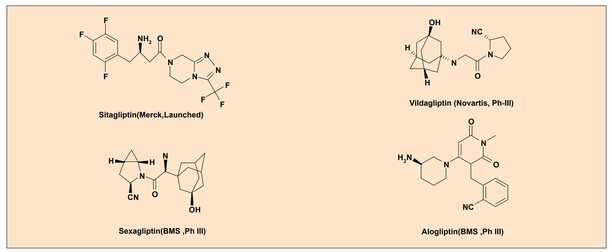


Fig.-2: Structure of DPP-IV inhibitors

At Torrent Research Centre, we have also initiated our program and succeeded to get novel, potent, selective, competitive and reversible DPP-IV inhibitors. The details of design, SAR and biological data are presented here.

2. Design and Synthesis

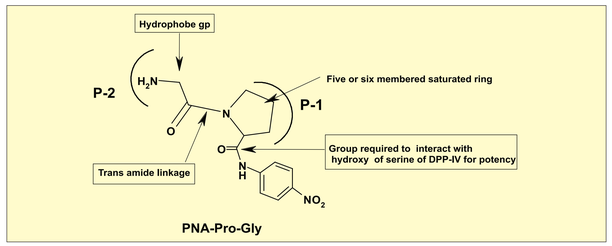
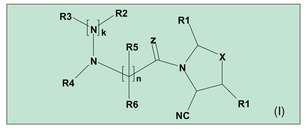
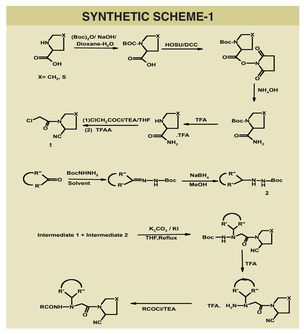


Fig.-3: Required feature for DPP-IV Inhibition[5]

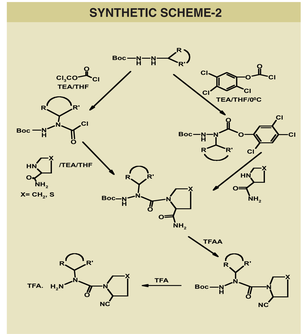
We have designed compounds based on the structural information of known ligand[3-4] (fig-3) and using molecular modeling technique. We have considered various heterocyclic like oxazolidines, pyrrolidines, piperazines; thiazolidines in designing with required pharmacophore.Various in house designed novel molecules were synthesized and screened for DPP-IV inhibitory assay. QSAR was developed on the series of pyrrolidine and thiazolidine class of molecules. Appropriate QSAR models were then chosen for activity predictions of the newly designed molecules. The synthesized novel molecules belong to following general formula (I) and their synthetic strategies are shown in schemes 1 to 3.



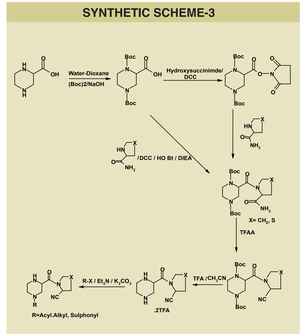
SYNTHETIC SCHEME-1



SYNTHETIC SCHEME-2



SYNTHETIC SCHEME-3



3. Results and Discussions

All the synthesized derivatives were screened for DPP-IV inhibition activity. Compounds having IC50 < 250 nM were listed in table-2. Strucure-activity relationship has been described in Fig-4. All the selected compounds have been subjected for selectivity studies. TRC8156 was found as a most selective derivative (table-3) and selected for pharmacokinetics and in vivo studies. Fig-5 shows the pharmacokinetic profile of TRC8156 at single dose treatment in n5-STZ diabetic rat. The in-vivo activity profile of TRC8156 has been described under fig-6. In all the studies activity profile was compared along with Vildagliptin (LAF237). TRC8156 showed encouraging results in vivo and selected for safety evaluation as shown in table-4. TRC8156 was found safe and has wide therapeutic window in 30 days toxicity studies in rat.

Table-2: In-Vitro Profile of TRC Compounds

Sr. No.	Code	IC50(nM)
1	TRC 8113 (LAF237)	32
2	TRC 8156	88
3	TRC 8161	90
4	TRC 8172	98
5	TRC 8179	104
6	TRC 8162	119
7	TRC 8136	120
8	TRC 8160	185
9	TRC 8140	200
10	TRC 8145	222
11	TRC 8158	226

Table-3: Selectivity Profile of TRC8156

	Concentration (100x IC50 of DPP-IV) (nM)	% Inhibition					
		PEP	DPP-II	Trypsin	Elastase	DPP8	DPP9
LAF-237	3200	Nil	Nil	Nil	Nil	Nil	50% inhibition at 10,000 nM
TRC 8156	8800	32.8	43.4	Nil	Nil	Nil	20% inhibition at 100,000 nM

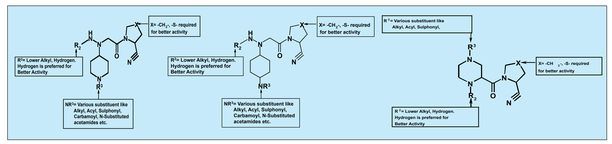


Fig-4: Strucure-activity relationship

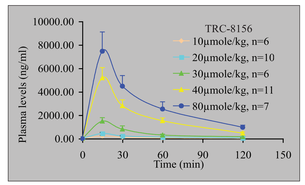


Fig-5: Pharmacokinetic Profile of TRC8156 at Single dose treatment in n5-STZ Diabetic Rats

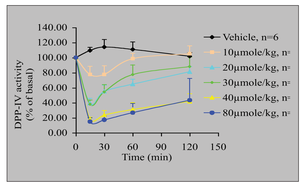


Fig-6a: Dose Response for DPP-IV Activity of TRC8156 in n5-STZ Diabetic Rats

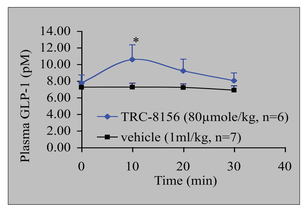


Fig-6b: Maximal efficacy dose of TRC8156 in n5-STZ Diabetic Rats

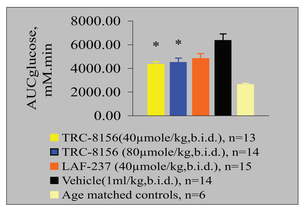
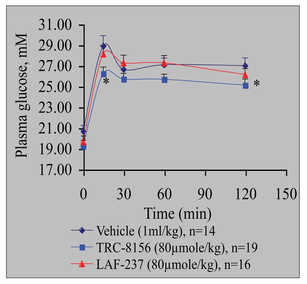


Fig-6c: 21 days treatment in n5-STZ diabetic rats aTRC 8156 vis-à-vis LAF-237



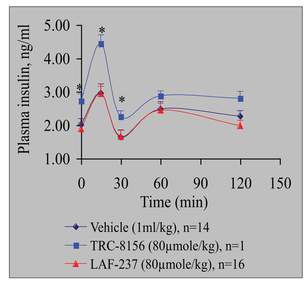
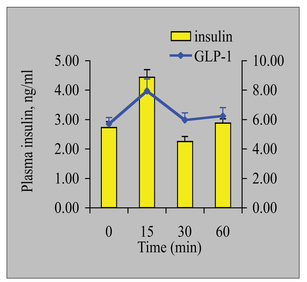
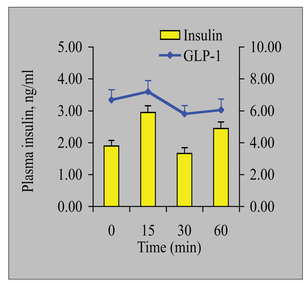


Fig-6d: 10-weeks treatment in ZDF rats of TRC8156



TRC-8156



LAF237

Fig-6e: OGTT at Week 8 (15-25 mM fasted glucose) in ZDF Rats TRC8156 and LAF237

Table-4: Safety profile of TRC8156

Sr. No.	Study Name	Results
1	hERG	Negative
2	AMES Test	Negative
3	Mouse micronucleus test	Negative
4	30 days rat toxicity studies at	175, 350 and 900 mg/kg b. wt.

4. Conclusions

Based on the biological response as discussed above the benefits and advantages offered by TRC8156 are summarized in the following way.

1. Improving glucose tolerance
2. Improving insulin secretion with better glucorecognition
3. Improves beta cells function
4. Effective across different stages of the progression of disease..
5. Maintains Phase-1 Insulin secretion indicating better functioning beta cells
6. Effective even in severe diabetes
7. No tachyphylaxis after long term treatment
8. Persistent benefit even after the drug washout.

Finally TRC8156 has been selected for further development as DPP-IV inhibitor in treatment of type 2 diabetes.

5. References

1. Knop, F. K., Vilsboll, T., Larsen, S., Madsbad, S., Holst, J. J. and Krarup, T., Diabetes Care, September 1, 2003; 26(9): 2581 - 2587.

2. Holst JJ, Adv Exp Med Biol 2003, 524:263-279

3. Borloo, M. and Demeester, J., Verhk Acad Geneerhunde Belg. 1994, 56(1) 57-88.

4. Villhaurer EB, Brinkman JA, Naderi GB, Dunning BE, Mangold BL, Mone MD, Russell ME, Weldon SC Hughes TE, J. Med. Chem., 2002, 45 (12), 2362-2365.

5. Villhaurer EB, Coppola GM, Weldon SC Hughes TE, Annual Reports in Medicinal Chemistry, 2001, 36, 191-200, Academic Press