Synthesis and DPP-IV Inhibition Activity of [1,3]Thiazolo[5,4-*D*]Pyrimidine Derivatives

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Abstract
The thiazolopyrimidine derivatives was designed, synthesized and screened for inhibition of Dipeptidyl Peptidase IV (DPP IV). Methyl-5-amino-1,3-thiazole -4-carboxylate were reacted with urea to form scaffold, it was chlorination, alkaline hydrolysis, N-alkylationand amination to obtained final compounds. The final compounds were obtained Compound **1,2,3** having more DPP-IV inhibition activity. The present study on substituted thiazolopyrimidine derivatives shows good to moderate inhibitory potential of DPP IV enzyme.

Key words
[1,3]Thiazolo[5,4-*D*]Pyrimidine, Dipeptidyl Peptidase IV, Fluorescence intensity

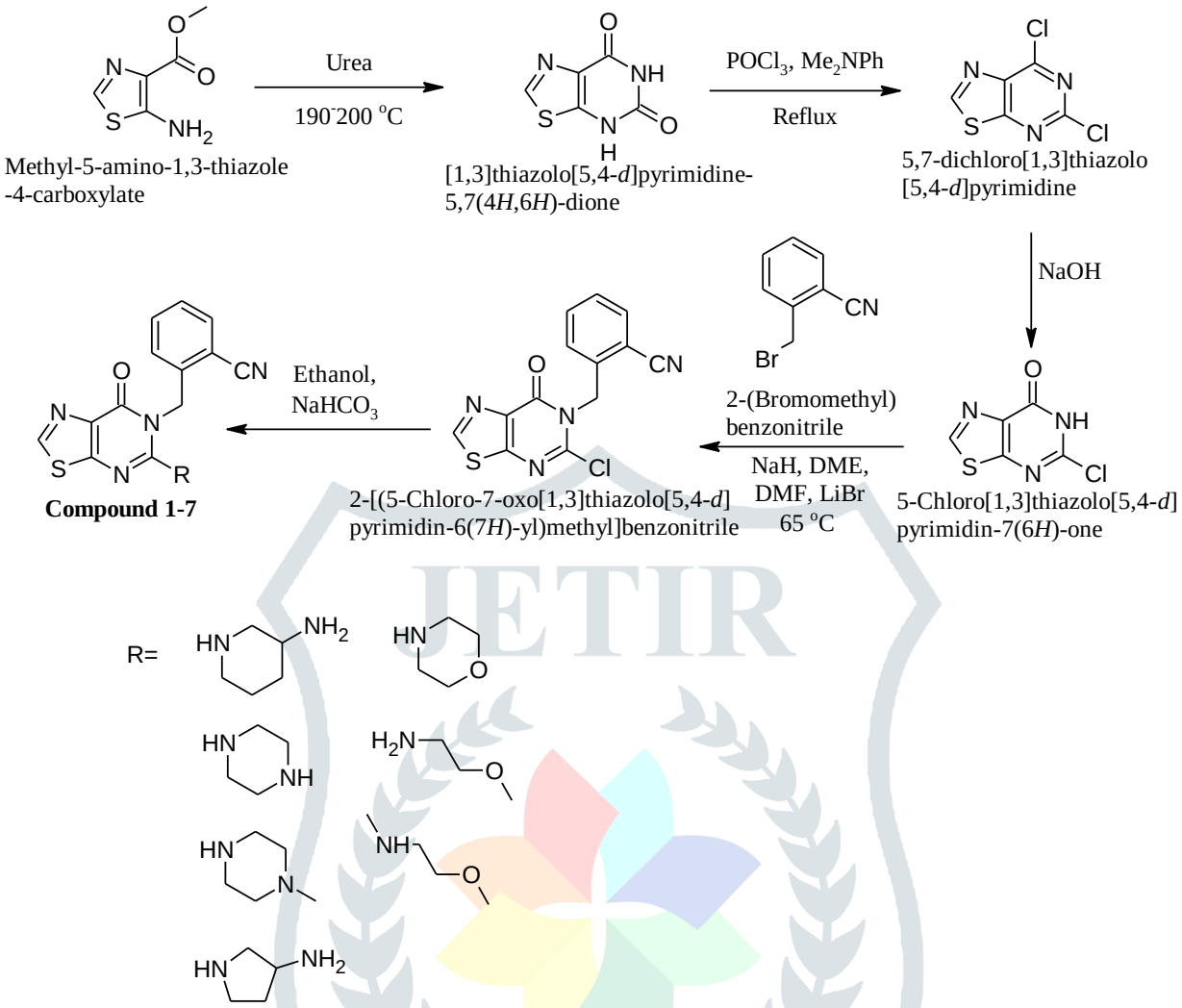
Introduction

The high prevalence of type 2 diabetes (T2D) is a major global public health concern and affects approximately 180 million patients worldwide, with yet more people still undiagnosed or at the pre-diabetes stage. T2D, known as non-insulin-dependent diabetes mellitus, is a chronic, severe and increasingly prevalent disease [1,2.] Although many standard therapies for T2D are available in the market, none of them can stop disease progression. Therefore, new therapeutic agents are still needed to combat diabetes. Dipeptidyl peptidase IV (DPP-IV) inhibitors have emerged as a major breakthrough in anti-diabetic drug discovery. By blocking the DPP-IV enzyme, these inhibitors can prolong the half-life of active glucagons like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) and thus stimulate insulin biosynthesis and secretion. These inhibitors finally represent away to effectively and safely control blood glucose in diabetic patients [3-5]. DPP-IV inhibitors possess several advantages over traditional anti-diabetic drugs, such as not leading to increased bodyweight or causing hypoglycemia, and are generally well-tolerated [6-8]. DPPIV inhibitors may be disease-modifying therapies because they can delay or prevent the loss of functional b-cell mass and, therefore, slow disease progression [9].

Chemistry

The synthesis of compounds 1 to 7 is outlined in Scheme[5].Briefly, the available starting materials Methyl-5-amino-1,3-thiazole -4-arboxylate were either heated with urea[10] between 190°C to 200°C, to generate the corresponding Int-1[11].Chlorination of Int-1 with phosphoryl trichloride yielded Int-2, which were hydrolyzed with aqueous sodium hydroxide to give key intermediates Int-3. Selective N-alkylation was performed using a previously published method [12-13] to produce compounds Int-4. The final compounds were obtained in high yields by the amination of the chloro precursors by using different amines.

Reaction Scheme



Procedure

Stage-1

A mixture of Methyl-5-amino-1,3-thiazole -4-carboxylate (15.0 g, 0.08 mol) and urea (24.3 g, 0.41 mol) was heated at 190-200°C for 4-5 h. The reaction mixture was cooled and poured into a 150 ml 4N sodium hydroxide solution, and any insoluble material was removed by filtration. The filtrate was then acidified with 2N HCl to give a white precipitate, which was collected by filtration, washed with 45 ml water and dried under vacuum at 50°C, obtained [1,3]thiazolo[5,4-*d*]pyrimidine-5,7(4*H*,6*H*)-dione (7.5 g, yield 47%).

Stage-2

A total of 1.5 mL N,N-dimethylaniline was added to 5 g of [1,3]thiazolo[5,4-*d*]pyrimidine-5,7(4*H*,6*H*)-dione in 30 mL POCl3. The mixture was then heated under reflux for 16 h. Excess $POCl_3$ was removed in vacuo, and the resulting residue was treated with ice water to yield a precipitate. The solid was collected by filtration, washed with water and dried over a funnel to afford solid 5,7-dichloro[1,3]thiazolo[5,4-*d*]pyrimidine (1.9 g, yield 31.1%).

Stage-3

A mixture of 3 g of 5,7-dichloro[1,3]thiazolo[5,4-*d*]pyrimidine, 45 mL 1N NaOH and 12 mL of THF was stirred at room temperature under for 5h. The solution was then chilled and adjusted to pH 4-5 with Acetic acid. The resulting precipitate was collected, washed with water and dried to afford a solid 5-Chloro[1,3]thiazolo[5,4-*d*]pyrimidin-7(6*H*)-one (1.8 g, yield 65.9%).

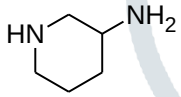
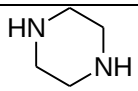
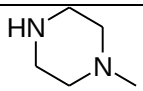
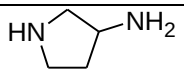
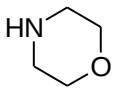
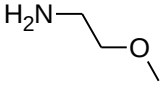
Stage-4

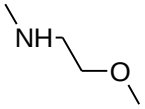
NaH (0.073 g, 3.0 mmol) was added to a stirred solution of 5-Chloro[1,3]thiazolo[5,4-*d*]pyrimidin-7(6*H*)-one (0.5 g, 2.6 mmol) in DME (7.5 mL) and DMF (2 mL) at 0°C, stir for 30 min, LiBr (0.46 g, 5.3 mmol) was added, and the mixture was allowed to warm to room temperature. After 15 min, 2-(Bromomethyl)benzonitrile (0.6 g, 3.0 mmol) was then added, and the mixture was heated at 65-70°C for overnight. After cooling, the mixture was poured into water (30 mL) with stirring to yield a precipitate. This solid was filtered and dried to give 2-[(5-Chloro-7-oxo[1,3]thiazolo[5,4-*d*]pyrimidin-6(7*H*)-yl)methyl]benzonitrile (0.6 g, yield 74.53%).

Stage-5

A mixture of 2-[(5-Chloro-7-oxo[1,3]thiazolo[5,4-*d*]pyrimidin-6(7*H*)-yl)methyl]benzonitrile (0.3 g, 0.9 mmol), Amine (1.1 mmol) and $NaHCO_3$ (0.33 g, 3.9 mmol) in 6 mL of ethanol in a sealed tube was heated at 120-130°C for 5-8 h. The reaction mixture was then cooled to room temperature and filtered. The resulting filtrate was concentrated in vacuo and then purified by column chromatography to give the title compound.

Table-1 Spectral data of Synthesized compounds

Compound No.	R	Molecular Formula	Molecular Weight (g/mol)	Yield (%)	Mass	NMR (δ, ppm)
1		**$C_{18}H_{18}N_6OS$**	**366.44**	**49**	367.5 [M^+]	8.8(s, 1H, ArH), 7.2-7.5 (m, 4H, ArH), 5.6 (d, 2H, CH_2), 1.4-4.46 (m, 9H, piperidine ring)
2		**$C_{17}H_{16}N_6OS$**	**352.41**	**53**	353.3 [M^+]	
3		**$C_{18}H_{18}N_6OS$**	**366.44**	**65**	367.4 [M^+]	
4		**$C_{17}H_{16}N_6OS$**	**352.41**	**45**	353.6 [M^+]	
5		**$C_{17}H_{15}N_5O_2S$**	**353.39**	**68**	354.4 [M^+]	8.8(s, 1H, ArH), 7.2-7.45 (m, 4H, ArH), 5.5 (d, 2H, CH_2), 2.9-3.7 (m, 8H, morpholine ring)
6		**$C_{16}H_{15}N_5O_2S$**	**341.38**	**56**	342.7 [M^+]	

7		**$C_{17}H_{17}N_5O_2S$**	**355.41**	**61**	356.5 [M^+]	8.8(s, 1H, ArH), 7.2-7.45 (m, 4H, ArH), 5.5 (d, 2H, CH_2), 3.3 (s, 3H, OCH_3), 2.5 (s,3H, NCH_3), 3.45-3.55 (t, 4H, $2CH_2$)

DPP-4 Inhibition activity

DPP-4 inhibition activity performed by using DPP-4 inhibition screening kit. DPP-4 inhibitor assay provides convenient fluorescence based method for screening DPP-IV inhibitors. The assay uses the fluorogenic substrate, Gly-Pro-Aminomethyl coumarin (AMC), to measure DPP-IV activity. Cleavage of peptide bond by DPP release the free AMC group, resulting fluorescence that can be analyzed using an excitation wavelength of 350-360nm and emission wavelength of 450-460nm

Table-2 DPP-4 Inhibition activity of synthesized compounds

Compound No.	Fluorescence intensity	% Inhibition	Concentration of compound (µM)
Initial	803.2±1.2	0%	--
1	306.4±1.4	61.85%	250
2	310.0±1.2	61.40%	250
3	352.3±0.8	56.14%	250
4	409.4±1.6	49.02%	250
5	460.8±1.2	42.63%	250
6	430.9±0.8	46.35%	250
7	458.5±1.5	42.91%	250

Conclusion

The main aim is to synthesis and evaluation of DPP-4 inhibition activity of some on Thaizolopyrimidine derivatives. We synthesized seven on Thaizolopyrimidine derivatives. Activity of all the on Thaizolopyrimidine derivatives were evaluated by DPP-4 inhibition screening kit. Out of seven synthesized compounds, the 1, 2, 3 showed DPP-4 activity better than rest of the other compounds.

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