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Discovery of novel 1,3,5-triazine-thiazolidine-2,4-diones as dipeptidyl peptidase-4 (DPP - 4) inhibitor targeting *S1* pocket for the treatment of type 2 diabetes along with antibacterial activity

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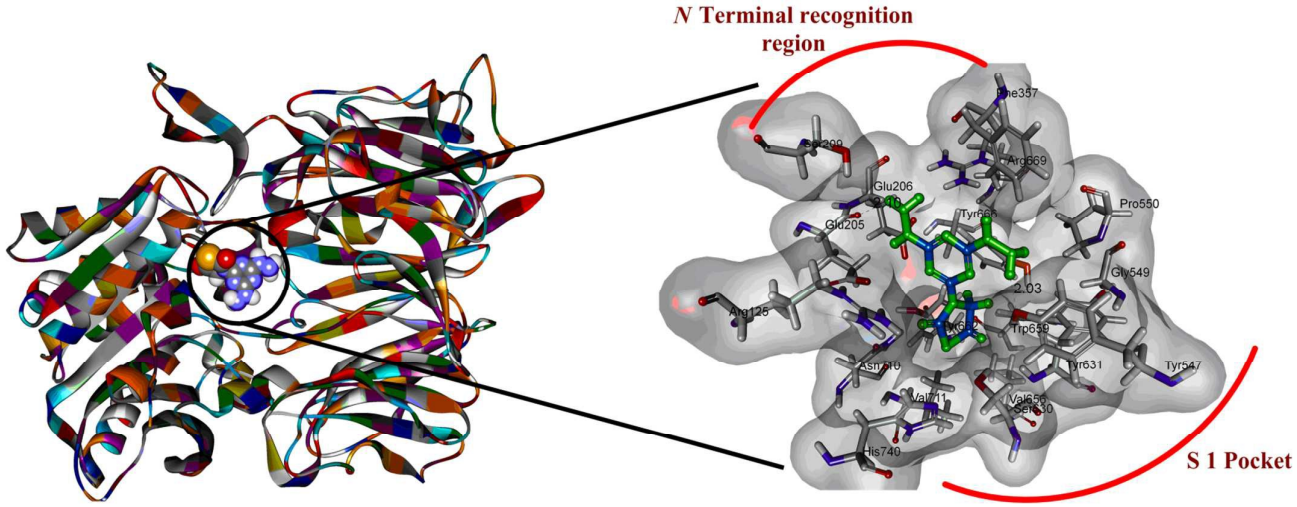
Graphical Abstract



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Discovery of novel 1,3,5-triazine-thiazolidine-2,4-diones as dipeptidyl peptidase-4 (DPP - 4) inhibitor targeting *S1* pocket for the treatment of type 2 diabetes along with antibacterial activity

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Abstract

A novel series of 1,3,5-triazine-thiazolidine-2,4-diones have been synthesized and characterized with the aid of numerous analytical and spectroscopic techniques. These molecules were screened for in vitro inhibition of dipeptidyl peptidase-4, where compound 7a showed most prominent inhibition with IC_{50} = 6.25 μM. The rest of the compound showed considerable inhibition (IC_{50} = 12.11 – 49.21 μM). Docking studies indicate that, lipophillic thiazolidine-2,4-dione fragment of the ligand 7a found oriented towards the tight liphophillic cavity of *S1* pocket of the active site formed by residues like, Tyr631, Val656, Trp659, Tyr662, Tyr666 and Val711 via formation of H-bond with Tyr547. Moreover, one of the amine present on the wings of triazine forms a hydrogen bond with Glu205, a vital residue for the N-terminal recognition site with efficient CDOCKER interaction energy. In bacterial inhibition study, the entire set of compounds showed excellent activity and even in some cases found equipotent to Cefixime as standard.

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Introduction

Diabetes is frequently concerned to by clinicians as diabetes mellitus, which further classified into type 1 (previously known as insulin-dependent) and type 2 (formerly called non-insulin-dependent). It depicts a group of metabolic diseases in which either the pancreas does not make adequate insulin or when the body cannot effectively use the insulin it produces, or both.[1] The burden of disease is increasing globally, especially in developing nations which contribute more than 80% of diabetes deaths.[2] By an estimate, in 2012, 1.5 million deaths were induced by diabetes and till 2030, it will be the 7th leading cause of morbidity and death rate across the world.[3]

In type 2 diabetic patients, besides classical insulin-based treatment, inhibition of the serine protease dipeptidyl peptidase 4 (DPP-4) has proven to be an effective treatment for improving glycemic control.[4] DPP-4 is also known as CD26 (cluster of differentiation 26 or T-cell activation antigen CD26) or is adenosine deaminase complexing protein 2 which selectively cleaves first two amino acids of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide (GLP-1) thereby makes it inactive. Accordingly, inhibition of DPP-4 by a chemotherapeutic agent may lead to increase the levels of circulating endogenous GLP-1 by prolonging its half-life and consequently enhances the beneficial effects of GLP-1 in glucose dependent insulin secretion and β-cell restoration. The DPP-4 inhibitor results in higher circulating concentrations of endogenous GLP-1 and subsequent decrease in plasma glucose, by enhancing β-cell glucose-stimulated insulin release and promoting insulin gene expression and its biosynthesis, fig 1.[5]

<Figure 1>

Imeglimin ((6R)-(+)-4-dimethylamino-2-imino-6methyl-1,2,5,6-tetrahydro-1,3,5-triazine hydrochloride) is the first in a new tetrahydrotriazine-containing class of oral antidiabetic agents, the glimins. It is currently in phase 2b clinical development.[6] In preclinical studies, imeglimin has been shown to reduce excessive hepatic glucose production, increase glucose uptake in skeletal muscle, and improve insulin secretion in response to glucose via acting on the liver, muscle and the pancreas, three key organs involved in the pathophysiology of type 2 diabetes. Imeglimin offers a unique mechanism of action that targets the mitochondria and is compatible with drugs that counter insulin resistance or enhance insulin secretion and β-cell protection.[7]

Thiazolidin-2,4-diones (TZDs), a class of oral insulin sensitizing agents that improves insulin resistance, are agonist of proxisome proliferator activated receptor- γ (PPAR - γ). These molecules amplify PPAR - γ expression in the adipose tissue thus increasing adipocytes and subcutaneous adipose tissue mass. Increased PPAR - γ expression in the adipose tissue results in increased fatty acid uptake and storage by increasing the transcription of fatty acid transport protein-1 and acyl-coenzyme A synthetase. Decreased circulating free fatty acid levels protect β

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cells, the liver, and the skeletal muscle from their toxic effects, thus improving insulin sensitivity.[8] Of TZDs, ciglitazone, pioglitazone (PGZ), rosiglitazone (RGZ), troglitazone (TGZ) (approved in 1997) and englitazone have been clinically examined.[9]

According to American Diabetes Association combinations of oral agents than monotherapy have been used to attack the pathophysiology of diabetes at multiple points in cases where insulin secretion is still moderate.[10] Combination therapy includes treatment with two or more agents with different, complementary mechanisms of action, for example, the combination of a thiazolidinedione and a biguanide improves insulin sensitivity and lowers blood glucose through complementary pathways, and therefore produces an additive effect. Nevertheless, sometimes, this strategy fails because of incomplete and altered pharmacokinetics of the combined drugs which affects their bioavailibity.[11] Molecular hybridization is an innovative approach gaining attention from medicinal chemists owing to resemblance with combination therapies where two diverse pharmacophoric groups were joined. These drugs can indeed be more powerful than either of their precursors because of dual drug targeting at more than one site and privileged activity when compared to the individual agent.[12]

Considering the clinical implication of DPP-4 inhibitors, advantages of molecular hybridisation and antidiabetic potential of 1,3,5-triazine and thiazolidine-2,4-diones, we report design, synthesis and biological evaluation of novel hybrid 1,3,5-triazine-thiazolidine-2,4-diones as DPP-4 inhibitor along with antibacterial activity.

Results and Discussion

Chemistry

In our approach, the synthesis of novel hybrid conjugates was accomplished in multi-step reaction as outlined in scheme 1. The synthesis of thiazolidin-2,4-dione (3) was achieved by reacting chloro acetic acid (1) and thiourea (2) in the presence of water via formation of the thiouronium salt, which later cyclises in the presence of an acid to afford the corresponding product 3 in good yield. Reaction of 2,4,6-tri chloro 1,3,5-triazine (4) with distinguished amines 5 (a-k) in the presence of activating base yielded mono chloro di-substituted 1,3,5-triazine 6 (a-k) upon stirring for desired time, step 2. The clubbing of previously obtained product from two different steps viz., 3 and 6 (a-k) leads to the generation of novel target hybrid conjugates 7 (a-k) via nucleophilic reaction in the presence of K_2CO_3 as a base.

<SCHEME 1>

Inhibitory activity against DPP-4 in vitro

The synthesized 1,3,5-triazine-thiazolidine-2,4-diones derivatives 7 (a–k) were evaluated in vitro for DPP-4 enzyme inhibitory activity, and results obtained are reported as micromolar inhibitory concentration, IC_{50} (μM) along with standard (P 32 / 98) in table 1. In a comparison test,

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compound 7a containing hydrazine as substituent was identified as very potent analogue of the series (IC_{50} = 6.25 μM). Whereas, the twofold decline in activity was reported on the introduction of bulky aromatic substitution (IC_{50} = 12.11 μM), compound 7b. A marginal progress in activity was indicated by compound 7c having *o*-NH_2 as substituent on the phenyl. While on the introduction of NO_2 (compound 7d) in place of NH_2 render compound less potent, and much more decline in activity was reported on insertion of Cl (compound 7g). The optimization strategy has been then focused to study the optimal placement of these functional groups. Compound 7e and 7f having *m*- and *p*-NO_2 has been led to further decline in activity. Moreover, the same pattern of activity was not being observed and showed comparative weak inhibition in the case of compounds 7h and 7i containing *m*- and *p*-Cl. A significant decline in activity was reported by compounds 7j and 7k having *p*-F and a *p*-Br substituent, IC_{50} = 38.37 μM and 49.21 μM, respectively.

Structural-activity relationship study suggests that, the presence of substituent has a substantial influence on the inhibitory activity. It has been clearly revealed that, the presence of bulky substituent endangers the activity and further decrease was observed on the introduction of the substituent. The pattern of the SAR has been clearly depicted in fig 2.

<Figure 2>

Molecular Docking Studies

Dipeptidyl peptidase-4 enzyme consists of two domains, a eight-stranded β-propeller domain at the N-terminus and an α/β-hydrolase domain at the C-terminus. The X-ray crystal study of various inhibitors in complex with DPP-4 revealed that the binding site of DPP-4 mainly comprises three parts: a S1 pocket, the N-terminal recognition region, and a S2 pocket.[13] The S1 hydrophobic pocket comprising residues Tyr631, Val656, Trp659, Tyr662, Tyr666 and Val711 adjacent to catalytic triad of active site. The active site is situated in the hydrolase domain and comprised of catalytic triad residues Ser630, Asp708 and His740. The N-terminal recognition region is formed by Glu205, Glu206, and Tyr662, while the hydrophobic S2 pocket, which is larger compared with *S1*, is surrounded by residues Phe357, Arg358, Ser209, and Tyr666.

With in-vitro results in-hand, it is worthwhile to perform the molecular docking study of most active compound 7a with DPP-4 inhibitor to explicate key molecular interactions (2FJP.pdb). Thereupon, as depicted in fig. 3, it has been found that one of the amine present on the wings of triazine forms a hydrogen bond with Glu205, a vital residue for the N-terminal recognition site. In the new orientation, amine present on the other wing protrudes towards the Ser630 and Tyr631 of S1 pocket by creating a hydrogen bond with neighboring Tyr547. The lipophillic thiazolidine-2,4-dione fragment of the ligand found

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oriented towards the *S1* pocket of the active site formed by residues like, Tyr631, Val656, Trp659, Tyr662, Tyr666 and Val711. Additionally, the creation of one pi-cation interaction was also reported between 1,3,5-triazine and Arg105 of *S1* pouch. Scoring parameters indicate that compound 7a bind more firmly to the *S1* pocket of the DPP-4 by the virtue of efficient CDOCKER interaction energy and CDOCKER energy. As a consequence of this tight binding, the residence time of the 7a increases in DPP-4 which may transform into higher inhibitory activity in-vitro. The engulfment of compound 7a in the *S1* pocket of DPP-4 was found in accordance with the earlier studies which states that, small fragments target tight liphophillic cavity of the *S1* pocket.[14]

The docking of least active compound 7k in the DPP-4 binding site revealed the reason for its non-activity. It was found that, thiazolidine-2,4-dione fragment of ligand 7k positioned towards the *S2* pocket of binding site by making H-bonds with Arg358 and Arg669 owing to its extensive hydrophobic character, fig 4. While, it also create H-bond with Glu205 of N-terminal recognition site with different CDOCKER energies.

The docking study suggested that optimum balance between hydrophobic-hydrophillic character favors to increase the bioactivity (7a), while extensive hydrophibic character as seen in compound 7k lead to loss of activity. It was also recommended that, for better activity, the size of molecule should be kept small enough to fit into S1 pocket rather that big which detoriates the bio-activity.

<**FIGURE 3**>

<**FIGURE 4**>

Antibacterial activity

The immune dysfunction in diabetes patients due to hyperglycemic environment leads to more frequent and/or serious infectious diseases, which potentially increases their morbimortality.[15] However the efficacy of the drugs used to treat these infections, so called antibiotics has been seriously compromised by the resistance and necessitates the discovery of newer agents.[16] In continuation of our research endeavours towards the discovery of novel antibacterial agents from 1,3,5-triazine derivatives,[17] these constitutive derivatives were tested against panel of diverse microorganisms.

The newly prepared compounds were screened for determination of their minimal inhibitory concentration (MIC) against selected Gram-positive organisms viz. *Bacillus subtilis* (NCIM-2063), *Bacillus cereus* (NCIM-2156), *Staphylococcus aureus* (NCIM-2079) and Gram-negative organism viz. *Escherichia coli* (NCIM-2065), *Proteus vulgaris* (NCIM-2027) and *Pseudomonas aeruginosa* (NCIM-2036), broth microdilution (in tubes) method of the Clinical and Laboratory Standards

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Institute (CLSI) with minor modifications using Cefixime as standard and results are mentioned in table 3.

In a comparison test, the entire set of hybrid derivative showed moderate to excellent activities against tested Gram-positive and Gram-negative microorganisms in comparison to a standard. Compound 7a showed moderate to no activity against tested organisms. The compound 7b showed equipotent activity to standard against *E. coli* and found moderately active against the rest of the strains. The compound 7c showed improved activity in comparison to standard against *E.coli, B. Subtilis* and *S. aureus* with moderate activity against rest of the strains. The presence of NO_2 (7d) renders compound more active against all the strains except *P. aeruginosa* and *P. vulgaris* where it displayed moderate activity in comparison to the previous analogue. The isomeric replacements of NO_2, as seen in the case of compound 7e and 7f does not display any significant change on the activity profile except in the case of *B. Subtilis* (for 7f), where it showed almost no activity. In the case of P. vulgaris, compound 7g-i showed considerable activity, while mild to no activity was reported by compound 7j and 7k. Moreover, these compounds, i.e. 7 (g-j) showed moderate activities against E. coli except compound 7k. In the case of *P. aeruginosa*, *m*-chloro substituted analogue (7h) showed prominent inhibition than their isomeric counterparts, compounds 7g and 7i. The compound 7j and 7k showed mild to no activity against *P. aeruginosa*, respectively. It is noteworthy to mention that, against entire tested Gram positive microorganism, halogen substituted compounds showed mild to moderate activity, 7 (g-k).

Experimental

Melting points of the synthesized compounds was determined in an open capillary tube Hicon Melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was performed on silica gel-G coated plates to detect the completion of the reaction. The diverse mobile phase was selected in different proportion according to the assumed polarity of the products. The spots was visualized by exposure to the Iodine vapor. Infra-Red (IR) spectra were recorded in KBr on Biored FTs spectrophotometer and the reported wave numbers are given in cm^{-1}. ^1H NMR spectra were recorded in DMSO on Bruker Model D9RX-300MHz spectrometer. Chemical shifts were reported as δ (ppm) relative to TMS as internal standard. Mass spectra were obtained on Waters Q-TOF MICROMA SS (LC-MS) .

General Procedure

Step 1

The synthesis of thiazolidinone (3) was performed according to a published procedure.[18]

View Article Online
DOI: 10.1039/C4RA16903D

Step 2

The synthesis of di-substituted 1,3,5-triazines 6 (a-k) were performed in accordance with the earlier reported procedure. [17a]

Step 3

6-Chloro-N^2,N^4-bis(substituted phenyl)-1,3,5-triazine-2,4-diamine 6 (a-k) (0.1 mol) was added into 50 mL of dioxane at temperature 40–45 °C. A solution of thiazolidine-2,4-dione (3) (0.1 mol) in 35 mL dioxane was added slowly to the above solution and stirred for 2 h followed by drop-wise addition of K_2CO_3 (0.1 mol), refluxed the reaction mixture at 120-135 °C for 3-6 h. The completion of the reaction was monitored by TLC using benzene and ethyl acetate (9:1) as mobile phase. The reaction mixture was washed thoroughly with water, filtered and further purified by column chromatography to afford pure products 7 (a-k).

3-(4,6-dihydrazinyl-1,3,5-triazin-2-yl)thiazolidine-2,5-dione, **7a**

Dark brown powder; M.P.: >300 °C; FT-IR (ν_{max}; cm^{-1}, in KBr): 3259.03 (-NH- Stretching), 1613.67 (-C=O Stretching), 1599.12-1445.77(-NH_2 bending), 1385.41-1222.59(C-N Stretching), 687.11 (C-S Stretching); ^{1}H NMR (300MHz, $CDCl_3$-d_6, TMS) δ ppm: 2.52-2.54 (d, 4H, 2 x –NH_2 -hydrazine), 3.32-3.34 (d, 4H, 2 x –NH- -hydrazine), 3.59 (s, 2H, -CH_2- thiazolidine); ^{13}C NMR (400MHz, $CDCl_3$) δ ppm: 163.84 (C=O, thiazolidine), 138.27 (C, triazine), 128.24 (C, triazine), 123.22 (C=O, thiazolidine), 78.34 (-CH_2-, thiazolidine); Mass Spectra (TOF MS ES+, m/z): Calculated 256.05; Observed 257.20 (M+1).

3-(4,6-bis(phenylamino)-1,3,5-triazin-2-yl)thiazolidine-2,5-dione, **7b**

White powder; M.P.: >300 °C; FT-IR (ν_{max}; cm^{-1}, in KBr): 3366.40 (-NH- Stretching), 1605.32 (-C=O Stretching), 1573.76-1426.76 (-NH_2 bending), 1338.47-1238.92 (C-N Stretching), 667.75 (C-S Stretching); ^{1}H NMR (300MHz, $CDCl_3$-d_6, TMS) δ ppm: 3.58 (s, 2H, 2 x -NH-Ar), 3.88 (s, 2H, -CH_2- thiazolidine), 6.59-6.61 (d, 4H, J=6Hz, 2 x Ar-H), 6.96-7.02 (m, 2H, 2 x Ar-H), 7.49-7.58 (m, 4H, 2 x Ar-H). ^{13}C NMR (400MHz, $CDCl_3$) δ ppm: 163.99 (C=O, thiazolidine), 147.91 (C, triazine), 140.81 (C, triazine), 135.37 (C=O, thiazolidine), 129.48 (2 x -NH-C, Ar), 126.17(2 x C, Ar), 119.80 (4 x C, Ar), 116.47 (4 x C, Ar), 109.67 (2 x C, Ar), 66.33 (-CH_2-, thiazolidine); Mass Spectra (TOF MS, m/z): Calculated 378.09; Observed 139.1 (100%), 85.0 (35.86%), 379.3 (20.03%, M+1), 93.1 (17.69%).

3-(4,6-bis(2-aminophenylamino)-1,3,5-triazin-2-yl)thiazolidine-2,5-dione, **7c**

Dark brown powder; M.P.: > 300 °C; FT-IR (ν_{max}; cm^{-1}, in KBr): 3230.15 (-NH- Stretching), 1611.78 (-C=O Stretching), 1566.21-1434.62 (-NH_2 bending), 1348.65-1249.96 (C-N Stretching),

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684.20 (C-S Stretching); ^{1}H NMR (300MHz, $CDCl_3$-d_6, TMS) δ ppm: 3.36 (s, 4H, 4 x –NH_2), 3.56 (s, 2H, -CH_2- thiazolidine), 6.79-6.81 (m, 4H, 2 x Ar-H), 7.01-7.07 (m, 4H, 2 x Ar-H); ^{13}C NMR (400MHz, $CDCl_3$) δ ppm: 163.65 (C=O, thiazolidine), 162.76 (C, triazine), 133.42 (2xC, triazine), 126.67 (C=O, thiazolidine), 126.03 (2 x -NH-C, Ar), 124.43 (2 x NH_2-C, Ar),117.29 (4 x C, Ar), 112.15 (4 x C, Ar), 78.50 (2 x C, Ar), 65.38 (-CH_2-, thiazolidine); Mass Spectra (TOF MS, m/z): Calculated 408.11; Observed 133.1 (100%), 189.2 (69.81%), 409.20 (09.17%, M+1), 134.1 (09.08%).

3-(4,6-bis(2-nitrophenylamino)-1,3,5-triazin-2-yl)thiazolidine-2,5-dione, **7d**

Yellow powder; M.P.: 121-123 °C; FT-IR (ν_{max}; cm^{-1}, in KBr): 3271.48 (-NH- Stretching), 1616.55 (-C=O Stretching), 1566.12-1422.45(-NH_2 bending), 1398.80(N=O Stretching, Ar-NO_2), 1386.64-1115.86(C-N Stretching), 819.61(C-N Stretching, Ar-NO_2), 617.06 (C-S Stretching); ^{1}H NMR (300MHz, $CDCl_3$-d_6, TMS) δ ppm: 3.58 (s, 2H, 2 x -NH-Ar), 3.80 (s, 2H, -CH_2- thiazolidine), 7.36-7.38 (d, 2H, J=6Hz, 2 x Ar-H), 7.40-7.43 (t, 2H, J=9 Hz, 2 x Ar-H), 7.47-7.49 (t, 2H, J=6Hz, 2 x Ar-H), 7.59-7.61 (d, 2H, J=6 Hz, 2 x Ar-H); ^{13}C NMR (400MHz, $CDCl_3$) δ ppm: 163.75 (C=O, thiazolidine), 162.16 (C, triazine), 131.00 (C, triazine), 122.67 (C=O, thiazolidine), 120.03 (2 x NO_2-C, Ar), 113.15(2 x -NH-C, Ar), 109.24(2 x C, Ar), 107.29 (2 x C, Ar), 84.15 (2 x C, Ar), 78.50 (2 x C, Ar), 66.33 (-CH_2-, thiazolidine); Mass Spectra (TOF MS, m/z): Calculated 468.06; Observed 454.1 (100%), 469.10 (66.89%, M+1), 510.1 (64.90%), 113.0 (24.88%).

3-(4,6-bis(3-nitrophenylamino)-1,3,5-triazin-2-yl)thiazolidine-2,5-dione, **7e**

Yellow powder; M.P.: > 300 °C; FT-IR (ν_{max}; cm^{-1}, in KBr): 3198.08 (-NH- Stretching), 1646.10 (-C=O Stretching), 1558.62-1456.79(-NH_2 bending), 1329.40(N=O Stretching, Ar-NO_2), 1287.17-1195.20(C-N Stretching), 782.96 (C-N Stretching, Ar-NO_2), 615.40 (C-S Stretching); ^{1}H NMR (300MHz, $CDCl_3$-d_6, TMS) δ ppm: 3.34 (s, 2H, 2 x -NH-Ar), 3.36 (s, 2H, -CH_2- thiazolidine), 6.82-6.83 (d, 2H, J=3Hz, 2 x Ar-H), 7.23-7.25 (t, 2H, J=6 Hz, 2 x Ar-H), 7.39 (s, 2H, 2 x Ar-H), 8.25-8.27 (d, 2H, J=6 Hz, 2 x Ar-H); ^{13}C NMR (400MHz, $CDCl_3$) δ ppm: 142.43 (C=O, thiazolidine), 134.35 (C, triazine), 131.52 (C, triazine), 123.25 (C=O, thiazolidine), 119.00 (2 x NO_2-C, Ar), 112.25 (2 x -NH-C, Ar), 110.14 (2 x C, Ar), 108.25 (2 x C, Ar), 82.12 (2 x C, Ar), 78.40 (2 x C, Ar), 42.36 (-CH_2-, thiazolidine); Mass Spectra (TOF MS, m/z): Calculated 468.06; Observed 175.0 (100%), 469.10 (40.85%, M+1), 259.0 (15.55%), 113.0 (14.55%).

3-(4,6-bis(4-nitrophenylamino)-1,3,5-triazin-2-yl)thiazolidine-2,5-dione, **7f**

Yellow powder; M.P.: 221-223 °C; FT-IR (ν_{max}; cm^{-1}, in KBr): 3341.72 (-NH- Stretching), 1622.48 (-C=O Stretching), 1592.65-1426.44(-NH_2 bending), 1343.29(N=O Stretching, Ar-NO_2), 1281.37-1008.75(C-N Stretching), 870.58 (C-N Stretching, Ar-NO_2), 729.41 (C-S Stretching); ^{1}H NMR

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(300MHz, $CDCl_3$-d_6, TMS) δ ppm: 3.33 (s, 2H, 2 x -NH-Ar), 3.36 (s, 2H, -CH_2- thiazolidine), 6.57-6.61 (t, 4H, J= 12 Hz, 2 x Ar-H), 6.99-7.02(d, 4H, J=12 Hz, 2 x Ar-H); ^{13}C NMR (400MHz, $CDCl_3$) δ ppm: 146.13 (C=O, thiazolidine), 135.35 (C, triazine), 130.32 (C, triazine), 125.24 (C=O, thiazolidine), 119.04 (2 x -NH-C, Ar), 115.23 (2 x NO_2-C, Ar), 79.12 (2 x C, Ar), 78.46 (2 x C, Ar), 40.16 (-CH_2-, thiazolidine); Mass Spectra (TOF MS, m/z): Calculated 468.06; Observed 121.1 (100%), 469.10 (91.11%, M+1), 85.0 (45.54%), 91.1 (29.93%).

3-(4,6-bis(2-chlorophenylamino)-1,3,5-triazin-2-yl)thiazolidine-2,5-dione, **7g**

White Powder; M.P.: 286-288 °C; FT-IR (ν_{max}; cm^{-1}, in KBr): 328.34 (-NH- Stretching), 1622.12 (-C=O Stretching), 1586.58-1416.81(-NH_2 bending), 1298.13-1180.52(C-N Stretching), 1007.74(C-Cl Stretching), 685.97 (C-S Stretching); ^{1}H NMR (300MHz, $CDCl_3$-d_6, TMS) δ ppm: 3.39 (s, 2H, 2 x -NH-Ar), 3.90(s, 2H, -CH_2- thiazolidine), 6.58-6.62 (m, 2H, 2 x Ar-H), 6.99-7.02 (d, 2H, J=12 Hz, 2 x Ar-H), 7.33-7.41 (m, 2H, 2 x Ar-H), 7.91-7.95 (m, 2H, 2 x Ar-H); ^{13}C NMR (400MHz, $CDCl_3$) δ ppm: 164.95 (C=O, thiazolidine), 163.73 (C, triazine), 155.68 (C, triazine), 146.13 (C=O, thiazolidine), 135.65 (2 x -NH-C, Ar), 130.20 (2 x C, Ar), 126.35 (2 x Cl-C, Ar), 125.32 (2 x C, Ar), 124.67 (2 x C, Ar),119.33 (2 x C, Ar), 112.32 (-CH_2-, thiazolidine); Mass Spectra (TOF MS, m/z): Calculated 446.01; Observed 386.2 (100%), 368.2 (90.99%), 447.20 (32.45%, M+1), 258.2 (25.36%).

3-(4,6-bis(3-chlorophenylamino)-1,3,5-triazin-2-yl)thiazolidine-2,5-dione, **7h**

White crystal; M.P.: 245-247 °C; FT-IR (ν_{max}; cm^{-1}, in KBr): 3374.84 (-NH- Stretching), 1613.45 (-C=O Stretching), 1569.83-1486.44 (-NH_2 bending), 1423.52-1178.53 (C-N Stretching), 1090.62 (C-Cl Stretching), 795.46 (C-S Stretching); ^{1}H NMR (300MHz, $CDCl_3$-d_6, TMS) δ ppm: 3.88 (s, 2H, -CH_2- thiazolidine), 3.77(s, 2H, 2 x -NH-Ar), 7.19 (s, 2H, 2 x Ar-H), 7.21-7.22(d, 2H, J=3 Hz, 2 x Ar-H), 7.24-7.25(d, 2H, J=3 Hz, 2 x Ar-H), 7.27-7.29(d, 2H, J=6 Hz, 2 x Ar-H); ^{13}C NMR (400MHz, $CDCl_3$) δ ppm: 163.73 (C=O, thiazolidine), 162.16 (C, triazine), 128.07 (C, triazine), 127.90 (C=O, thiazolidine), 122.16 (2 x -NH-C, Ar), 78.70 (2 x C, Ar), 78.57 (2 x C, Ar), 78.37(2 x C, Ar),78.04(2 x C, Ar), 40.21-39.16 (2 x Cl-C, Ar), 38.85 (-CH_2-, thiazolidine); Mass Spectra (TOF MS, m/z): Calculated 446.01; Observed 347.2 (100%), 349.2(63.92%), 447.10 (40.67%, M+1), 368.1(37.74%).

3-(4,6-bis(4-chlorophenylamino)-1,3,5-triazin-2-yl)thiazolidine-2,5-dione, **7i**

Light pink powder; M.P.: 281-283 °C; FT-IR (ν_{max}; cm^{-1}, in KBr): 3399.59 (-NH- Stretching), 1620.96(-C=O Stretching), 1580.88-1495.08(-NH_2 bending), 1431.00-1155.52(C-N Stretching), 986.80(C-Cl Stretching), 792.14 (C-S Stretching); ^{1}H NMR (300MHz, $CDCl_3$-d_6, TMS) δ ppm:

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3.83 (s, 2H, 2 x -NH-Ar), 3.77 (s, 2H, -CH_2- thiazolidine), 6.99 (m, 4H, 2 x Ar-H), 7.60-7.75(d, 4H, J=4.5 Hz, 2 x Ar-H). ^{13}C NMR (400MHz, $CDCl_3$) δ ppm: 163.83 (C=O, thiazolidine), 162.23 (C, triazine), 135.58 (C, triazine), 134.54(C=O, thiazolidine), 122.81-121.98 (2 x -NH-C, Ar), 115.23-114.63 (4 x C, Ar), 79.16-78.50 (2 x Cl-C, Ar), 40.12-38.87 (4 x C, Ar), 35.95 (-CH_2-, thiazolidine); Mass Spectra (TOF MS, m/z): Calculated 446.01; Observed 316.2 (100%), 390.2 (51.65%), 315.2 (40.57%), 447.10 (30.09%, M+1), 372.2 (26.03%).

3-(4,6-bis(4-fluorophenylamino)-1,3,5-triazin-2-yl)thiazolidine-2,5-dione, **7j**

White powder; M.P.: 123-125 °C; FT-IR (ν_{max}; cm^{-1}, in KBr): 3297.56 (-NH- Stretching), 1619.16-1600.60 (-C=O Stretching), 1568.73-1480.86 (-NH_2 bending), 1426.75-1169.27 (C-N Stretching), 988.78 (C-F Stretching), 714.56 (C-S Stretching); ^{1}H NMR (300MHz, $CDCl_3$-d_6, TMS) δ ppm: 3.35 (s, 2H, 2 x -NH-Ar), 3.58 (s, 2H, -CH_2- thiazolidine), 7.08-7.11(d, 4H, J=9 Hz, 2 x Ar-H), 7.31-7.35(d, 4H, J=12 Hz, 2 x Ar-H); ^{13}C NMR (400MHz, $CDCl_3$) δ ppm: 163.84 (C=O, thiazolidine), 139.69 (C, triazine), 133.15(C=O, thiazolidine), 130.01 (C, triazine), 123.07 (2 x F-C, Ar), 120.29-119.06 (2 x -NH-C, Ar), 79.16-78.50 (4 x C, Ar), 63.33 (4 x C, Ar), 40.15(-CH_2-, thiazolidine); Mass Spectra (TOF MS, m/z): Calculated 414.07; Observed 85.0 (100%), 366.1 (88.11%), 415.1 (74.86%, M+1), 475.5 (54.79%).

3-(4,6-bis(4-bromophenylamino)-1,3,5-triazin-2-yl)thiazolidine-2,5-dione, **7k**

Off-white crystals; M.P.: 167-169 °C; FT-IR (ν_{max}; cm^{-1}, in KBr): 3388.40 (-NH- Stretching), 1586.84 (-C=O Stretching), 1556.09-1434.25 (-NH_2 bending), 1372.76-1129.67 (C-N Stretching), 794.78 (C-Br Stretching), 729.98 (C-S Stretching);); ^{1}H NMR (300MHz, $CDCl_3$-d_6, TMS) δ ppm: 3.45 (s, 2H, 2 x -NH-Ar), 3.68 (s, 2H, -CH_2- thiazolidine), 7.42-7.44(d, 4H, J=6 Hz, 2 x Ar-H), 7.73-7.75(d, 4H, J=6 Hz, 2 x Ar-H); ^{13}C NMR (400MHz, $CDCl_3$) δ ppm: 181.18 (C=O, thiazolidine), 170.31(C, triazine), 162.96(C=O, thiazolidine), 135.17(C, triazine), 129.13-129.00(2 x -NH-C, Ar), 127.39-126.73(4 x C, Ar), 79.21-78.55 (4 x C, Ar), 40.13-38.88 (2 x Br-C, Ar), 36.90(-CH_2-, thiazolidine); Mass Spectra (TOF MS, m/z): Calculated 535.91; Observed 347.2 (100%), 348.2 (74.59%), 537.10 (70.91%, M+1), 349.2 (70.83%)

Biological Activity

In vitro DPP-4 Inhibitory activity

The compounds synthesized were evaluated for in vitro DPP-4 enzyme inhibition. The enzyme assay was performed using DPP-4 drug discovery kit (BML-AK 499; Enzo Life Sciences, Plymouth Meeting, PA, USA). The activity of test compounds was assayed using human recombinant DPP-4 enzyme, chromogenic substrate (H-Gly-Pro-AMC, Km 114 μM), DPP-4 inhibitor (P32/98), assay

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buffer and calibration standard as provided in the kit. The assay was performed using 96-well flat-bottomed microtitre plate followed by addition of assay buffer, DPP-4 enzyme and chromogenic substrate (HGly-Pro-pNA). The assay principle and procedure was followed as per manufacturer's guidelines. Solutions of the test compounds were made in dimethyl sulphoxide (DMSO) at different concentrations of 25, 50, 100, 200 µg/mL, and 20 µL were added to each well after further dilutions. The plate was incubated at 37 °C for 10 min to allow enzyme-inhibitor reaction and read continuously at A-405 nm using Bio-Rad Elisa Plate Reader. IC_{50} values were determined using non-linear regression analysis.

Antibacterial Activity

All synthesized compounds were screened for their minimum inhibitory concentration (MIC, µg/mL) against selected Gram-positive organisms viz. *Bacillus subtilis* (NCIM-2063), *Bacillus cereus* (NCIM-2156), *Staphylococcus aureus* (NCIM-2079) and Gram-negative organism viz. *Escherichia coli* (NCIM-2065), *Proteus vulgaris* (NCIM-2027) and *Pseudomonas aeruginosa* (NCIM-2036), by the broth dilution method as recommended by the National Committee for Clinical Laboratory Standards with minor modifications.[19] Cefixime was used as a standard antibacterial agent. Solutions of the test compounds and reference drug were prepared in dimethyl sulfoxide (DMSO) at concentrations of 125, 62.5, 31.25, 15.62, 7.81, 3.91, 1.95, 0.97 µg/mL. Ten tubes were made in parallel with the second set being used as MIC reference controls (16–24 h visual). After sample preparation, the controls were placed in a 37 °C incubator and read for macroscopic growth (clear or turbid) the next day. Into each tube, 0.8 mL of nutrient broth was pipette (tubes 2–7), tube 1 (negative control) received 1.0 mL of nutrient broth and tube 10 (positive control) received 0.9 mL of nutrient. Tube 1, the negative control, did not hold bacteria or antibiotic. The positive control, tube 10, received 0.9 mL of nutrient broth since it contained bacteria but not an antibiotic. The test compound was dissolved in DMSO (125 µg/mL), 0.1 mL of increasing concentration of the prepared test compounds which are serially diluted from tube 2 to tube 9 (tube 2–9 containing 125, 62.5, 31.25, 15.62, 7.81, 3.91, 1.95, 0.97 µg/mL, respectively). After this process, each tube was inoculated with 0.1 mL of the bacterial suspension whose concentration corresponded to 0.5 McFarland scale (9×10^8 cells/mL) and each bacterium was incubated at 37 °C for 24 h at 150 rpm. The final volume in each tube was 1.0 mL. The incubation chamber was kept humid. At the end of the incubation period, MIC values were recorded as the lowest concentration of the substance that gave no visible turbidity, i.e. no growth of inoculated bacteria.

Docking Study

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The computational studies were carried out with the X-ray crystal structure of DPP-4 (PDB Id: 2FJP, Chain A) in complex with (2S,3S)-3-Amino-4-(3,3-difluoropyrrolidin-1-yl)-N,N-dimethyl-4-oxo-2-(4-[1,2,4]triazolo[1,5-a]-pyridin-6-ylphenyl)butanamide: a selective alpha-amino amide dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. Docking of the ligand 7a was carried out in the binding pocket of DPP-4 with CDOCKER protocol of DS 2.5 following the protocol given elsewhere.[20] CDOCKER is a grid-based MD-simulated, annealing-based algorithm that uses CHARMm. The receptor (protein) was held rigid, while the ligands were flexible during the refinement.[21,22] The basic strategy involves the generation of several initial ligand orientations in the allosteric site of the target protein followed by MD-based simulated annealing, and final refinement by minimization

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Figures and schemes legends

Figure 1: Classical role of Dipeptidyl peptidase-4 (DPP - 4) and implication of its inhibition by DPP-4 inhibitors.

Scheme 1: Synthesis of novel 1,3,5-triazine-thiazolidine-2,4-diones: Reagents and condition: a) Conc. H_2SO_4, water, reflux; b) NaOH, stirring, 40-45 °C; c) K_2CO_3, reflux, 120-135 °C.

Figure 2: Structure-activity relationships of target analogues as DPP-4 Inhibitors.

Figure 3: The docked complex of compound 7a in the active of in the binding pocket of dipeptidyl peptidase (DPP) 4.

Figure 4: The docked complex of compound 7k in the active of in the binding pocket of dipeptidyl peptidase (DPP) 4.

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TABLES

Table 1: DPP-4 inhibitory effect of novel 1,3,5-triazine-thiazolidine-2,4-diones derivatives

Compound	IC_{50} (in μM)[a]
7a	6.35
7b	12.11
7c	10.76
7d	14.64
7e	15.38
7f	17.85
7g	27.32
7h	23.25
7i	29.53
7j	38.37
7k	49.21
P 32 /98 (Standard)	2.5

[a]IC_{50} represents inhibitory concentration determined by non-linear regression analysis using GRAPHPAD PRISM software. Values are expressed as mean of three independent experiments.

Table 2: Docking interaction and scoring of 7a (most active) and 7k (least active).

Compound	CDOCKER INTERACTION ENERGY	CDOCKER ENERGY	H-bonding residues	Pi-cation interaction
7a	31.90	44.31	Glu205, Tyr547	Arg125
7k	44.27	43.51	Arg358, Arg669, Glu205	Arg125

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Table 3 Antibacterial activity of compound 7(a-k).

Compound	Minimum Inhibitory Concentration (MIC, in µg/mL)					
	P. vulgaris	E.coli	P. aeruginosa	B. subtilis	S. aureus	B. cerus
7a	31.25	62.5	62.5	125	62.5	62.5
7b	15.62	15.62	15.62	31.25	62.5	62.5
7c	125	125	3.91	15.62	3.91	125
7d	32.25	15.62	15.62	7.81	7.81	15.62
7e	15.62	15.62	3.91	3.91	31.25	3.91
7f	62.5	62.5	15.62	125	15.62	7.81
7g	7.81	62.5	125	7.81	125	31.25
7h	3.91	62.5	15.62	62.5	7.81	62.5
7i	15.62	62.5	31.25	31.25	31.25	125
7j	125	125	62.5	31.25	31.25	7.81
7k	62.5	31.25	31.25	62.5	31.25	31.25
Cefixime (Standard)	7.81	15.62	3.91	31.25	7.81	31.25

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FIGURES

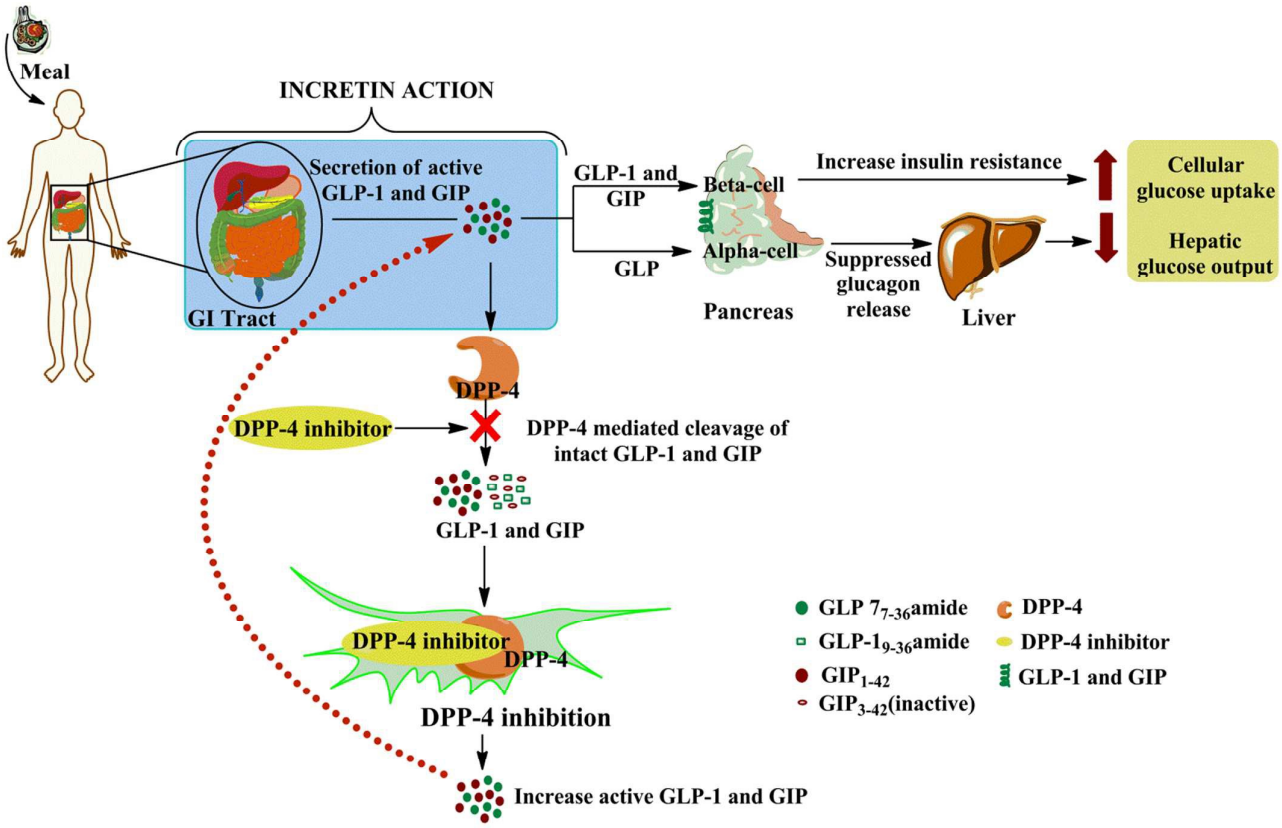

Figure 1: Classical role of Dipeptidyl peptidase-4 (DPP - 4) and implication of its inhibition by DPP-4 inhibitors.

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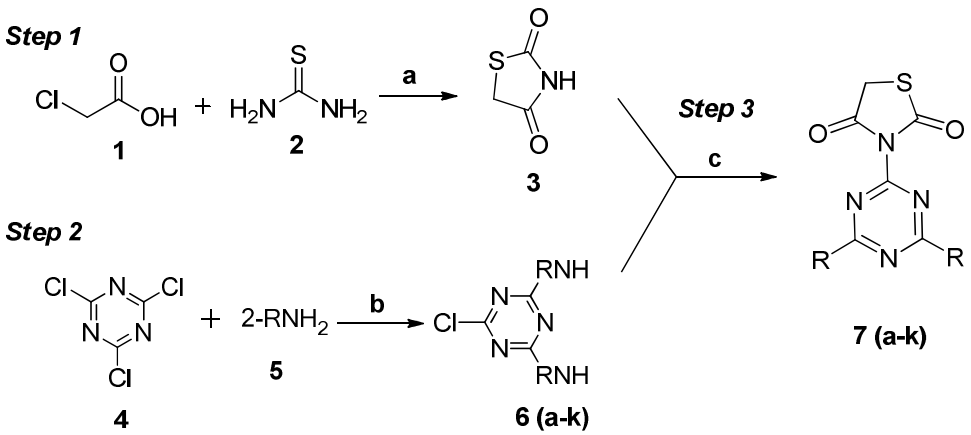

*Where **R**=*

7a = $NHNH_2$
7b = NH C_6H_5
7c = NH C_6H_5 2-NH_2
7d = NHC_6H_5 2-NO_2
7e = NHC_6H_5 3-NO_2
7f = NHC_6H5 4-NO_2
7g = NHC_6H_5 2-Cl
7h = NHC_6H_5 3-Cl
7i = NHC_6H5 4-Cl
7j = NHC_6H_5 4-F
7k = NHC_6H_5 4-Br

Scheme 1: Synthesis of novel 1,3,5-triazine-thiazolidine-2,4-diones: Reagents and condition: a) Conc. H_2SO_4, water, reflux; b) NaOH, stirring, 40-45 °C; c) K_2CO_3, reflux, 120-135 °C.

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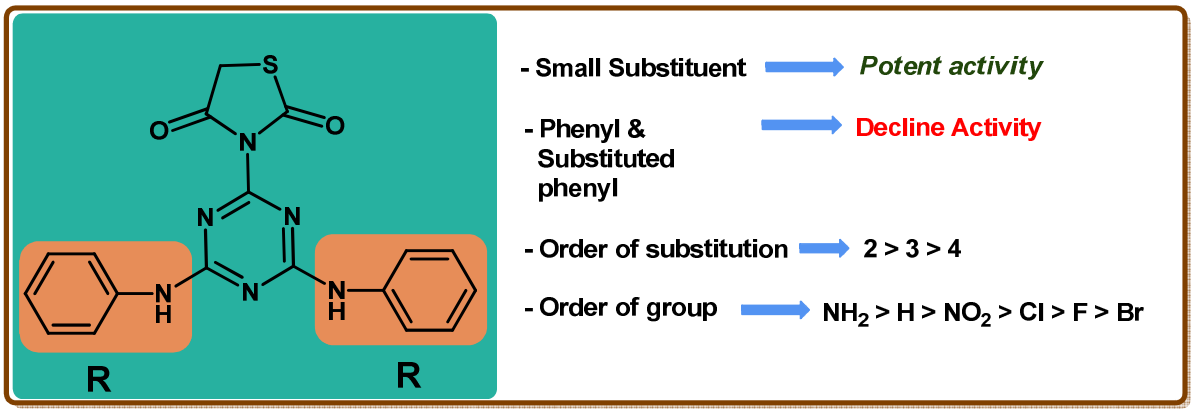

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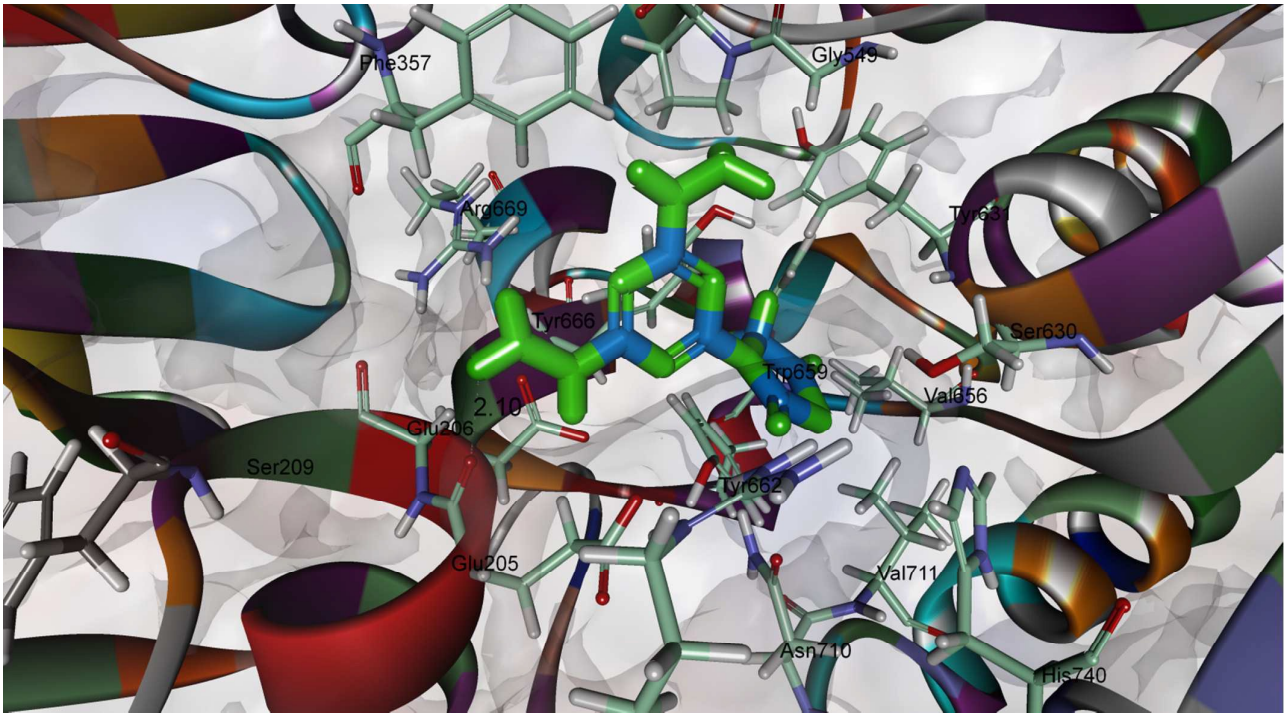

Figure 3: The docked complex of compound 7a in the active of in the binding pocket of dipeptidyl peptidase (DPP) 4.

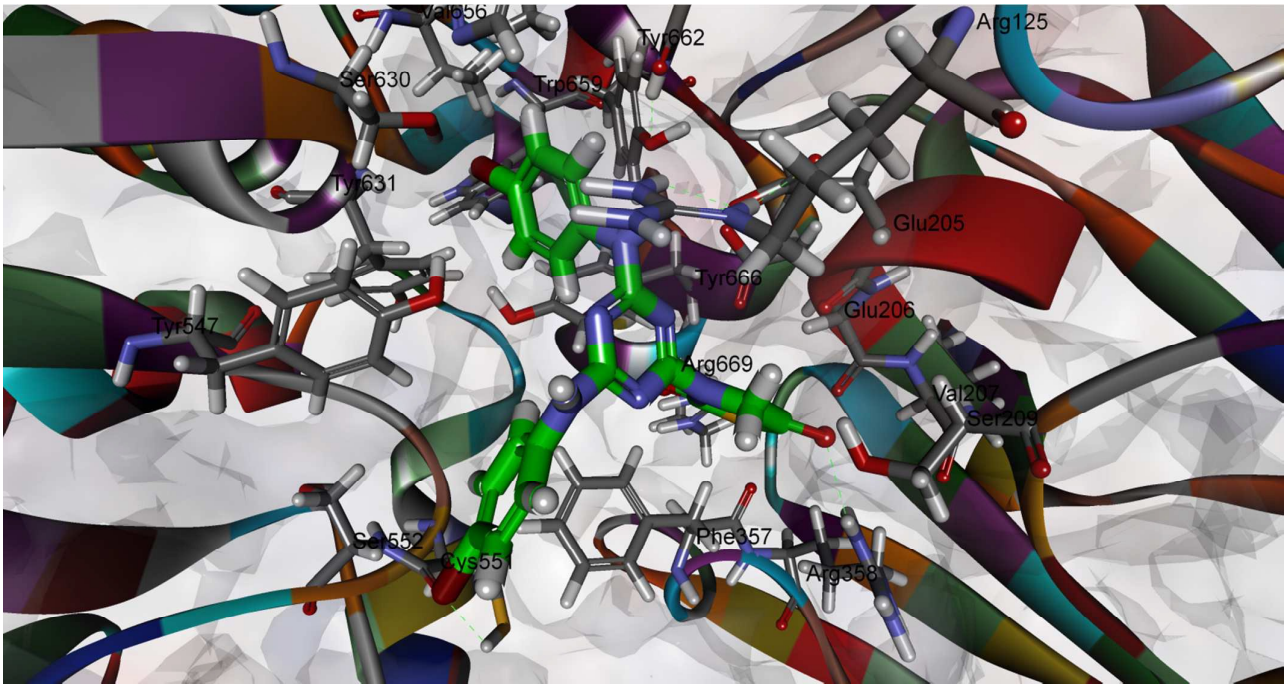

Figure 4: The docked complex of compound 7k in the active of in the binding pocket of dipeptidyl peptidase (DPP) 4.