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Design, synthesis and biological evaluation of glycolamide, glycinamide, and β-amino carbonyl 1,2,4-triazole derivatives as DPP-4 inhibitors

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ABSTRACT

Through modification of the skeleton of Sitagliptin and Vildagliptin, we successfully synthesized and built-up four series of 1,2,4-triazole derivatives, containing *N,O*-disubstituted glycolamide, *N,N′*-disubstituted glycinamide, β-amino ester, and β-amino amide as linkers, for the development of new dipeptidyl peptidase 4 (DPP-4) inhibitors. The synthetic strategy for glycolamides or glycinamides involved convenient two-steps reaction: functionalized transformation of 2-chloro-*N*-(2,4,5-triflurophenyl)acetamide **9** (hydroxylation or amination) and esterification or amidation of 1,2,4-triazole-3-carboxylic acid. On the other hand, the one-pot synthesis procedure, including substitution and deprotection, was developed for the preparation of β-amino carbonyl 1,2,4-triazoles from (1*H*-1,2,4-triazol-3-yl)methanol **12** or (1*H*-1,2,4-triazol-3-yl)methanamine **13** and Boc-(*R*)-3-amino-4-(2,4,5-trifluoro-phenyl)-butyric acid **14**. All of glycolamides, glycinamides, and β-amino carbonyl 1,2,4-triazoles were also evaluated against DPP-4 inhibitory activity. Based on the SAR study of DPP-4 inhibitory capacity, β-amino ester **5n** and β-amino amide 1,2,4-triazoles **6d** and **6p** possessed the significant inhibition of DPP-4 (IC_{50} < 51.0 nM), particularly for compound **6d** (IC_{50} = 34.4 nM). The selectivity evaluation indicated compound **5n** and **6p** had excellent selectivity over QPP, DPP-8, and DPP-9. In addition, the docking results revealed compounds **5n** and **6p** provided stronger π–π stacking interaction with residue Phe357 than 1,5-disubstituted 1,2,4-triazole **6d** and Sitagliptin **1**. In summary, compounds **5n** and **6p** could be promising lead compounds for further development of DPP-4 inhibitor.

1. Introduction

Dipeptidyl peptidase IV (DPP-4) inhibitors are a new type of oral hypoglycemic drugs that inhibit the enzyme DPP-4 for treating patients with type 2 diabetes [1–4]. DPP-4 is an abundant enzyme existing in most cell types. It can deactivate glucagon-like peptide 1 (GLP-1) by cleaving peptide bonds [5]. The GLP-1 has multiple physiological functions, including insulin stimulation, glucagon inhibition, and delayed gastric emptying [6]. Inhibition of DPP-4 can elevate the concentration of active GLP-1 to increase insulin secretion and reduction in HbAlc without weight gain and have a low risk of hypoglycemia [7–11]. Hence, DPP-4 inhibitors have been gained attention due to the well-tolerated and minimal side effects.

Sitagliptin **1** is the first marketed selective inhibitor of DPP-4 [12], followed by Vildagliptin, Saxagliptin, Linagliptin, and Alogliptin [13,14]. The chemical structure of Sitagliptin **1** is divided into three parts: 2,4,5-triflorophenyl fragment pharmacophore, enantiomerically pure β-amino carbonyl linker, and the 5,6,7,8-tetrahydro-[1,2,4]triazolo[4,3-*a*]pyridine fragment (Fig. 1) [15]. Although numerous literatures on the synthesis of Sitagliptin have appeared [16–18], the enantioselective enamine reduction via efficient catalytic route was widely utilized to generate the pure β-amino carbonyl linker in Sitagliptin scaffold [19].

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However, this method is an expensive and non-green procedure due to using the metal reagent-based catalysts. Herein, we revaluated that the β-amino carbonyl linker necessitated a simplified modification.

Sitagliptin **1** is structurally a non-substrate-like DPP-4 inhibitor, which is non-peptide mimetic compound that occupies the hydrophobic pocket of the enzyme binding site via the trifluorophenyl moiety. On the other hand, Vildagliptin **2** is a substrate-like DPP-4 inhibitor with a cyanopyrrolidine ring that mimic DPP-4 substrates to occupy the same hydrophobic pocket of the enzyme binding site [20]. Despite the diverse chemical structures of DPP-4 inhibitors, the β-amino carbonyl linker of Sitagliptin **1** and glycinamide linker of Vildagliptin **2** have common interactions with key residues that play an important role in the inhibitory activity through hydrogen bonds [20,21]. Based on the different structures but similar binding sites results of Sitagliptin **1** and Vildagliptin **2**, we assumed that the linker structure and length of DPP-4 inhibitors might be a key factor in the binding mode. Moreover, functional glycinamide linkers also play a crucial role in the investigation of biological processes in modern pharmacology, biochemistry, and medicinal chemistry [22,23]. Thus, we designed four series of compounds **3**–**6** with different linkers, including glycolamide, glycinamide, β-amino amide, and β-amino ester groups in a design of functional 1,2,4-triazole modules in which 2,4,5-triflorophenyl fragment was maintained into the architecture for modulating the heterocyclic moiety to structural variation (Fig. 2). Based on the considerable attracted academic interest, these designed 1,2,4-triazole compounds with various linkers were evaluated DPP-4 inhibitory activity compared with Sitagliptin **1** [24]. The selectivity of DPP-4 over QPP, DPP-8, and DPP-9 for each compound were also determined by the IC_{50} values in a plaque assay to discovery novel DPP-4 inhibitors with increasing safety and durability for the treatment of T2DM.

2. Result and discussion

2.1. Design of N,O-disubstituted glycolamides 3, N,N-disubstituted glycinamides 4, and β-amino carbonyl 1,2,4-triazoles 5 and 6

To investigate the binding structural mode of Sitagliptin **1** and Vildagliptin **2** to DPP-4, the advanced docking program iGEMDOCK v2.1 was used to simulate the binding domain between inhibitors and DPP-4 enzyme [25]. The design approach was followed the rational Sitagliptin **1** and Vildagliptin **2** and carried out by structural optimization to pursue high in vitro activity, high selectivity and low cytotoxicity. To earn these benefits, we calculated the length of the linker and the binding mode of the kinase domain of DPP-4 to generate the new designed structures of *N,O*-disubstituted glycolamides **3**, *N,N*-disubstituted glycinamides **4**, and β-amino carbonyl 1,2,4-triazoles **5** and **6** grafting 2,4,5-trifloro-phenyl fragment (Fig. 2). The linker length is ranged between 4.6 Å and 8.5 Å from the standard drugs Sitagliptin **1** and Vildagliptin **2**.

Glycolamides **3** and glycinamides **4** were divided into two fragments, *N*-(2,4,5-trifluorophenyl)-2-hydroxyacetamide or *N*-(2,4,5-trifluorophenyl)-2-aminoacetamide and 1,2,4-triazole heterocyclic moiety **7** (Scheme 1 and Fig. 2). At first, both 1,3-disubstituted 1,2,4-triazoles **7a**–**g** [26a] and 1,3,5-trisubstituted 1,2,4-triazoles **7h**–**q** [26b] were synthesized by following our previous published literature procedure [26]. Their preliminary DPP-4 inhibition data were presented in Table 1. Based on the inhibitory activities against DPP-4, 1,3-disubstituted 1,2,4-triazoles **7a**–**e** and 1,3,5-trisubstituted 1,2,4-triazoles **7l**–**q** were found possessed better inhibition rates (DPP-4 Inh@1mM ≧35%). Therefore, compounds **7a**–**e** and **7l**–**q** were further selected as promising targets for synthesis of *N,O*-disubstituted glycolamides **3**, *N,N*-disubstituted glycinamides **4**, and β-amino carbonyl 1,2,4-triazoles **5** and **6**.

2.2. Chemistry

2.2.1. Synthesis of N,O-disubstituted glycolamides 3 and N,N-disubstituted glycinamides 4

Because the triazolopiperazine moiety of Sitagliptin **1** was recognized as an important pharmacophore that contributed to its good pharmacokinetic profile, potency, and selectivity [12,27], a new series of heterocycle moieties were investigated. Preliminarily, we focused on the evaluation of the following variations on 1,2,4-triazole structure. As outlined retrosynthetically in Scheme 1, both product targets including 1,2,4-triazole moiety *N,O*-disubstituted glycolamides **3** and *N,N*-disubstituted glycinamides **4** could be accessed from key intermediates *N*-(2,4,5-trifluorophenyl)-2-hydroxyacetamide **10** or *N*-(2,4,5-trifluorophenyl)-2-aminoacetamide **11** treated with 1*H*-1,2,4-triazole-3-carboxylic acids **8** [28], which were hydrolyzed from methyl 1*H*-1,2,4-triazole-3-carboxylates **7** [26]. Compounds **10** and **11** were obtained from their α-chloro *N*-arylacetamide precursor **9** through hydrolysis with cesium formate [29] or amination with aqueous ammonium hydroxide [30]. α-Chloro *N*-arylacetamide **9** was prepared from 2,4,5-trifluorobenzenamine reacted towards 2-chloroacetyl chloride via our previous published procedure [31].

With an effective synthesis of intermediates 2-hydroxyacetamide **10** and 2-aminoacetamide **11**, we examined the two-steps synthesies involving amidation and hydrolysis or amination from 2,4,5-trifluorobenzenamine (Scheme 2). At the outset, the reaction of 2,4,5-trifluorobenzenamine and 2-chloroacetyl chloride proceeded successfully in refluxing toluene for 3 h to give α-chloro *N*-arylacetamide **9** in 92% high yield [31]. Subsequently, the hydrolysis of *N*-arylacetamide **9** which was performed at reflux for 6 h on treatment with 3.0 equivalents of cesium formate (HCO_2Cs) [29] in EtOH solution to afford 2-hydroxyacetamide **10** efficiently in 86% yield. We then evaluated the scope of the amination using *N*-arylacetamide **9** at 60 °C for 3 h under the EtOH/aqueous ammonium hydroxide condition [30]. The desired 2-aminoacetamide product **11** was obtained in 88% yield (Scheme 2). Subsequently, a series of methyl 1*H*-1,2,4-triazole-3-carboxylates **7** were prepared by our reported literature procedures [26] and hydrolyzed in presence of DBU/MeOH at room temperature for 6 h to afford the corresponding 1*H*-1,2,4-triazole-3-carboxylic acids **8** in > 90% high yields [28]. Crude compounds **8** were extracted with CH_2Cl_2 and washed with 10% $HCl_{(aq)}$ without purification technique for further investigations.

To evaluate the activated forms of carboxylic acid in optimization study [32,33], compound **8a** (1.0 equiv) and 2-hydroxyacetamide **10** (1.1 equiv) was reacted in the presence of activating reagents (2.0 equiv), such as acid halides (Oxalyl chloride $(COCl)_2$ and thionyl chloride $(SOCl_2)$) [34], activated amides (1,1′-carbonyldiimidazole, CDI) [35], activeesters (carbodiimides: *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC) [36], and dicyclohexylcarbodiimide (DCC)) [37], and re

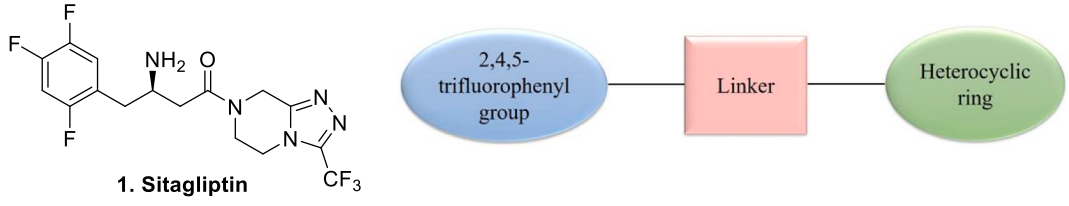


Fig. 1. The structure of Sitagliptin **1**.

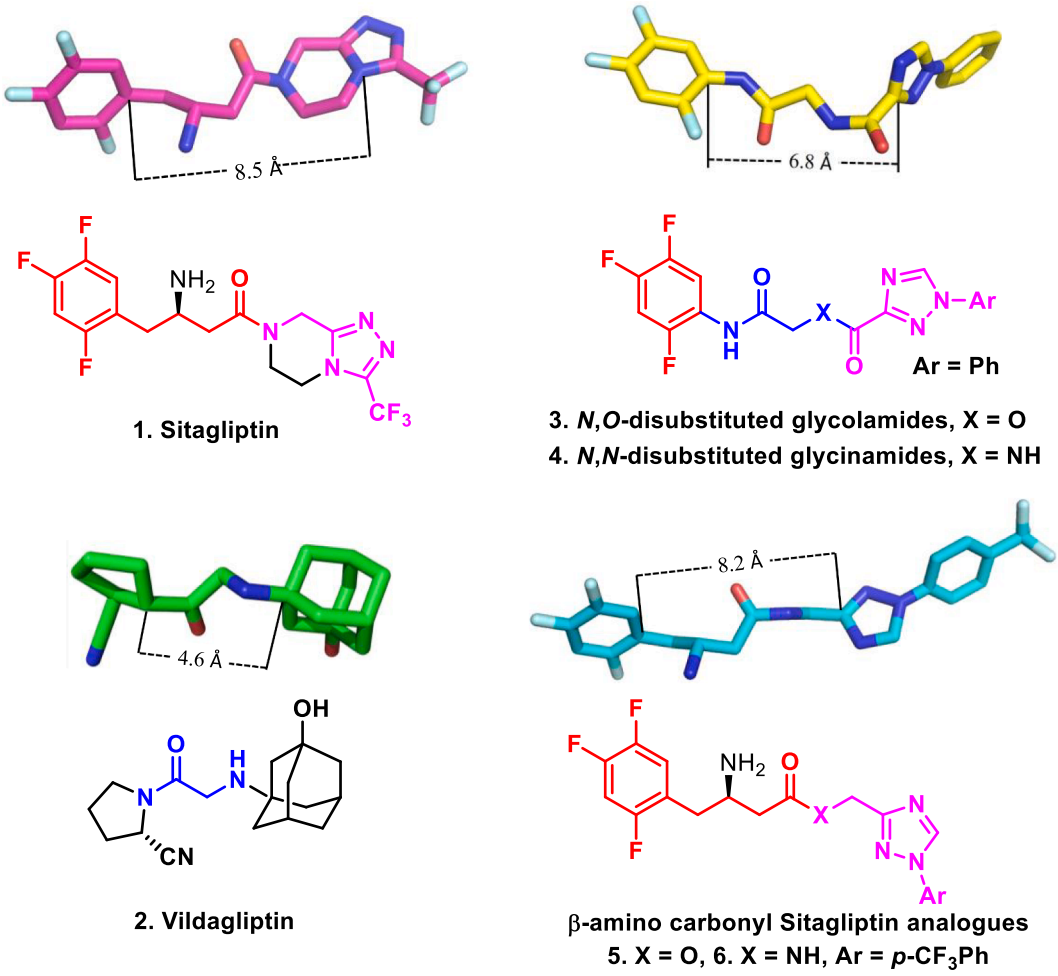
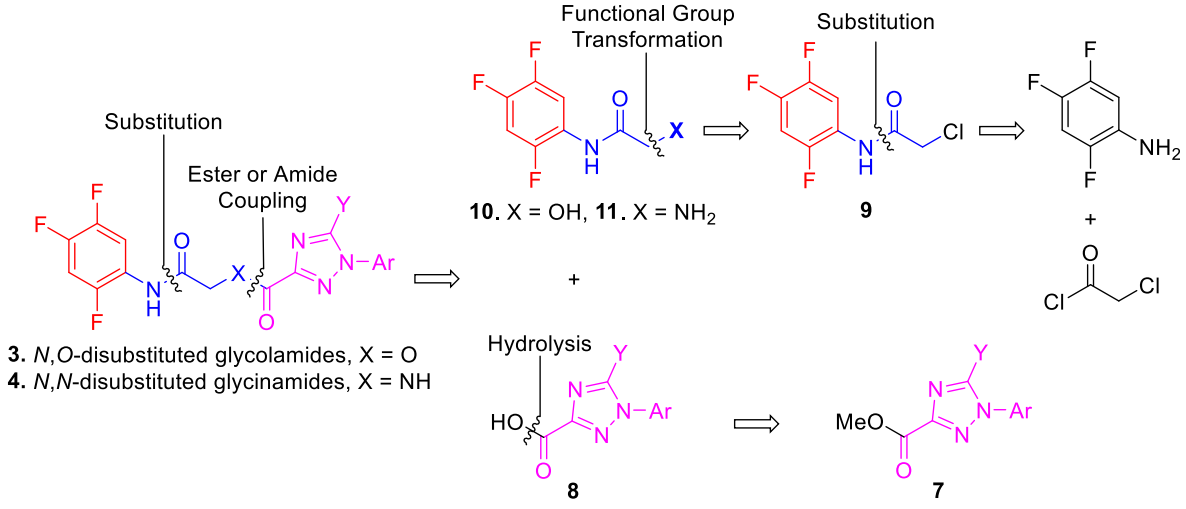


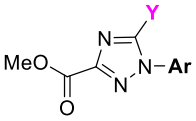
Fig. 2. The new designed structures of *N,O*-disubstituted glycolamides **3**, *N,N*-disubstituted glycinamides **4**, and β-amino carbonyl 1,2,4-triazoles **5** and **6** in comparison with Sitagliptin **1** and Vildagliptin **2**.



Scheme 1. The retrosynthesis route of *N,O*-disubstituted glycolamide 1,2,4-triazoles **3** and *N,N*-disubstituted glycinamide 1,2,4-triazoles **4**.

active phosphate agents (benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) [38], and *N,N*-bis[2-*oxo*-3-*oxazolidinyl*] phosphorodiamide chloride (BOP-Cl)) [39] in anhydrous THF or CH_2Cl_2 solution under basic condition at 0 °C to room temperature for 6 h [33]. However, most of these reactions afforded *N,O*-disubstituted glycolamides product **3a** in moderate yields (<72%). Remarkably, significant and successful reaction was allowed to stir with BOP-Cl activating reagent (2.0 equiv) and 2.0 equiv of NEt_3, and the yield of product **3a** was significantly increased to 85% good yield. Since, *N,N*-bis[2-*oxo*-3-*oxazolidinyl*]phosphorodiamide chloride (BOP-Cl) could be served as the best convenient and efficient activating carboxyl groups reagent that has been employed in the further esterification and amidation transformation to prepare *N,O*-

Table 1
The inhibitory activity results against DPP-4 of 1,3-disubstituted 1,2,4-triazoles **7a–g** and 1,3,5-trisubstituted 1,2,4-triazoles **7h–q**.



7a-q

Compounds No.	Y	Ar	DPP-4% Inh@1mM[a]
7a	H	Ph	33.9
7b	H	*p*-Me-Ph	37.0
7c	H	*p*-Cl-Ph	34.6
7d	H	*p*-CF_3-Ph	37.8
7e	H	*m*-CF_3-Ph	40.1
7f	H	*o*-CF_3-Ph	32.3
7g	H	*p*-F-Ph	10.1
7h	Me	*p*-Cl-Ph	19.2
7i	Me	*p*-Br-Ph	1.01
7j	Et	*p*-Cl-Ph	4.13
7k	*i*-Pr	*p*-Cl-Ph	24.4
7l	Furanyl	*p*-Cl-Ph	43.7
7m	Cyclopentyl	*p*-Cl-Ph	85.1
7n	Pyrrolyl	*m*-CF_3-Ph	94.5
7o	*n*-Bu	*p*-Cl-Ph	100
7p	Ph	Ph	100
7q	Ph	*p*-Me-Ph	100

[a] Concentration of each compound is 1.0 mmol L^{-1} in DMSO.

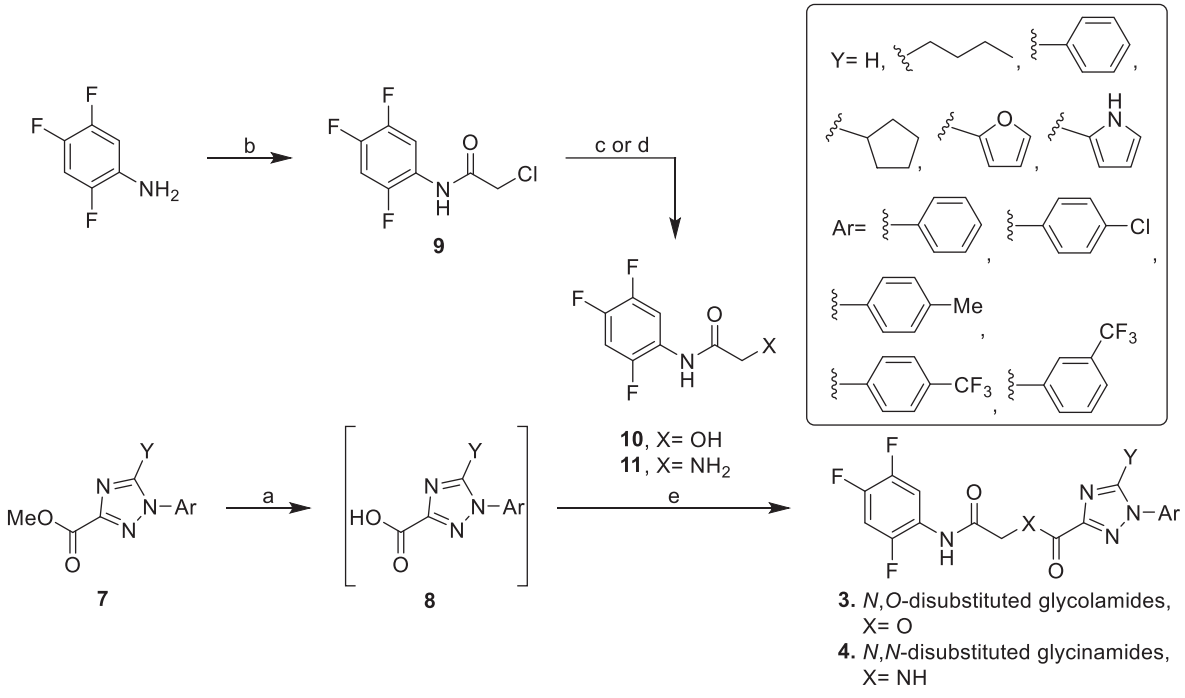
disubstituted glycolamides **3** and *N,N*-disubstituted glycinamides **4**.

Following the above activated carboxylic acid optimization study, the 1,2,4-triazole-3-carboxylates **7a–e** and **7l–q** were preliminarily converted to the corresponding 1,2,4-triazole-3-carboxylic acids **8a–e** and **8l–q**. Without further purification, compounds **8** were directly reacted with 2-hydroxyacetamide **10** or 2-aminoacetamide **11** and 2.0 equiv of BOP-Cl in presence of 2.0 equiv of NEt_3 to give the corresponding glycolamides **3a–e** and **3m** and glycinamides products **4a–e** and **4l–q** in moderate to good yields (71–88% for **3** and 72–92% for **4**, Table 2). In general, 2-aminoacetamide **11** possessed better substitution reactivity with the activated acid **8** than 2-hydroxyacetamide **10**. All structures of *N,O*-disubstituted glycolamides **3** and *N,N*-disubstituted glycinamides **4** were fully characterized by spectroscopic methods, for example, compound **4m** presents one doublet peak at δ 4.39 ppm of α-carbon methylene(—CH_2—) in ^{1}H NMR and characteristic absorption at δ 43.9 ppm for methylene carbon —$^{13}CH_2$—, at δ 167.3 ppm for glycinamide carbon N—(^{13}C=O)—CH_2, and at δ 161.9 ppm for amide carbon N—(^{13}C=O) in the ^{13}C NMR spectrum. Glycinamide **4m** was also further characterized by X-rays crystallographic analysis (as the single-crystal X-ray diffraction study, ORTEP) and shown in Fig. 3.

2.2.2. *Synthesis of β-amino carbonyl 1,2,4-triazoles 5 and 6*

The enantiomerically β-amino carbonyl linker intensively plays an important role in examining the designed structure. Therefore, this result drove us to synthesize β-amino carbonyl Sitagliptin analogues containing 1,2,4-triazole moiety **5** and **6** (Scheme 3 and 4). Based on the preliminary DPP-4 inhibition data of substituted 1,2,4-triazole-3-carboxylates in Table 1, compounds **7d** (DPP-4 Inh@1mM 37.8%) and **7m–q** with good inhibition rates (DPP-4 Inh@1mM ≧85%) were selected as promising targets. Initially, we chose 1,2,4-triazole-3-carboxylate **7d** (1.0 equiv) as the model substrates to carry out the reduction reaction with $NaBH_4$ (1.5 equiv) in THF/MeOH at room temperature for 5 h. The desired 1*H*-1,2,4-triazol-3-ylmethanol product **12d** was obtained in 91% yield (Scheme 3) [40]. The subsequent chlorination was performed using thionylchloride ($SOCl_2$, 1.5 equiv) to yield the chloried intermediate [41]. Without further purification, the intermediate 3-chloromethyl-1,2,4-triazole was treated with $NH_4OH_{(aq)}$ in EtOH solution and smoothly converted to the amination (1*H*-1,2,4-triazol-3-yl) methanamine product **13d** in 73% yield for two steps (Scheme 3) [42].

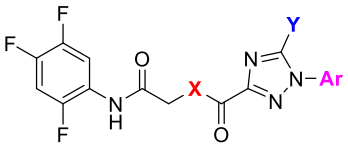
Finally, β-amino carbonyl targets **5d** and **6d** were individually synthesized from Boc-(*R*)-3-amino-4-(2,4,5-trifluoro-phenyl)-butyric acid **14** with (1*H*-1,2,4-triazol-3-yl)methanol **12d** or (1*H*-1,2,4-triazol-3-yl) methanamine **13d** by employing *N,N*-bis[2-*oxo*-3-*oxazolidinyl*]phosphorodiamide chloride (BOP-Cl, 2.0 equiv) as activating agent in presence of 2.0 equiv of NEt_3 in CH_2Cl_2 solution to afford the Boc-protected intermediates **15d** and **16d** (Scheme 4) [39]. In this key substitution



Scheme 2. Synthesis of *N,O*-disubstituted glycolamides **3** and *N,N*-disubstituted glycinamides **4**. Reagents and conditions: (a) DBU, MeOH, r.t., 6 h, > 90%; (b) 2-chloroacetyl chloride, toluene, reflux, 3 h, 92%; (c) For **10**: cesium formate, EtOH, reflux, 6 h, 86%; (d) For **11**: NH_4OH, EtOH, 40 °C, 4 h, 88%; (e) BOP-Cl, NEt_3, CH_2Cl_2, 4 h, 71–92%.

Table 2
The results of *N,O*-disubstituted glycolamide 1,2,4-triazoles **3a–q** and *N,N*-disubstituted glycinamide 1,2,4-triazoles

4a–q.



Compounds No.	X	Y	Ar	Yields (%)[a]
3a	O	H	Ph	85
4a	NH			87
3b	O	H	*p*-Me-Ph	87
4b	NH			92
3c	O	H	*p*-Cl-Ph	88
4c	NH			89
3d	O	H	*p*-CF_3-Ph	83
4d	NH			84
3e	O	H	*m*-CF_3-Ph	71
4e	NH			72
4l	NH	Furanyl	*p*-Cl-Ph	83
3m	O	Cyclopentyl	*p*-Cl-Ph	73
4m	NH			74
4n	NH	Pyrrolyl	*m*-CF_3-Ph	73
4o	NH	*n*-Bu	*p*-Cl-Ph	82
4p	NH	Ph	Ph	82
4q	NH	Ph	*p*-Me-Ph	84

[a] The calculated isolation yield was based on the amount of starting materials 2-hydroxyacetamide **10** or 2-aminoacetamide **11**.

reaction, we found that the amino group of compound **13d** was more nucleophilic reactivity than the hydroxyl group of **12d**. Without further purification, the one-pot synthesis procedure was proceeded to prepare β-amino carbonyl target compounds **5d** and **6d**. The deprotection of Boc-protected group of intermediates **15d** and **16d** was carried out in TFA/CH_2Cl_2 acidic solution to obtain β-amino ester product **5d** and β-amino amide product **6d** in 76% and 79% yields for two steps, respectively (Scheme 4 and Table 3) [43]. On the other hand, Boc-protected β-amino carbonyl product **15d** was tried to isolate for further identification of structural skeleton (Scheme 4). Compound **15d** presents two singlet peaks at δ 1.33 ppm for the *tert*-Boc group and δ 8.62 ppm for the 1,2,4-triazolic ring (N═C*H*—N═N) and one doublet peak δ 5.32 ppm of the ester (O—CH_2—) in ^{1}H NMR. In ^{13}C NMR spectrum, compound **15d** possessed characteristic absorptions at δ 170.8 ppm for ^{13}C═O, at δ 59.2 ppm for O—$^{13}CH_2$—, and at δ 28.2 and 79.5 ppm for *tert*-Boc group O—$^{13}C(^{13}CH_3)_3$.

Employing the above reliable procedure for synthesis of **12d** and **13d**, we converted compounds **7m–q** to 1*H*-1,2,4-triazol-3-ylmethanols **12m–q** in 92–96% yields and 1*H*-1,2,4-triazol-3-ylmethanamines **13m–q** in 76–84% yields (Scheme 3) [40–42]. Subsequently, compound **14** was directly reacted with 1*H*-1,2,4-triazol-3-ylmethanols **12m–q** or 1*H*-1,2,4-triazol-3-ylmethanamines **13m–q** to carried out the substitution reaction for generation of intermediates **15m–q** and **16m–q** [39]. Without further purification, the deprotection of Boc-protected group of intermediates **15m–q** and **16m–q** was carried out in TFA/CH_2Cl_2 acidic solution [43]. The β-amino ester products **5m–q** were provided from 74% to 84% yields and β-amino amide products **6m–q** were obtained from 74% to 88% yields in two steps, respectively (Table 3 and Scheme 4).

2.3. Biological activity

2.3.1. Pharmacological evaluation in vitro DPP-4 inhibition studies and SAR analysis of N,O-disubstituted glycolamides 3, N,N-disubstituted glycinamides 4, and β-amino carbonyl 1,2,4-triazoles 5 and 6

Screening of glycolamides **3a–e** and glycinamides **4a–e** with 1,3-disubstituted 1,2,4-triazole moiety were evaluated in vitro against a panel of standard DPP-4. The inhibitory activity was executed using the

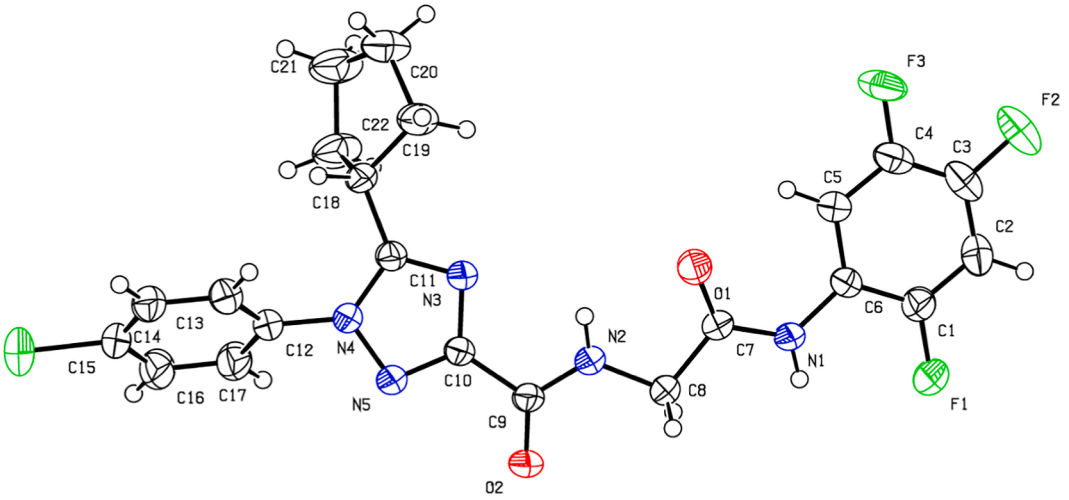
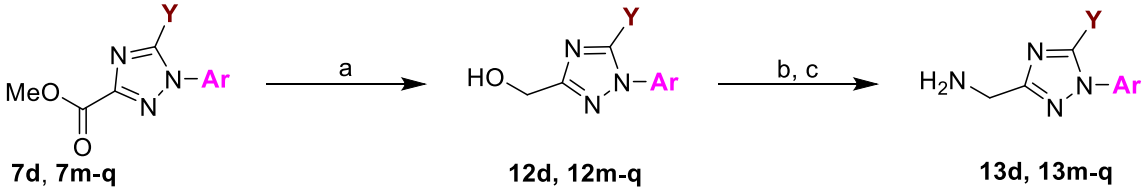
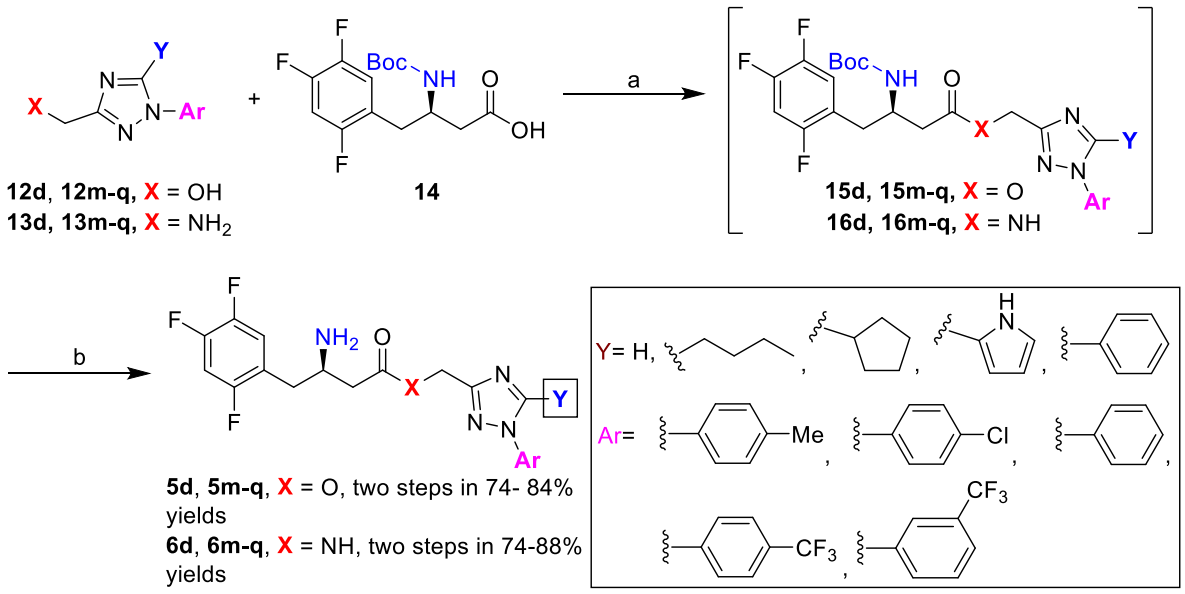


Fig. 3. The ORTEP diagram of 1-(4-chlorophenyl)-5-cyclopentyl-*N*-(2-oxo-2((2,4,5-trifluorophenyl)amino)ethyl)-1*H*-1,2,4-triazole-3-carboxamide **4m** (CCDC No. 1973637).

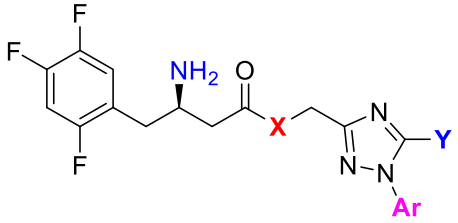


Scheme 3. Synthesis of (1*H*-1,2,4-triazol-3-yl)methanols **12d**, **12m–q** and (1*H*-1,2,4-triazol-3-yl)methanamine **13d**, **13m–q**. Reagents and conditions: (a) $NaBH_4$, THF, MeOH, r.t., 4 h, 91–96%; (b) $SOCl_2$, CH_2Cl_2, r.t., 2 h; (c) NH_4OH, EtOH, 30 °C, 6 h, 73–84%.



Scheme 4. Synthesis of β-amino carbonyl target compounds containing 1,2,4-triazole analogues **5d**, **5m–q** and **6d**, **6m–q**. Reagents and conditions: (a) BOP-Cl, NEt_3, CH_2Cl_2, r.t., 4 h; (b) CF_3COOH, CH_2Cl_2, r.t., 3 h, 74–88%.

Table 3
The synthesis results of β-amino carbonyl 1,2,4-triazoles **5d**, **5m–q** and **6d**, **6m–q**.



Compounds No.	X	Y	Ar	Yields (%)[a]
5d	O	H	*p*-CF_3-Ph	76
6d	NH			79
5m	O	Cyclopentyl	*p*-Cl-Ph	79
6m	NH			83
5n	O	Pyrrolyl	*m*-CF_3-Ph	74
6n	NH			74
5o	O	*n*-Bu	*p*-Cl-Ph	78
6o	NH			81
5p	O	Ph	Ph	83
6p	NH			88
5q	O	Ph	*p*-Me-Ph	84
6q	NH			86

[a] The calculated isolation yield was based on the amount of reactant Boc-(*R*)-3-amino-4-(2,4,5-trifluoro-phenyl)-butyric acid **14**.

disc diffusion method and the measurement of the zone of growth inhibition for a concentration 1.0 mmol/L (DMSO) for each glycolamides **3a–e** and glycinamides products **4a–e**. Sitagliptin **1** was used as comparison model for the inhibitory activity study (100%, Table 4). Noteworthily, compounds **3d** and **4d** with *p*-CF_3Ph substituents on *N*-1 position of triazole ring possessed better inhibitory activity against DPP-4 (57.2% and 58.2%, Table 4). Moreover, the inhibitory activity tendency of DPP 4 was observed that most of glycinamides **4a–e** presented better inhibitory activity than glycolamides **3a–e**. Based on this result, glycinamides **4l–q** with 1,3,5-trisubstituted 1,2,4-triazole moiety bearing *n*-butyl, cyclopentyl, furanyl, and pyrrolyl on *C*-5 position and *p*-Cl-Ph, *m*-CF_3-Ph, Phenyl, and *p*-Me-Ph on *N*-1 position of triazole ring were significantly synthesized for further investigation. However, the poor inhibitory data was obtained from glycinamides **4l–q** (≤48.8%, Table 4). The unsatisfactory inhibitory results led to the conclusion that glycolamides **3** and glycinamides **4** were unfavorable linkers related to the β-amino carbonyl of Sitagliptin **1**. To sum up, we observed that the enantiomerically β-amino carbonyl linker seems to intensively play an important role. Therefore, the results drove us to the discovery of β-amino carbonyl 1,2,4-triazoles **5** and **6**.

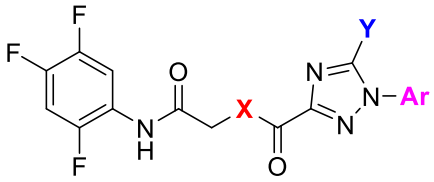
For further optimization, introducing β-amino carbonyl as a linker became a primary goal. Glycolamide **3d** and glycinamide **4d** possessed better inhibitory activity in Table 4. Thus, β-amino carbonyl 1,3-disubstituteds 1,2,4-triazoles **5d** and **6d** were selected to optimize linkage property for evaluating DPP-4 inhibitory capacity. Fortunately, β-amino amide **6d** showed superior inhibitory activity (IC_{50} = 34.4 nM, Table 5). On the other hand, no significant improvement was observed for β-amino ester **5d** (IC_{50} = 775 nM, Table 5). For further demonstration of bioactivity potency, two series of compounds including β-amino esters **5m–q** and β-amino amides **6m–q** were synthesized in parallel to modify the linker and 1,3,5-trisubstituted 1,2,4-triazoles (Table 5). Most of compounds **5m–q** and **6m–q** showed the moderate inhibitory activity and their IC_{50} values were ranging from 49.9 nM to 497 nM (Table 5). However, these results could not provide us conclusive potential inhibitory tendency order between β-amino esters **5m–q** and β-amino amides **6m–q** (Table 5). To sum up, compounds **5n** and **6p** also exhibited the significant potent anti-DPP-4 activity with IC_{50} value of 49.9 nM and 50.4 nM, respectively (Table 5).

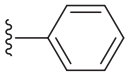
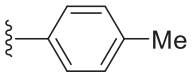
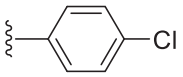
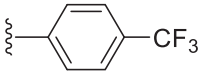
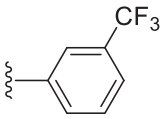
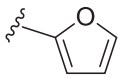
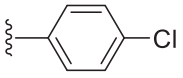
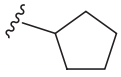
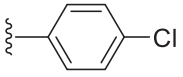
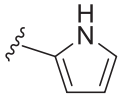
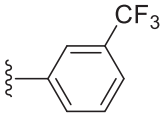
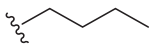
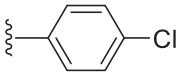
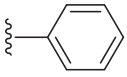
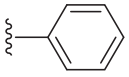
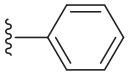
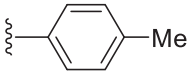
*2.3.2. Selectivity DPP-4 over DPP-7, DPP-8, and DPP-9 of β-amino ester **5n** and β-amino amides **6d** and **6p***

The DPP-4 activity and/or structural homologue (DASH) family, including quiescent cell proline dipeptidase (QPP, also known as DPP-2 or DPP-7), DPP-8, DPP-9, and FAP [45]. As reported in previous study, the DPP-4 inhibitors containing β-amino acid fragment in the structure resulted in the QPP off-target activity [12,46]. In addition, selective inhibition of DPP-8/DPP-9 was associated with toxicity, such as alopecia, thrombocytopenia, anemia, and animal mortality [47]. Therefore, selectivity against QPP, DPP-8, DPP-9 was important for the safety of DPP-4 inhibitors. Based on the potency of DPP-4 inhibition, we selected β-amino ester **5n** and β-amino amides **6d** and **6p** (IC_{50} < 51 nM) to evaluate selectivity across enzymes QPP, DPP-8, and DPP-9. As shown in Table 6, both β-amino carbonyl 1,3,5-trisubstituted 1,2,4-triazoles **5n** and **6p** showed excellent selectivity over QPP, DPP-8, and DPP-9 (>1950-fold), however, the selectivity of 1,5-disubstituted 1,2,4-triazole **6d** was lower (135-, 31-, and 105-fold selectivity over QPP, DPP-8,

Table 4
The DPP-4 inhibitory activity results of *N,O*-disubstituted glycolamide 1,2,4-triazoles **3**, *N,N*-disubstituted glycinamide 1,2,4-triazoles **4**, and β-amino carbonyls **5** and

6.



Compounds No.	X	Y	Ar	DPP-4% Inh@1mM[a]
3a	O	H		43.4
4a	NH			37.6
3b	O	H		28.4
4b	NH			34.1
3c	O	H		39.4
4c	NH			47.2
3d	O	H		57.2
4d	NH			58.2
3e	O	H		37.0
4e	NH			42.5
4l	NH			33.5
3m	OH			39.3
4m	NH			48.8
4n	NH			34.0
4o	NH			36.6
4p	NH			32.5
4q	NH			39.2
Sitagliptin **1**				100

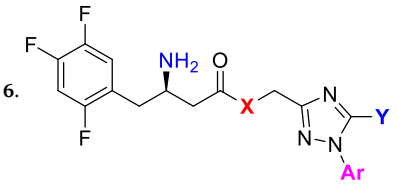
[a] Concentration of each used compound is 1.0 mmol L^{-1} (DMSO).

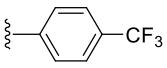
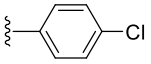
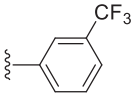
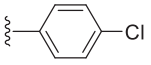
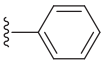
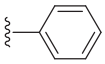
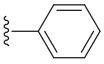
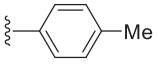
and DPP-9, respectively). This result indicated that an aryl group appeared to be essential for the 5-position of 1,2,4-triazole. Importantly, the selectivity over DPP8 of compounds **5n** and **6d** (>1984-fold) was better than Sitagliptin (**1**, 787-fold), making compounds **5n** and **6d** suitable for further study.

*2.4. Docking study of β-amino carbonyl target **5n, 6d,** and **6p** into the active site of DPP-4*

In order to gain an insight of the possible binding interactions of β-amino ester **5n** and β-amino amides **6d** and **6p** in the active site of DPP-4 enzyme, molecular modelling was performed. The X-ray structure of DPP-4 cocrystallized with Sitagliptin **1** (PDB ID 1X70, resolution 2.10 Å) was used to perform the docking study through docking program iGEMDOCK v2.1 [12,25]. Re-docking of the co-crystallized ligand Sitagliptin **1** into DPP-4 active site via iGEMDOCK was performed, and the RMSD was calculated by using LigRMSD [48]. Sitagliptin **1** docked in the DPP-4 binding site almost at the same position (RMSD = 0.53 Å) as the co-crystallized data (PDB ID 1X70), which validated the iGEMDOCK protocol. The DPP-4 enzyme was docked with compounds **5n**, **6d**, and **6p** in Sitagliptin **1** binding site. As depicted in Fig. 4, the docking model revealed that compounds **5n**, **6d**, and **6p** were stabilized at DPP-4 active site similarly to Sitagliptin **1** through three hydrogen bond interactions with residues Tyr662, Glu205, and Glu206, and two π–π stacking interaction with residues Tyr662 and Phe357. β-amino amide **6d** with superior DPP-4 inhibition achieves lower docking scores of –150.4 kcal/mol (Table S2), because 4-(trifluoromethyl)phenyl group of compound **6d** possesses additional van der Waals interaction with residue Arg358 in comparison with Sitagliptin **1** (Fig. 4A). On the other hand, it has been reported that neither DPP-8 nor DPP-9 has a residue corresponding to Phe357 of DPP-4 [49]. Based on the ducking result, we found that 1,3,5-trisubstituted 1,2,4-triazoles **5n** (−26.4 kcal/mol) and

Table 5
The DPP-4 inhibitory activity results of β-amino carbonyls **5** and **6**.



Compounds No.	X	Y	Ar	DPP-4 IC_{50} (nM)[a]
5d	O	H		775 (nM)
6d	NH			34.4 (nM)
5m	O			80.3 (nM)
6m	NH			131 (nM)
5n	O			49.9 (nM)
6n	NH			99.8 (nM)
5o	O			81.6 (nM)
6o	NH			119 (nM)
5p	O			91.3 (nM)
6p	NH			50.4 (nM)
5q	O			497 (nM)
6q	NH			138 (nM)
Sitagliptin **1**				28 (18–38 nM)[b]

[a] IC_{50} is the concentration (nM) needed to cause 50% inhibition human recombinant DPP-4 enzymatic activity. Values represent the mean of double determinations.
[b] The IC_{50} value of Sitagliptin was between 18 and 38 nM in reported literature [12,44].

6p (−23.8 kcal/mol) with additional aromatic ring on the triazole ring provided stronger π–π stacking interaction with residue Phe357 than 1,5-disubstituted 1,2,4-triazole **6d** (−11.8 kcal/mol) and Sitagliptin **1** (−19.4 kcal/mol, Fig. 4B and Table S3), which might explain compounds **5n** and **6p** improved selectivity inhibition of DPP-4 over DPP-8 and DPP-9.

3. Conclusion

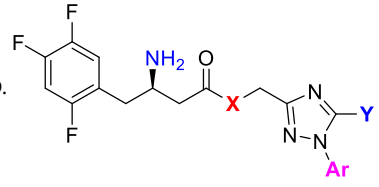
We have successfully synthesized and built-up four series of 1,2,4-triazole derivatives **3**–**6** containing *N,O*-disubstituted glycolamide, *N,N′*-disubstituted glycinamides, β-amino ester, and β-amino amide as linkers through modifying Sitagliptin **1** and Vildagliptin **2**. Different linkers grafted the same 2,4,5-triflorophenyl fragment into the architecture, and 1,2,4-triazole heterocyclic moiety was modulated for structural variation. The synthetic strategy for glycolamides or glycinamides involved functionalized transformation of α-chloro *N*-arylacetamides (hydroxylation or amination) and esterification or amidation of 1,2,4-triazole-3-carboxylic acid by activating with BOP-Cl reagent. We also developed one-pot synthesis procedure, including treating (1*H*-1,2,4-triazol-3-yl)methanol or (1*H*-1,2,4-triazol-3-yl)methylamine with Boc-(*R*)-3-amino-4-(2,4,5-trifluoro-phenyl)-butyric acid and deprotection of *tert*-Butyloxycarbonyl (BOC) group, for the preparation of β-amino carbonyl 1,2,4-triazoles. For the preliminary screening in vitro, glycolamides **3** and glycinamides **4** showed poor dipeptidyl peptidase 4 inhibitory activity (≦58.2% DPP-4 Inh@1mM). Most of β-amino ester **5** and β-amino amide 1,2,4-triazoles **6** possessed the moderate inhibition of DPP 4 (IC_{50} < 775 nM). Especially, β-amino carbonyl **5n**, **6d**, and **6p** had satisfactory DPP-4 inhibitory activity (50.4, 34.4, 49.9 nM, respectively). Docking study also revealed compounds **5n**, **6d** and **6p** have favorable binding mode with DPP-4 enzyme. Selectivity evaluation indicated β-amino carbonyl **5n** and **6p** hold excellent selectivity over QPP, DPP-8, and DPP-9 and superior to reference Sitagliptin. Furthermore, compounds **5n** and **6p** provided stronger π–π stacking interaction with residue Phe357 than 1,5-disubstituted 1,2,4-triazole **6d** and Sitagliptin **1** based on the molecular docking results. In summary, β-amino carbonyl **5n** and **6p** exhibited good activity in vitro and excellent selectively and might be a promising lead for the treatment of diabetes mellitus.

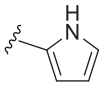
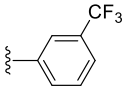
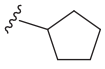
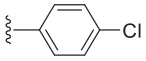
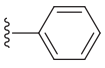
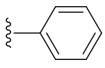
4. Experimental section

4.1. Chemistry

All chemicals were purchased with reagent grade. Both 1,3-disubstituted 1,2,4-triazoles **7a**–**g** [26a] and 1,3,5-trisubstituted 1,2,4-triazoles **7h**–**q** [26b] were prepared following our reported procedure [26]. All reactions were carried out under argon or nitrogen atmosphere and monitored by TLC. Flash column chromatography was carried out on

Table 6
Selectivity of *β-amino ester* ***5n****, β-amino amides* ***6d*** *and* ***6p****, Sitagliptin* ***1*** against QPP, DPP-8, and DPP-9.



Compounds No.	X	Y	Ar	IC_{50} (nM)[a]			
				DPP-4	QPP	DPP-8	DPP-9
5n	O			49.9	$>10^5$	$>10^5$	$>10^5$
6d	NH			34.4	4630	1060	3610
6p	NH			50.4	$>10^5$	$>10^5$	$>10^5$
Sitagliptin **1**[b]				28	$>10^5$	22030	$>10^5$

[a] IC_{50} is the concentration (nM) needed to cause 50% inhibition human recombinant DPP-4 enzymatic activity. Values represent the mean of double determinations.
[b] Sitagliptin was used as the reference drug in previous literature [12].

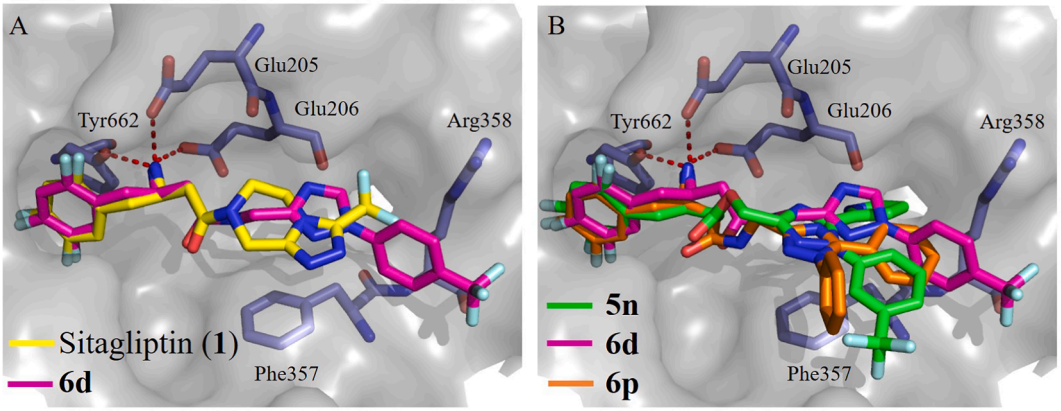


Fig. 4. Docked pose of the active site of DPP-4 enzyme with (A) Sitagliptin **1** (yellow) and β-amino amide **6d** (magenta) (B) β-amino ester **5n** (green), β-amino amides **6d** (magenta) and **6p** (orange). The red dashed lines indicate hydrogen-bonding interactions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

silica gel (230–400 mesh). Analytical thin-layer chromatography (TLC) was performed on precoated plates (silica gel 60F-254) purchased from Merck Inc. Commercially available reagents were used without further purification unless otherwise noted. Dichloromethane, ethyl acetate, and hexane were purchased from Mallinckrodt Chemical Co. Dimethyl sulfone ($DMSO_2$, purity = 100%) was purchased from Tokyo Chemical Industry Co. Proton NMR spectra were obtained on a Bruker (400 MHz or 500 MHz) spectrometer by use of $CDCl_3$ or DMSO-d_6 as solvent. Carbon-13 NMR spectra were obtained on a Bruker (100 MHz or 125 MHz) spectrometer by used of $CDCl_3$ or DMSO-d_6 as solvent. The following abbreviations are used for spin multiplicity: s, singlet; d, doublet; t, triplet; q, quartet; p, quintet/pentet; sept, septet; dd, doublet of doublets; dt, triplet of doublets; m, multiplet; *J*, coupling constant (Hz). The purities of the compounds **3**–**6** were determined by using the q^1HNMR method (absolute q^1HNMR with internal calibration) [50]. Most of purities were more than > 98%, for example, compound **6n** was 98.80% (Figure S91). The purity of compound **6n** was calculated by following equation:

$$p(\%) = \frac{n_{ic}.int_t.MW_t.m_{ic}}{n_t.int_{ic}.MW_{ic}.m_s}.p_{ic}$$

where MW is the molecular weight, *P* is the purity of internal calibrant, m_{ic} is the amount of internal calibrant, m_s is the amount of sample, int is integral, and *n* is the number of protons giving rise to a given NMR signal. The subscript ic and t represents the internal calibrant and target analyte, respectively. Infrared (IR) spectra were measured on a Bomem Michelson Series FT-IR spectrometer. The wavenumbers reported are referenced to the polystyrene absorption at 1601 cm^{-1}. Flash column chromatography purification of compounds was carried out by gradient elution using hexanes in ethyl acetate (EA) unless otherwise stated. High-resolution mass spectra were obtained from a JEOL JMS-HX110 mass spectrometer.

*4.1.1. Standard procedure for synthesis of N,O-disubstituted glycolamides **3a**–**e** and **3m** [26,28,39]*

A series of methyl 1*H*-1,2,4-triazole-3-carboxylates **7a–e** and **7m** were prepared as the starting martials by following our previous published procedure [26]. 1*H*-1,2,4-triazole-3-carboxylates **7a–e** and **7m** (1.0 equiv, 5.5 mmol) was dissolved in MeOH (15 mL) and stirred at room temperature. 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 3.0 equiv) [28] was slowly added to the reaction mixture at room temperature. Then the mixture was stirred for 6 h. When the reaction was completed, the reaction mixture was added to water (15 mL) and extracted with ethyl acetate (15 mL × 3). The combined organic layers were washed sodium bicarbonate (15 mL × 3), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford the corresponding crude 1*H*-1,2,4-triazole-3-carboxylic acids **8a**–**e** and **8m**.

A solution of crude 1*H*-1,2,4-triazole-3-carboxylic acids **8a**–**e** and **8m** (1.0 equiv from **7a–e** and **7m**) and 2-hydroxy-*N*-(2,4,5-trifluorophenyl)acetamide **10** (1.1 equiv) was treated with *N*,*N*-bis[2-*oxo*-3-*oxazolidinyl*]phosphorodiamide chloride (BOP-Cl, 2.0 equiv) in presence of NEt_3 (2.0 equiv) in CH_2Cl_2 solution (20 mL) [39]. The reaction mixture was stirred at room temperature for 4 h. When the reaction was completed, the reaction mixture was washed with water (15 mL) and extracted with CH_2Cl_2 (15 mL × 3). The combined organic layers were washed sodium bicarbonate (15 mL × 3), dried over $MgSO_4$ to give crude *N,O*-disubstituted glycolamides **3a–e** and **3m**. The crude products **3a–e** and **3m** were purified by column chromatography on silica gel to give the pure corresponding *N,O*-disubstituted glycolamides **3a–e** and **3m** in 71–88% yields.

*4.1.1.1. 2-Oxo-2-((2,4,5-trifluorophenyl)amino)ethyl 1-phenyl-1H-1,2,4-triazole-3- carboxylate (**3a**).* Yield 85%, white solid, m.p. 184–186 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.08 (s, 2H, OC*H*$_2$), 7.50 (t, *J* = 7.38 Hz, 1H, Ar*H*), 7.62 (t, *J* = 7.86 Hz, 2H, Ar*H*), 7.66–7.71 (m, 1H, Ar*H*), 7.93 (d, *J* = 8.00 Hz, 2H, Ar*H*), 8.03 (dt, *J* = 15.95, 6.15 Hz, 1H, Ar*H*), 9.50 (s, 1H, Ar*H*), 10.34 (br, 1H, N*H*). ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6) δ 63.12, 106.01 (dd, *J* = 25.63, 22.73 Hz), 111.60 (d, *J* = 24.08 Hz), 120.01 (2 × C), 122.37, 128.79, 129.93 (2 × C), 136.30, 144.27, 145.17 (d, *J* = 238.67 Hz), 147.55, 149.92, 154.11, 158.83, 165.75. IR (KBr): 3460, 3164, 1750, 1692, 1427, 1202, 883, 750 cm^{-1}. EIMS *m/z*: 376 (M^+, 5), 231 (25), 173 (20), 172 (100), 145 (22), 104 (11), 77 (13). HRMS (EI) *m/z*: $[M]^+$ Calcd for $C_{17}H_{11}F_3N_4O_3$: 376.0783; Found 376.0785.

*4.1.1.2. 2-Oxo-2-((2,4,5-trifluorophenyl)amino)ethyl 1-(p-tolyl)-1H-1,2,4-triazole- 3-carboxylate (**3b**).* Yield 87%, light yellow solid, m.p. 186–188 °C, ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.39 (s, 3H, C*H*$_3$), 5.06 (s, 2H, OC*H*$_2$), 7.42 (d, *J* = 8.40 Hz, 2H, Ar*H*), 7.68 (td, *J* = 10.72, 7.45 Hz, 1H, Ar*H*), 7.80 (d, *J* = 8.40 Hz, 2H, Ar*H*), 8.02 (dt, *J* = 15.96, 6.30 Hz, 1H, Ar*H*), 9.44 (s, 1H, Ar*H*), 10.34 (br, 1H, N*H*). ^{13}C{^{1}H} NMR (125 MHz, DMSO-d_6) δ 20.58, 63.12, 106.04 (t, *J* = 23.76 Hz), 111.65 (d, *J* = 23.52 Hz), 119.92 (2 × C), 120.19, 130.30 (2 × C), 134.08, 138.51, 144.06, 144.37, 146.04, 147.79, 153.97, 158.87, 165.78. IR (KBr): 3449, 3160, 2929, 1736, 1685, 1427, 1202, 883, 815 cm^{-1}. EIMS *m/z*: 390 (M^+, 6), 244 (22), 187 (20), 186 (100), 159 (30), 118 (15), 91 (12). HRMS (EI) *m/z*: $[M]^+$ Calcd for $C_{18}H_{14}F_3N_4O_3$: 390.0940; Found 390.0945.

*4.1.1.3. 2-Oxo-2-((2,4,5-trifluorophenyl)amino)ethyl 1-(4-chlorophenyl)-1H-1,2,4- triazole-3-carboxylate (**3c**).* Yield: 88%, light yellow solid; m. p. 182–183 °C, ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.07 (s, 2H, OC*H*$_2$),

7.65–7.67 (m, 1H, Ar*H*), 7.69 (d, *J* = 8.91 Hz, 2H, Ar*H*), 7.97 (d, *J* = 8.74 Hz, 2H, Ar*H*), 8.02 (dt, *J* = 16.03, 6.19 Hz, 1H, Ar*H*), 9.52 (s, 1H, Ar*H*), 10.33 (br, 1H, N*H*). $^{13}C\{^1H\}$ NMR (125 MHz, DMSO-d_6) δ 63.14, 106.01 (dd, *J* = 25.91, 22.40 Hz), 111.59 (d, *J* = 23.85 Hz), 121.76 (2 × C), 122.34 (t, *J* = 11.36 Hz), 129.88 (2 × C), 130.05, 135.14, 144.47, 145.24 (dd, *J* = 240.59, 12.65 Hz), 147.75 (d, *J* = 8.48 Hz), 149.70 (d, *J* = 9.61 Hz), 154.17, 158.72, 165.69. IR (KBr): 3456, 3160, 2919, 2851, 1746, 1682, 1427, 1199, 883, 828 cm^{-1}. EIMS *m/z*: 410 (M^+, 6), 264 (26), 208 (35), 207 (10), 206 (100), 179 (20), 147 (11), 138 (11). HRMS (EI) *m/z*: $[M]^+$ Calcd for $C_{17}H_{10}ClF_3N_4O_3$: 410.0394; Found 410.0390.

*4.1.1.4. 2-Oxo-2-((2,4,5-trifluorophenyl)amino)ethyl 1-(4-(trifluoromethyl) phenyl)- 1H- 1,2,4-triazole-3-carboxylate (**3d**).* Yield 83%, yellow solid, m. p. 112–113 °C, ^1H NMR (400 MHz, DMSO-d_6) δ 5.09 (s, 2H, OCH_2), 7.69 (td, *J* = 10.65, 7.51 Hz, 1H, Ar*H*), 8.00–8.07 (m, 3H, Ar*H*), 8.19 (d, *J* = 8.46 Hz, 2H, Ar*H*), 9.65 (s, 1H, Ar*H*), 10.36 (br, 1H, N*H*). $^{13}C\{^1H\}$ NMR (100 MHz, DMSO-d_6) δ 63.23, 106.07 (dd, *J* = 25.58, 22.85 Hz), 111.59 (d, *J* = 24.22 Hz), 120.53(2 × C), 122.36, 123.82 (d, *J* = 272.31 Hz), 127.26, 127.30, 128.75 (q, *J* = 31.49 Hz), 139.20, 144.98, 145.28 (dd, *J* = 241.78, 12.67 Hz), 147.55, 149.94 (d, *J* = 7.37 Hz), 154.44, 158.70, 165.71. IR (KBr): 3439, 3221, 2953, 1746, 1678, 1430, 1321, 1127, 1066, 849 cm^{-1}. EIMS *m/z*: 444 (M^+, 2), 271 (15), 256 (13), 241 (13), 240 (100), 213 (26), 172 (16), 159 (22), 147 (14), 145 (18). HRMS (EI) *m/z*: $[M]^+$ Calcd for $C_{18}H_{10}F_6N_4O_3$:444.0657; Found 444.0649.

*4.1.1.5. 2-Oxo-2-((2,4,5-trifluorophenyl)amino)ethyl 1-(3-(trifluoromethyl) phenyl)- 1H-1,2,4-triazole-3-carboxylate (**3e**).* Yield: 71%, white solid, m. p. 163–164 °C, ^1H NMR (500 MHz, DMSO-d_6) δ 5.08 (s, 2H, OCH_2), 7.67 (td, *J* = 10.69, 7.44 Hz, 1H, Ar*H*), 7.85–7.90 (m, 2H, Ar*H*), 8.03 (dt, *J* = 15.98, 6.19 Hz, 1H, Ar*H*), 8.25–8.27 (m, 1H, Ar*H*), 8.31 (s, 1H, Ar*H*), 9.65 (s, 1H, Ar*H*), 10.35 (br, 1H, N*H*). $^{13}C\{^1H\}$ NMR (125 MHz, DMSO-d_6) δ 63.22, 106.05 (dd, *J* = 25.86, 22.09 Hz), 111.65 (d, *J* = 22.87 Hz), 116.76, 116.79, 123.59 (d, *J* = 272.83 Hz), 124.04, 125.34, 130.55 (q, *J* = 32.81 Hz), 131.42, 136.87, 144.91, 145.28 (dd, *J* = 241.05, 12.57 Hz), 147.85, 149.72, 154.31, 158.72, 165.71. IR (KBr): 3470, 3160, 1746, 1692, 1508, 1427, 1202, 1131, 883 cm^{-1}. EIMS *m/z*: 444 (M^+, 4), 298 (18), 241 (14), 240 (100), 213 (14), 172 (10), 147 (11), 145 (14). HRMS (EI) *m/z*: $[M]^+$ Calcd for $C_{18}H_{10}F_6N_4O_3$: 444.0657; Found 444.0665.

*4.1.1.6. 2-Oxo-2-((2,4,5-trifluorophenyl)amino)ethyl 1-(4-chlorophenyl)-5- cyclopentyl-1H-1,2,4-triazole-3-carboxylate (**3m**).* Yield 73%, white solid, m.p. 68–69 °C, ^1H NMR (400 MHz, $CDCl_3$) δ 1.62–1.68 (m, 2H, CH_2), 1.85–1.94 (m, 2H, CH_2), 1.96–2.01 (m, 4H, CH_2), 3.13 (quint, *J* = 8.28 Hz, 1H, C*H*), 5.00 (s, 2H, OCH_2), 6.97 (td, *J* = 9.97, 6.99 Hz, 1H, Ar*H*), 7.40 (d, *J* = 8.71 Hz, 2H, Ar*H*), 7.53 (d, *J* = 8.72 Hz, 2H, Ar*H*), 8.31 (dt, *J* = 15.73, 5.92 Hz, 1H, Ar*H*), 8.46 (br, 1H, N*H*).$^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 25.77 (2 × C), 33.00 (2 × C), 36.26, 63.38, 104.93 (t, *J* = 23.47 Hz), 111.56 (d, *J* = 25.00 Hz), 121.64, 126.73 (2 × C), 129.88 (2 × C), 135.10, 136.05, 142.95, 145.05, 148.77, 152.91, 158.12, 162.87, 164.69. IR (KBr): 3303, 3072, 2960, 2875, 1756, 1715, 1549, 1501, 1426, 1192, 1097, 876, 839 cm^{-1}. EIMS *m/z*: 480 (M^+ + 2, 5), 479 (M^+ + 1, 4), 478 (M^+, 16), 305 (16), 277 (18), 276 (37), 275 (40), 274 (100), 249 (13), 248 (30), 247 (39), 246 (70), 232 (14), 206 (17), 160 (22), 153 (16), 151 (44), 147 (46), 146 (16), 138 (13), 129 (22), 127 (75), 125 (33), 119 (19), 113 (11), 111 (26), 99 (22). HRMS (EI) *m/z*: $[M]^+$ Calcd for $C_{22}H_{18}ClF_3N_4O_3$: 478.1020; Found 478.1016.

*4.1.2. Standard procedure for synthesis of N,N-disubstituted glycinamides **4a–e** and **4l–q** [26,28,39]*

A series of methyl 1*H*-1,2,4-triazole-3-carboxylates **7a–e** and **7l–q** were prepared as the starting martials by following our previous published procedure [26]. 1*H*-1,2,4-Triazole-3-carboxylates **7a–e** and **7l–q** (1.0 equiv, 5.5 mmol) was dissolved in MeOH (15 mL) and stirred at room temperature. 1,8-diazabicyclo(5.4.0)undec-7-ene (DBU, 3.0 equiv) [28] was slowly added to the reaction mixture at room temperature. Then the mixture was stirred for 6 h. When the reaction was completed, the reaction mixture was added to water (15 mL) and extracted with ethyl acetate (15 mL × 3). The combined organic layers were washed sodium bicarbonate (15 mL × 3), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford the corresponding crude 1*H*-1,2,4-triazole-3-carboxylic acids **8a–e** and **8–q**.

A solution of crude 1*H*-1,2,4-triazole-3-carboxylic acids **8a–e** and **8l–q** (1.0 equiv, from 7**a–e** and **7l–q**) and 2-amino-*N*-(2,4,5-trifluorophenyl)acetamide **11** (1.1 equiv) was treated with *N*,*N*-bis[2-*oxo*-3-*oxazolidinyl*]phosphorodiamide chloride (BOP-Cl, 2.0 equiv) in presence of NEt_3 (2.0 equiv) in CH_2Cl_2 solution (20 mL) [39]. The reaction mixture was stirred at room temperature for 4 h. When the reaction was completed, the reaction mixture was washed with water (15 mL) and extracted with CH_2Cl_2 (15 mL × 3). The combined organic layers were washed sodium bicarbonate (15 mL × 3), dried over $MgSO_4$ to give crude *N*,*N*-disubstituted glycinamides **4a–e** and **4l–q**. The crude products **4a–e** and **4l–q** were purified by column chromatography on silica gel to give the pure corresponding *N*,*N*-disubstituted glycinamides **4a–e** and **4l–q** in 72–92% yields.

*4.1.2.1. 2-Oxo-2-((2,4,5-trifluorophenyl)amino)ethyl-1-phenyl-1H-1,2,4-triazole-3- carboxylate (**4a**).* Yield 87%, white solid, m.p. 245–246 °C, ^1H NMR (500 MHz, DMSO-d_6) δ 4.17 (d, *J* = 3.34 Hz, 2H, —NHCH_2—), 7.48 (t, *J* = 7.44 Hz, 1H, Ar*H*), 7.61 (t, *J* = 7.97 Hz, 2H, Ar*H*), 7.64–7.68 (m, 1H, Ar*H*), 7.92 (d, *J* = 7.53 Hz, 2H, Ar*H*), 8.02 (dt, *J* = 16.03, 6.25 Hz, 1H, Ar*H*), 8.85 (t, *J* = 5.44 Hz, 1H, N*H*), 9.43 (s, 1H, Ar*H*), 10.14 (br, 1H, NH). $^{13}C\{^1H\}$ NMR (125 MHz, DMSO-d_6) δ 42.60, 105.91 (dd, *J* = 26.21, 22.28 Hz), 111.58 (d, *J* = 22.53 Hz), 119.82 (2 × C), 122.88 (t, *J* = 11.12 Hz), 128.47, 129.87 (2 × C), 136.49, 143.48, 144.19, 146.09, 147.68, 157.08, 158.83, 168.04. IR (KBr): 3401, 3290, 2922, 1671, 1542, 1505, 1433, 1202, 873 cm^{-1}. EIMS *m/z*: 375 (M^+, 2), 230 (14), 229 (58), 202 (16), 201 (34), 173 (15), 172 (100), 147 (36), 145 (21), 104 (12), 77 (16). HRMS (EI) *m/z*: $[M]^+$ Calcd for $C_{17}H_{12}F_3N_5O_2$: 375.0943; Found 375.0951.

*4.1.2.2. N-(2-Oxo-2-((2,4,5-trifluorophenyl)amino)ethyl)-1-(p-tolyl)-1H-1,2,4- triazole-3-carboxamide (**4b**).* Yield 92%, light yellow solid, m.p. 251–252 °C, ^1H NMR (500 MHz, DMSO-d_6) δ 2.38 (s, 3H, CH_3), 4.17 (d, *J* = 5.95 Hz, 2H, OCH_2), 7.41 (d, *J* = 8.30 Hz, 2H, Ar*H*), 7.64 (td, *J* = 15.98, 7.45 Hz, 1H, Ar*H*), 7.80 (d, *J* = 8.45 Hz, 2H, Ar*H*), 8.02 (dt, *J* = 16.01, 6.23 Hz, 1H, Ar*H*), 8.19 (t, *J* = 5.90 Hz, 1H, N*H*), 9.37 (s, 1H, Ar*H*), 10.11 (br, 1H, N*H*). $^{13}C\{^1H\}$ NMR (125 MHz, DMSO-d_6,) δ 20.54, 42.58, 105.91 (t, *J* = 24.20 Hz), 111.56 (d, *J* = 24.48 Hz), 119.71 (2 × C), 122.82, 130.20 (2 × C), 134.22, 138.10, 143.23, 145.20 (dd, *J* = 237.34, 12.60 Hz), 147.73, 149.60, 156.92, 158.87, 168.05. IR (KBr): 3395, 3286, 3113, 2919, 2854, 1671, 1546, 1513, 1430, 1202, 866 cm^{-1}. EIMS *m/z*: 389 (M^+, 2), 243 (51), 187 (13), 186 (100), 159 (24), 118 (14), 91 (14). HRMS (EI) *m/z*: $[M]^+$ Calcd for $C_{18}H_{14}F_3N_5O_2$: 389.1110; Found 389.1103.

*4.1.2.3. 1-(4-Chlorophenyl)-N-(2-oxo-2-((2,4,5-trifluorophenyl)amino) ethyl)-1H- 1,2,4-triazole-3-carboxamide (**4c**).* Yield 89%, white solid, m. p. 245–246 °C, ^1H NMR (500 MHz, DMSO-d_6) δ 4.17 (d, *J* = 5.99 Hz, 2H, NHCH_2), 7.48 (t, *J* = 7.44 Hz, 1H, Ar*H*), 7.63–7.67 (m, 1H, Ar*H*), 7.69 (dd, *J* = 6.82, 2.12 Hz, Ar*H*), 7.96 (dd, *J* = 6.82, 2.12 Hz, Ar*H*), 8.02 (dt, *J* = 16.02, 6.23 Hz, 1H, Ar*H*), 8.86 (t, *J* = 5.97 Hz, 1H, N*H*), 9.45 (s, 1H, Ar*H*), 10.11 (br, 1H, N*H*). $^{13}C\{^1H\}$ NMR (125 MHz, DMSO-d_6) δ 42.58, 105.91 (dd, *J* = 25.86, 22.23 Hz), 111.54 (d, *J* = 24.94 Hz), 121.53 (2 × C), 122.79, 128.47, 129.82 (2 × C), 135.32, 143.70, 145.19 (dd, *J* = 240.40, 15.64 Hz), 147.72, 149.57, 157.14, 158.71, 168.00. IR (KBr): 3334, 3228, 3116, 2919, 1712, 1678, 1525, 1495, 825 cm^{-1}. EIMS *m/z*: 412 (M^+ + 2, 2), 411 (M^+ + 1, 3), (M^+, 4), 238 (13), 237 (22), 236 (26), 235 (38), 208 (41), 207 (20), 206 (100), 180 (10), 179 (20), 151 (11), 149 (12), 148 (47), 147 (68), 138 (21), 119 (12), 111 (18). HRMS (EI) *m/z*: $[M]^+$ Calcd for $C_{17}H_{11}ClF_3N_5O_2$: 409.0553; Found 409.0545.

*4.1.2.4. N-(2-Oxo-2-((2,4,5-trifluorophenyl)amino)ethyl)-1-(4-(trifluoromethyl) phenyl)-1H-1,2,4-triazole-3-carboxamide (**4d**).* Yield 84%, light yellow solid, m.p. 177–178 °C, ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.18 (d, J = 5.50 Hz, 2H, NHCH_2), 7.65 (q, J = 9.61 Hz, 1H, ArH), 8.01 (d, J = 7.65 Hz, 2H, ArH), 8.03–8.04 (m, 1H, ArH), 8.17 (d, J = 7.90 Hz, 2H, ArH), 8.92 (t, J = 5.30 Hz, 1H, NH), 9.57 (s, 1H, ArH), 10.12 (br, 1H, NH). ^{13}C{^{1}H} NMR (125 MHz, DMSO-d_6) δ 42.63, 105.94 (dd, J = 25.98, 22.30 Hz), 111.62 (d, J = 23.94 Hz), 120.30 (2 × C), 122.78, 124.94, 127.21, 127.23, 128.49 (q, J = 32.50 Hz), 139.38, 144.19, 145.18 (dt, J = 243.33, 13.50 Hz), 147.78, 149.64, 157.42, 158.69, 168.01. IR (KBr): 3426, 3293, 3099, 2929, 1691, 1671, 1512, 1437, 1321, 1121, 1063, 842. cm^{-1}. EIMS m/z: 443 (M$^+$, 2), 297 (16), 269 (27), 241 (10), 240 (100), 213 (12), 172 (16), 147 (78), 145 (21). HRMS (EI) m/z: [M]$^+$ Calcd for $C_{18}H_{11}F_6N_5O_2$: 443.0817; Found 443.0812.

*4.1.2.5. N-(2-Oxo-2-((2,4,5-trifluorophenyl)amino)ethyl)-1-(3-(trifluoromethyl) phenyl)-1H-1,2,4-triazole-3-carboxamide (**4e**).* Yield 72%, white solid, m.p. 288–289 °C, ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.18 (d, J = 6.05 Hz, 2H, NHCH_2), 7.66 (td, J = 10.71, 7.50 Hz, 1H, ArH), 7.86–7.87 (m, 2H, ArH), 8.03 (dt, J = 15.98, 6.24 Hz, 1H, ArH), 8.25–8.28 (m, 1H, ArH), 8.32 (s, 1H, ArH), 8.92 (t, J = 6.03 Hz, 1H, NH), 9.59 (s, 1H, ArH), 10.11 (br, 1H, NH). ^{13}C{^{1}H} NMR (125 MHz, DMSO-d_6) δ 42.57, 105.92 (dd, J = 25.85, 22.19 Hz), 111.54 (d, J = 23.37 Hz), 116.46, 116.49, 123.60 (d, J = 272.57 Hz), 123.67, 124.92, 130.50 (q, J = 32.79 Hz), 131.29, 137.02, 144.10, 145.21 (dt, J = 223.08, 14.88 Hz), 147.69, 149.64, 157.23, 158.65, 167.99. IR (KBr): 3402, 3230, 3106, 1671, 1560, 1505, 1433, 1321, 1131, 801 cm^{-1}. EIMS m/z: 443 (M$^+$, 2), 391 (21), 297 (15), 269 (23), 257 (50), 241 (10), 240 (87), 217 (92), 213 (14), 172 (26), 165 (95), 159 (68), 147 (100), 146 (18), 145 (21), 139 (11), 119 (21). HRMS (EI) m/z: [M]$^+$ Calcd for $C_{18}H_{11}F_6N_5O_2$: 443.0817; Found 443.0812.

*4.1.2.6. 1-(4-Chlorophenyl)-5-(furan-2-yl)-N-(2-oxo-2-((2,4,5-trifluorophenyl) amino) ethyl)-1H-1,2,4-triazole-3-carboxamide (**4l**).* Yield 83%, white solid, m.p. 188–189 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.32 (d, J = 5.91 Hz, 2H, NHCH_2), 6.48 (d, J = 1.94 Hz, 1H, ArH), 6.77 (d, J = 2.97 Hz, 1H, ArH), 6.95 (td, J = 9.78, 6.90 Hz, 1H, ArH), 7.41 (d, J = 8.35 Hz, 1H, ArH), 7.46 (s, 1H, ArH), 7.49 (d, J = 8.62 Hz, 2H, ArH), 7.87 (br, 1H, NH), 8.22 (dt, J = 15.59, 5.85 Hz, 1H, ArH), 8.37 (br, 1H, NH). ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$) δ 44.74, 105.91 (t, J = 24.23 Hz), 110.61 (d, J = 24.39 Hz), 112.05, 114.55, 122.14, 127.25 (2 × C), 129.60 (2 × C), 135.82, 136.23, 141.28, 145.14, 145.33, 146.90, 147.34, 147.38, 155.88, 159.94, 166.74. IR (KBr): 3405, 3123, 2926, 1708, 1678, 1613, 1549, 1491,1436, 1230, 1090, 835 cm^{-1}. EIMS m/z: 475 (M$^+$, 5), 331 (16), 329 (45),304 (17), 303 (19), 302 (52), 301 (33), 274 (33), 273 (18), 272 (100),247 (17), 245 (50), 206 (12), 204 (35), 151 (22), 147 (24), 111 (23). HRMS (EI) m/z: [M]$^+$ Calcd for $C_{21}H_{13}ClF_3N_5O_3$: 475.0659; Found 475.0653.

*4.1.2.7. 1-(4-Chlorophenyl)-5-cyclopentyl-N-(2-oxo-2-((2,4,5-trifluorophenyl) amino)ethyl)-1H-1,2,4-triazole-3-carboxamide (**4m**).* Yield 74%, yellow solid, m.p. 159–160 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.55–1.57 (m, 2H, CH_2), 1.77–1.92 (m, 6H, CH_2), 3.05 (quint, J = 8.20 Hz, 1H, CH), 4.39 (d, J = 5.60 Hz, 2H, NHCH_2), 6.84 (td, J = 9.96, 7.07 Hz, 1H, ArH), 7.31 (dd, J = 6.78, 1.90 Hz, 2H, ArH), 7.43 (dd, J = 6.72, 1.92 Hz, 2H, ArH), 8.06 (dt, J = 15.45, 6.12 Hz, 1H, ArH), 9.12 (br, 1H, NH). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 25.65 (2 × C), 32.80 (2 × C), 36.03, 43.93, 104.77 (t, J = 23.28 Hz), 111.06 (d, J = 24.19 Hz), 121.15, 126.62 (2 × C), 129.60 (2 × C), 135.18, 135.54, 145.60 (d, J = 250.82 Hz), 145.86 (dt, J = 247.30, 14.53 Hz), 149.26 (d, J = 10.65 Hz), 155.28, 160.21, 161.89, 167.33. IR (KBr): 3402, 3293, 2960, 1682, 1542, 1495, 1433, 1209, 835 cm^{-1}. EIMS m/z: 477 (M$^+$, 5), 333 (12), 331 (36), 306 (26), 305 (24), 304 (82), 303 (32), 291 (11), 276 (24), 275 (15), 274 (74), 252 (30), 251 (12), 250 (100), 248 (17), 247 (18), 246 (37), 232 (28), 206 (13), 151 (27), 147 (16), 141 (22), 138 (10), 129 (12), 127 (57), 125 (62), 111 (19). HRMS (EI) m/z: [M]$^+$ Calcd for $C_{22}H_{19}ClF_3N_5O_2$: 477.1171; Found 477.1179.

*4.1.2.8. N-(2-Oxo-2-((2,4,5-trifluorophenyl)amino)ethyl)-5-(1H-pyrrol-2-yl)-1- (3-(trifluoromethyl)phenyl)-1H-1,2,4-triazole-3-carboxamide (**4n**).* Yield 73%, light yellow solid, m.p. 181–182 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.38 (d, J = 5.36 Hz, 2H, NHCH_2), 5.83 (s, 1H, ArH), 6.01 (dd, J = 6.00, 2.55 Hz, 1H, ArH), 6.90–6.96 (m, 2H, ArH), 7.65–7.71 (m, 2H, ArH), 7.80–7.83 (m, 2H, ArH), 7.94 (t, J = 5.20 Hz, 1H, NH), 8.16 (dt, J = 16.03, 5.60 Hz, 1H, ArH), 8.60 (br, 1H, NH), 9.78 (br, 1H, NH). ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$) δ 44.38, 104.95 (t, J = 23.49 Hz), 110.60, 110.82 (d, J = 23.88 Hz), 111.49, 117.77, 122.02, 122.31, 123. 63 (d, J = 3.43 Hz), 124.19, 127.07 (d, J = 3.81 Hz), 129.68, 130.36, 132.47 (q, J = 33.77 Hz), 137.88,145.28, 146.92, 148.80, 150.06, 155.47, 160.12, 166.93. IR (KBr): 3276, 3079, 2926, 1675, 1549, 1501, 1437, 1328, 1131 cm^{-1}. EIMS m/z: 508 (M$^+$, 10), 362 (34), 361 (24), 335 (40), 334 (28), 306 (18), 305 (100), 278 (32), 277 (25), 237 (42), 185 (13), 147 (15), 145 (22), 92 (13). HRMS (EI) m/z: [M]$^+$ Calcd for $C_{22}H_{14}F_6N_6O_2$: 508.1082; Found 508.1088.

*4.1.2.9. 5-Butyl-1-(4-chlorophenyl)-N-(2-oxo-2-((2,4,5-trifluorophenyl) amino) ethyl)-1H-1,2,4-triazole-3-carboxamide (**4o**).* Yield 82%, white solid, m.p. 131–132 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85 (t, J = 7.35 Hz, 3H, CH_3), 1.30 (sext, J = 7.52 Hz, 2H, CH_2), 1.70 (quint, J = 7.64 Hz, 2H, CH_2), 2.74 (t, J = 7.73 Hz, 2H, CH_2), 4.35 (d, J = 5.80 Hz, 2H, NHCH_2), 6.90 (td, J = 9.82, 7.21 Hz, 1H, ArH), 7.35 (d, J = 8.60 Hz, 2H, ArH), 7.47 (d, J = 8.60 Hz, 2H, ArH), 7.94 (t, J = 5.50 Hz, 1H, NH), 8.14 (dt, J = 15.86, 5.85 Hz), 8.77 (br, 1H, NH). ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$) δ 13.54, 22.17, 26.20, 29.51, 44.34, 104.85 (t, J = 23.61 Hz), 111.89 (d, J = 24.60 Hz), 122.18, 126.38 (2 × C), 129.75 (2 × C), 135.19, 135.67, 145.83 (dt, J = 249.22, 12.94 Hz), 146.14 (ddd, J = 243.81, 12.74, 2.93 Hz), 148.81, 155.36, 157.87, 160.26, 167.13. IR (KBr): 3313, 2963, 2933, 2871, 1729, 1678, 1546, 1495, 1433, 1216, 1090, 1015, 835 cm^{-1}. EIMS m/z: 465 (M$^+$, 6), 321 (18), 319 (51), 294 (26), 293 (27), 292 (76), 291 (47), 264 (33), 263 (20), 262 (100), 237 (13), 236 (11), 234 (29), 194 (13), 193 (19), 192 (12). HRMS (EI) m/z: [M]$^+$ Calcd for $C_{21}H_{19}ClF_3N_5O_2$: 465.1179; Found 465.1176.

*4.1.2.10. N-(2-Oxo-2-((2,4,5-trifluorophenyl)amino)ethyl)-1,5-diphenyl-1H-1,2,4- triazole-3-carboxamide (**4p**).* Yield 82%, white solid, m.p. 210–212 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.34 (d, J = 6.04 Hz, 2H, NHCH_2), 6.94 (td, J = 9.99, 7.02 Hz, 1H, ArH), 7.32–7.38 (m, 4H, ArH), 7.41–7.46 (m, 4H, ArH), 7.47–7.50 (m, 2H, ArH), 7.96 (t, J = 5.92 Hz, 1H, NH), 8.21 (dt, J = 15.77, 5.98 Hz, 1H, ArH), 8.55 (br, 1H, NH). ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$) δ 44.70, 104.89 (t, J = 23.29 Hz), 110.66 (d, J = 24.61 Hz), 122.18, 125.45 (2 × C), 126.72, 128.72 (2 × C), 128.96 (2 × C), 129.51 (2 × C), 129.64, 130.70, 137.55,145.82 (d, J = 249.18 Hz), 146.41, 148.93, 155.24, 155.50, 160.40, 166.98. IR (KBr): 3286, 3069, 1671, 1546, 1498, 1433, 1209, 692 cm^{-1}. EIMS m/z: 451 (M$^+$, 5), 305 (39), 279 (14), 278 (92), 277 (31), 249 (19), 248 (100), 222 (13), 221 (58), 180 (56), 147 (12), 117 (19), 77 (22). HRMS (EI) m/z: [M]$^+$ Calcd for $C_{23}H_{16}F_3N_5O_2$: 451.1256; Found 451.1251.

*4.1.2.11. N-(2-Oxo-2-((2,4,5-trifluorophenyl)amino)ethyl)-5-phenyl-1-(p-tolyl)-1H- 1,2,4- triazole-3-carboxamide (**4q**).* Yield 84%, yellow solid, m.p. 121–122 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.34 (s, 3H, CH_3), 4.44 (d, J = 5.64 Hz, 2H, NHCH_2), 6.80 (td, J = 9.91, 7.15 Hz, 1H, ArH), 7.14 (s, 4H, ArH), 7.26 (t, J = 7.52 Hz, 2H, ArH), 7.35 (t, J = 7.38 Hz, 1H, ArH), 7.42 (d, J = 7.80 Hz, 2H, ArH), 8.02 (dt, J = 15.79, 5.95 Hz, 1H, ArH), 8.21 (t, J = 5.62 Hz, 1H, NH), 9.22 (br, 1H, NH). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 21.07, 43.88, 104.69 (t, J = 23.40 Hz), 111.25 (d, J = 23.75 Hz), 122.13, 125.03 (2 × C), 126.68, 128.45 (2 × C), 128.75 (2 × C), 129.87 (2 × C), 130.36, 134.92, 139.64, 144.67, 146.70, 149.45, 154.85, 155.45, 160.18, 167.44. IR (KBr): 3286, 3058, 2922, 1739, 1695, 1549, 1512, 1430, 1209 cm^{-1}. EIMS m/z: 465 (M$^+$, 5), 447

(13), 391 (12), 319 (32), 293 (19), 292 (91), 291 (18), 279 (12), 263 (25), 262 (100), 236 (19), 235 (84), 195 (15), 194 (87), 147 (16), 132 (23), 131 (40), 129 (13), 105 (54), 104 (29), 103 (12). HRMS (EI) *m/z*: $[M]^+$ Calcd for $C_{24}H_{18}F_3N_5O_2$: 465.1413; Found 465.1406.

4.1.3. Standard procedure for synthesis of β-amino esters ***5d*** *and* ***5m–q*** *[39,43]*

The 1*H*-1,2,4-triazol-3-ylmethanols **12d**, and **12m–q** (1.0 equiv, 3.0 mmol) were treated with Boc-(*R*)-3-amino-4-(2,4,5-trifluoro-phenyl)-butyric acid **14** (1.1 equiv) and *N,N*-bis[2-*oxo*-3-*oxazolidinyl*]phosphorodiamide chloride (BOP-Cl, 2.0 equiv) in presence of NEt_3 (2.0 equiv) in CH_2Cl_2 solution (20 mL) [39]. The reaction mixture was stirred at room temperature for 4 h. When the reaction was completed, the reaction mixture was washed with water (15 mL) and extracted with CH_2Cl_2 (15 mL × 3). The combined organic layers were washed sodium bicarbonate (15 mL × 3), dried over $MgSO_4$ to give crude Boc-protected β-amino esters **15d** and **15m–q**. The reaction mixture of crude Boc-protected β-amino esters **15d** was purified by column chromatography on silica gel to give the pure corresponding β-amino esters **15d** in 84% yield. The crude intermediates **15m–q** were directly carried out the deprotection without the further purification.

Boc-Protected β-amino esters **15d** and **15m–q** (1.0 equiv) were added with trifluoroacetic acid (TFA, 1.5 equiv) in CH_2Cl_2 solution (15 mL) [43]. The reaction mixture was stirred at room temperature for 3 h. When the reaction was completed, the reaction mixture was nertulized with sodium bicarbonate (15 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford the corresponding crude β-amino esters **5d** and **5m–q**. The crude products **5d** and **5m–q** were purified by column chromatography on silica gel to give the pure corresponding β-amino esters **5d** and **5m–q** in 74–84% yields.

4.1.3.1. (1-(4-(Trifluoromethyl)phenyl)-1H-1,2,4-triazol-3-yl)methyl (R)-3-((tert- butoxycarbonyl)amino)-4-(2,4,5-trifluorophenyl)butanoate ***(15d)***. Yield 84%, light yellow solid, m.p. 89–91 °C, ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.33 (s, 9H, C*H*$_3$), 2.60–2.69 (m, 2H, C*H*$_2$), 2.86 (br, 2H, C*H*$_2$), 4.16 (br, 1H, C*H*), 5.32 (d, J = 6.15 Hz, 2H, C*H*$_2$), 6.84 (dd, J = 15.36, 8.79 Hz, 1H, Ar*H*), 7.03 (dd, J = 15.62, 8.73 Hz, 1H, Ar*H*), 7.75 (d, J = 7.92 Hz, 2H, Ar*H*), 7.80 (d, J = 8.30 Hz, 2H,Ar*H*), 8.62 (s, 1H, Ar*H*). ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 28.18 (3 × C), 32.90, 37.92, 47.69, 59.16, 79.52, 105.27 (dd, J = 28.60, 20.84 Hz), 119.07 (d, J = 18.93 Hz), 119.85 (2 × C), 121.32 (d, J = 18.72 Hz), 123.47 (d, J = 272.17 Hz), 127.12, 127.14, 130.37 (q, J = 33.06 Hz), 139.14, 141.87, 145.57 (dd, J = 244.16, 12.51 Hz), 148.83 (dt, J = 251.26, 13.54 Hz), 155.14, 156.20 (dd, J = 244.02, 8.84 Hz), 160.78, 170.78. IR (KBr): 3354, 2977, 2929, 1739, 1705, 1692, 1522, 1328, 1168, 842 cm^{-1}. ESIMS *m/z*: 581 ($[M+Na]^+$, 15), 560 (25), 559 ($[M+H]^+$, 100), 395 (11), 377 (14). HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{25}H_{25}F_6N_4O_4$: 559.1775; Found 559.1785.

4.1.3.2. (1-(4-(Trifluoromethyl)phenyl)-1H-1,2,4-triazol-3-yl)methyl (R)-3-amino-4- (2,4,5-trifluorophenyl)butanoate ***(5d)***. Yield: 76%, light yellow solid, m.p. 135–136 °C, ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.36 (dd, J = 15.78, 8.63 Hz, 1H, C*H*$_2$), 2.51 (dd, J = 15.76, 4.10 Hz, 1H, C*H*$_2$), 2.59 (dd, J = 13.71, 7.65 Hz, 1H, C*H*$_2$), 2.67 (dd, J = 13.76, 6.00 Hz, 1H, C*H*$_2$), 3.42 (quint, J = 6.53 Hz, 1H, C*H*), 5.22 (d, J = 1.60 Hz, 2H, OC*H*$_2$), 6.78 (td, J = 9.52, 6.80 Hz, 1H, Ar*H*), 6.98 (dd, J = 16.93, 8.98 Hz, 1H, Ar*H*), 7.65 (d, J = 8.60 Hz, 2H, Ar*H*), 7.72 (d, J = 8.50 Hz, 2H, Ar*H*), 8.60 (s, 1H, Ar*H*). ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 35.14, 41.34, 48.58, 58.84, 105.18 (dd, J = 28.77, 20.72 Hz), 118.79 (dd, J = 18.88, 6.00 Hz), 119.48 (2 × C), 121.60 (dt, J = 18.17, 4.55 Hz), 123.33 (d, J = 272.19 Hz), 126.82, 126.85, 129.87 (q, J = 33.17 Hz), 138.97, 141.2, 146.33 (ddd, J = 244.43, 12.33, 3.25 Hz), 148.51 (dt, J = 249.67, 13.35 Hz), 155.91 (dd, J = 244.11, 7.28 Hz), 160.65, 171.22. IR (KBr): 3221, 3116, 2922, 1729, 1617, 1549, 1522, 1335, 1179, 1138, 1049, 845 cm^{-1}. ESIMS *m/z*: 460 (22), 459 (M + H$^+$, 100), 317 (3), 258 (4), 244 (7), 173 (3). HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{20}H_{16}F_6N_4O_2$: 459.1177; Found 459.1244.

4.1.3.3. (1-(4-Chlorophenyl)-5-cyclopentyl-1H-1,2,4-triazol-3-yl)methyl (R)-3- amino-4-(2,4,5-trifluorophenyl)butanoate ***(5m)***. Yield 79%, deep yellow liquid ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.57 (t, J = 5.50 Hz, 2H, CP), 1.64 (br, 2H, N*H*$_2$), 1.80–1.93 (m, 6H, CP), 2.37 (dd, J = 15.69, 8.58 Hz, 1H, C*H*$_2$), 2.54 (dd, J = 15.69, 4.04 Hz, 1H, C*H*$_2$), 2.62 (dd, J = 13.66, 7.67 Hz, 1H, C*H*$_2$), 2.71 (dd, J = 13.73, 5.87 Hz, 1H, C*H*$_2$), 3.04 (quint, J = 8.19 Hz, 1H, CP), 3.45 (quint, J = 6.50 Hz, 1H, C*H*), 5.19 (d, J = 2.19 Hz, 2H, OC*H*$_2$), 6.86 (dd, J = 9.42, 6.86 Hz, 1H, Ar*H*), 7.02 (dd, J = 16.91, 8.99 Hz, 1H, Ar*H*), 7.32 (d, J = 8.56 Hz, 2H, Ar*H*), 7.44 (d, J = 8.55 Hz, 2H, Ar*H*). ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$) δ 25.68 (2 × C), 32.90 (2 × C), 36.15, 36.20, 41.77, 48.74, 59.48, 105.37 (dd, J = 28.74, 20.68 Hz), 118.96 (dd, J = 18.86, 5.99 Hz), 121.88 (dt, J = 18.14, 4.49 Hz), 126 63 (2 × C), 129.59 (2 × C), 135.07, 135.70, 146.53 (ddd, J = 244.68, 12.43, 3.30 Hz), 148.70 (dt, J = 250.01, 13.44 Hz), 156.09 (dd, J = 244.24, 9.06 Hz), 158.53, 161.37, 171.41. IR (KBr): 3374, 3296, 3057, 2958, 2869, 1736, 1519, 1488, 1151, 1093, 836 cm^{-1}. ESIMS *m/z*: 492 ($[M+H]^+$ + 2, 34),494 (26), 493 ($[M+H]^+$, 100), 381 (3), 292 (4). HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{24}H_{25}ClF_3N_4O_2$: 493.1613; Found 493.1601.

4.1.3.4. (5-(1H-Pyrrol-2-yl)-1-(3-(trifluoromethyl)phenyl)-1H-1,2,4-triazol-3-yl) methyl(R)-3-amino-4-(2,4,5-trifluorophenyl)butanoate ***(5n)***. Yield 74%, yellow liquid, ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.01 (br, 2H, N*H*$_2$), 2.41 (dd, J = 15.61, 8.80 Hz, 1H, C*H*$_2$), 2.59 (dd, J = 15.58, 3.98 Hz, 1H, C*H*$_2$), 2.66 (dd, J = 13.73, 7.73 Hz, 1H, C*H*$_2$), 2.74 (dd, J = 13.76, 5.90 Hz, 1H, C*H*$_2$), 3.51 (quint, J = 6.58 Hz, 1H, C*H*), 5.25 (d, J = 6.55 Hz, 2H, OC*H*$_2$), 5.81 (d, J = 3.35 Hz, 1H, Ar*H*), 6.07 (t, J = 2.83 Hz, 1H, Ar*H*), 6.84–6.89 (m, 2H, Ar*H*), 7.02 (dd, J = 17.05, 8.85 Hz, 1H, Ar*H*), 7.64 (t, J = 7.80 Hz, 1H, Ar*H*), 7.70 (d, J = 8.10 Hz, 1H, Ar*H*), 7.78 (d, J = 7.90 Hz, 1H, Ar*H*), 7.80 (s, 1H, Ar*H*), 10.20 (br, 1H, N*H*). ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$) δ 36.21, 41.85, 48.82, 59.07, 105.44 (dd, J = 28.74, 20.71 Hz), 110.23, 110.87, 118.39, 118.96 (dd, J = 18.86, 5.87 Hz), 121.65, 121.83 (t, J = 4.51 Hz), 123.20 (d, J = 272.65 Hz), 123.58 (d, J = 3.52 Hz), 126.52 (d, J = 3.37 Hz), 129.68, 130.20, 132.29 (q, J = 33.46 Hz), 138.33, 146.58 (ddd, J = 244.74, 12.39, 3.26 Hz), 148.78 (dt, J = 250.09, 13.40 Hz), 149.50, 156.12 (dd, J = 243.94, 9.22 Hz), 158.96, 171.50. IR (KBr): 3360, 3289, 3071, 2926, 2855, 1738, 1599, 1520, 1329, 1175, 1134, 739 cm^{-1}. EIMS *m/z*: 523 (M$^+$, 5), 379 (17), 378 (100), 310 (14), 309 (95), 307 (20), 292 (21), 291 (95), 199 (13), 170 (23), 151 (41), 145 (24). HRMS (EI) *m/z*: $[M]^+$ Calcd for $C_{24}H_{19}F_6N_5O_2$: 523.1443; Found 523.1448.

4.1.3.5. (5-Butyl-1-(4-chlorophenyl)-1H-1,2,4-triazol-3-yl)methyl (R)-3-amino-4- (2,4,5-trifluorophenyl)butanoate ***(5o)***. Yield: 78%, orange liquid, ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.79 (t, J = 7.36 Hz, 3H, C*H*$_3$), 1.25 (sext, J = 7.44 Hz, 2H, C*H*$_2$CH$_3$), 1.63 (quint, J = 7.67 Hz, 2H, C*H*$_2$CH$_2$CH$_3$), 1.83 (br, 2H, N*H*$_2$), 2.34 (dd, J = 15.75, 8.58 Hz, 1H, C*H*$_2$), 2.51 (dd, J = 15.79, 3.90 Hz, 1H, C*H*$_2$), 2.59 (dd, J = 13.56, 7.64 Hz, 1H, C*H*$_2$), 2.65–2.70 (m, 3H, C*H*$_2$CH$_2$CH$_2$CH$_3$, C*H*$_2$), 3.42 (br, 1H, C*H*), 5.15 (s, 2H, OC*H*$_2$), 6.81 (td, J = 9.57, 6.69 Hz, 1H, Ar*H*), 6.98 (dd, J = 17.05, 8.89 Hz, 1H, Ar*H*), 7.28 (d, J = 8.70 Hz, 2H, Ar*H*), 7.40 (d, J = 8.68 Hz, 2H, Ar*H*). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 13.40, 22.10, 26.06, 29.53, 36.03, 41.52, 48.58, 59.23, 105.25 (dd, J = 28.62, 20.78 Hz), 118.85 (dd, J = 18.71, 5.51 Hz), 121.73 (dt, J = 18.01, 4.22 Hz), 126 11 (2 × C), 129.50 (2 × C), 134.86, 135.45, 146.39 (dd, J = 244.75, 12.70 Hz), 148.55 (dt, J = 249.64, 13.41 Hz), 155.91 (d, J = 244.68 Hz), 157.21, 158.47, 171.30. IR (KBr): 3378, 3289, 3057, 2960, 2933, 1738, 1519, 1489, 1152, 1093, 837 cm^{-1}. ESIMS *m/z*: 483 ($[M+H]^+$ + 2, 35), 482 (26), 481 ($[M+H]^+$, 100), 289 (4), 280 (5). HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{23}H_{25}ClF_3N_4O_2$: 481.1613; Found 481.1601.

*4.1.3.6. (1,5-Diphenyl-1H-1,2,4-triazol-3-yl)methyl (R)-3-amino-4-(2,4,5-trifluorophenyl)butanoate (**5p**).* Yield: 83%, orange liquid, ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.42 (dd, J = 15.76, 8.60 Hz, 1H, CH_2), 2.58 (dd, J = 15.78, 4.08 Hz, 1H, CH_2), 2.64 (dd, J = 13.76, 7.65 Hz, 1H, CH_2), 2.73 (dd, J = 13.76, 5.90 Hz, 1H, CH_2), 3.48 (quint, J = 6.55 Hz, 1H, CH), 5.30 (d, J = 4.35 Hz, 2H, OCH_2), 6.85 (td, J = 9.60, 6.65 Hz, 1H, ArH), 7.02 (dd, J = 17.11, 8.85 Hz, 1H, ArH), 7.29–7.31 (m, 4H, ArH), 7.36–7.39 (m, 4H, ArH), 7.43–7.45 (m, 2H, ArH). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 36.20, 41.73, 48.71, 59.41, 105.36 (dd, J = 28.82, 20.59 Hz), 118.97 (dd, J = 18.81, 6.12 Hz), 121.86 (d, J = 18.45 Hz), 125.25 (2 × C), 127.36, 128.51 (2 × C), 128.82 (2 × C), 129.00, 129.35 (2 × C), 130.16, 137.84, 146.52 (dd, J = 244.77, 12.59 Hz), 148.68 (dt, J = 249.87, 13.35 Hz), 154.84, 156.06 (dd, J = 243.48, 9.19 Hz), 158.92, 163.24, 171.46. IR (KBr): 3370, 3299, 3064, 2929, 2858, 1738, 1519, 1424, 1152, 766, 694 cm^{-1}. EsIMS m/z: 478 (27), 467 (M^+, 100), 381 (2), 266 (6). HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{25}H_{22}F_3N_4O_2$: 467.1689; Found 467.1680.

*4.1.3.7. (5-Phenyl-1-(p-tolyl)-1H-1,2,4-triazol-3-yl)methyl (R)-3-amino-4-(2,4,5- trifluorophenyl)butanoate (**5q**).* Yield: 84%, light yellow solid, m.p. 71–73 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.34 (s, 3H, CH_3), 2.40 (dd, J = 15.71, 8.60 Hz, 1H, CH_2), 2.57 (dd, J = 15.68, 4.08 Hz, 1H, CH_2), 2.62 (dd, J = 13.77, 7.70 Hz, 1H, CH_2), 2.72 (dd, J = 13.76, 5.85 Hz, 1H, CH_2), 3.47 (quint, J = 6.47 Hz, 1H, CH), 5.28 (d, J = 4.90 Hz, 2H, OCH_2), 6.83 (td, J = 14.32, 6.69 Hz, 1H, ArH), 7.01 (dd, J = 16.98, 8.98 Hz, 1H, ArH), 7.16 (s, 4H, ArH), 7.27 (t, J = 7.50 Hz, 2H, ArH), 7.34 (t, J = 7.38 Hz, 1H, ArH), 7.43 (d, J = 7.30 Hz, 2H, ArH). ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$) δ 20.91, 36.03, 41.65, 48.61, 59.27, 105.17 (dd, J = 28.78, 20.72 Hz), 118.83 (dd, J = 18.82, 6.02 Hz), 121.81 (dt, J = 18.11, 4.55 Hz), 124.93 (2 × C), 127.34, 128.31 (2 × C), 128.63 (2 × C), 129.76 (2 × C), 129.90, 135.26, 139.00, 146.33 (dd, J = 245.24, 12.39 Hz), 148.49 (dt, J = 249.95, 13.62 Hz), 154.56, 155.92 (dd, J = 241.75, 8.93 Hz), 158.63, 171.29. IR (KBr): 3449, 3374, 3299, 3050, 2954, 2926, 2858, 1738, 1519, 1423, 1151, 823, 732, 696 cm^{-1}. ESIMS m/z: 482, (28), 481 ($[M+H]^+$, 100), 468 (2), 280 (6). HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{26}H_{24}F_3N_4O_2$: 481.1846; Found 481.1845.

*4.1.4. Standard procedure for synthesis of β-amino amides **6d** and **6m–q** [39,43]*

The 1*H*-1,2,4-triazol-3-ylmethanamines **13d** and **13m–q** (1.0 equiv, 3.0 mmol) were treated with Boc-(*R*)-3-amino-4-(2,4,5-trifluoro-phenyl)-butyric acid **14** (1.1 equiv) and *N*,*N*-bis[2-*oxo*-3-*oxazolidinyl*]phosphorodiamide chloride (BOP-Cl, 2.0 equiv) in presence of NEt_3 (2.0 equiv) in CH_2Cl_2 solution (20 mL) [39]. The reaction mixture was stirred at room temperature for 4 h. When the reaction was completed, the reaction mixture was washed with water (15 mL) and extracted with CH_2Cl_2 (15 mL × 3). The combined organic layers were washed sodium bicarbonate (15 mL × 3), dried over $MgSO_4$ to give crude Boc-protected β-amino amides **16d** and **16m–q**. Without further purification, the crude intermediates **16d** and **16m–q** were directly carried out the deprotection.

Boc-Protected β-amino amides **16d** and **16m–q** (1.0 equiv) were added with trifluoroacetic acid (TFA, 1.5 equiv) in CH_2Cl_2 solution (15 mL) [43]. The reaction mixture was stirred at room temperature for 3 h. When the reaction was completed, the reaction mixture was nertulized with sodium bicarbonate (15 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford the corresponding crude β-amino amides **6d** and **6m–q**. The crude products **6d** and **6m–q** were purified by column chromatography on silica gel to give the pure corresponding β-amino esters **6d** and **6m–q** in 74–88% yields.

*4.1.4.1. (R)-3-Amino-N-((1-(4-(trifluoromethyl)phenyl)-1H-1,2,4-triazol-3-yl) methyl)-4-(2,4,5-trifluorophenyl)butanamide (**6d**).* Yield 79%, light yellow solid, m.p. 50–51 °C, ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.25 (dd, J = 15.09, 9.28 Hz, 1H, CH_2), 2.46 (dd, J = 15.19, 1.90 Hz, 1H, CH_2), 2.66 (dd, J = 13.41, 7.88 Hz, 1H, CH_2), 2.76 (dd, J = 13.77, 5.35 Hz, 1H, CH_2), 3.44 (d, J = 6.71 Hz, CH), 4.63 (d, J = 5.15 Hz, 2H, NHCH_2), 6.89 (q, J = 8.47 Hz, 1H, ArH), 7.03 (q, J = 8.55 Hz, 1H, ArH), 7.72–7.77 (m, 4H, ArH), 8.54 (s, 1H, ArH). ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz): δ 37.19 (2 × C), 42.66, 49.09, 105.55 (dd, J = 28.73, 20.45 Hz), 118.99 (dd, J = 18.82, 5.92 Hz), 119.60 (2 × C), 121.57 (d, J = 17.93 Hz), 123.50 (d, J = 273.01 Hz), 127.06, 127.10, 130.06 (d, J = 33.53 Hz), 139.21, 141.58, 146.54 (d, J = 243.56 Hz), 148.74 (dd, J = 249.08, 15.03 Hz), 156.06 (d, J = 245.32 Hz), 162.74, 171.46. IR (KBr): 3364, 3092, 2926, 1617, 1535, 1325, 1165, 1124, 841 cm^{-1}. ESIMS m/z: 459 (23), 458 ($[M+H]^+$, 100), 285 (17), 243 (20). HRMS (EI) m/z: $[M+H]^+$ Calcd for $C_{20}H_{18}F_6N_5O$: 458.1410; Found 458.1414

*4.1.4.2. (R)-3-Amino-N-((1-(4-chlorophenyl)-5-cyclopentyl-1H-1,2,4-triazol-3-yl) methyl)-4-(2,4,5-trifluorophenyl)butanamide (**6m**).* Yield 83%, yellow liquid, ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.59–1.61 (m, 2H, CH_2), 1.85–1.90 (m, 4H, CH_2), 1.94–1.96 (m, 2H, CH_2), 2.29 (dd, J = 14.73, 8.78 Hz, 1H, CH_2), 2.46 (dd, J = 14.66, 2.25 Hz, 1H, CH_2), 2.68–2.71 (m, 1H, CH_2), 2.73 (br, 2H, NH_2), 2.79 (dd, J = 13.53, 5.63 Hz, 1H, CH_2), 3.06 (quint, J = 7.95 Hz, 1H, CH), 3.49 (br, 1H, CH), 4.55 (t, J = 4.13 Hz, 2H, NCH_2), 6.90 (dt, J = 9.43, 6.74 Hz, 1H, ArH), 7.07 (dd, J = 16.73, 8.98 Hz, 1H, ArH), 7.34 (d, J = 8.45 Hz, 2H, ArH), 7.47 (d, J = 8.45 Hz, 2H, ArH), 7.65 (br, 1H, NH_2). ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$) δ 25.54 (2 × C), 32.77 (2 × C), 36.01, 36.25, 37.25, 42.31, 48.98, 105.29 (dd, J = 28.66, 20.70 Hz), 118.94 (dd, J = 18.86, 5.89 Hz), 121.56 (d, J = 18.37 Hz), 126 49 (2 × C), 129.46 (2 × C), 134.84, 135.62, 146.44 (dd, J = 245, 12.10 Hz), 148.61 (dt, J = 250.07, 13.34 Hz), 155.97 (dd, J = 243.82, 7.98 Hz), 160.12, 161.03, 171.18. IR (KBr): 3434, 3349, 3289, 3054, 2957, 2872, 1659, 1518, 1424, 1210, 1093, 837 cm^{-1}. EIMS m/z: 491 (M^+, 1), 346 (10), 277 (21), 261 (18), 260 (21). HRMS (EI) m/z: $[M]^+$ Calcd for $C_{24}H_{25}ClF_3N_5O$: 491.1700; Found 491.1704.

*4.1.4.3. (R)-N-((5-(1H-Pyrrol-2-yl)-1-(3-(trifluoromethyl)phenyl)-1H-1,2,4-triazol- 3-yl)methyl)-3-amino-4-(2,4,5-trifluorophenyl)butanamide (**6n**).*. Yield 74%, light yellow liquid, ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.30 (dd, J = 14.86, 9.25 Hz, 1H, CH_2), 2.45 (dd, J = 14.86, 3.10 Hz, 1H, CH_2), 2.62–2.66 (m, 3H, CH_2 and NH_2), 2.73 (dd, J = 13.73, 5.83 Hz, 1H, CH_2), 3.49 (d, J = 6.75 Hz, 1H, CH), 4.56 (qd, J = 15.91, 5.32 Hz, 2H, NCH_2), 5.76 (d, J = 3.10 Hz, 1H, ArH), 6.03 (t, J = 3.10 Hz, 1H, ArH), 6.81–6.86 (m, 2H, ArH), 6.97 (dd, J = 16.81, 8.90 Hz, 1H, ArH), 7.57–7.63 (m, 2H, ArH), 7.72–7.74 (m, 2H, ArH), 7.97 (t, J = 5.10 Hz, 1H, NH), 10.57 (br, 1H, NH). ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$) δ 36.48, 37.11, 42.52, 49.13, 105.45 (dd, J = 28.70, 20.68 Hz), 110.08, 110.89, 118.24, 118.94 (dd, J = 18.81, 5.86 Hz), 121.48 (d, J = 18.07 Hz), 121.71, 123.19 (d, J = 272.62 Hz), 123.43 (d, J = 3.52 Hz), 126.31 (d, J = 3.26 Hz), 129.57, 130.10, 132.14 (q, J = 33.41 Hz), 138.34, 146.57 (ddd, J = 244.91, 12.23, 3.43 Hz), 148.78 (dt, J = 250.27, 13.42 Hz), 149.32, 156.08 (dd, J = 242.58, 8.84 Hz), 160.63, 171.76. IR (KBr): 3358, 3285, 3075, 2926, 2855, 1738, 1649, 1597, 1519, 1329, 1176, 1133, 1070, 738 cm^{-1}. EIMS m/z: 523 (M^+, 3), 378 (14), 377 (80), 309 (17), 308 (100), 306 (27), 292 (20), 291 (57), 171 (10), 151 (18), 145 (11). HRMS (EI) m/z: $[M]^+$ Calcd for $C_{24}H_{20}F_6N_6O$: 522.1603; Found 522.1612.

*4.1.4.4. (R)-3-Amino-N-((5-butyl-1-(4-chlorophenyl)-1H-1,2,4-triazol-3-yl)methyl)- 4-(2,4,5-trifluorophenyl)butanamide (**6o**).* Yield: 81%, yellow liquid, ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.82 (t, J = 7.33 Hz, 3H, CH_3), 1.28 (sext, J = 7.60 Hz, 2H, CH_2CH$_3$), 1.62 (quint, J = 7.63 Hz, 2H, CH_2CH$_2$CH$_3$), 2.41 (br, 1H, CH_2), 2.50–2.52 (m, 1H, CH_2), 2.68 (t, J = 7.67 Hz, 2H, CH_2), 2.86 (br, 2H, CH_2), 3.58 (br, 1H, CH), 4.11 (br, 2H, NH_2), 4.52 (s, 2H, NCH_2), 6.86 (td, J = 16.05, 9.32 Hz, 1H, ArH), 6.98 (dd, J = 15.14, 7.88 Hz, 1H, ArH), 7.30 (d, J = 8.41 Hz, 2H, ArH), 7.44 (d, J = 8.47 Hz, 2H, ArH) 7.60 (br, 1H, NH). ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$) δ 13.53, 22.22, 26.15, 29.78, 36.14, 37.35, 42.04, 49.18, 105.53 (dd, J = 28.50, 20.65 Hz), 119.13 (dd, J = 18.82, 5.74 Hz), 121.35 (d, J = 20.24 Hz), 126 25 (2 × C), 129.67 (2 × C), 135.00, 135.67, 146.63 (d,

J = 260.46 Hz), 148.88 (d, J = 250.23 Hz), 156.13 (d, J = 243.66 Hz), 157.12, 160.36, 171.15. IR (KBr): 3273, 3054, 2961, 2933, 2874, 1667, 1519, 1424, 1211, 1151, 1094, 838, 731 cm^{-1}. EIMS *m/z*: 479 (M^+, 1), 336 (14), 332 (42), 267 (30), 266 (16), 265 (100), 263 (16), 250 (11), 249 (18), 248 (26), 165 (15), 137 (13), 125 (10). HRMS (EI) *m/z*: $[M]^+$ Calcd for $C_{23}H_{25}F_3N_5O$: 479.1700; Found 479.1705.

4.1.4.5. (R)-3-Amino-N-((1,5-diphenyl-1H-1,2,4-triazol-3-yl)methyl)-4-(2,4,5- trifluorophenyl)butanamide ***(6p)***. Yield 88%, orange liquid, ^1H NMR (500 MHz, $CDCl_3$) δ 2.25 (dd, J = 14.98, 8.93 Hz, 1H, CH_2), 2.45 (d, 2.58 J = 14.91 Hz, 1H, CH_2), 2.65 (dd, J = 15.53, 7.93 Hz, 1H, CH_2), 2.76 (dd, J = 13.68, 5.34 Hz, 1H, CH_2), 3.45 (br, 1H, CH), 4.64 (d, J = 5.20 Hz, 2H, NCH_2), 6.84–6.89 (m, 1H, ArH), 7.01 (dd, J = 17.08, 8.78 Hz, 1H, ArH), 7.28–7.31 (m, 4H, ArH), 7.35–7.38 (m, 4H, ArH), 7.41–7.42 (m, 2H, ArH), 7.46 (br, 1H, NH). $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$) δ 36.85, 37.42, 42.89, 49.07, 105.44 (dd, J = 28.77, 20.81 Hz), 119.03 (dd, J = 18.73, 6.30 Hz), 121.79 (d, J = 18.16 Hz), 125.26 (2 × C), 127.54, 128.57 (2 × C), 128.79 (2 × C), 128.93, 129.37 (2 × C), 130.15, 137.94, 146.63 (d, J = 241.14 Hz), 148.75 (d, J = 249.40 Hz), 154.63, 156.15 (dd, J = 244.04 Hz), 160.64, 171.35. IR (KBr): 3430, 3285, 3061, 2929, 1652, 1517, 1423, 1210, 1154, 765, 695 cm^{-1}. EIMS *m/z*: 465 (M^+, 2), 321 (13), 321 (74), 252 (17), 251 (100), 249 (19), 235 (21), 234 (37), 131 (12), 103 (20). HRMS (EI) *m/z*: $[M]^+$ Calcd for $C_{25}H_{22}F_3N_5O$: 465.1776; Found 465.1769.

4.1.4.6. (R)-3-Amino-N-((5-phenyl-1-(p-tolyl)-1H-1,2,4-triazol-3-yl) methyl)-4- (2,4,5-trifluorophenyl)butanamide ***(6q)***. Yield 86%, yellow liquid, ^1H NMR (500 MHz, $CDCl_3$) δ 2.35 (dd, J = 14.88, 8.93 Hz, 1H, CH_2), 2.47 (s, 3H, CH_3), 2.54 (dd, J = 14.91, 3.30 Hz, 1H, CH_2), 2.74 (dd, J = 13.71, 7.90 Hz, 1H, CH_2), 2.85 (dd, J = 13.68, 5.73 Hz, 1H, CH_2), 3.53–3.59 (m, 1H, CH), 4.74 (d, J = 5.30 Hz, 2H, NCH_2), 6.97 (td, J = 9.42, 6.84 Hz, 1H, ArH), 7.12 (dd, J = 16.51, 9.35 Hz, 1H, ArH), 7.27 (s, 4H, ArH), 7.38–7.42 (m, 2H, ArH), 7.47 (t, J = 7.08 Hz, 1H, ArH), 7.53 (d, J = 7.75 Hz, 2H, ArH), 7.69 (t, J = 4.83 Hz, 1H, NH). $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$) δ 21.02, 36.71, 37.30, 42.85, 49.00, 105.30 (dd, J = 28.69, 20.68 Hz), 118.93 (dd, J = 18.77, 6.01 Hz), 121.82 (dt, J = 18.20, 4.49 Hz), 124.99 (2 × C), 127.53, 128.43 (2 × C), 128.65 (2 × C), 129.84 (2 × C), 129.95, 135.38, 139.01, 146.44 (dd, J = 245.28, 12.82 Hz), 148.60 (dt, J = 250.12, 13.59 Hz), 154.41, 156.00 (dd, J = 244.24, 9.02 Hz), 160.41, 171.31. IR (KBr): 3424, 3286, 3050, 2926, 2865, 1651, 1518, 1423, 1210, 1151, 823, 698 cm^{-1}. EIMS *m/z*: 479 (M^+, 2), 335 (18), 334 (87), 266 (22), 265 (100), 263 (24), 249 (26), 248 (44), 145 (21), 117 (17), 105 (12). HRMS (EI) *m/z*: $[M]^+$ Calcd for $C_{26}H_{24}F_3N_5O$: 479.1933; Found 479.1941.

4.1.5. Standard procedure for synthesis of 2-chloro-N-(2,4,5-trifluorophenyl)acetamide ***9*** *[31,51]*

2,4,5-Trifluorobenzenamine (1.0 equiv, 5.0 mmol) was dissolved in toluene (20 mL) and stirred in an ice-bath. 2-Chloroacetyl chloride (1.1 equiv.) was slowly added to the reaction mixture under N_2. Then the mixture was heated and stirred at refliux (ca. 108 °C) within 3 h. When the reaction was completed, the reaction mixture was added to water (15 mL) and extracted with ethyl acetate (15 mL × 3). The combined organic layers were washed sodium bicarbonate (15 mL × 3), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford the corresponding crude 2-chloro-*N*-(2,4,5-trifluorophenyl)acetamide **9**. The crude desired products **9** was recrystallized in EtOH/hexane (3/1) solution to obtain the pure 2-chloro-*N*-(2,4,5-trifluorophenyl)acetamide **9** in 92% yield; ^1H NMR ($CDCl_3$, 500 MHz): δ 4.19 (s, CH_2NH$_2$), 6.99 (td, J = 9.96, 7.00 Hz, 1H, ArH), 8.22 (dt, J = 15.81, 8.78 Hz, 1H, ArH), 8.44 (br. 1H, NH). $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$): δ 42.69, 105.01 (t, J = 23.55 Hz), 110.30 (d, J = 24.81 Hz), 121.55 (t, J = 8.73 Hz), 146.07 (d, J = 249.56 Hz), 146.27 (dt, J = 247.84, 11.89 Hz), 147.73 (ddd, J = 243.43, 9.11, 2.73 Hz), 163.88. Physical and spectral data were consistent with those previously reported [51].

4.1.6. Standard procedure for synthesis of 2-hydroxy-N-(2,4,5-trifluorophenyl)acetamide ***10*** *[29]*

2-Chloro-*N*-(2,4,5-trifluorophenyl)acetamide **9** (1.0 equiv, 4.0 mmol) and cesium formate (HCO_2Cs, 3.0 equiv) in dry EtOH (10 mL) was heated at reflux (ca. 77 °C) for 6 h. When the reaction was completed, the solution was filtered to remove the excess amount of HCO_2Cs and the filtrate was concentrated under reduced pressure. corresponding crude 2-hydroxyacetamide **10**. The crude product **10** was recrystallized in EtOH/hexane (3/1) solution to obtain the pure 2-hydroxyacetamide **10** in 86% yield; white solid; m.p. 89–90 °C, ^1H NMR ($CDCl_3$, 500 MHz): δ 2.93 (t, J = 5.15 Hz,1H, OH), 4.26 (d, J = 5.15 Hz, 2H, CH_2NH$_2$), 6.98 (td, J = 10.03, 7.00 Hz, 1H, ArH), 8.29 (dt, J = 15.86, 5.95 Hz, 1H, ArH), 8.60 (br. 1H, NH). $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$): δ 62.52, 104.95 (t, J = 23.53 Hz), 110.24 (d, J = 24.68 Hz), 121.79 (t, J = 10.82 Hz), 145.84 (ddd, J = 249.12, 14.60, 11.75 Hz), 146.34 (ddd, J = 243.43, 12.72, 3.63 Hz), 147.57 (ddd, J = 243.43, 8.98, 2.70 Hz), 169.53. IR (KBr): 3429, 3374, 3286, 1688, 1552, 1437, 1199, 876 cm^{-1}. EIMS *m/z*: 205 (M^+, 32), 147 (100), 146 (18), 119 (21), 71 (19). HRMS (EI) *m/z*: $[M]^+$ Calcd for $C_8H_6F_3NO_2$: 205.0351; Found 205.0349.

4.1.7. Standard procedure for synthesis of 2-amino-N-(2,4,5-trifluorophenyl)acetamide ***11*** *[30]*

2-Chloro-*N*-(2,4,5-trifluorophenyl)acetamide **9** (1.0 equiv, 4.0 mmol) was added with ammonium hydroxide solution (NH_4OH, 15 mL) in ethanol solution (15 mL). The mixture was heated at 60 °C under sealed conditions for 3 h. When the reaction was completed, the reaction mixture was concentrated and nertulized with 3% $HCl_{(aq)}$ and extracted with CH_2Cl_2 (3 × 30 mL). The organic extracts were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford the corresponding crude 2-amino-*N*-(2,4,5-trifluorophenyl)acetamide **11**. The crude product **11** was recrystallized in EtOH/hexane (3/1) solution to obtain the pure 2-aminoacetamide **11** in 88% yield; white solid; m.p. 69–70 °C, ^1H NMR ($CDCl_3$, 500 MHz): δ 3.44 (s, 2H, CH_2NH$_2$), 6.92 (q, J = 9.07 Hz, 1H, ArH), 8.30 (dt, J = 16.38, 5.89 Hz, 1H, ArH), 9.73 (s, 2H, NH_2). $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$): δ 44.98, 104.65 (t, J = 23.52 Hz), 109.53 (d, J = 24.65 Hz), 122.47 (t, J = 8.89 Hz), 145.22 (dt, J = 247.25, 12.96 Hz), 146.17 (dd, J = 242.46, 9.04 Hz), 147.42 (dd, J = 245.87, 8.97 Hz), 171.00. IR (KBr): 3503, 3364, 1678, 1569, 1501, 1426, 1206, 862 cm^{-1}. EIMS *m/z*: 205 (11), 204 (M^+, 46), 173 (26), 148 (16), 147 (100), 146 (18), 145 (15), 81 (11), 69 (16). HRMS (EI) *m/z*: $[M]^+$ Calcd for $C_8H_7F_3N_2O$: 204.0510; Found 287.1163.

4.1.8. Standard procedure for synthesis of (1H-1,2,4-triazol-3-yl)methanol ***(12d,*** *and* ***12m–q)*** *[40]*

The reliable procedure involved the treatment of 1*H*-1,2,4-triazole-3-carboxylates **7d** and **7m–q** (1.0 equiv, 5.0 mmol) with $NaBH_4$ (1.5 equiv, 7.5 mmol) in THF/MeOH (1/3, 12 mL) [40]. The reaction mixture was stirred at room temperature for 5 h. When the reaction was completed, the reaction mixture was concentrated under reduced pressure to afford the crude residue. The residue was added water (15 mL) and extracted with ethyl acetate (15 mL × 3). The combined organic layers were washed sodium bicarbonate (15 mL × 3), dried over $MgSO_4$ to give crude (1*H*-1,2,4-triazol-3-yl)methanol product **12d** and **12m–q**. The crude desired products **12d** and **12m–q** were recrystallized in *n*-hexane/methanol (1/4) solution to obtain the pure (1*H*-1,2,4-triazol-3-yl) methanol product **12d** and **12m–q** in 91–96% yields.

4.1.8.1. (1-(4-(Trifluoromethyl)phenyl)-1H-1,2,4-triazol-3-yl)methanol ***(12d)***. Yield 91%, white solid, m.p. 111–113 °C, ^1H NMR (400 MHz, $CDCl_3$) δ 2.84 (t, J = 5.97 Hz, 1H, OH), 4.85 (d, J = 5.55 Hz, 2H, CH_2OH), 7.76 (d, J = 8.79 Hz, 2H, ArH), 7.81 (d, J = 8.70 Hz, 2H, ArH), 8.59 (s, 1H, ArH). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 58.44, 119.70 (2 × C), 123.17 (d, J = 272.03 Hz), 127.13, 127.17, 130.17 (q, J = 33.02 Hz), 139.27, 141.59, 164.97. IR (KBr): 3210, 3114, 2922, 2851, 1335, 1176,

1133, 1111, 1047, 848 cm^{-1}. EIMS *m/z*: 243 (M^+, 70), 242 (85), 224 (18), 214 (58), 187 (10), 172 (21), 159 (100), 145 (42), 140 (15), 139 (30), 109 (15). HRMS (EI) *m/z*: $[M]^+$ Calcd for $C_{10}H_8F_3N_3O$: 243.0619; Found 243.0625.

*4.1.8.2. (1-(4-Chlorophenyl)-5-cyclopentyl-1H-1,2,4-triazol-3-yl)methanol (**12m**).* Yield 94%, light yellow solid, m.p. 100–102 °C, ^1H NMR (500 MHz, $CDCl_3$) δ 1.59 (t, J = 5.35 Hz, 2H, CP), 1.83–1.95 (m, 6H, CP), 3.05 (quint, J = 8.18 Hz, 1H, CP), 3.41 (br, 1H, O*H*), 4.74 (s, 2H, CH_2OH), 7.34 (d, J = 8.47 Hz, 2H, Ar*H*), 7.46 (d, J = 8.50 Hz, 2H, Ar*H*). $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$) δ 25.75 (2 × C), 32.96 (2 × C), 36.19, 58.29, 126.71 (2 × C), 129.64 (2 × C), 135.05, 135.82, 161.19, 162.83. IR (KBr): 3264, 2955, 2869, 1519, 1486, 1092, 1059, 1011, 838 cm^{-1}. EIMS *m/z*: 279 (M^+ + 2, 5), 277 (M^+, 14), 276 (14), 238 (24), 237 (10), 236 (77), 182 (18), 127 (32), 126 (13), 125 (100), 90 (22). HRMS (EI) *m/z*: $[M]^+$ Calcd for $C_{14}H_{16}ClN_3O$: 277.0982; Found 277.0975.

*4.1.8.3. (5-(1H-Pyrrol-2-yl)-1-(3-(trifluoromethyl)phenyl)-1H-1,2,4-triazol-3-yl) methanol (**12n**).* Yield 92%, white solid, m.p. 183–184 °C, ^1H NMR (500 MHz, $CDCl_3$) δ 4.59 (br, 1H, O*H*), 4.83 (s, 1H, CH_2OH), 5.81 (s, 1H, Ar*H*), 6.09–6.11 (m, 1H, Ar*H*), 6.92–6.93 (m, 1H, Ar*H*), 7.65–7.71 (m, 2H, Ar*H*), 7.80 (br, 2H, Ar*H*), 10.01 (br, 2H, N*H*). $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$) δ 57.99, 110.36, 111.07, 118.49, 121.74, 123.25 (d, J = 272.61 Hz), 123.73, 126.59, 129.84, 130.28, 132.43 (d, J = 33.19 Hz), 138.49, 149.38, 162.88. IR (KBr): 3317, 3128, 3114, 3054, 1499, 1327, 1161, 1112, 1070, 806, 753, 698 cm^{-1}. EIMS *m/z*: 309 (19), 308 (M^+, 93), 307 (40), 289 (53), 159 (100), 145 (14), 139 (11). HRMS (EI) *m/z*: $[M]^+$ Calcd for $C_{14}H_{11}F_3N_4O$: 308.0885; Found 308.8092.

*4.1.8.4. (5-Butyl-1-(4-chlorophenyl)-1H-1,2,4-triazol-3-yl)methanol (**12o**).* Yield 93%, orange solid, m.p. 48–50 °C, ^1H NMR (500 MHz, $CDCl_3$) δ 0.85 (t, J = 7.35 Hz, 3H, CH_3), 1.30 (sext, J = 7.44 Hz, 2H, CH_2CH$_3$), 1.69 (q, J = 7.68 Hz, 2H, CH_2CH$_2$CH$_3$), 2.72 (t, J = 7.78 Hz, 2H, CH_2CH$_2$CH$_2$CH$_3$), 3.97 (br, 1H, O*H*), 4.74 (s, 2H, CH_2OH), 7.33 (dt, J = 9.06, 2.60 Hz, 2H, Ar*H*), 7.45 (dt, J = 9.45, 2.35 Hz, 2H, Ar*H*). $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$) δ 13.56, 22.24, 26.15, 29.72, 57.99, 126.32 (2 × C), 129.66 (2 × C), 134.98, 135.70, 157.14, 162.98. IR (KBr): 3292, 2958, 2931, 1520, 1487, 1092, 1055, 1012, 837 cm^{-1}. EIMS *m/z*: 265 (M^+, 5), 236 (18), 225 (32), 224 (12), 223 (100), 182 (13), 170 (35), 127 (22), 125 (54), 90 (19). HRMS (EI) *m/z*: $[M]^+$ Calcd for $C_{13}H_{16}ClN_3O$: 265.0982; Found 265.0989.

*4.1.8.5. (1,5-Diphenyl-1H-1,2,4-triazol-3-yl)methanol (**12p**).* Yield 96%, light yellow solid, m.p. 137–139 °C, ^1H NMR (400 MHz, $CDCl_3$) δ 3.70 (br, 1H, O*H*), 4.84 (s, 2H, CH_2OH), 7.29–7.33 (m, 4H, Ar*H*), 7.36–7.40 (m, 4H, Ar*H*), 7.45–7.47 (m, 2H, Ar*H*). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 58.22, 125.33 (2 × C), 127.45, 128.59 (2 × C), 128.85 (2 × C), 128.96, 129.40 (2 × C), 130.17, 137.93, 154.65, 163.24. IR (KBr): 3307, 3061, 2922, 1595, 1511, 1449, 1050, 990, 774, 694 cm^{-1}. EIMS *m/z*: 251 (M^+, 46), 148 (36), 91 (100), 64 (11). HRMS (EI) *m/z*: $[M]^+$ Calcd for $C_{15}H_{13}N_3O$: 251.1059; Found 251.1056.

*4.1.8.6. (5-Phenyl-1-(p-tolyl)-1H-1,2,4-triazol-3-yl)methanol (**12q**).* Yield 96%, light yellow solid, m.p. 112–114 °C, ^1H NMR (500 MHz, $CDCl_3$) δ 2.38 (s, 3H, CH_3), 4.83 (s, 2H, CH_2OH), 7.20 (s, 4H, Ar*H*), 7.32 (t, J = 7.43 Hz, 2H, Ar*H*), 7.38 (t, J = 7.35 Hz, 1H, Ar*H*), 7.46–7.48 (m, 2H, Ar*H*). $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$) δ 21.18, 58.55, 125.18 (2 × C), 127.67, 128.56 (2 × C), 128.83 (2 × C), 129.99 (2 × C), 130.07, 135.55, 139.13, 154.57, 162.99. IR (KBr): 3209, 3039, 2922, 2858, 1519, 1453, 1050, 992, 825, 778, 696 cm^{-1}. EIMS *m/z*: 266 (19), 265 (M^+, 91), 162 (59), 106 (12), 105 (100), 104 (29). HRMS (EI) *m/z*: $[M]^+$ Calcd for $C_{16}H_{15}N_3O$: 265.1215; Found 265.1210.

*4.1.9. Standard procedure for synthesis of (1H-1,2,4-triazol-3-yl) methanamine (**13d, and 13m–q**) [41,42]*

The reliable procedure involved the treatment of 1*H*-1,2,4-triazol-3-ylmethanol **12d**, and **12m–q** (1.0 equiv, 5.0 mmol) with thionyl chloride ($SOCl_2$, 1.5 equiv, 7.5 mmol) in CH_2Cl_2 solution (15 mL) [41]. The reaction mixture was stirred at room temperature for 2 h under N_2. When the reaction was completed, the reaction mixture was concentrated under reduced pressure, added with CH_2Cl_2 (15 mL), neutralized with saturated sodium bicarbonate solution (15 mL × 3). The organic layer was dried over $MgSO_4$, filtered, then concentrated under reduced pressure to afford crude residue. The residue was added with ammonium hydroxide solution (NH_4OH, 15 mL) in methanol solution (15 mL). The mixture was heated at 60 °C under sealed conditions for 6.0 h [42]. When the amination reaction was completed, the reaction mixture was concentrated and nertulized with 3% $HCl_{(aq)}$ and extracted with CH_2Cl_2 (3 × 30 mL). The organic extracts were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford the corresponding crude 1*H*-1,2,4-triazol-3-ylmethanamines **13d** and **13m–q**. The crude products **13d** and **13m–q** were purified by column chromatography on silica gel to give the pure corresponding β-amino esters **13d** and **13m–q** in 73–84% yields.

*4.1.9.1. (1-(4-(Trifluoromethyl)phenyl)-1H-1,2,4-triazol-3-yl)methanamine (**13d**).* Yield 73%, light yellow solid, m.p. 75–77 °C ^1H NMR (500 MHz, $CDCl_3$) δ 1.98 (s, 2H, NH_2), 3.98 (s, 2H, CH_2NH$_2$), 7.69 (d, J = 7.90 Hz, 2H, Ar*H*), 7.75 (d, J = 7.85 Hz, 2H, Ar*H*), 8.53 (s, 1H, Ar*H*). $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$) δ 39.76, 119.42 (2 × C), 123.42 (d, J = 272.15 Hz), 126.94, 126.97, 129.74 (q, J = 33.40 Hz), 139.37, 141.39, 166.82. IR (KBr): 3370, 3306, 3093, 2926, 2858, 1618, 1534, 1324, 1125, 983, 844 cm^{-1}. EIMS *m/z*: 242 (M^+, 54), 241 (100), 214 (77), 172 (12), 145 (14). HRMS (EI) *m/z*: $[M]^+$ Calcd for $C_{10}H_9F_3N_4$: 242.0779; Found 242.0770.

*4.1.9.2. (1-(4-Chlorophenyl)-5-cyclopentyl-1H-1,2,4-triazol-3-yl)methanamine (**13m**).* Yield 79%, orange liquid, ^1H NMR (500 MHz, $CDCl_3$) δ 1.37–1.42 (m, 2H, CP), 1.64–1.77 (m, 6H, CP), 2.09 (br, 2H, NH_2), 2.89 (quint, J = 8.24 Hz, 1H, CP), 3.76 (br, 2H, CH_2NH$_2$), 7.18 (d, J = 8.79 Hz, 2H, Ar*H*), 7.27 (d, J = 8.65 Hz, 2H, Ar*H*). $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$) δ 25.31 (2 × C), 32.51 (2 × C), 35.76, 39.37, 126.22 (2 × C), 129.12 (2 × C), 134.28, 135.60, 160.42, 163.98; IR (KBr): 3372, 3302, 3050, 2957, 2870, 1513, 1487, 1092, 1012, 835 cm^{-1}. EIMS *m/z*: 278 (M^+ + 2, 26), 277 (30), 276 (M^+, 92), 275 (57), 250 (15), 248 (53), 237 (27), 235 (100), 224 (12), 218 (20), 182 (14), 180 (45), 152 (22), 127 (20), 125 (62), 111 (13), 90 (15). HRMS (EI) *m/z*: $[M]^+$ Calcd for $C_{14}H_{17}ClN_4$: 276.1142; Found 2776.1147.

*4.1.9.3. (5-(1H-Pyrrol-2-yl)-1-(3-(trifluoromethyl)phenyl)-1H-1,2,4-triazol-3-yl) methanamine (**13n**).* Yield 76%, light yellow liquid, ^1H NMR (500 MHz, $CDCl_3$) δ 2.65 (br, 2H, NH_2), 4.00 (br, 2H, CH_2NH$_2$), 5.80 (d, J = 2.90 Hz, 1H, Ar*H*), 6.05 (s, 1H, Ar*H*), 6.87 (s, 1H, Ar*H*), 7.60 (t, J = 7.85 Hz, 1H, Ar*H*), 7.67 (d, J = 7.85 Hz, 1H, Ar*H*), 7.75 (d, J = 7.70 Hz, 1H, Ar*H*), 7.79 (br, 1H, Ar*H*), 11.39 (s, 1H, N*H*). $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$) δ 39.18, 19.84, 110.72, 118.62, 121.55, 123.18 (d, J = 272.61 Hz), 123.53, 126.18, 129.67, 130.05, 132.10 (q, J = 33.37 Hz), 138.55, 149.37, 164.59. IR (KBr): 3356, 3292, 2922, 2855, 1597, 1508, 1328, 1126, 1069, 748, 697 cm^{-1}. EIMS *m/z*: 308 (19), 307 (M^+, 100), 306 (53), 289 (26), 279 (20), 237 (12), 222 (18), 221 (13), 145 (12). HRMS (EI) *m/z*: $[M]^+$ Calcd for $C_{14}H_{12}F_3N_5$: 307.1045; Found 307.1039.

*4.1.9.4. (5-Butyl-1-(4-chlorophenyl)-1H-1,2,4-triazol-3-yl)methanamine (**13o**).* Yield: 81%, orange liquid, ^1H NMR (500 MHz, $CDCl_3$) δ 0.80 (t, J = 7.35 Hz, 3H, CH_3), 1.25 (sext, J = 7.40 Hz, 2H, CH_2CH$_3$), 1.63 (q, J = 7.67 Hz, 2H, CH_2CH$_2$CH$_3$), 2.65 (t, J = 7.83 Hz, 2H, CH_2CH$_2$CH$_2$CH$_3$), 3.05 (br, 1H, NH_2), 3.92 (br, 2H, CH_2NH$_2$), 7.29 (d, J = 8.70 Hz, 2H, Ar*H*), 7.39 (d, J = 8.65 Hz, 2H, Ar*H*). $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$) δ

13.46, 22.16, 26.10, 29.68, 39.28, 126.14 (2 × C), 129.48 (2 × C), 134.61, 135.75, 156.82, 163.61. IR (KBr): 3406, 3296, 3046, 2959, 2930, 2630, 1504, 1488, 1092, 1012, 836 cm^{-1}. EIMS *m/z*: 266 (M^+ + 2, 19), 265 (26), 264 (57), 263 (53), 249 (19), 238 (19), 237 (15), 236 (59), 235 (21), 224 (33), 223 (25), 222 (100), 205 (25), 191 (32), 182 (15), 180 (40), 170 (63), 152 (28), 130 (21), 129 (18), 128 (64), 127 (62), 125 (94), 111 (25), 90 (31). HRMS (EI) *m/z*: $[M]^+$ Calcd for $C_{13}H_{17}ClN_4$: 264.1142; Found 264.1134.

*4.1.9.5. (1,5-Diphenyl-1H-1,2,4-triazol-3-yl)methanamine (**13p**).*. Yield 82%, yellow liquid, ^1H NMR (500 MHz, $CDCl_3$) δ 1.94 (s, 2H, N*H*$_2$), 3.99 (s, 2H, C*H*$_2$NH$_2$), 7.25–7.30 (m, 4H, Ar*H*), 7.31–7.36 (m, 4H, Ar*H*), 7.41–7.43 (m, 2H, Ar*H*). $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$) δ 39.62, 124.93 (2 × C), 127.53, 128.18(2 × C), 128.39, 128.44 (2 × C), 128.99 (2 × C), 129.61, 137.81, 154.06, 164.78. IR (KBr): 3371, 3303, 3064, 2923, 2851, 1596, 1508, 1448, 989, 770, 694 cm^{-1}. EIMS *m/z*: 251 (19), 250 (M^+, 100), 249 (69), 235 (35). 222 (53), 180 (30), 146 (31), 118 (19), 104 (14), 103 (14), 92 (12), 91 (62). HRMS (EI) *m/z*: $[M]^+$ Calcd for $C_{15}H_{14}N_4$: 250.1218; Found 250.1223.

*4.1.9.6. (5-Phenyl-1-(p-tolyl)-1H-1,2,4-triazol-3-yl)methanamine (**13q**).* Yield 84%, yellow liquid, ^1H NMR (500 MHz, $CDCl_3$) δ 2.09 (br, 2H, N*H*$_2$), 2.37 (s, 3H, C*H*$_3$), 4.03 (s, 2H, C*H*$_2$NH$_2$), 7.18 (d, J = 8.43 Hz, 2H, Ar*H*), 7.21 (d, J = 8.52 Hz, 2H, Ar*H*), 7.31 (t, J = 7.39 Hz, 2H, Ar*H*), 7.36 (t, J = 7.33 Hz, 1H, Ar*H*), 749 (dd, J = 8.28, 1.10 Hz, 2H, Ar*H*). $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$) δ 20.82, 39.58, 124.80 (2 × C), 127.61, 128.17 (2 × C), 128.45 (2 × C), 129.57, 129.60 (2 × C), 135.36, 138.53, 154.00, 164.54. IR (KBr): 3367, 3292, 3068, 2923, 2853, 1517, 1451, 990, 821, 696 cm^{-1}. EIMS *m/z*: 265 (24), 264 (M^+, 100), 263 (56), 249 (31), 236 (49), 194 (24), 160 (29), 132 (27), 106(13), 105 (87), 104 (32). HRMS (EI) *m/z*: $[M]^+$ Calcd for $C_{16}H_{16}N_4$: 264.1375; Found 264.1385.

4.2. In vitro dipeptidyl peptidase 4 assay

4.2.1. Preparation of the DPP-4 enzyme

Human recombinant dipeptidyl peptidase IV and its substrate, Gly-Pro-7-amido-4-methylcoumarin (GP- AMC) and its natural inhibitor, Ile-Pro-Ile tripeptide (diprotin A), were purchased from R&D Systems (Minneapolis, MN). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

4.2.2. Enzyme-based assay of DPP-4 [24]

The reaction was carried out at pH 7.5 in the presence of 50 mM HEPES, 140 mM NaCl, 10 mM KCl, and 15 μM of G-P-AMC (the Km for substrate G-P-AMC is about 15 μM). The reaction volume is 200 μl with 20 ng of recombinant DPP-4 at 25 °C. The reaction rate was monitored by a fluorometer with 380-nm excitation and 460-nm emission filters, and determined by the linear slope of the fluorescence accumulation. For inhibition kinetic, substrate and inhibitor concentrations varied and encompassed the Km and Ki values, respectively, and the data were analyzed by the EZ-FIT program, which uses a nonlinear regression method to obtain kinetic parameters. Inhibition constants (IC_{50}) were calculated from enzyme progress curves using standard mathematical models.

4.3. In vitro QPP, DPP-8 and DPP-9 assay [52]

DPP-7, DPP-8 and DPP-9 inhibitions were measured using fluorogenic DPP-7 assay kit, fluorogenic DPP-8 assay kit, and fluorogenic DPP-9 assay kit (BPS Bioscience, San Diego, CA, USA) following the manufacturer's instructions. Briefly, solutions of test compounds in varying concentrations were prepared in dimethyl sulfoxide (DMSO) and then diluted into assay buffer. Human recombinant DPP-7, DPP-8, or DPP-9 was added to the dilutions and pre-incubated for 10 min at 25 °C before the reaction was initiated by the addition of fluorogenic DPP substrate (AMC). The reaction rate was monitored by a fluorometer with 380-nm excitation and 460-nm emission filters, and determined by the linear slope of the fluorescence accumulation. Inhibition constants (IC_{50}) were calculated from enzyme progress curves using standard mathematical models.

4.4. Molecular modeling [12,25]

Computational studies were performed with Sitagliptin **1** and β-amino carbonyl target **5n**, **6d**, and **6p**. The lowest energy conformer for chemical structure was saved in mol2 files by Chemdraw before use in docking studies. The structure of DPP-4 enzyme, encoded PDB ID: 1X70 was downloaded from the protein data bank (PDB) before performing docking studies [12]. Molecular docking studies were performed using iGemdock 2.1 [25]. iGemdock docking calculations were performed at the drug screening docking accuracy setting with parameters set for population size, generation, and number of solutions as 800, 80, and 10, respectively, and a Gemdock score function of hydrophobic and electrostatic (1:1 preference). Residue interaction figure was produced by Pymol to demonstrate the binding pocket.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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