The 8th International Electronic Conference on Medicinal Chemistry (ECMC 2022)
01–30 NOVEMBER 2022 | ONLINE

Synthesis and evaluation of novel 1-(2-(5-aryl-1,3,4-Thiadiazol-2-Ylamino)acetyl)Pyrrolidine-2-Carbonitrile derivatives for theirDPP-4 inhibiting activity

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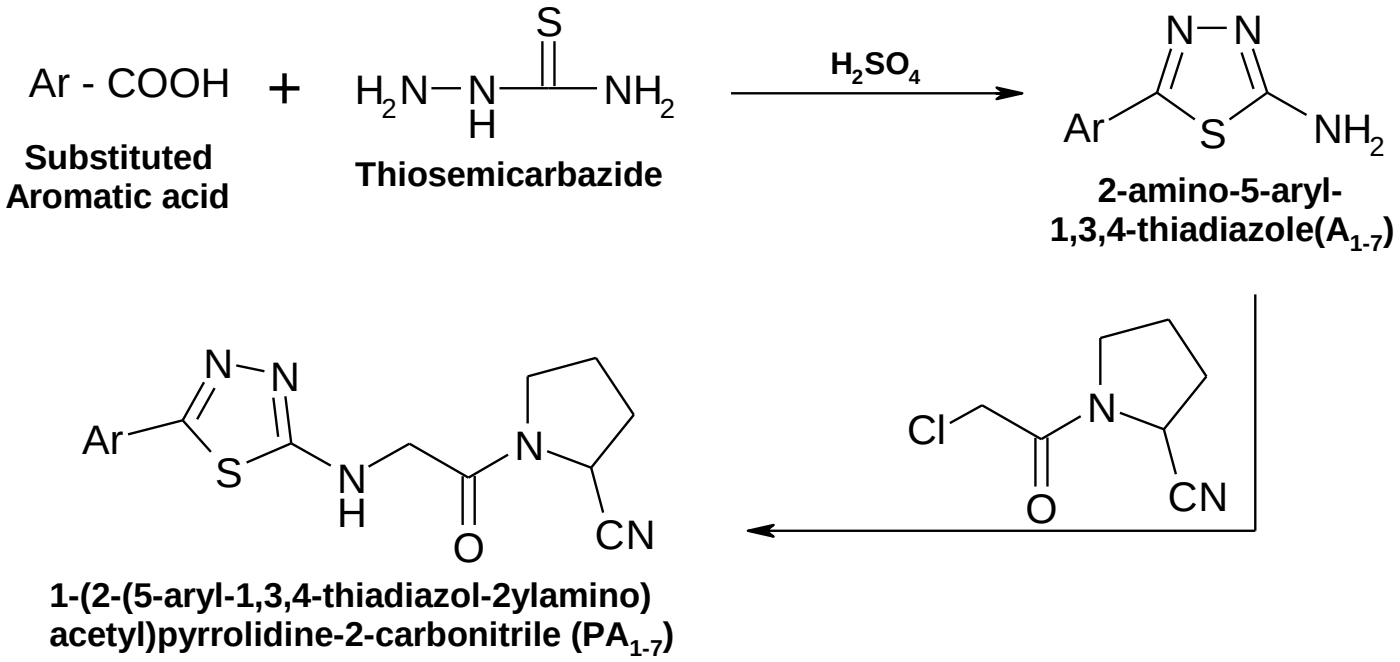
Graphical Abstract



Abstract

The current therapeutic agents for Type 2 diabetes (like Insulin, Sulphonylureas, Biguanides, α-Glucosidase inhibitors, PPAR agonist and GLP-1 agonist), although effective in increasing insulin secretion, are associated with some safety issue and undesirable side effects, including hypoglycemia, abnormalities in cardiovascular responses and β-cell apoptosis. DPP-4 inhibitors offer several potential advantages over existing therapies including decreased risk of hypoglycemia, potential for weight loss, and the potential for regeneration and differentiation of pancreatic β-cells. Moreover, DPP-4 inhibitors can also be administered orally. Among all DPP-4 inhibitor derivatives, 2-Cyano pyrrolidine-based inhibitors have been studied most extensively. Apart from behaving as a proline mimic, the presence of the nitrile on the five-membered ring was shown to provide (i) nanomolar inhibition of DPP-4 and (ii) chemical stability adequate for oral administration. These intermediate was fused with 2-amino-5-aryl-1, 3, 4-thiadiazole derivative to get series of novel 1-(2-(5-aryl-1,3,4-thiadiazol-2-ylamino)acetyl)pyrrolidine-2-carbonitrile derivatives. The synthesized DPP-4 inhibitor derivatives were evaluated by fluorescence assay using Gly-Pro-AMC as a DPP-4-specific fluorescent substrate.

Keywords: 2-cyano pyrrolidine derivatives, 1,3,4-thiadiazol, Dipeptidyl peptidase-4, Fluorescence assay

Introduction

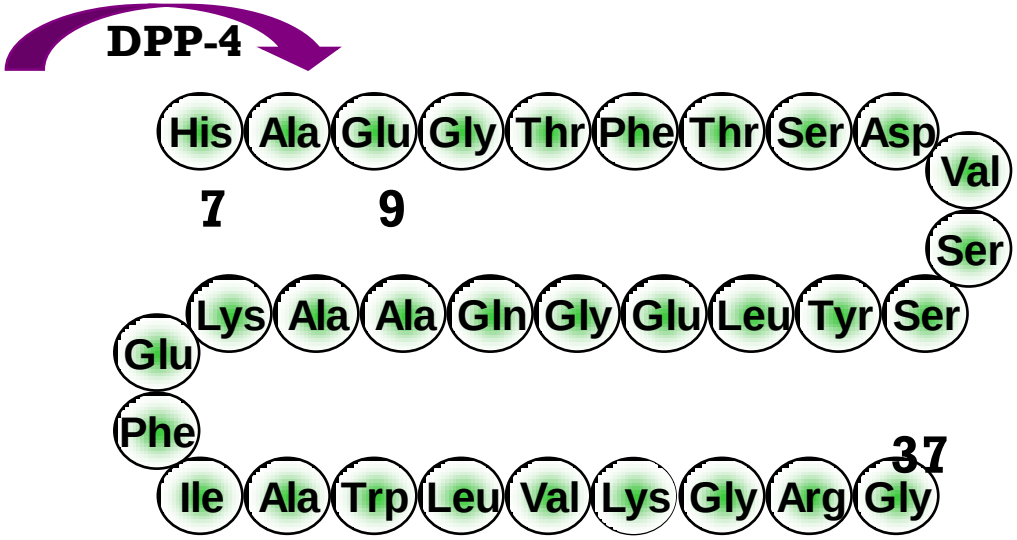
DPP-4 (serine exopeptidase or serine aminodipeptidase) is a membrane-associated peptidase of 766 amino acids, that is, widely distributed in numerous tissues for example, intestine, liver, lung, kidney and placenta. Serine proteases are a class of enzymes that cleave peptide bonds in proteins and as implicated by the name, one of the critical amino acids in the active site of the enzyme that cleaves peptide bonds is serine. Researchers have found that the activity of two potent stimulators of insulin secretion, glucagon like peptide-1 (GLP-1) and glucose- dependent insulinotropic polypeptide (GIP), which is rapidly cleaved by DPP-4. Inhibition of DPP-4, has been shown to be a new approach for the treatment of T2D.

DPP-4 inhibitors offer several potential advantages over existing therapies including decreased risk of hypoglycemia, potential for weight loss, and the potential for regeneration and differentiation of pancreatic β-cells. More importantly, DPP-4 inhibitors can be administered orally. Apart from T2D, DPP-4 inhibitors are also believed to be useful for several other related disease conditions such as diabetic dyslipidemia, conditions of impaired glucose tolerance (IGT), conditions of impaired fasting plasma glucose (IFG), metabolic acidosis, ketosis, appetite regulation and obesity.

Introduction

The active form of glucagon-like peptide-1 (GLP-1), an incretin hormone secreted by intestinal L-cells in response to food intake , is a 30-amino acid peptide that stimulates insulin release, inhibits glucagon release, and slows gastric emptying.

However, in the presence of plasma DPP-4 the active form of GLP-1 is inactivated rapidly ($t_{1/2}$~1 min) due to the cleavage of a dipeptide from the N-terminus.

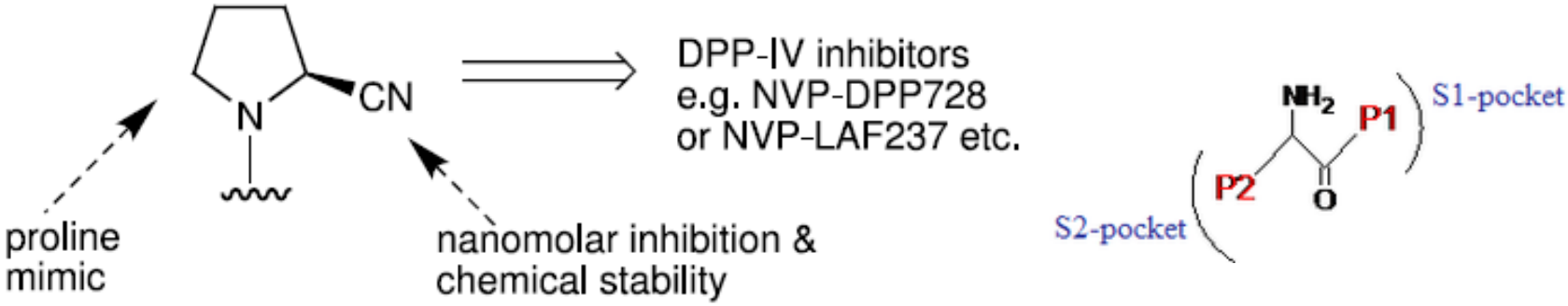


Thus inhibition of DPP-IV extends the half-life of endogenously secreted GLP-1, which in turn enhances insulin secretion and improves the glucose tolerance.

2-cyano pyrrolidine-based inhibitors have been studied most extensively because apart from behaving as a proline mimic, the presence of the nitrile on the five-membered ring provided,

(i) Nanomolar inhibition of DPP-4 and

(ii) Chemical stability adequate for oral administration



Cyanopyrrolidines have two key interactions to the DPP-4 complex:

P-1 site: Nitrile group which is important for high potency, forms reversible covalent bond with the catalytically active serine hydroxyl (Ser630).

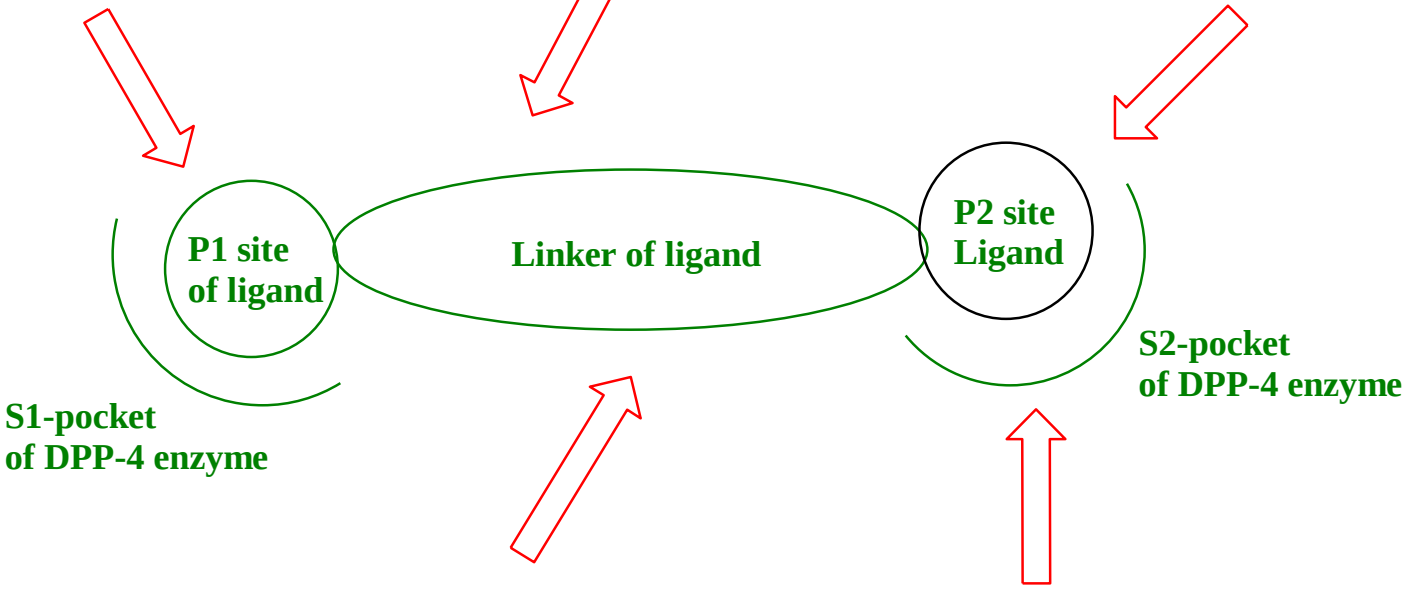
P-2 site: Hydrogen bonding network between the protonated amino group and a negatively charged region of the protein surface, Glu205, Glu206 and Tyr662.

Designing strategy for novel DPP-4 inhibitors

Five or six membered hydrophobic groups only can accommodate and required for all DPPs activity

The polar Nitrogen(basicity) is mandatory for all DPPs activity

Bulkier aromatic/heterocyclic (mono or fused) rings are required to gain selectivity for DPP-4 and showed pi-pi interaction with Phe_{357}



In place of carbonyl group substitution of aromatic/heterocyclic rings (angular) with suitable linker (eg. amide) helps in additional gain in DPP4 potency

Aromatic heterocyclic rings with electron -withdrawing groups (N, O, acidic) are compulsory for gaining DPP-4 selectivity and showed interaction with Arg_{358}

METHODOLOGY

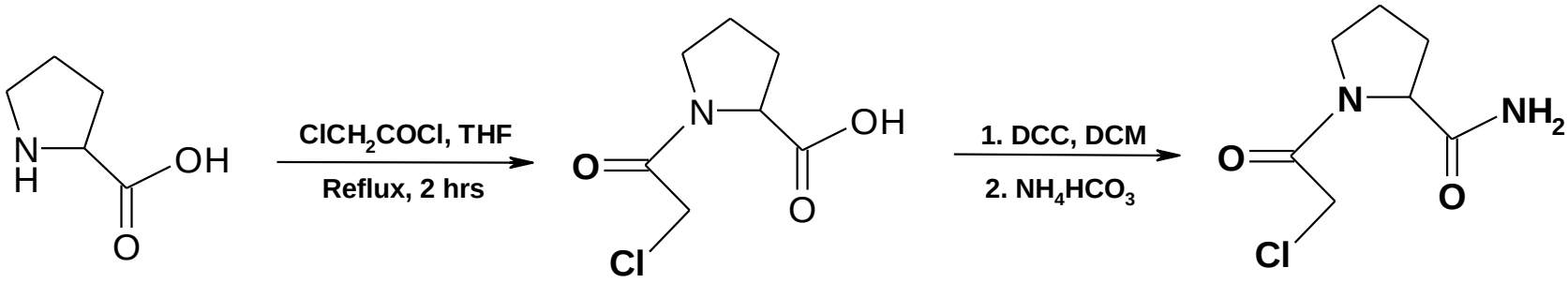
Synthesis of 1-(2- chloroacetyl)pyrrolidine-2-carbonitrile(1)

Incorporation of a 2-cyanopyrrolidine moiety into a molecule can be carried out by using 1-(2-chloroacetyl) pyrrolidine-2-carbonitrile as a reactant. Therefore, this reactant has become a widely used key intermediate for the synthesis of many DPP-4 inhibitors including Vildagliptin.

By alternative synthetic method, 1-(2-chloroacetyl)pyrrolidine- 2-carbonitrile(1), synthesized from L-proline(2) which is less expensive and readily available. This synthetic route neither involves N-protection-/ deprotection strategy nor a complicated isolation method as it was in earlier routes.

In this method, chloroacetyl group play the role of a protecting group so that the use of an additional protecting group and its removal (i.e. deprotection) can be avoided. Moreover, chloroacetyl group is also a part of the key intermediate. Therefore, L-proline(2) was N-acylated with chloroacetyl chloride to prepare 1-(2-chloroacetyl)pyrrolidine-2-carboxylic acid(3) in good yield. We also observed that compound(3) can be prepared with 78 % within 2 hours.

Synthesis of 1-(2- chloroacetyl)pyrrolidine-2-carbonitrile(1)

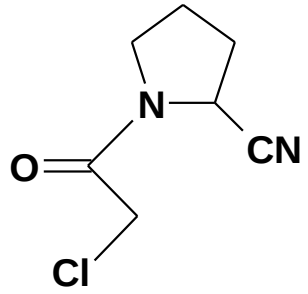


L-Proline (2)

1-(2-Chloroacetyl) pyrrolidine -2-carboxylic acid(3)

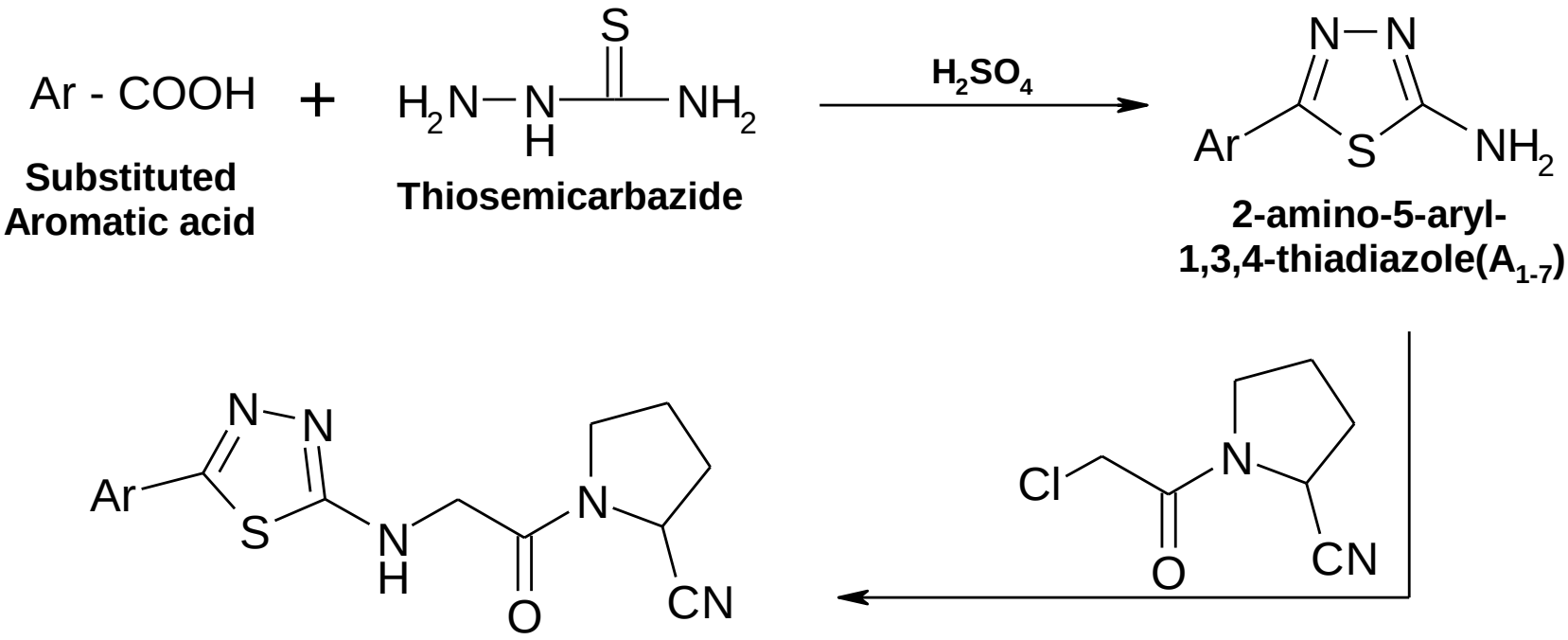
1-(2-chloroacetyl)pyrrolidine -2-carboxamide (4)

1. TFAA, THF
2. NH_4HCO_3, toluene



1-(2-chloroacetyl)pyrrolidine-2-carbonitrile (1)

Synthesis of 1-(2-(5-aryl-1,3,4-thiadiazol-2-ylamino)acetyl)pyrrolidine-2-carbonitrile derivatives (PA_{1-7})



General procedure for synthesis of 2-amino-5-aryl-1, 3, 4-thiadiazole (A_{1-7})

In round bottomed flask aromatic carboxylic acid (0.05 M) and thiosemicarbazide (0.05 M) were added and dissolved in ethanol (25 mL) by shaking. Concentrated sulphuric acid (1 mL) was added to this flask with shaking and the reaction mixture was heated under reflux for 2 hrs on a boiling water bath. Ethanol was removed after completion of the reaction (monitored by TLC) to a possible extent by distillation and the residue was cooled and triturated with crushed ice. The solid crude product was filtered, washed and collected.

Representative procedure for preparation of 1-(2-(5-phenyl-1,3,4-thiadiazol-2-ylamino) acetyl)pyrrolidine-2-carbonitrile (PA_1):

A solution of (S)-1-(2-chloroacetyl) pyrrolidine-2-carbonitrile (2.70 mmol) in THF (5.0 mL) was added drop-by-drop to an ice-cooled stirred suspension of A1 (2.50 mmol) and K_2CO_3 (2.70 mmol), in acetone (20 mL). The reaction mixture was stirred at room temperature overnight. The resulting mixture was filtered to remove insoluble materials, and concentrated under reduced pressure. To this ice cooled solution 1, 4 - dioxane (5.0 mL) was added. The reaction mixture was stirred at 0°C for 1 h and then evaporated to yield the title compound.

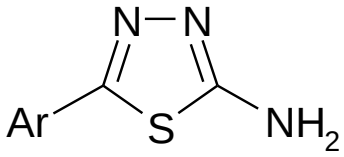
DPP 4 Inhibition Assay

DPP-4 inhibitor screening assay employs fluorogenic substrate, Gly-Pro-Aminomethylcoumarin (AMC), to measure DPP 4 activity. Cleavage of the peptide bond by DPP releases the free AMC group, resulting in fluorescence that can be analyzed using an excitation wavelength of 350-360 nm and emission wavelength of 450-465 nm.

Assay was carried out using DPP-4 inhibitor screening assay kit (Cayman's DPP 4 Inhibitor Screening Assay Kit, USA) as per the manufacturer's instructions. Briefly the experiment was performed in a white half volume 96 well plate. 30 µL of assay buffer, 10 µL of DPP-4 enzyme, 10 µL of dimethoxy sulphoxide (DMSO) and 50 µL of DPP substrate were added to 100% initial activity wells. 40 µL of assay buffer, 10 µL of DMSO and 50 µL of DPP substrate were added to background wells. 30 µL of assay buffer, 10 µL of DPP-4 enzyme, 10 µL of extract (sample) and 50 µL of DPP substrate were added to inhibitor wells. The plate was incubated for 30 min at 37 °C, and the fluorescence was recorded using multimode micro plate reader at excitation and emission wavelengths of 355 and 455 nm respectively.

Results and discussion

Table 1: Physical Data of 2-amino-5-ary-1, 3, 4-thiadiazole derivatives (A_{1-7})

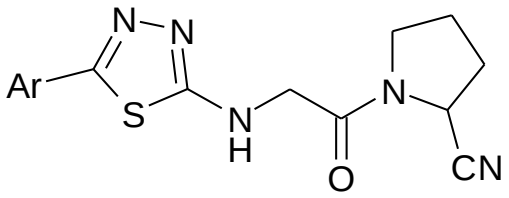


2-amino-5-aryl-1,3,4-thiadiazole

Compound	Ar	Molecular Formula	Molecular Weight	Rf*	Melting Point (°C)
A_1	C_6H_5-	$C_8H_7N_3S$	177	0.34	223-225
A_2	4-Cl-C_6H_5-	$C_8H_6N_3SCl$	211	0.56	230-232
A_3	4-CH_3-C_6H_5-	$C_9H_9N_3S$	191	0.39	215-217
A_4	4-OCH_3-C_6H_5-	$C_9H_9N_3SO$	207	0.53	186-188
A_5	4-F-C_6H_5-	$C_8H_6N_3SF$	195	0.47	236-238
A_6	4-OH-C_6H_5-	$C_8H_7N_3SO$	193	0.68	139-141
A_7	4-Br-C_6H_5-	$C_8H_6N_3SBr$	256	0.26	226-228

Results and discussion

Table 2: Physical Data of 1-(2-(5-aryl-1,3,4-thiadiazol-2-ylamino) acetyl) pyrrolidine-2-carbonitrile (PA_{1-7})



1-(2-(5-aryl-1,3,4-thiadiazol-2-ylamino)acetyl) pyrrolidine-2-carbonitrile (PA_{1-7})

Compound	Ar	Molecular Formula	Molecular Weight	R_f	Melting Point (°C)	% Yield
PA_1	C_6H_5-	$C_{15}H_{15}N_5OS$	313	0.64	264-266	58
PA_2	4-Cl-C_6H_5-	$C_{15}H_{14}N_5OSCl$	347	0.53	258-260	60
PA_3	4-CH_3-C_6H_5-	$C_{16}H_{17}N_5OS$	327	0.57	271-273	63
PA_4	4-OCH_3-C_6H_5-	$C_{16}H_{17}N_5O_2S$	343	0.37	227-229	78
PA_5	4-F-C_6H_5-	$C_{15}H_{14}N_5OSF$	331	0.58	282-284	53
PA_6	4-OH-C_6H_5-	$C_{15}H_{15}N_5O_2S$	329	0.41	190-192	58
PA_7	4-Br-C_6H_5-	$C_{15}H_{14}N_5OSBr$	392	0.49	258-260	53

Results and discussion

Spectral characterization of 1-(2- chloroacetyl)pyrrolidine-2-carbonitrile (1): (Yield: 82 %). M.P. 53 °C. R_f : 0.48, IR (KBr, cm^{-1}): 2925, 2812 (>CH_2 of CH_2Cl), 2870 (>CH-), 2229(CN), 1687(C=O of $COCH_2Cl$), 1412(C-N), 1492 (>CH_2 of Pyrrolidine), 1302, 754 (C-Cl). ^{1}H NMR (400 MHz, DMSO) δ: 2.1 (2H, C_4 of Pyrrolidine), 2.25-2.26 (2H, C_3 of Pyrrolidine), 3.5–3.36 (2H, C_5 of Pyrrolidine), 4.76 (2H, CH_2Cl), 4.05-4.06 (1H, C*H*CN) m/z = 173 $[M+H]^+$

Spectral characterization of 1-(2-(5-phenyl-1,3,4-thiadiazol-2-ylamino) acetyl)pyrrolidine-2-carbonitrile (PA_1): (Yield: 79 %), M.P. 189-190 ^oC **FT-IR (KBr, cm^{-1}):** 3339 (-NH-), 3060 (Aromatic CH), 2937 (>CH_2 of -$COCH_2$-), 2887 (>CH-), 2236 (CN), 1689(C=O of -$COCH_2$-), 1613 (C=N), 1590(Aromatic C=C), 1452 (>CH_2 of Pyrrolidine), 676 (C-S) ^{1}H NMR (400 MHz, DMSO) δ: 1.48 (2H, C_4 of Pyrrolidine), 2.1 (2H, C_5 of Pyrrolidine), 2.24-2.29 (2H, C_3 of Pyrrolidine), 3.8 (1H, C*H*CN), 3.45 (2H, CH_2CO), 8.05-8.39 (5H, Aro. CH), 8.7 (1H, NH) m/z =313 $[M + H]^+$

Results and discussion

The percentage of residual activity of DPP 4 was determined for 1-(2-(5-phenyl -1,3,4-thiadiazol-2-ylamino) acetyl)pyrrolidine-2-carbonitrile by comparing the activity of DPP 4 in the presence and absence of the tested compound. DPP 4 activity was not affected at the used DMSO concentration. Negative controls lacking DPP 4 were used as background.

A standard DPP 4 inhibitor, Vildagliptin, was used as a positive control. All measurements were conducted in duplicates. The percentage DPP-4 inhibition was calculated at 1 µM and 10 µM as given below.

The results shows in vitro screening of molecules for DPP-4 inhibition and the values indicated that the synthesized molecule have weak to moderate inhibitory action against DPP 4 enzyme compared to Vildagliptin. Compound PA_2, PA_4 and PA_7 showed comparatively good activity and produce more inhibition of enzyme.

Results and discussion

Table 3: In vitro screening of molecules for DPP-4 inhibition

Sr. No.	Compound	R	% DPP-4 inhibition	
			1 µM	10 µM
1	PA_1	C_6H_5-	5.4	11.6
2	PA_2	4-Cl-C_6H_5-	7.4	13.9
3	PA_3	4-CH_3-C_6H_5-	5.9	12.1
4	PA_4	4-OCH_3-C_6H_5-	4.4	8.3
5	PA_5	4-F-C_6H_5-	8.7	21.5
6	PA_6	4-OH-C_6H_5-	4.9	11.7
7	PA_7	4-Br-C_6H_5-	7.7	14.6
8	**Standard**	**Vidagliptin**	**12.8**	**68.1**

Conclusions

- In this research work, we have synthesized seven novel DPP-4 inhibitors. All the synthesized compounds were assayed by in vitro biological screening and were found to give less than 25 % inhibition of DPP 4 during in-vitro enzyme inhibition assay up to 10 µM concentration except compound PA_5. This compound gave 8.7% and 21.5% DPP 4 inhibition at 1 µM and 10 µM concentrations respectively. It is also reveal that increasing electron withdrawing efficacy at -4 the position of aromatic ring increases the activity.

- Further, 1-(2-(5-phenyl-1,3,4-thiadiazol-2-ylamino) acetyl)pyrrolidine-2-carbonitrile derivative could be served as useful clues for developing next generation of antidiabetes medicines via inhibiting DPP-4 activity. The information provides new scaffold which may also lead to the discovery of other possible DPP-4 inhibitors, which could improve treatment of diabetes.

Acknowledgments

- The authors are thankful to the Atmiya University for providing necessary facilities to carryout this research work.

- Authors are highly thankful to organizing team of ECMC 2022 to provide a platform to share research carried out on synthesis and evaluation of novel 1-(2-(5-aryl-1,3,4-Thiadiazol-2-Ylamino)acetyl)Pyrrolidine-2-Carbonitrile derivatives for theirDPP-4 inhibiting activity .