

***In Silico* Design, Synthesis and  
*In Vitro* Evaluation of Quinazolinone Derivatives as  
Dipeptidyl Peptidase-4 (DPP-IV) Inhibitors**

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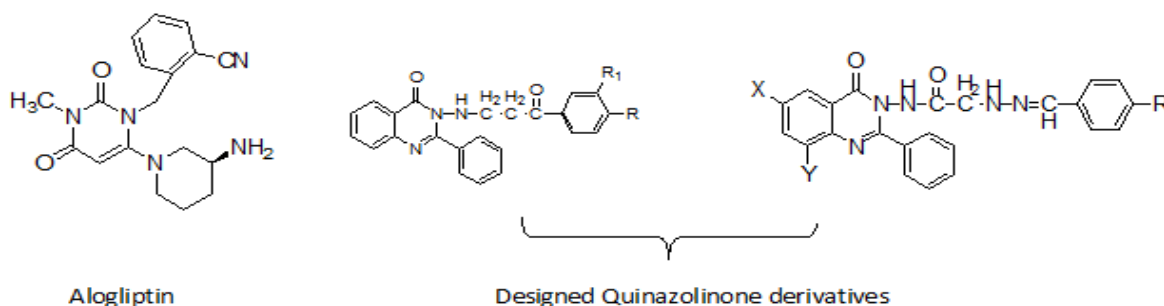
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**Abstract :** Taking into account the important role of DPP-IV in diabetes mellitus, inhibitors for DPP-IV were designed, synthesized and evaluated for the activity. From amongst various nitrogen containing heterocycles, quinazolinones were selected for testing the DPP-IV inhibition. The synthesized molecules were characterized by melting point, infrared spectra and <sup>1</sup>H-NMR, elemental analysis and <sup>13</sup>C-NMR. Two best molecules [PS-3 & PS-6] were chosen on the basis of Vlife score of -45.78 and -51.72 respectively and when tested for *in vitro* DPP-IV inhibition, the activity was concomitant to the docking scores. These molecules may therefore serve as the lead for further modification to design potent DPP-IV inhibitors.

**Keywords:** Key words: *In silico*, Quinazolinone, In-Vitro, DPP-IV inhibition activity.

Graphical Abstract:

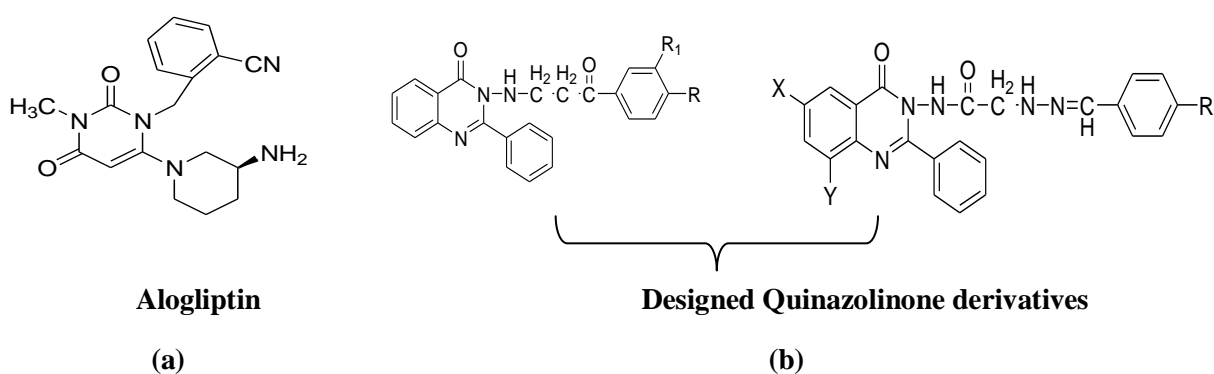


## 1. Introduction

Diabetes Mellitus (DM) is defined as a group of metabolic disorders, characterized by high blood glucose levels, significant enough to increase the incidence of microangiopathy (retinopathy, nephropathy and neuropathy)<sup>1,2</sup>. DM is a chronic metabolic disorder that represents a serious public health concern. It is characterized by defective insulin secretion or deficiencies in the action of insulin. Its prevalence has been reported to rise to an epidemic level, afflicting more than 366 million people worldwide which is expected to rise to 552 million by 2030<sup>3</sup>. Diabetes mellitus is becoming one of the major health problems in the developing countries. As the International Diabetes Federation suggests that the number of adults living with diabetes worldwide was increasing from time to time. The disease has gained the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the disease. 31.7 million population was suffering with this disease in India in the year 2000 awarding top most rank to the country followed by China (20.8 million) with the United States (17.7 million) in second and third place respectively<sup>4</sup>. International Diabetes Federation reports 72 million cases of diabetes in the country in year 2017 [<https://www.idf.org/our-network/regions-members/south-east-asia/.../94-india.html>]. Management of diabetes has been challenging, particularly in the presence of the enormous prevalence of obesity. The need of the day is to design newer agents to treat diabetes and hence, we focused our work on DPP-4 (Dipeptidyl peptidase-IV) inhibitors.

DPP-IV inhibitors work by blocking the action of DPP-IV, an enzyme which destroys the hormone incretin, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP)<sup>5,6</sup>. Inhibition of this enzyme results in an increase in the half-life and the sustained physiologic action of incretins, leading to an improvement in hyperglycaemia<sup>6</sup>. Incretins help the body produce more insulin only when it is needed and reduce the amount of glucose being produced by the liver when it is not needed. These hormones are released throughout the day and levels are increased at meal times<sup>7</sup>. The first available DPP-IV inhibitors were sitagliptin and vildagliptin. Dipeptidyl peptidase-IV (DPP-IV) inhibitors also known as gliptins and it is a new class of oral hypoglycaemic that block DPP-IV. In type-2 diabetes mellitus, DPP-IV inhibitors are administered in those patients having HbA<sub>1c</sub>>7% despite treatment with either metformin or a sulfonylurea and agents like metformin, sulfonylurea or glitazone when taken alone as single dose that does not helps in controlling glycaemia control<sup>8-10</sup>.

Here in our study, quinazolinones (figure 1) were evaluated for DPP-IV inhibition. Quinazolinones<sup>11,12</sup> have natural drug likeliness for anti hyperglycemic activity as exemplified by drug development of gliptins and therefore chosen for this research. Quinazolinone molecules were synthesized from the series of 3-(3-oxo-3-substituted phenylpropylamino)-2-phenylquinazolin-4(3H)-one and N-(4-oxo-2-phenyl quinazolin-3-yl) 2-[2-(substituted benzylidene) hydrazine-1-yl] acetamide and 2-(2-(4-substituted benzylidene)hydrazinyl)-N-(6,8-dibromo-4-oxo-2-phenylquinazolin-3(4H)-yl)acetamide to arrive at a molecules with better *in silico* inhibition scores and were synthesized. Molecular docking was carried using Vlife MDS 4.3 software. Synthesized molecules were characterized by melting point, FT-IR, <sup>1</sup>H-NMR, elemental analysis and these complied with spectral assignment. These molecules were further evaluated for *in vitro* DPP-IV enzyme inhibition assay.



**Figure 1: Structure of alogliptin (a) and (b) designed quinazolinone derivative**

## 2. Material & methods

### 2.1 Docking

**Hardware and Software:** All Docking studies and conformational analysis were performed using the Molecular Design Suite (VLife MDS software package, version 4.3; from VLife Sciences, Pune, M.S., India)

**Conformation Generation:** Structures of compounds were sketched using the 2D structure draw application Vlife2Ddraw and converted to 3D structures. All the structures were minimized and optimized with the AMBER force field with root mean square gradient (RMS) of 0.01 kcal/molA° and the iteration limit to 10,000. Conformers for each structure were generated using Monte Carlo applying AMBER force field method and least energy conformer was selected for further study.

**Preparation of Protein:** The PDB structures (www.rcsb.org) [2QOE] were downloaded and energy minimized. All the bound water molecules, ligands, and cofactors were removed (preprocess) from the proteins which were taken in.pdb format. The tool neutralized the side chains that were not close to the binding cavity and did not participate in salt bridges. This step was then followed by restrained minimization of co-crystallized complex, which reoriented side-chain hydroxyl groups and alleviated potential steric clashes. The complex obtained was minimized using AMBER force field. The minimization was terminated after either completion of 10,000 steps or after the energy gradient converged below 0.05 kcal/mol.

**Preparation of Ligands:** Structures were sketched using built Vlife2D draw taken in.mol2 format. AMBER Force Fields with default settings were used for the ligand minimization. VlifeMDS software was used to prepare the ligand for docking. Lowest energy conformer was selected and used for docking. Docking was done by “Batch” based docking. Dock score was generated only and reported. The docking scores for the designed compounds are shown in **table 1**.

### 2.2 Synthesis

All the chemicals used were of synthetic (puresis) grade. Thin Layer Chromatography (TLC) was carried out and monitored for all the reactions to ensure completion. It was carried out on pre-coated silica gel GF254 aluminium sheets (Merck 5554). Iodine vapors and UV light were used to visualize the spots on TLC chromatograms. Column chromatography was performed for purification of compounds on Spectrochem silica gel (60-120 mesh FT-IR spectrophotometer using KBr pellets on Shimadzu IR Affinity-1 spectrophotometer using KBr disc. <sup>1</sup>H & <sup>13</sup>C Nuclear magnetic resonance (NMR) spectra of the pure compounds in DMSO-*d*<sub>6</sub>/CDCl<sub>3</sub> were recorded 300MHz and 400MHz Varian Mercury advanced instrument. Perkin-Elmer 240 analyzer was used for elemental analyses (C, H, N). The melting points were determined in open capillary method on Veego electronic digital apparatus. The compounds PS-3 to PS-6 were synthesized using Scheme -1 and compounds PS-8 to PS-13 were synthesized using Scheme-2.

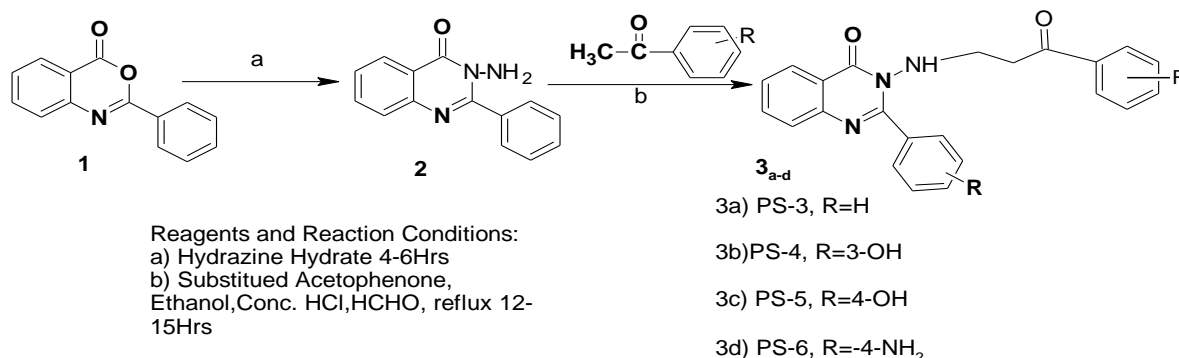
### 2.3 *In vitro* DPP-IV enzyme inhibition assay

The study was performed using DPP-IV inhibition assay kit of Cayman chemicals item no. 700210. Inhibition concentration values of compounds for DPP-IV inhibition were determined at concentration range of 0.001 μm-10μm and it was compared with sitagliptin, used as standard in *in vitro* evaluations.

### 3. Experimental

#### Synthesis

##### 3.1 Synthesis of compounds from Series 1:



Scheme 1: Synthetic Scheme for the compounds PS3, PS4, PS-5 and PS-6

##### 3.1.1 2-phenyl-4-H-benzo[d][1,3]oxazin-4-one (1)

10 gm of anthranilic acid was dissolved in 200 ml pyridine. 40 ml benzoyl chloride was added dropwise to this solution. The reaction was maintained at cold condition. After completion of reaction, ice was added and solid precipitated out. The solid was washed with 50mL of 5% sodium bicarbonate solution and then with 30mL of 5% sodium chloride solution. The precipitate was separated by filtration and dried to obtain 2-phenyl-4-H-benzo[d][1,3]oxazin-4-one (1). Yield: 80% m.p. 120 C. IR: (KBr)  $\nu$  (cm<sup>-1</sup>) 3077 (Ar C-H), 1751 (C,O), 1625 (C,N), 1616 (C,C), 1038 (C-O-C). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) ppm:  $\delta$  6.95–7.78 (9H, m, Ar-CH). Anal. Cald for C<sub>14</sub>H<sub>9</sub>NO<sub>2</sub>: C, 75.33; H, 4.06; N, 6.27. Found: C, 75.42; H, 4.05; N, 6.29

##### 3.1.2 3-amino-2-phenyl quinazolinone (2):

2-phenyl-4-H-benzo[d][1,3]oxazin-4-one (1) (0.001 mol) was dissolved in a mixture of 50 ml pyridine and 8 ml of hydrazine hydrate, it was further heated and refluxed at 80°C for 4-6hr. Reaction was allowed to cool at room temperature. Ice was added to obtain solid precipitate. The solid was washed with 50mL of 5% w/v NaCl solution and recrystallized from ethanol. The solid precipitated was separated by filtration and dried to obtain 3-amino-2-phenyl quinazolinone (2). Yield 40%; mp 172 °C; IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3307, 3215 (N-H), 3062 (ArC-H), 1662 (C=O), 1564, 1471 (ArC=C), 1338 (C-N); <sup>1</sup>H NMR (400MHz, DMSO-d<sub>6</sub>):  $\delta$  8.15–8.25 (d, 1H, J=7.0Hz, ArH), 7.8-8.1 (m, 3H, ArH), 7.71-7.73 (d, 1H, J=8.0Hz, ArH), 7.55-7.6 (t, 1H, J=5.6Hz, ArH), 7.45-7.5 (d, 3H, J=6.73Hz, ArH), 5.2 (s, 2H, NH<sub>2</sub>). Anal. Calcd for C<sub>14</sub>H<sub>11</sub>N<sub>3</sub>O (237.3): C, 70.87; H, 4.67; N, 17.71. Found: C, 70.78; H, 4.76; N, 17.80.

**3.1.3 General Procedure for 3-(3-oxo-3-substituted phenyl propyl amino)-2-phenyl quinazolin-4(3H)-one (3a-d) (PS-3 to PS-6):** 3-amino-2-phenyl quinazolinone (2) (1 Eq. mole) was dissolved in 50ml of ethanol and then 0.5 mL of concentrated HCl (35.5%) & formaldehyde (1.2 Eq. mole) with substituted acetophenone (1 Eq. mole) was added and refluxed for 12-15 hrs and reaction was monitored by TLC. After completion of reaction, 20mL of 40% NaOH solution was added to obtain solid precipitate and compound was further purified by column chromatography to obtain 3-(3-oxo-3-substituted phenyl propyl amino)-2-phenyl quinazolin-4(3H)-one.

**3.1.3.1 3-(3-oxo-3-phenylpropylamino)-2-phenylquinazolin-4(3H)-one (3a) (PS-3):** Yield 60%; mp 147°C; IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3216 (NH of NH<sub>2</sub>); 3071 (C-H,Ar.); 1768 (C=O,ketone); 1495 (C-C,Ar.); 1302 (C-N,Ar.); 1079 (C-H). <sup>1</sup>H-NMR (300MHz, DMSO-d<sub>6</sub>):  $\delta$  6.19 (m, 9H, Ar-H), 6.64-6.69 (m, 5H, Ar - H), 6.92-6.98 (s, 1H, NH), 7.27-7.32 (d, 2H, J = 15Hz, CH<sub>2</sub>), 7.64-7.67(d, 2H, J = 9Hz, CH<sub>2</sub>). <sup>13</sup>C NMR (300MHz, DMSO,  $\delta$ ): 36.0, 39.3, 49.2, 119.9, 125.0, 126.4, 126.8, 127.0, 127.1, 128.9, 133.0, 145.8, 152.8, 160.5, 197. Anal. Calcd for C<sub>23</sub>H<sub>19</sub>N<sub>3</sub>O<sub>2</sub> (369): C, 74.14; H, 5.09; N, 11.79. Found: C, 74.63; H, 5.49; N, 11.39.

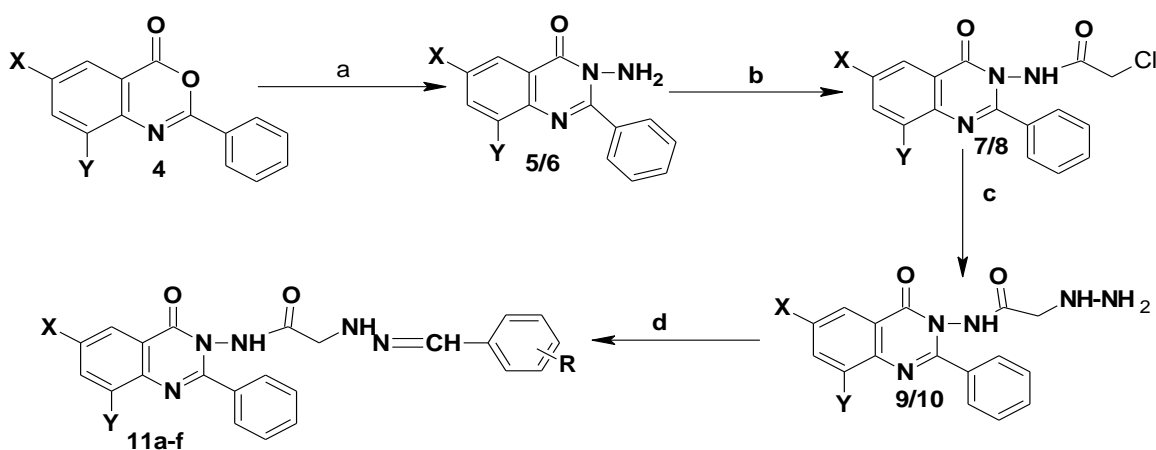
**3.1.3.2 3-(3-(3-hydroxyphenyl)-3-oxopropylamino)-2-phenylquinazolin-4(3H)-one(3b) (PS-4):** Yield 70%; mp 162°C; IR (KBr)  $\nu$  (cm-1): 3308 (OH, str.), 3281 (NH of NH<sub>2</sub>), 2932 (C-H,Ar.), 1683 (C=O,ketone), 1476

(C-C, Ar.), 1251 (C-N,Ar.), 1232 (C-H), <sup>1</sup>H-NMR (300MHz, DMSO-d<sub>6</sub>): δ 7.48 - 7.54 ( m , 4 H , Ar-H ) , 7.56 -7.59 ( d , 2H , Ar-H ) , 7.70 - 7.73 ( d , 2H , Ar-H ) , 7.79 – 7.87 ( m , 5H , Ar-H ) , 8.18 -8.20 ( d , 1H , OH ) , 2.40 ( s , 1H , NH ) , 3.80 ( s , 2H , CH<sub>2</sub> ) , 2.05 ( s , 2H , CH<sub>2</sub>). <sup>13</sup>C NMR (300MHz, DMSO, δ): 39.3, 49.2, 109.2, 116.5, 119.9, 125.0, 126.4, 126.8, 127.0, 127.1, 128.9, 128.9, 130.1, 133.0, 145.8, 152.8, 157.1, 160.5, 197.0. Anal. Calcd for C<sub>23</sub>H<sub>19</sub>N<sub>3</sub>O<sub>3</sub> (385.4): C, 71.67; H, 4.97; N, 10.90. Found: C, 72.07; H, 4.37; N, 10.30.

**3.1.3.3 3-(3-(4-hydroxyphenyl)-3-oxopropylamino)-2-phenylquinazolin-4(3H)-one (3c) (PS-5):** Yield 70%; mp 192°C; IR (KBr) ν (cm<sup>-1</sup>): 3308 (OH, str.), 3281 (NH of NH<sub>2</sub>), 3031 (C-H,Ar.), 1734 (C=O, ketone), 1495 (C-C,Ar.), 1251 (C-N,Ar.), 1215 (C-H). <sup>1</sup>H-NMR (300MHz, DMSO-d<sub>6</sub>): δ 2.02 ( s , 2H , CH<sub>2</sub> ) , 2.40 ( s , 1H , NH ) , 3.50 ( s , 2H , CH<sub>2</sub> ) , 7.48-7.54 ( m , 4H , Ar-H ) , 7.56-7.59 ( d , 2H , Ar-H ) , 7.70-7.73 ( d , 2H , Ar-H ) , 7.79-7.87 ( m , 5H , Ar-H ) , 8.18 -8.20 ( d , 1H , OH ) . <sup>13</sup>C NMR (300MHz, DMSO, δ): 49.2, 39.3, 115.4, 115.4, 119.9, 125.0, 126.4, 126.8, 127.0, 127.1, 128.9, 129.5, 133.0, 134.9, 145.8, 152.8, 157.8, 160.5, 197.0. Anal. Calcd for C<sub>23</sub>H<sub>19</sub>N<sub>3</sub>O<sub>3</sub> (385.42): C, 71.67; H, 4.97; N, 10.90; O, 12.45. Found: C, 72.17; H, 4.97; N, 11.45.

**3.1.3.4 3-(3-(4-aminophenyl)-3-oxopropylamino)-2-phenylquinazolin-4(3H)-one (3d) (PS-6):** Yield 80%; mp 210°C; IR (KBr) ν (cm<sup>-1</sup>): 3309 (NH of NH<sub>2</sub>), 3064 (C-H, Ar.), 1739 (C=O, ketone), 1495 (C-C,Ar.), 1251 (C-N, Ar.), 1079 (C-H). <sup>1</sup>H-NMR (300MHz, DMSO-d<sub>6</sub>): δ 2.05 ( s , 2H , CH<sub>2</sub> ) , 2.40 ( s , 2H , NH ) , 3.50 ( s , 2H , CH<sub>2</sub> ) , 7.47 - 7.54 ( m , 4H , Ar-CH ) , 7.56 - 7.59 ( d , 2H , Ar-CH ) , 7.70-7.73 ( d , 2H , Ar-CH ) , 7.79 – 7.87 ( m , 5H , Ar – CH ) , 8.1- 8.20 ( d , 2H , NH). <sup>13</sup>C NMR (300MHz, DMSO, δ): 39.3, 49.2, 113.1, 119.9, 125.0, 126.4, 126.8, 127.0, 127.1, 128.9, 130.3, 133.0, 134.9, 145.8, 149.0, 152.8, 160.5, 197.0. Anal. Calcd for C<sub>23</sub>H<sub>20</sub>N<sub>4</sub>O<sub>2</sub> (384.4) : C, 71.86; H, 5.24; N, 14.57. Found: C, 71.66; H, 5.18; N, 14.48.

### 3.2 Synthesis of compounds from Series 2



#### Reagents and Reaction Conditions:

a) Hydrazine Hydrate reflux , 4-6Hrs

b) Chloroacetyl Chloride reflux , 4-5 Hrs

c) Hydrazine Hydrate reflux , 4-6Hrs

d) Substituted Aromatic Aldehyde, H<sub>2</sub>SO<sub>4</sub>,  
Reflux , 4 hrs

	Code	R	X	Y
5	--	--	Br	Br
6	--	--	H	H
7	--	--	Br	Br
8	--	--	H	H
9	--	--	Br	Br
10	--	--	H	H
11a	PS-8	4-F	H	H
11b	PS-9	4-Cl	H	H
11c	PS-10	4-OH	H	H
11d	PS-11	4-F	Br	Br
11e	PS-12	4-Cl	Br	Br
11f	PS-13	4-OH	Br	Br

Scheme 2: synthetic Scheme for the compounds 11<sub>a-f</sub>

**3.2.1 Unsubstituted /6,8-dibromo-3-amino-2-phenyl quinazolinone (5/6):** 2-phenyl-4-H-benzo[d][1,3]oxazin-4-one / 6,8-dibromo-2-phenyl-4H-benzo[d][1,3]oxazin-4-one (0.01 moles each) was dissolved in

pyridine separately and 8 ml of hydrazine hydrate was added to it, heated and refluxed at 80 °C for 4-6 hrs. Reaction was allowed to cool at room temperature. Ice was added to obtain solid precipitate. The solid was washed with 50 mL of 5% NaCl solution and recrystallized with ethanol. The solid precipitated was separated by filtration and dried to obtain separately 3-amino-2-phenylquinazolinone (1a) / 6,8-dibromo-3-amino-2-phenylquinazolinone (1b).

**3.2.1.1 2-phenyl-4-H-benzo[d][1,3]oxazin-4-one (5):** Yield 40%, mp 172 °C; IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3307, 3215 (N-H), 3062 (ArC-H), 1662 (C=O), 1564, 1471 (ArC=C), 1338 (C-N), <sup>1</sup>H NMR (400MHz, DMSO-d<sub>6</sub>):  $\delta$  8.15–8.25 (d, 1H, J=7.0Hz, ArH), 7.8-8.1 (m, 3H, ArH), 7.71-7.73 (d, 1H, J=8.0Hz, ArH), 7.55-7.6 (t, 1H, J=5.6Hz, ArH), 7.45-7.5 (d, 3H, J=6.73Hz, ArH), 5.2 (s, 2H, NH<sub>2</sub>). Anal. Calcd for C<sub>14</sub>H<sub>11</sub>N<sub>3</sub>O (237.3): C, 70.87; H, 4.67; N, 17.71. Found: C, 70.93; H, 4.57; N, 17.69.

**3.2.1.2 6,8-dibromo-3-amino-2-phenylquinazolinone (6):** Yield 65%, mp 235 °C, IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3311, 3274 (N-H), 3082 (ArC-H), 1670 (C=O), 1568 (ArC=C), 694 (C-Br), <sup>1</sup>H NMR (400MHz, DMSO-d<sub>6</sub>):  $\delta$  8.4 (s, 1H, ArH), 8.2-8.3 (s, 1H, ArH), 7.8-7.9 (d, 2H, J=6.4Hz, ArH), 7.4-7.6 (m, 3H, ArH), 5.72-5.75 (s, 2H, NH<sub>2</sub>). Anal. Calcd for C<sub>14</sub>H<sub>9</sub>Br<sub>2</sub>N<sub>3</sub>O (395.0): C, 42.56; H, 2.30; Br, 40.45; N, 10.64. Found: C, 42.56; H, 2.30; N, 10.64.

**N-(6,8-dibromo-4-oxo-2-phenylquinazolin-3(4H)-yl)-2-chloroacetamide(7)/2-chloro-N-(4-oxo-2-phenylquinazolin-3(4H)-yl)acetamide (8):** Equimolar (0.001 moles) quantities of **5/6** were taken in RBF separately and to it 0.004 moles of chloroacetyl chloride was added to it separately and refluxed for 4-5 hrs and reaction was monitored with TLC, after the product has been obtained it was washed with 100 ml of water. The solid precipitated was separated by filtration and dried to obtain separately / N-(6,8-dibromo-4-oxo-2-phenylquinazolin-3(4H)-yl)-2-chloroacetamide (**7**) / 2-chloro-N-(4-oxo-2-phenylquinazolin-3(4H)-yl)acetamide (**8**).

**3.2.1.3 N-(6,8-dibromo-4-oxo-2-phenylquinazolin-3(4H)-yl)-2-chloroacetamide (7):** Yield 58%; mp 238 °C; IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3230 (N-H), 3070 (ArC-H), 2943 (CH<sub>2</sub>), 1710 (C=O), 1542, 1488 (ArC=C), 700 (C-Cl), 694 (C-Br), <sup>1</sup>H NMR (400MHz, DMSO-d<sub>6</sub>):  $\delta$  11.6-11.8 (s, 1H, Enol), 8.4-8.5 (s, 1H, ArH), 8.2-8.3 (s, 1H, ArH), 7.4-7.7 (m, 5H, ArH), 4.05-4.20 (dd, 2H, J=13.7Hz, diastereotopic-CH<sub>2</sub>). Anal. Calcd for C<sub>16</sub>H<sub>10</sub>Br<sub>2</sub>ClN<sub>3</sub>O<sub>2</sub> (471.5): C, 40.75; H, 2.14; 7.52; N, 8.91. Found: C, 42.75; H, 2.74; N, 8.78.

**3.2.1.4 2-chloro-N-(4-oxo-2-phenylquinazolin-3(4H)-yl)acetamide (8):** Yield 55%, mp 148 °C; IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3205 (N-H), 3010 (Ar-C-H), 2935 (CH<sub>2</sub>), 1690 (C=O), 1568, 1475 (Ar-C=C), 1328 (C-N), 775 (C-Cl), <sup>1</sup>H NMR (400MHz, DMSO-d<sub>6</sub>):  $\delta$  11.5 (s, 1H, Enol), 8.1-8.2 (d, 2H, J=6.7Hz, ArH), 7.89-7.90 (t, 1H, J=7.0Hz, ArH), 7.75- 7.77 (d, 1H, J=7.9Hz, ArH), 7.48-7.54 (m, 5H, ArH), 4.08-4.18 (dd, 2H, J=13.6Hz, -CH<sub>2</sub>).

**N-(6,8-dibromo-4-oxo-2-phenylquinazolin-3(4H)-yl)-2-hydrazinylacetamide (9) / 2-hydrazinyl-N-(4-oxo-2-phenylquinazolin-3(4H)-yl)acetamide (10):** Equimolar quantities of **7** and **8** were taken in RBF separately, and hydrazine hydrate was added to it and refluxed for 4-6 hrs until the product has been obtained. The product obtained was washed with 50mL of 5% NaCl and recrystallized with ethanol. The solid precipitated was separated by filtration and dried to obtain N-(6,8-dibromo-4-oxo-2-phenylquinazolin-3(4H)-yl)-2-hydrazinyl-acetamide (**9**) / 2-hydrazinyl-N-(4-oxo-2-phenylquinazolin-3(4H)-yl)acetamide (**10**).

**3.2.1.5 N-(6,8-dibromo-4-oxo-2-phenylquinazolin-3(4H)-yl)-2-hydrazinylacetamide (9):** Yield 70%, mp: 223 °C; IR (KBr)  $\nu$  (cm<sup>-1</sup>): <sup>1</sup>H-NMR (400Mhz, DMSO-d<sub>6</sub>): 2.00 (s, 3H, NH-NH<sub>2</sub>), 3.54 (q, 2H, CH<sub>2</sub>), 7.29-7.52 (m, 3H, 2-phenyl), 7.69-7.83 (d, 2H, 2-phenyl), 8.00 (s, 1H, NH) 8.16 (d, 1H, quinazolinyl), 8.25 (d, 1H, quinazolinyl). <sup>13</sup>C-NMR (200MHz, DMSO): 59.3, 113.2, 122.0, 125.2, 128.2, 128.6, 128.8, 130.1, 131.3, 139.4, 154.2, 156.2, 160.6, 170.3. Anal. Calcd for C<sub>16</sub>H<sub>13</sub>Br<sub>2</sub>N<sub>5</sub>O<sub>2</sub> (467.1): C, 41.14; H, 2.81, N, 14.99. Found: C, 40.94; H, 2.93; N, 14.99.

**3.2.1.6 2-hydrazinyl-N-(4-oxo-2-phenylquinazolin-3(4H)-yl)acetamide (10)** Yield 73%; mp: 285 °C; IR (KBr)  $\nu$  (cm<sup>-1</sup>): <sup>1</sup>H-NMR (400MHz, DMSO-d<sub>6</sub>): 2.00 (s, 3H, NH-NH<sub>2</sub>), 3.54 (q, 2H, CH<sub>2</sub>), 7.52-7.83 (m, 8H, Ar-H), 8.00 (s, 1H, NH). Anal. Calcd for C<sub>16</sub>H<sub>15</sub>N<sub>5</sub>O<sub>2</sub> (309.3): C, 62.13; H, 4.89; N, 22.64. Found: C, 62.53; H, 4.39; N, 21.64.

**3.2.2 General procedure for 2-(2-(substituted benzylidene)hydrazinyl)-N-(4-oxo-2-phenylquinazolin-3(4H)-yl) acetamide (11<sub>a-c</sub>): (PS-8, PS-9, PS-10):** Equimolar (0.001 mole ) quantities of **10** was taken in RBF and dissolved in ethanol separately , and 0.001 moles substituted (-F, -Cl and -OH) benzaldehydes were added to it with 0.2 ml of concentrated H<sub>2</sub>SO<sub>4</sub> and refluxed for 4 hrs and monitored with TLC. The compound was purified by column chromatography to obtain 2-(2-substituted benzylidenehydrazinyl)-N-(4-oxo-2-substituted phenylquinazolin-3(4H)-yl) acetamide (11<sub>a-c</sub>).

**3.2.2.1 2-(2-(4-fluorobenzylidene)hydrazinyl)-N-(4-oxo-2-phenylquinazolin-3(4H)-yl)acetamide (PS-8):** Yield 65%; mp 127°C; IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3080(C-H,Ar.), 1722 (C=O, ketone), 1590 (C-C,Ar.), 1269 (C-N,Ar.), 1180 (CH-F). <sup>1</sup>H-NMR (300MHz, DMSO-d<sub>6</sub>):  $\delta$  1.30 (s,1H,-NH), 1.60-1.72 (d,2H,-CH<sub>2</sub>), 7.07-7.13 (d, 2H, Ar-H), 7.43-7.45 (d, 2H, Ar-H), 7.51-7.56 (q, 2H, Ar-H), 7.66-7.72 (m, 5H, Ar-H), 7.77-7.84 (m, 2H, Ar-H), 8.34-8.37 (d, 1H, -NH), 9.05 (d,1H,=CH). <sup>13</sup>C NMR (300MHz, DMSO,  $\delta$ ): 39.3, 49.2, 115.4, 119.9, 125.06, 126.4, 127.05, 127.1, 128.9, 129.5, 133.05, 134.9, 145.8, 152.8, 157.8, 160.5, 197.09. Anal. Calcd for C<sub>23</sub>H<sub>17</sub>O<sub>2</sub>N<sub>5</sub>F (415.42): C, 66.50; H, 4.37; 4.57; N, 16.86. Found: C, 65.53; H, 4.29; N, 17.86.

**3.2.2.2 2-(2-(4-chlorobenzylidene)hydrazinyl)-N-(4-oxo-2-phenylquinazolin-3(4H)-yl) acetamide (PS-9):** Yield : 80%., mp 160°C; IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3280 (NH of NH<sub>2</sub>), 3098 (C-H, Ar.), 1683 (C=O, ketone), 1489 (C-C,Ar.), 1292 (C-N,Ar.), 840 (CH-Cl). <sup>1</sup>H-NMR(300 MHz, CDCl<sub>3</sub>):  $\delta$  1.30 (s,1H,-NH), 1.60-1.72 (d,2H,-CH<sub>2</sub>), 7.37-7.51 (m,7H,Ar-H), 7.53-7.56 (d,1H,Ar-H), 7.59-7.62 (d,2H,Ar-H), 7.68-7.71 (d,2H,Ar-H), 7.79-7.81 (d,1H,Ar-H), 8.34-8.37 (d,1H,=CH), 9.10 (s,1H,-NH). <sup>13</sup>C NMR (300MHz, DMSO,  $\delta$ ): 49.2, 115.7, 119.9, 125.06, 126.4, 126.8, 127.05, 127.1, 128.9, 130.2, 133.05, 134.2, 142.5, 145.8, 152.8, 160.5, 163.3, 165.8. Anal. Calcd for C<sub>23</sub>H<sub>17</sub>O<sub>2</sub>N<sub>5</sub>Cl (431.11): C, 63.96; H, 4.20; N, 16.22. Found: C, 63.36; H, 4.50; N, 16.24.

**3.2.2.3 2-(2-(4-hydroxybenzylidene)hydrazinyl)-N-(4-oxo-2-phenylquinazolin-3(4H)-yl)acetamide (PS-10):** Yield: 75%., mp 192°C; IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3172 (OH, str.), 3074 (NH of NH<sub>2</sub>), 2899 (C-H,Ar.), 1734 (C=O, ketone), 1473 (C-C,Ar.), 1293 (C-N,Ar.). <sup>1</sup>H-NMR(300 MHz, DMSO):  $\delta$  1.08-1.12 (s,1H,-NH), 3.70-3.77 (t,2H,-CH<sub>2</sub>), 6.84-6.87 (d,2H,Ar-H), 7.43-7.45 (d,3H,Ar-H), 7.56-7.63 (t,4H,Ar-H), 7.68-7.70 (d,2H,Ar-H), 7.76-7.79 (s,1H,Ar-H), 7.86-7.91 (t,1H,Ar-H), 8.21-8.24 (d,1H,-NH), 8.80 (s,1H,=CH), 10.35 (s,1H,-OH). <sup>13</sup>C NMR (300MHz, DMSO,  $\delta$ ): 49.2, 119.9, 125.06, 126.4, 126.8, 127.05, 127.1, 128.9, 129.2, 129.4, 133.05, 134.2, 135.6, 142.5, 145.8, 152.8, 160.5, 165.8. Anal. Calcd for C<sub>23</sub>H<sub>18</sub>O<sub>3</sub>N<sub>5</sub> (413.15): C, 66.82; H, 4.63; N, 16.94. Found: C, 65.82; H, 4.93; N, 17.94.

**3.3 General procedure for synthesis of 2-(2-(4-substituted benzylidene)hydrazinyl)-N-(6,8-dibromo-4-oxo-2-phenylquinazolin-3(4H)-yl)acetamide (11<sub>d-f</sub>): ( PS-11, PS-12, PS-13 ) :** Equimolar quantities (0.001 moles) N-(6,8-dibromo-4-oxo-2-phenylquinazolin-3(4H)-yl)-2-hydrazinyl-acetamide (**9**) and 4-substituted (-F, -Cl and -OH) -benzaldehyde were taken in RBF separately and dissolved in ethanol and 0.2 ml of concentrated H<sub>2</sub>SO<sub>4</sub> was added to it . Reaction mixture was refluxed for 4 hrs and monitored with TLC. After completion of reaction solid was obtained and was filtered to obtain 2-(2-(4-substituted benzylidene)hydrazinyl)-N-(6,8-dibromo-4-oxo-2-phenylquinazolin-3(4H)-yl)acetamide (11<sub>d-f</sub>). The compound was purified by column chromatography.

**3.3.1: 2-(2-(4-fluorobenzylidene)hydrazinyl)-N-(6,8-dibromo-4-oxo-2-phenylquinazolin-3(4H)-yl)acetamide (PS-11):** Yield: 70%; mp 177°C ; IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3085 (C-H,Ar), 1764 (C=O, ketone), 1603 (C-C,Ar.), 1269 (C-N,Ar.), 1237 (CH-F), 688 (C-Br). <sup>1</sup>H-NMR(300 MHz, CDCl<sub>3</sub>):  $\delta$  1.03 (s,1H,-NH), 1.60-1.72 (d,2H,-CH<sub>2</sub>), 7.09-7.14 (t,2H,Ar-H), 7.40-7.48 (q,4H,Ar-H), 7.69-7.73 (q, 2H,Ar), 7.80-7.82 (d,2H,Ar-H), 8.16-8.17 (d,1H,Ar-H), 8.41-8.41 (d,1H,-NH), 8.96 (s,1H,=CH). <sup>13</sup>C NMR (300MHz, DMSO,  $\delta$ ): 49.2, 112.7, 115.7, 116.2, 120.3, 126.8, 127.1, 128.9, 130.2, 130.6, 134.2, 134.4, 140.4, 142.5, 152.8, 160.5, 163.3, 165.8. Anal. Calcd for C<sub>23</sub>H<sub>15</sub>O<sub>2</sub>N<sub>5</sub>Br<sub>2</sub>F (573.2): C, 48.19; H, 2.81; N, 12.22. Found: C, 48.50 ; H, 2.81; N, 11.06.

**3.3.2: 2-(2-(4-chlorobenzylidene)hydrazinyl)-N-(6,8-dibromo-4-oxo-2-phenylquinazolin-3(4H)-yl)acetamide(PS-12):** Yield: 65%; mp 272°C ; IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3079 (C-H,Ar), 1734 (C=O), 1448 (C-C,Ar), 1311 (C-N,Ar.), 849 (CH-Cl), 691cm<sup>-1</sup>(C-Br). <sup>1</sup>H-NMR(300 MHz, CDCl<sub>3</sub>):  $\delta$  1.30 (s,1H,-NH), 1.60-1.72 (d,2H,-CH<sub>2</sub>), 7.40-7.48 (m,6H,Ar-H), 7.63-7.66 (d,2H,Ar-H), 7.79-7.81 (d,2H,Ar-H), 8.15-8.18 (s,1H,Ar-H), 8.43-8.42 (d,1H,-NH), 9.01 (d,1H,=CH). <sup>13</sup>C NMR (300MHz, DMSO,  $\delta$ ): 49.2, 112.7, 116.2, 120.3, 126.8, 127.1, 128.9, 129.2, 129.4, 130.6, 134.2, 134.4, 135.6, 140.4, 142.5, 152.8, 160.5, 165.8.

**3.3.3: 2-(2-(4-hydroxybenzylidene)hydrazinyl)-N-(6,8-dibromo-4-oxo-2-phenylquinazolin-3(4H)-yl)acetamide (PS-13):** Yield 70%; mp 280°C; IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3214 (OH, str.), 3208 (NH of NH<sub>2</sub>), 3021 (C-

H), 1766 (C=O), 1448 (C-C,Ar), 1290 (C-N,Ar), 688 (C-Br). <sup>1</sup>H-NMR(300 MHz, DMSO):  $\delta$  2.46 (s,1H,-NH), 3.34 (s,1H, -CH<sub>2</sub>), 6.85-6.88 (d,2H,Ar-H), 7.45-7.80 (q,4H,Ar-H), 7.57-7.59 (d,2H,Ar-H), 7.72-7.74 (d,2H,Ar-H), 8.28 (s,1H,Ar-H), 8.42 (s,1H,-NH), 8.76 (s,1H, =CH), 10.38 (s,1H,-OH). <sup>13</sup>C NMR (300MHz, DMSO,  $\delta$ ): 49.2, 112.7, 115.01, 116.2, 120.3, 126.8, 127.1, 128.7, 128.9, 130.6, 134.2, 134.4, 140.4, 142.5, 152.8, 157.8, 160.5, 165.8. Anal. Calcd for C<sub>23</sub>H<sub>16</sub>O<sub>3</sub>N<sub>5</sub>Br<sub>2</sub> (571.22): C, 46.85; H, 2.73; N, 11.88. Found: C, 47.85; H, 2.53; N, 12.88.

#### 4. *In vitro* DPP-IV inhibition Assay

Rat splenocytes were treated with 0.001-10 $\mu$ M test samples or sitagliptin. Untreated wells received vehicle only. Cells were incubated in CO<sub>2</sub> (5%) incubator, at 37°C for 1 hour. DPP-IV substrate was added according to Kit protocol (Sigma MAK088) and incubated in CO<sub>2</sub> (5%) incubator, at 37°C for 2 hours. Cells were centrifuged and supernatant was transferred to 96 well plates. Plates were examined on BMG Fluostar (Germany). Values were auto calculated by Mars Omega software and represented as percentage inhibition. **Table. 1** depicts the IC<sub>50</sub> values of various synthesized compounds sitagliptin and algoliptin.

**Table 1: IC<sub>50</sub> values and docking scores of synthesized molecules**

Synthesized Compounds and sitagliptin	Concentration ( $\mu$ M)					IC <sub>50</sub> Value in $\mu$ M	Docking score
	10	1	0.1	0.01	0.001		
<b>PS 3</b>	53.1	48.75	33.68	15.74	3.65	1	-45.78
<b>PS4</b>	11.54	8.32	7.89	4.65	3.95	>10	-46.51
<b>PS5</b>	19.67	9.20	7.70	4.53	3.40	>10	-51.32
<b>PS 6</b>	51.33	48.2	32.88	20.67	4.78	1	-51.72
<b>PS 8</b>	18.34	8.77	7.8	6.3	7.47	>10	-50.38
<b>PS 9</b>	8.51	7.71	6.83	4.82	3.68	>10	-53.21
<b>PS 10</b>	11.4	8.28	7.45	4.12	3.88	>10	-53.51
<b>PS 11</b>	9.80	8.65	8.4	7.8	5.78	>10	-50.53
<b>PS 12</b>	12.24	8.40	7.80	5.13	4.76	>10	-48.22
<b>PS 13</b>	12.33	9.45	8.6	5.42	4.63	>10	-49.95
<b>Sitagliptin</b>	-	-	--	-	-	0.06	-37.41
<b>Algoliptin</b>	-	-	-	-	-	0.1	-56.47



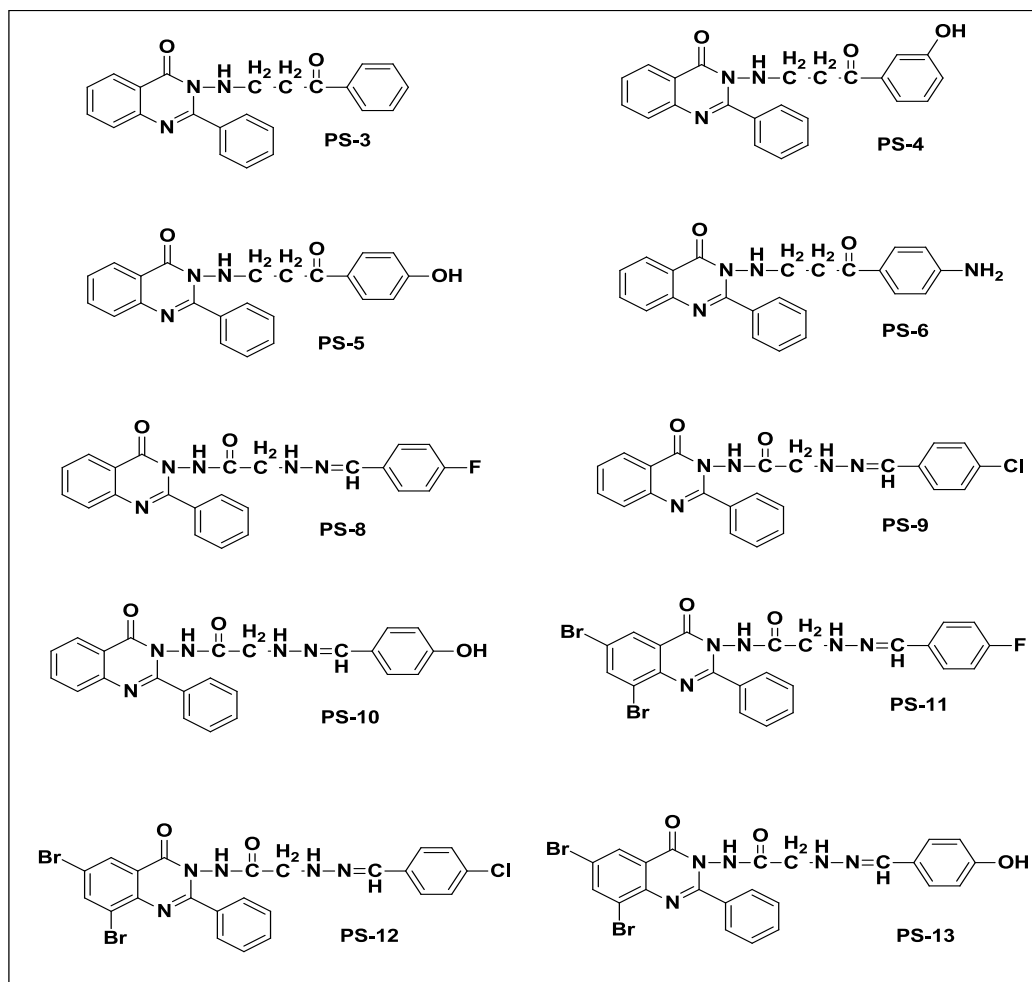


Figure 2: Structures of synthesized compounds

## 5. Results and Discussion

Gliptins are known to be anti-diabetic drugs which work by DPP-IV inhibition. Saxagliptin, sitagliptin and alogliptin are examples of drugs falling in this class which are used to lower blood sugar in Type II diabetes. A recent report by U.S. Food and Drug Administration (FDA) safety 2016 reported these drugs to increase the risk of heart failures<sup>13</sup>. Based upon our previous work and recent literature for search of better heterocycles as DPP-IV inhibitors<sup>14,15</sup> we aimed to synthesize newer agents using pyrimidinones as pharmacophore. The pyrimidinedione heterocycle moiety of alogliptin is recognized as a key pharmacophore that contributes to its good pharmacokinetic profile, potency and selectivity. Using this information, we designed a new series of quinazolinones, which are benzopyrimidinones and hypothesized that these may show potent inhibition as compared with alogliptin and sitagliptin. Also Alogliptin was discovered from the modification of quinazolinones<sup>16</sup>. The structure-based design also helped us to hypothesize that a quinazolinone scaffold could effectively display groups known to interact with the active site residues of DPP-IV. Sitagliptin and Alogliptin were used as the standard molecule for the docking and *in vitro* activity comparison.

Compounds 3a-d were prepared by fusion of 2-phenyl benzoxazin-4-one (1) with hydrazine hydrate for 4-6 hours which yielded 2-phenyl-3-amino quinazolin-4-one derivatives (2) where amine derivatives act as nucleophiles. The compound (2) was further treated with substituted acetophenones, formaldehyde in presence of ethanol and conc. HCl and refluxed for 12-15 hours. This afforded the desired compounds 3<sub>a-d</sub> (PS-3, PS-4, PS-5 and PS-6) bearing different substituents. This final step was based on Mannich reaction wherein formaldehyde reacts with primary or secondary amine and substituted acetophenones (source of active hydrogen) to cause insertion of a CH<sub>2</sub> on amine (R-NH-CH<sub>2</sub><sup>+</sup>) forming carbonium ion and hydrogen abstraction from acetophenone gives carbanion. The two then combine to form the mannich base thus yielding PS-3, 4, 5, 6. The structure was confirmed by characteristics IR stretching signals at 3216 cm<sup>-1</sup> for -NH stretching, 1768

cm<sup>-1</sup> for carbonyl stretch. The nmr data also confirmed the structure. In second scheme, substituted (position 6 and 8) and unsubstituted quinazolinone ring system were allowed to undergo amidation on the primary amine. Compound 5 and 6 were treated with chloroacetyl chloride under reflux for 4-5 hours. The reaction involves nucleophilic substitution reaction of chloroacetylchloride with amines to yield compound 7 and 8. These on treatment with hydrazine hydrate afforded to give compound 9 and 10 as hydrazine derivatives. Compound 9 and 10 were then reacted with aromatic aldehydes bearing different substituents at para position on phenyl ring. This reaction formed the imines called as schiffs base. The reaction is acid catalyzed and proceeds with water elimination. Purification through column yielded the final compounds PS-8, PS-9, PS-10, PS-11, PS-12 and PS-13. The compounds were confirmed through IR and proton nmr spectroscopy. Designed and evaluated molecules are shown in figure 2.

The synthesized compounds displayed fair DPP-IV inhibition profile. Compound PS-3 and PS-6 revealed good inhibition potential with IC<sub>50</sub> values below 1µM. The compound in the second series displayed low activity despite of high docking scores. The docking scores were seen to be better than the standard but the correlation between the scores and the activity could not be extracted because sitagliptin used as standard had high potency despite the low docking score.

## 6. Conclusion

Two series of substituted and unsubstituted quinazolinones as DPP-IV inhibitors were designed and synthesized. The results were compared with sitagliptin as standard molecule. The study revealed that from amongst all the compounds, PS-3 and PS-6 displayed good inhibition profile. Correlation between docking and activity could not be sought for but study dictates that quinazolinones can be used as lead molecules for development for potent DPP-IV inhibitors.

## Conflict of Interest

There is no conflict of Interests.

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