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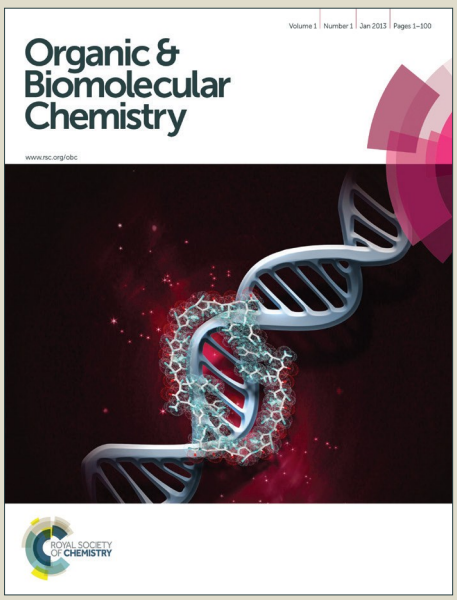

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ARTICLE

Synthesis and Biological Evaluation of Triazole Based Uracil Derivatives as Novel DPP-4 inhibitors

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A series of triazole based uracil derivatives were designed and synthesized as novel DPP-4 inhibitors. Compound **A01** was firstly identified as a lead compound for SAR studies, which focused on structural modification in S2' subsite of DPP-4. Then novel analogues **A02-A25** by modifying substituents at phenyl group and **B01-B09** by introducing carbonyl group were designed. By screening in DPP-4, compounds **B03**, **B04** and **B08** showed a significant improvement in DPP-4 inhibitory activities compared with compound **A01** and a comparable activities with marketed DPP-4 inhibitor alogliptin. Docking study revealed a new favorable binding modes of designed compounds in S_2' subsite, and proved structural modifications in S2' subsite were an effective option to increase the inhibition of DPP-4. In vitro DPP-8 and DPP-9 tests displayed that all compounds showed excellent selectivity against DPP-8 and DPP-9. Further in vivo evaluation showed compound **B04** could significantly improve oral glucose tolerance in ICR mice and dose-dependently reduced glucose levels in type 2 diabetic C57BL/6 mice. These data suggest that compound **B04** could be a promising DPP-4 inhibitor for future treatment of T2DM. .

Introduction

Type 2 diabetes mellitus (T2DM) is growing metabolic disorder, which is expected to affect 366 million people by 2030 worldwide.[1] Currently, Dipeptidyl peptidase-IV (DPP-4) has been validated as one of the most effective targets for T2DM treatment. DPP-4 is a serine peptidase, which rapidly inactivates endogenous glucagon-like peptide-1 (GLP-1) with a very short half-life 1-2 min.[2, 3] GLP-1 secreted by intestinal endocrine cells in response to the presence of nutrients can increase the production and release of insulin from pancreatic β cells.[4, 5] Inhibition of DPP-4 can prevent the degradation of GLP-1, and thus enhance insulin secretion and improve the glucose tolerance.[6] Various DPP-4 inhibitors, such as aloglitptin and linagliptin (**Fig. 1**), have already been released as therapeutic drugs for T2DM.[7] However, some of DPP-4 inhibitors has been reported to increase risk of hospitalisation for heart failure, [8, 9] and also may be associated with severe joint pain side effect.[10] Thus it is necessity to develop novel DPP-4 inhibitors with more safety and less side effects for the treatment of T2DM.

The binding site of DPP-4 enzyme has four subsites, including S_1, S_2, S_1', and S_2' subsites (**Fig. 2**).[11] The S_1 subsite is highly hydrophobic pocket consists of catalytic triad (Ser630, Asn710 and His740) and the S2 subsite involves key interactions with Glu205 and Glu206 and Arg125. The S_1 and S2 subsites have been extensively studied, but the S_1', and S_2' subsites have not been clearly defined.[12] The crystallographic structure (PDB ID: 2RGU) indicates that the S_1' subsite is made up of Tyr547 and Ser630. The S_2' subsite is a larger cavity surrounded by residues of Trp629 Trp627, Tyr752, His740, Ile742 and His748 (**Fig. 2B**). Only few inhibitors can bind into this S_2' subsite to increase their inhibitory activities, and thus leaving more to be explored. [11]

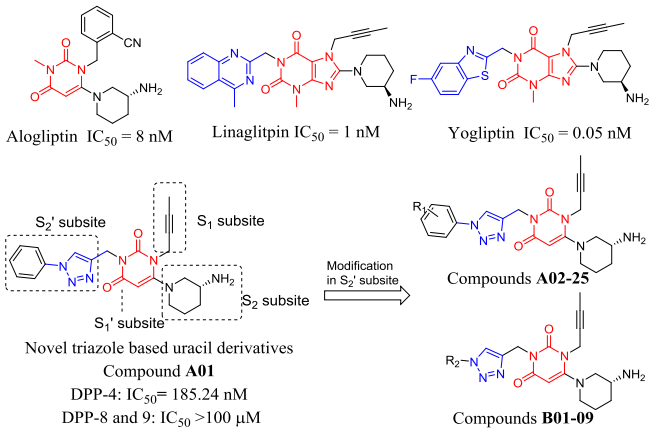

Fig. 1 The structures of alogliptin, linagliptin and yogliptin, and the design of triazole based uracil derivatives.

Among all the DPP-4 inhibitors, alogliptin with IC_{50} value of 8 nM and linagliptin with IC_{50} value of 1 nM are approved by US FDA (**Fig. 1**).[13, 14] The aligned crystallographic structures (PDB ID: 3G0B and 2RGU) revealed that alogliptin and linagliptin have the similar binding modes in S_1, S_2, and S_1' subsites. Both incorporate (*R*)-3-aminopiperidine to form salt bridges with Glu205 and Glu206 in S_2 subsite, hydrophobic cyanobenzyl group and butynyl group bind to

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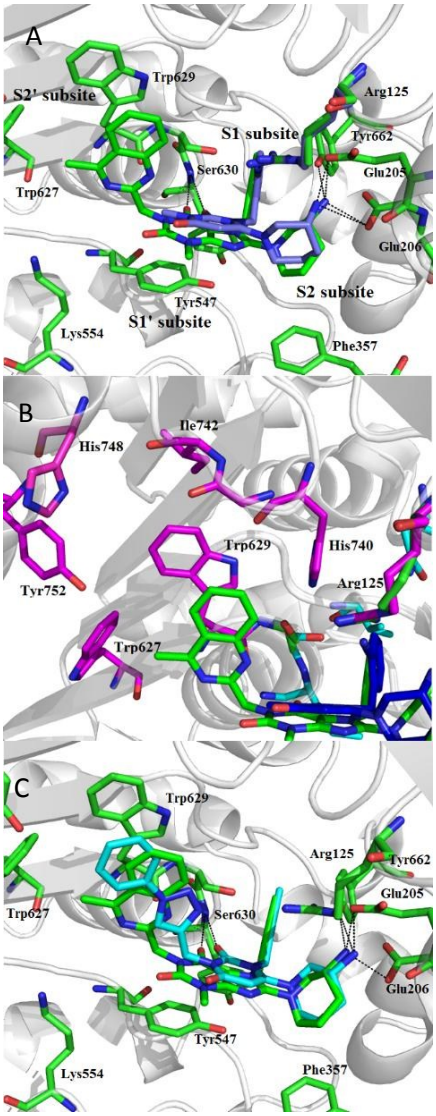

Fig. 2 A, the aligned crystallographic structures of alogliptin (blue) and linagliptin (green); **B**, the residues (purple) of the S_2' subsite; **C**, the docking binding modes of compound **A01** (cyan).

S_1 subsite (**Fig. 2**). This salt bridge interaction in S_2 subsite and hydrophobic binding with S_1 subsite are two key features of DPP-4 inhibitors. [11] In addition, the uracil and purine rings form same π-π interactions with Tyr547 and hydrogen bond with Ser630 in the S_1' subsite. However, they have different binding interactions in the S_2' subsite. Alogliptin does not have binding interaction with S_2' subsite, while the quinazoline substituent of linagliptin forms π-π interaction with Trp629 in the S_2' subsite. Yogliptin is discovered as a more potent (IC_{50} = 0.05 nM), long-acting DPP-4 inhibitor,[15, 16] which may take more efficient binding with S_2' subsite via benzo[d]thiazole ring than quinazoline of linagliptin. The reason why linagliptin has 8-fold higher activity than alogliptin and yogliptin are 20-fold more potent than linagliptin may be because of the different binding interactions in the S_2' subsite, which suggested that modification of substituent in S_2' subsite can be a good strategy for design of new DPP-4 inhibitors.

With those information in mind, we chose the uracil ring as scaffold to interact with S_1' subsite, and 2-butynyl group of linagliptin was introduced at N3-position of uracil to bind with S_1 subsite, while 3-(*R*)-aminopiperidine group at C6-position of uracil was remained to form salt bridges with Glu205 and Glu206 in S_2 subsite. 1,2,3-triazole ring was introduced to N3-position of uracil to bind with the S_2' subsite (**Fig. 1**) because of two reasons: one is the 1,2,3-triazole itself may provide π-π interaction with Trp629 as quinazoline ring of linagliptin does, and the substituent in 1-position of 1,2,3-triazole can be easily modified for structure-activity studies. Compound **A01** with 1-phenyl-1,2,3-triazole group was firstly synthesized and showed a DPP-4 potency of 185.24 nM and excellent selectivity against DPP-8 and DPP-9 (both >100 μM, **Fig. 1**). Docking study reavealed that compound **A01** displayed expected binding modes with DPP-4. As shown in **Fig. 2**, the uracil ring, 2-butynyl group and 3-(*R*)-aminopiperidine group interacted with DPP-4 in a similar way with linagliptin, 1-phenyl-1,2,3-triazole provided π-π interaction with Trp629 in the S_2' subsite. DPP-4 potency of compound **A01** was less potent than that of marketed alogliptin and linagliptin. However, modification of 1-phenyl-1,2,3-triazole group of compound **A01** in S_2' subsite might be a good way to increase their inhibitory activities.Thus we continued to design and synthesize novel analogues **A02-A25** and **B01-B09** with diverse groups in N-1 position of triazole rings. Herein, we reported the design, synthesis and biological evaluation those 1,2,3-triazole based uracil derivatives as novel DPP-4 inhibitors.

Results and discussion

Chemistry

The synthetic route adopted to obtain compounds **A01-A25** is depicted in **Scheme 1**. The selective alkylation of commercially available material 6-chlorouracil with 1-bromo-2-butyne and ethyldiisopropylamine at room temperature produced compound **C**. Compound **C** was further alkylated by 3-bromopropyne to afford compound **D**. Amination of compound **D** with 3-(R)-Boc-amino-piperidine at 60 °C furnished compound **E.** Compounds **H01-22** were obtained by treating compounds **G01-22** with $NaNO_2$ followed by NaN_3 in 6 M hydrochloric acid according to literature procedures.[17] Subsequently, compound **E** was treated with compounds **H01-22** at room temperature in the presence of catalytic amount of copper sulfate and sodium ascorbate in methanol and water (v/v, 4/1) for 12 h to provide compounds **F01-22**. Deprotection of Boc group of compounds **F01-22** with HCl gas at 0 °C furnished the target compounds **A01-A22.** The compounds **A23-25** were prepared from **F20-22** by cleavage of Boc and ester groups.The structures of target compounds **A01-A25** obtained were listed in **Table 1** and **Table 2.**

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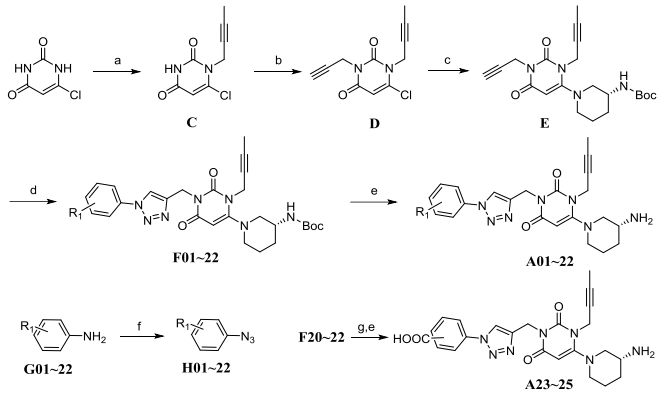
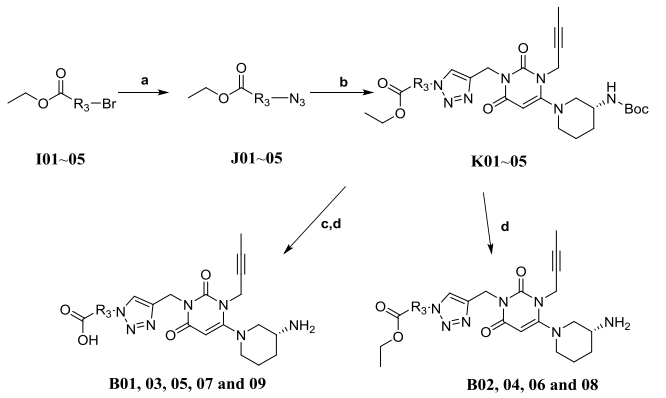


Scheme 1 Synthesis of compounds **A01-A25**. Reagents and conditions: (a) 1-bromobut-2-yne, DIPEA, DMF, r.t., 12 h; (b) 3-bromoprop-1-yne, K_2CO_3, DMF, r.t. 12 h; (c) tert-butyl (*R*)-piperidin-3-ylcarbamate, K_2CO_3, DMF, 60 °C, 6h; (d) compounds **H**, $CuSO_4{\cdot}5H_2O$, Sodium ascorbate, MeOH/H_2O (4:1), r.t., 12 h; (e) HCl gas, EA/ether, 0 °C; (f) (i) $NaNO_2$, HCl, H_2O, 0 °C; (ii) NaN_3, H_2O , 0 °C, 2h; (g) NaOH, H_2O/MeOH, 6h.

The synthetic route to compounds **B01-09** is outlined in **Scheme 2**. The starting materials compounds **I01-05** were treated with NaN_3 in acetone/H_2O to give compounds **J01-05**. Compounds **J01-05** reacted with compound E in similar condition depicted in **Scheme 1** to give compounds **K01-05**. Compounds **K01-05** were hydrolyzed with NaOH and removed protection group with HCl gas to afford the target compounds **B01**, **03**, **05**, **07** and **09**. Deprotection of compounds **K01-05** furnished the target compounds **B02**, **04**, **06** and **08**.The structures of target compounds **B01-B09** were listed in **Table 2.**

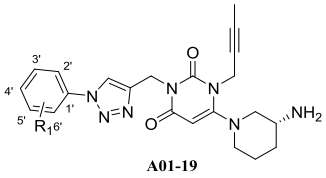
In vitro DPP-4 inhibition studies and SAR analysis of target compounds

The synthesized compounds were evaluated in vitro for their capacity to inhibit human recombinant DPP-4 using linagliptin and alogliptin as the positive controls. Inhibitory potency were measuredby following the increase of fluorimetric intensity at 460 nm upon hydrolysis of H-Gly-Pro-aminomethylcoumarin (H-Gly-Pro-AMC). The compounds with good inhibition rates at 100 nM were further selected to determine their IC_{50} values. The inhibitory activities were depicted in **Table 1**.



Scheme 2 Synthesis of compounds **B01-B09**. Reagents and conditions: (a) NaN_3, Acetone/H_2O, 60 °C, 8h; (b) **compound E**, $CuSO_4{\cdot}5H_2O$, Sodium ascorbate, MeOH/H_2O (4:1), r.t., 12h; (c) NaOH, H_2O/MeOH, 6h; (d) HCl gas, EA/ether, 0 °C.

Table 1 *In vitro* DPP-4 inhibitory activities of compounds **A01-19**



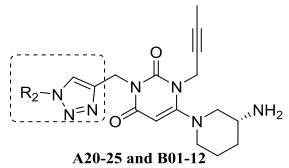
Compounds	R_1	%Inhibition at 100nM	IC_{50} (nM) [a,b]
A01	H	37.26±4.31	185.24
A02	2'-F	75.56±2.76	64.05
A03	3'-F	25.03±2.48	NT
A04	4'-F	46.44±1.71	135.45
A05	2',4'-diF	35.00±2.91	243.67
A06	2'-Me, 4'-F	26.88±0.78	NT
A07	2'-Cl	8.18±2.44	NT
A08	3'-Cl	46.90±1.48	168.63
A09	4'-Cl	3.72±0.71	NT
A10	2',5'-diCl	23.51±5.22	NT
A11	2'-Me	13.71±13.86	NT
A12	4'- Me	32.12±9.64	NT
A13	2',5'-diMe	30.94±16.65	NT
A14	2'-Me, 5-Cl	28.88±7.12	NT
A15	2'-OH, 4'-Me	23.29±6.41	NT
A16	4'-t-Bu	23.72±3.10	NT
A17	2'-MeO	40.61±0.70	219.42
A18	3'-MeO	52.94±0.00	88.53
A19	4'-MeO	23.79±6.87	NT
alogliptin	-	86.46±5.22	6.85
linagliptin	-	98.46±5.22	1.25

[a] Measured in three independent experiments.

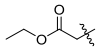
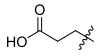
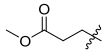
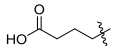
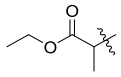
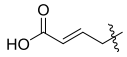
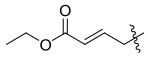
[b] NT: not tested

Compound **A01** with the IC_{50} of 185.24 nM was selected as starting point for our initial SAR studies, which focused on phenyl group in N-1 position of triazole ring (compounds **A01-19**). A wide variety of substituents were introduced to benzene ring. As shown in **Table 1**, most of the synthesized compounds demonstrated significant in vitro DPP-4 inhibitory activities. The mono-fluoro substituted at *ortho* or *para* position of benzene ring (compounds **A02** and **A04)** led to increase of activities, compound **A02** showed 3-fold more potent inhibitory activity (with the IC_{50} of 64.05 nM) compared to compound **A01**. Compound **A04** exhibited DPP-4 potency of 135.45 nM, which was slightly more potent than compound **A01**. However, addition of mono-fluorine atom at *meta* position or introduction of two fluorine atoms at *ortho*- or *para*-position of benzene ring reduced the DPP-4 inhibitory potency (**A03** and **A05**). Chloro group at *meta* position of the benzene ring tolerated DPP-4 inhibition (compound **A08**, IC_{50} = 168.63 nM), introduction of chloro group at other positions led to decrease of activities (compound **A07, A09** and **A10)**. In addition, presence of methyl group at benzene ring slightly reduced inhibitory potency (compounds **A11-15**). The methoxy at *meta*-position (compound

Table 2 *In vitro* DPP-4 inhibitory activities of compounds **A20-25** and **B01-09**



A20-25 and B01-12

Compounds	R_2	%Inhibition at 100nM	IC_{50}(nM)[a,b]
A20		28.94±1.53	345.32
A21		24.07±6.16	NT
A22		36.84±13.30	172.53
A23		72.73±1.32	65.63
A24		17.88±0.00	NT
A25		20.96±2.60	NT
B01		58.73±2.86	84.72
B02		22.84±8.30	NT
B03		**79.55±2.24**	**12.45**
B04		58.39±0.50	64.31
B05		77.28±1.14	26.81
B06		59.24±2.08	71.62
B07		28.82±0.09	NT
B08		**83.82±0.93**	**9.56**
B09		47.24±2.5	105.56
alogliptin	-	86.46±5.22	8.85
linagliptin	-	98.46±5.22	1.47

[a] Measured in three independent experiments.

[b] NT: not tested

A18) exhibited 2-fold more potent inhibitory activity (IC_{50} = 88.53 nM) compared to compound **A01.** The methoxy at *ortho*- or *para*-position reduced inhibitory potency. Finally, an investigation of the inhibitory activities of these N-1 phenyl triazole-based uracil derivatives showed that compound **A02 and A18** exhibited desirable inhibitory, but their acitivities were still less potent than reference drugs linagliptin (IC_{50} = 1.25 nM) and alogliptin (IC_{50} = 6.85 nM). Thus further optimization was performed to identify a compound with enhanced inhibitory activity.

Published X-ray co-crystal structure suggested that some DPP-4 inhibitors, such as aloglitpin[18] and nicotinic acid derivative[19], were interacted with Arg125 residue of DPP-4 in S_2 subsite, and docking study revealed Arg125 is located near the triazole ring of compound **A01 (Fig. 1)**. Thus Arg125 is a potential target residue for achieving potent DPP-4 inhibitory activity in our design. Some known DPP-4 inhibitors generate a hydrogen-bond between the carbonyl oxygen and Arg125[20]. Therefore, we further designed a series of triazole-based uracil derivatives by introducing an additional carbonyl oxygen that could make this desired hydrogen-bond interaction (compound **A20-25 and B01-09)**. The results were shown in **Table 2**.

Carbonyl groups were firstly introduced to the benzene ring of compound **A01** to afford compounds **A20-25**, in which compounds **A23-25** were added with carboxylic acid group and compound **A20-22** with corresponding carboxylic ester. Compound **A23** with carboxylic acid at *ortho*-position showed IC_{50} value of 65.63 nM, manifested 3-fold more potent inhibitory activity compared with compound **A01**. The carboxylic acid at *meta*- and *para*-positions and corresponding ester led to decrease of potency.

Some aliphatic carboxylic acids with different carbon chain lengths (2-4) and corresponding esters were also introduced at 1-position of triazole ring (**B01-09**). As shown in **Table 2,** introduction of aliphatic carboxylic acids led to a significant increase of DPP-4 potency. Compounds bearing acetic acid (**B01**, IC_{50} = 84.72 nM) or 2-propanoic acid (**B06**, IC_{50} = 71.62 nM) exhibited an increase in potency by more than 2- and 3-fold compared to compound **A01**. Remarkably, compound **B03** with 3-propanoic acid group exhibited low nanomolar inhibitory activity with the IC_{50} of 12.45 nM, which was 15-fold more potent than that of compound **A01.** Compound **B08** bearing (*E*)-but-2-enoic acid exhibited the most potent activity with the IC_{50} of 9.56 nM, its activity was comparable with alogliptin. These results clearly indicated the importance of chain lengths of the carboxylic acid for the activity. Replacement of carboxylic acids with their corresponding ester resulted in substantial potency loss**.** Compound **B04** (IC_{50} = 64.31 nM), which has methyl propionate, showed better potency relative to the rest of the ester.

Docking Study

To understand the binding modes of the triazole-based derivatives in S_2' subsite, compounds **A02**, **B03** and **B08** were selected for docking study (Fig.34). We used the molecular docking program GLIDE 5.9 to dock compounds into a DPP-4 crystal structure (PDB ID: 2RGU). The docking results were shown in **Fig. 3**, all compunds display favorable binding modes. As shown in **Fig. 3-A**, the overlay of **A02** against linagliptin shows the uracil ring, 2-butynyl group and (3R)-aminopiperidine group interact with DPP-4 in a similar way with linagliptin. However, compared with conjugated phenyl moiety of the quinazoline ring in linagliptin, nonconjugated 1-phenyl-triazole moiety of compound **A02** take poor π-π stacking interaction with Trp629. The difference may explain the observed potency decrease

of compounds **A01-19** compared to linagliptin. Docking results of compounds **B03** and **B08** (**Fig. 3-B)** showed that the uracil ring, 2-butynyl and (3*R*)-aminopiperidine groups also have similar binding modes to those shown by linaglitpin, but the carboxy groups with different carbon chain lengths form different binding modes in S_2' subsite. The carboxy group of compound **B03** with two carbon lengths can interact with Arg125 in S_2 subsite by hydrogen bond as we expected. However, compound **B08** with three carbon atoms lengths displays new conformations in S_2' subsite that the carboxy groups can interact with Tyr752 with hydrogen bond, and ethenyl chain has hydrophobic interaction with Trp629. The most potent activity of compound **B08** can be explained by the hydrogen-bonding interaction and the increase in hydrophobic interaction in S_2' subsite. Therefore, analysis of compounds **A01-25** and **B01-09** yielded the novel and important finding that modifications of substituent in S2' subsite were an effective and useful option to increase the inhibition of DPP-4.

Selectivity over DPP-8 and DPP-9

Selected compounds were evaluated for its inhibition of DPP-4 homologues, including DPP-8 and DPP-9, and the results were compared to those of marketed DPP-4 inhibitors. As shown in **Table 3**, Saxagliptin inhibited both DPP-8 and DPP-9 with a selectivity ratio for DPP-4 over DPP-9 of only about 30-fold. On the other hand, all selected compounds showed no inhibition of DPP-8 or DPP-9 with the IC_{50} > 100 μM, which showed excellent selectivity against DPP-8 and DPP-9.

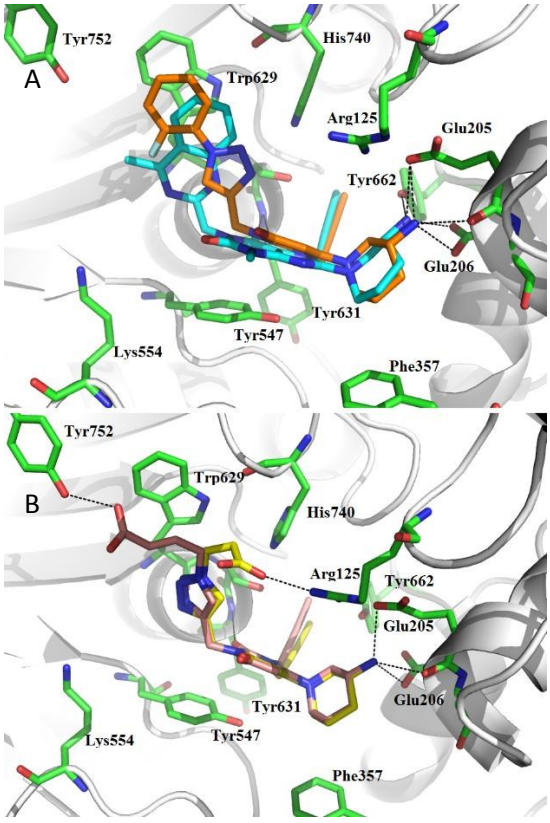


Fig. 3. A docking study of DPP-4 protein and compounds **A02** (orange carbons), **B03** (yellow carbons) and **B08** (salmon carbons). Linaglipitin (blue carbons) and the amino acids (green carbons, white text) are indicated. The hydrogen bonds are shown as black dotted lines.

Table 3 Selectivity for DPP-4 over DPP-8 and DPP-9.

Compounds	DPP-4 (nM)	DPP-8 (μM)	DPP-9 (μM)	DPP-8/DPP-4	DPP-9/DPP-4
A01	185.24	>100	>100	>540	>540
A02	64.05	>100	>100	>1560	>1560
A23	65.63	>100	>100	>1540	>1540
B01	84.72	>100	>100	>1190	>1190
B03	12.45	>100	>100	>8000	>8000
B04	64.31	>100	>100	>1560	>1560
B08	9.56	>100	>100	>10,000	>10,000
B09	105.56	>100	>100	>950	>950
Alogliptin	6.85	>100	>100	>14,000	>14,000
Saxagliptin	6.00	0.4	0.2	66	33

Hypoglycemic effect of B03, B04, B08 and B09 in ICR mice

Based on in vitro potency and selectivity analysis, compounds **B03**, **B04**, **B08** and **B09** were selected for acute efficacy evaluation by the oral glucose tolerance test (OGTT) in ICR mice. A single dose of compounds **B03**, **B04**, **B08** and **B09** (3 mg/kg) were administered to ICR mice. As shown in **Fig. 4**, the OGTT produced a significant decrease in glucose level after half hour compared with the vehicle group. Alogliptin, which was used as a positive control, reduced the area under curve from 0 to 120 min (AUC) $_{0\text{-}120}$ min to 38.2% (alogliptin, 842±103, vehicle control, 1,363±157). Compared compound **B03** with **B04,** compound **B03** showed more potent in vitro activity than compound **B04**, but compound **B04** was more potent in vivo. Compound **B04** reduced the value to 31.4% (935±125), and compound **B03** reduced only 21.2% (1150±130). The reason may be low membrane permeability of compound **B03** caused by the formation of a zwitterion between the carboxyl group and the amino group. Compound **B04** was the methyl ester of compound **B03**, the more lipophilic compound **B04** have higher membrane permeability to improve the bioavailability, and compound **B04** can probably act as a prodrug that metabolized into compound **B03,** as reported in literature,[21-23] both improved in vivo activity of compound **B04**. In addtion, compounds **B08** and **B09** showed similar potency in vivo, **B08** and **B09** reduced the value to 28.1% (**B08**, 981±98) and 25.2% (1091±120), respectively.

In order to verify the hypoglycemic effect, compounds **B04** and **B09** were selected for acute efficacy evaluation in ICR mice at a higher dose of 10 mg/kg (**Fig. 5**). Compound **B04** reduced area under the curve from 0 to 120 min $(AUC)_{0\text{-}120}$ min to 40.4% (B04, 947±85; vehicle control, 1,589±139), which was slightly less potent than the hypoglycemic effect of alogliptin (42.5%, 913±73). But the activity of compound **B09** (29.2%, 1105±124) was still much lower than that of

alogliptin at higher dose. Thus compound **B04** with best potency in vivo was then considered for further evaluation.

Antihyperglycemic effect of compound B04 in type 2 diabetic C57BL/6 mice

The antihyperglycemic effect of **B04** was evaluated in type 2 diabetic C57BL/6 mice. As shown in **Fig. 6**, Compund **B04** at dose of 1-10 mg/kg was orally administrated in diabetic C57BL/6 mice prior to glucose challenge. The results indicated that **B04** significantly reduced blood glucose at a dose of 3 mg/kg and oral administration of B04 dose-dependently reduced blood glucose in type 2 diabetic C57BL/6 mice.

The toxicity evaluation of compound B04

To evaluation of the potential toxicity of compound **B04**, the ICR mice and diabetic C57BL/6 mice after OGTT at the dose of 10 mg/kg were kept under conventional conditions of controlled temperature, humidity, and lighting for additional 1 week. All animals survived until the end of the study. The results suggested that the compound was non-toxic at the tested dose.

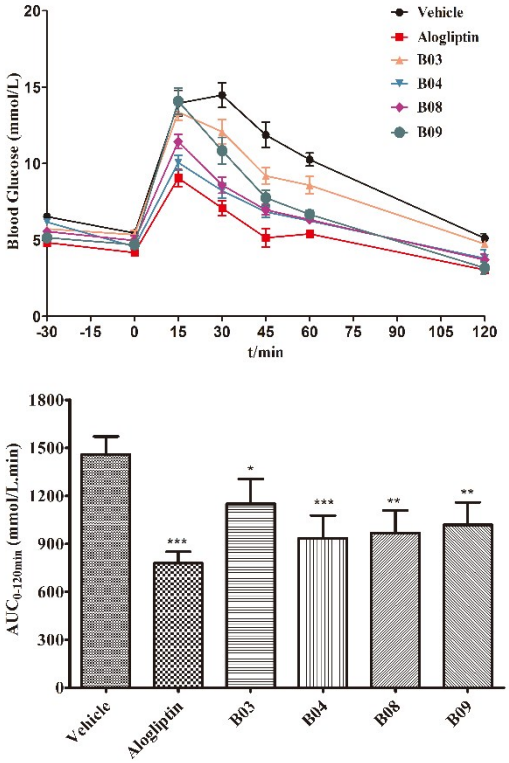


Fig. 4 Effect of Compounds **B03**, **B04, B08** and **B09** (3 mg/kg) during an OGTT in male ICR mice and $AUC_{0–120\ min}$ of blood glucose levels. Values are mean ± SEM (n = 8). * P≤0.05, **P≤0.01, ***P ≤0.001 Compared to vehicle-treated ICR mice by Student's t test.

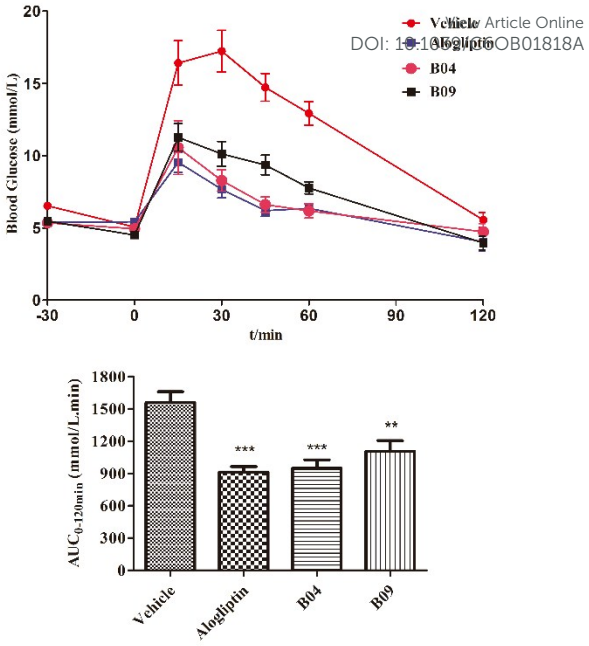


Fig. 5 Effect of Compounds **B04** and **B09** (10 mg/kg) during an OGTT in male ICR mice and $AUC_{0–120\ min}$ of blood glucose levels. Values are mean ± SEM (n = 8). **P≤0.01 and ***P ≤0.001 compared to vehicle-treated ICR mice by Student's t test.

Conclusions

In summary, we designed and optimized 1,2,3-triazole based uracil derivatives to obtain novel DPP-4 inhibitors. Firstly, Compound **A01** with phenyl group was firstly identified as a lead compound, then substituents were introduced to phenyl group at N-1 position of 1,2,3-triazole, and the resulting compounds manifested weak to moderate DPP-4 inhibitory activities. Then we designed the introduction of a carboxy group that could increase the inhibitory activities by interacting with Arg125. Compounds **B03**, **B04** and **B08** possessing a carboxy group actually showed improved DPP-4 inhibitory activities. Docking study revealed that the carboxy group at 1,2,3-triazole ring show new favorable conformations in S_2' subsite, and suggested modifications of substituent in S2' subsite were an effective way to increase the inhibition of DPP-4. Furthermore, Compounds **B03**, **B04** and **B08** also showed good selectivity over DPP-8 and DPP-9. In addtion, compound **B04** could significantly improve oral glucose tolerance in ICR mice and dose-dependently reduced glucose levels in type 2 diabetic C57BL/6 mice. These data suggest that compound **B04** could be a promising DPP-4 inhibitor for future treatment of T2DM.

Experimental

General chemistry

All reagents were purchased from commercial sources and used without further purification. Reactions were monitored by TLC on silica gel 60 F254 plates (Qingdao Ocean Chemical Company, China). Column chromatography was performed on silica gel (200-300 mesh, Qingdao Ocean Chemical Company, China). Melting points were

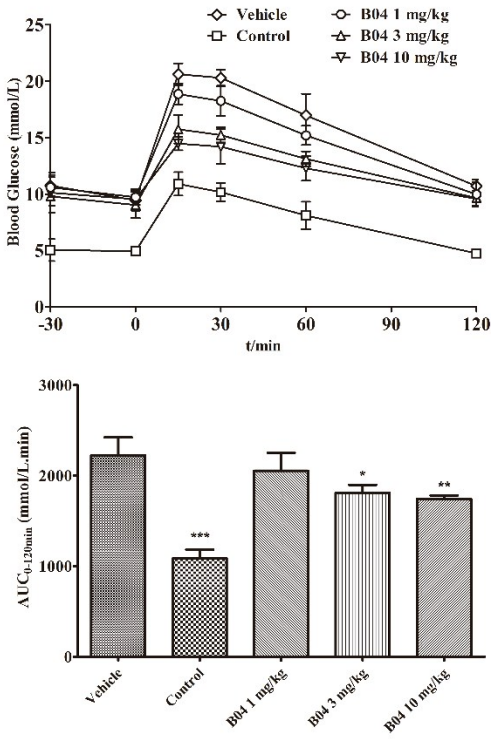


Fig. 6 Effect of **B04** during an OGTT in diabetic C57BL/6 mice. (A) show time-dependent changes of blood glucose after oral administration of **B04**, followed by 1 g/kg oral glucose challenge. Date in (B) represent $AUC_{0–120}$ min of blood glucose levels. Values are mean ± SEM (n = 6). *P≤0.05, **P ≤0.01 and ***P ≤0.001 compared to vehicle-treated C57BL/6 mice by Student's t test.

measured on capillary tube and were uncorrected. IR spectra (in KBr pellets) were taken using Shimadzu FT-IR-8400S spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra (DMSO-d_6, $CDCl_3$) were recorded with a Bruker AV-300 spectrometer in the indicated solvents (TMS as internal standard): the values of the chemical shifts are expressed in δ values (ppm) and the coupling constants (J) in Hz. High-resolution mass spectra were recorded using an Agilent QTOF 6520.

1-(but-2-ynyl)-6-chloropyrimidine-2,4(1H,3H)-dione (C)

To a mixture of 6-chlorouracil (10 g, 69 mmol) and DIPEA (9.7 g, 75 mmol) in DMF (30 mL) was added 1-bromo-2-butyne (9.9 g, 75 mmol). The reaction mixture was stirred at r.t for 12 h. Water (150 mL) was added. The precipitate was collected by filtration, washed

with water and EtOH, and dried to give compound **C** as a light yellow solid (10.8 g, 80%). mp: 216-217 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.71 (s, 1H), 5.99 (s, 1H), 4.65 (s, 2H), 1.80 (s, 3H). HR-MS (ESI) m/z: calculated $C_8H_8N_2O_2Cl$ $[M+H]^+$ 199.0274, found 199.0276.

1-(but-2-ynyl)-6-chloro-3-(prop-2-ynyl)pyrimidine-2,4(1H,3H)-dione (D)

To a suspension of **B** (9 g, 45.5 mmol) and K_2CO_3 (14.4 g, 104.53 mmol) in DMF (36 mL) was added 3-bromopropyne (6.5 g, 54.5 mmol). The reaction mixture was stirred at r.t for 12 h. The reaction mixture was poured into water. The precipitate was collected by filtration, washed with water, and dried to give compound **D** as a yellow solid (8.48 g, 79%). mp: 147-149 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.21 (s, 1H), 4.74 (s, 2H), 4.51 (s, 2H), 3.17 (s, 1H), 1.80 (s, 3H). HR-MS (ESI) m/z: calculated $C_{11}H_{10}N_2O_2Cl$ $[M+H]^+$ 237.1324, found 237.1318.

Tert-butyl (R)-(1-(3-(but-2-yn-1-yl)-2,6-dioxo-1-(prop-2-yn-1-yl)-1,2,3,6-tetra- hydropyrimidin-4-yl)piperidin-3-yl)carbamate (E)

A mixture of **C** (8.0 g, 33.9 mmol), (*R*)-3-(N-Boc-amino)piperidine (8.13 g, 40.68 mmol) and K_2CO_3 (10.76 g, 77.90 mmol) in DMF (40 mL) was stirred at 65 °C for 5 h. After cooling to r.t., the mixture was poured into water (1000 mL). The precipitate was collected by filtration, washed with water, and dried to give compound **E** as a brown solid (13.5 g, 99 %). mp: 87-89 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.27 (s, 1H), 4.92-4.76 (m, 1H), 4.70-4.66 (m, 3H), 4.49 (d, J = 17.1 Hz, 1H), 3.88-3.82 (m, 1H), 3.35-3.25 (m, 1H), 3.17-3.02 (m, 1H), 2.94-2.78 (m, 1H), 2.75-2.54 (m, 1H), 2.18 (t, J = 2.4 Hz, 1H), 1.97-1.85 (m, 1H), 1.82 (s, 3H), 1.74-1.67 (m, 2H), 1.45 (s, 9H). HR-MS (ESI) m/z: calculated $C_{21}H_{29}N_4O_4$[M+H]$^+$ 401.4327, found 401.4319.

azidobenzene (H01)

Aniline (5.62 g, 18 mmol) was dissolved in 6 mol/L hydrochloric acid (20 mL). To this solution, sodium nitrite (0.89 g, 12.9 mmol) was slowly added at 0 to 5 °C within 30 min. The solution was vigorously stirred at 0-5 °C for 30 min. Sodium azide (0.91 g, 13.9 mmol, dissolved in 2 mL of water) was slowly added into the reaction mixture at 0 °C. The resulting solution was stirred at 0 °C for 2 h followed by diluting with ice water (50 mL) and extracting with EtOAc (3 × 50 mL). The combined organic layer was washed with water (3 × 50 mL), saturated aqueous $NaHCO_3$ (50 mL × 3) and brine (100 mL), dried over anhydrous Na_2SO_4, filtered and concentrated to afford yellow oil (0.84 g, 65%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.27 (t, J = 7.2 Hz, 2H), 7.08-7.05 (m, 1 H), 6.96 (m, 2 H).

tert-butyl (*R*)-(1-(3-(but-2-yn-1-yl)-2,6-dioxo-1-((1-phenyl-1H-1,2,3-triazol-4-yl) methyl)-1,2,3,6-tetrahydropyrimidin-4-yl)piperidin-3-yl)carbamate (F01)

To the solution of compund **E** (212 mg, 0.53 mmol) and **H01** (76 mg, 0.64 mmol) in 80% methanol (10 mL), $CuSO_4{\cdot}5H_2O$ (6.6 mg, 0.027 mmol) and ascorbate sodium (30 mg) were added, respectively. The reaction solution was stirred at room temperature for 12 h. The mixture was conentrated under reduced presure, water was added and (20 mL) and extracted with DCM (3 × 20 mL). The combined organic layer was washed with saturated brine, dried over anhydrous Na_2SO_4, and concentrated. The crude product was purified by flash chromatography (petroleum ether/ethyl acetate, 10:1~ 3:1) to give compound **F01** as a light yellow solid (234 mg, 85%).^{1}H NMR (300 MHz, $CDCl_3$): δ 7.71 (s, 1H), 7.28-7.25 (m, 2H), 7.08-7.00 (m, 3H), 5.32 (s, 2H), 5.28 (s, 1H), 4.83-4.74 (m, 1H), 4.67 (d, J = 15.9 Hz, 1H), 4.49 (d, J = 15.9 Hz, 1H), 3.87-3.78 (m, 1H), 3.33-3.25 (m, 1H), 3.14-3.03 (m, 1H), 2.88-2.81 (m, 1H), 2.71-2.58 (m, 1H), 1.97-1.85 (m, 1H), 1.82 (s, 3H), 1.74-1.67 (m, 2H), 1.45 (s, 9H).

(*R*)-6-(3-aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((1-phenyl-1H-1,2,3-triazol-4-yl)methyl)pyrimidine-2,4(1H,3H)-dione hydrochloride (A01)

Compund **F01** (200 mg, 0.38 mmol) was dissolved in EtOAc (5 mL) and ether (30 mL). Freshly prepared HCl gas was bubbled into the solution at °C. After TLC analysis indicated the completed consumption of starting materials, the precipitate was collected by

filtration, dried in vacuo to afford compound **A01** as a white solid (135 mg, 77%). mp: 83-86 °C; IR (ν_{max} cm^{-1}): 3438, 2949, 1704, 1654, 1503, 1441, 805, 762; ^{1}H NMR (300MHz, DMSO-d_6): δ 8.64 (s, 1H), 8.34 (brs, 3H), 7.89-7.86 (m, 2H), 7.61-7.55 (m, 2H), 7.50-7.45 (m, 1H), 5.28 (s, 1H), 5.09 (s, 2H), 4.70-4.64 (m, 1H), 4.50-4.44 (m, 1H), 3.38-3.30 (m, 2H), 3.12-3.01 (m, 2H), 2.92-2.85 (m, 1H), 1.97-1.88 (m, 2H), 1.78 (s, 3H), 1.68-1.62 (s, 2H); ^{13}C NMR (75MHz, DMSO-d_6): δ 161.40, 159.04, 151.39, 144.03, 136.51, 129.82, 128.58, 121.45, 119.96, 88.33, 79.72, 74.57, 52.04, 51.41, 45.94, 35.79, 35.55, 26.91, 21.21, 3.13; HR-MS (ESI) m/z: calculated for $C_{22}H_{25}N_7O_2$ $[M+H]^+$: 420.2142, found: 420.2140.

(*R*)-6-(3-aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((1-(2-fluoro phenyl)-1H-1,2,3-triazol-4-yl)methyl)pyrimidine-2,4(1H,3H)-dione hydrochloride (A02)

Following a similar procedure for the preparation of **A01**, **A02** was prepared starting from 2-fluoroaniline. White solid (140 mg). mp: 71-75 °C; IR (ν_{max} cm^{-1}): 3438, 1704, 1654, 1600, 1510, 1474, 1441, 764; ^{1}H NMR (300MHz, DMSO-d_6): δ 8.41 (s, 1H), 8.28 (brs, 3H), 7.83-7.78 (m, 1H), 7.63-7.51 (m, 2H), 7.45-7.40 m, 1H), 5.29 (s, 1H), 5.12 (s, 2H), 4.66 (d, *J* = 18.0 Hz, 1H), 4.47 (d, *J* = 18.0 Hz, 1H), 3.35-3.27 (m, 2H), 3.12-2.98 (m, 2H), 2.92-2.84 (m, 1H), 1.98-1.88 (m, 2H), 1.78 (s, 3H), 1.69-1.61 (m, 2H); ^{13}C NMR (75MHz, DMSO-d_6): δ 161.40, 159.05, 151.40, 143.42, 131.12, 125.84, 125.51, 125.46, 124.75, 117.19, 116.93, 88.45, 79.72, 74.52, 52.12, 51.37, 46.00, 35.64, 35.52, 26.94, 21.28, 3.09; HR-MS (ESI) m/z: calculated for $C_{22}H_{24}FN_7O_2$ $[M+H]^+$:438.2048, found: 438.2056.

(*R*)-6-(3-aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((1-(3-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)pyrimidine-2,4(1H,3H)-dione hydrochloride (A03)

Following a similar procedure for the preparation of **A01**, **A03** was prepared starting from 3-fluoroaniline. White solid (135 mg); mp: 73-77 °C; IR (ν_{max} cm^{-1}): 3435, 2950, 1705, 1651, 1604, 871, 784; ^{1}H NMR (300MHz, DMSO-d_6) δ 8.71 (brs, 1H), 8.30 (s, 3H), 7.87-7.75 (m, 2H), 7.67- 7.59(m, 1H), 7.37-7.30 (m, 1H), 5.29 (s, 1H), 5.10 (s, 2H), 4.67 (d, *J* = 18.0 Hz, 1H), 4.48 (d, *J* = 18.0 Hz, 1H), 3.40-3.29 m, 2H), 3.15-2.98 (m, 2H), 2.92-2.85 (m, 1H), 1.97-1.88 (m, 2H), 1.78 (s, 3H), 1.69-1.62 (m, 2H). ^{13}C NMR (75MHz, DMSO-d_6): δ 161.40, 159.04, 151.39, 144.03, 136.51, 129.82, 128.58, 121.45, 119.96, 88.33, 79.72, 74.57, 52.04, 51.41, 45.94, 35.79, 35.54, 26.91, 21.21, 3.13; HR-MS (ESI) m/z: calculated for $C_{22}H_{24}FN_7O_2$ $[M+H]^+$: 438.2048, found: 438.2048.

(*R*)-6-(3-aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((1-(4-fluoro phenyl)-1H-1,2,3-triazol-4-yl)methyl)pyrimidine-2,4(1H,3H)-dione hydrochloride (A04)

Following a similar procedure for the preparation of **A01**, **A04** was prepared starting from 4-fluoroaniline. White solid (127 mg); mp: 83-87 °C; IR (ν_{max} cm^{-1}): 3442, 1705, 1651, 1517, 1441, 1047, 807; ^{1}H NMR (300MHz, DMSO-d_6) δ 8.62 (s, 1H), 8.28 (brs, 3H), 7.94-7.90 (m, 2H), 7.45-7.41 (m, 2H), 5.29 (s, 1H), 5.09 (s, 2H), 4.66 (d, *J* = 15.0 Hz, 1H), 4.48 (d, *J* = 15.0 Hz, 1H), 3.33-3.28 (m, 2H), 3.14-2.99 (m, 2H), 2.92-2.84 (m, 1H), 1.97-1.87 (m, 2H), 1.78 (s, 3H), 1.69-1.60 (m, 2H); ^{13}C NMR (75MHz, DMSO-d_6): δ 161.39, 159.04, 151.39, 144.03, 133.09, 122.43, 121.74, 116.77, 116.46, 88.44, 79.73, 74.53, 52.10, 51.40, 46.01, 35.76, 35.52, 26.96, 21.29, 3.10; HR-MS (ESI) m/z: calculated for $C_{22}H_{24}FN_7O_2$ $[M+H]^+$:438.2048, found:438.2050.

(*R*)-6-(3-aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((1-(2,4-difluoro phenyl)-1H-1,2,3-triazol-4-yl)methyl)pyrimidine-2,4(1H,3H)-dione hydrochloride(A05)

Following a similar procedure for the preparation of **A01**, **A05** was prepared starting from 2,4-difluoroaniline. White solid (112 mg); mp: 84-86 °C; IR (ν_{max} cm^{-1}): 3442, 2922, 1706, 1654, 1609, 1440, 808; ^{1}H NMR (300MHz, DMSO-d_6): δ 8.41 (s, 1H), 8.34 (brs, 3H), 7.91-7.83 (m, 1H), 7.71-7.63 (m, 1H), 7.38-7.30 (m, 1H), 5.28 (s, 1H), 5.11 (s, 2H), 4.67 (d, *J* = 17.4 Hz, 1H), 4.47 (d, *J* = 17.4 Hz, 1H), 3.39-3.28 (m, 2H), 3.15-2.98 (m, 2H), 2.93-2.84 (m, 1H), 1.97-1.88 (m, 2H), 1.78 (s, 3H), 1.70-1.59 (m, 2H); ^{13}C NMR (75MHz, DMSO-d_6): δ 161.38, 159.05, 151.38, 143.39, 127.54, 124.95, 112.81, 112.51, 105.96, 105.60, 105.28, 88.34, 79.72, 74.54, 52.07, 51.38, 45.93, 35.63, 35.54, 26.89, 21.18, 3.12; HR-MS (ESI) m/z: calculated for $C_{22}H_{23}F_2N_7O_2$ $[M+H]^+$:456.1954, found:456.1953.

(*R*)-6-(3-aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((1-(4-fluoro-2-methylphenyl)-1H-1,2,3-triazol-4-yl)methyl)pyrimidine-2,4(1H,3H)-dione hydrochloride (A06)

Following a similar procedure for the preparation of **A01**, **A06** was prepared starting from 4-fluoro-2-methylaniline. White solid (125 mg); mp: 92-95°C; IR (ν_{max} cm^{-1}): 3442, 1705, 1651, 1508, 1441, 1231, 809; ^{1}H NMR (300MHz, DMSO-d_6) δ: 8.33-8.29 (m, 4H), 7.50-7.45 (m, 1H), 7.39-7.35 (m, 1H), 7.27-7.21 (m, 1H), 5.29 (s, 1H), 5.11 (s, 2H), 4.67 (d, *J* = 15.0 Hz, 1H), 4.47 (d, *J* = 15.0 Hz, 1H), 3.38-3.27 (m, 2H), 3.13-3.00 (m, 2H), 2.93-2.86 (m, 1H), 2.11 (s, 3H), 1.97-1.86 (m, 2H), 1.78 (s, 3H), 1.70-1.61 (m, 2H); ^{13}C NMR (75MHz, DMSO-d_6): δ 161.40, 159.01, 151.39, 142.89, 132.66, 128.24, 125.14, 117.85, 117.54, 113.82, 113.52, 88.43, 79.69, 74.54, 52.10, 51.38, 45.99, 35.71, 35.50, 26.93, 21.27, 17.34, 3.10; HR-MS (ESI) m/z: calculated for $C_{23}H_{26}FN_7O_2$ $[M+H]^+$: 452.2205, found: 452.2200

(*R*)-6-(3-aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((1-(2-chloro phenyl)-1H-1,2,3-triazol-4-yl)methyl)pyrimidine-2,4(1H,3H)-dione hydrochloride (A07)

Following a similar procedure for the preparation of **A01**, **A07** was prepared starting from 2-chloroaniline. White solid (112 mg); mp:80-83 °C; IR (ν_{max} cm^{-1}):3442, 2949, 1704, 1654, 1440, 766; ^{1}H NMR (300MHz, DMSO-d_6) δ 8.37 (s, 1H), 8.27 (brs, 3H), 7.77-7.74 (m, 1H), 7.67-7.62 (m, 2H), 7.60-7.55 (m, 1H), 5.29 (s, 1H), 5.12 (s, 2H), 4.66 (d, *J* =15.0 Hz, 1H), 4.47 (d, *J* = 15.0 Hz, 1H), 3.33-3.27 (m, 2H), 3.13-2.97 (m, 2H), 2.91-2.84 (m, 1H), 1.98-1.88 (m, 2H), 1.78 (s, 3H), 1.69-1.60 (m, 2H); ^{13}C NMR (75MHz, DMSO-d_6): δ 161.37, 159.03, 151.40, 142.87, 134.44, 131.52, 130.50, 128.38, 128.28, 125.46, 88.46, 79.70, 74.54, 52.13, 51.37, 46.00, 35.66, 35.50, 26.95, 21.29, 3.10; HR-MS (ESI) m/z: calculated for $C_{22}H_{24}ClN_7O_2$ $[M+H]^+$:454.1753, found:454.1756.

(*R*)-6-(3-aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((1-(3-chlro phenyl)-1H-1,2,3-triazol-4-yl)methyl)pyrimidine-2,4(1H,3H)-dione hydrochloride (A08)

Following a similar procedure for the preparation of **A01**, **A08** was prepared starting from 3-chloroaniline. White solid (108 mg, 49%), mp:83-87 °C; IR (ν_{max} cm^{-1}):3439, 2921, 1704, 1652, 1595, 1441, 790; ^{1}H NMR (300MHz, DMSO-d_6) δ 8.74 (s, 1H), 8.32 (brs, 3H), 8.04-8.02 (m, 1H), 7.92-7.90 (m, 1H), 7.64-7.54 (m, 2H), 5.29 (s, 1H), 5.10

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(s, 2H), 4.67 (d, *J* = 17.1 Hz, 1H), 4.48 (d, *J* = 17.1 Hz, 1H), 3.40-3.29 (m, 2H), 3.14-2.99 (m, 2H), 2.93-2.85 (m, 1H), 1.98-1.89 (m, 2H), 1.79 (s, 3H), 1.70-1.61 (m, 2H); ^{13}C NMR (75MHz, DMSO-d_6): δ 161.37, 159.05, 151.39, 144.26, 137.58, 134.13, 131.53, 128.36, 121.63, 119.72, 118.55, 88.42, 79.73, 74.53, 52.10, 51.41, 46.00, 35.79, 35.55, 26.96, 21.29, 3.11; HR-MS (ESI) m/z: calculated for $C_{22}H_{24}ClN_7O_2$ $[M+H]^+$: 454.1753, found:454.1751.

(*R*)-6-(3-aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((1-(4-chloro phenyl)-1H-1,2,3-triazol-4-yl)methyl)pyrimidine-2,4(1H,3H)-dione hydrochloride(A09)

Following a similar procedure for the preparation of **A01**, **A09** was prepared starting from 4-chloroaniline. White solid (150 mg); mp: 88-91 °C; IR (ν_{max} cm^{-1}): 3442, 1705, 1651, 1502, 1442, 1232; ^{1}H NMR (300MHz, DMSO-d_6) δ 8.68 (s, 1H), 8.33 (brs, 3H), 7.93 (d, *J* = 9.0 Hz, 2H), 7.66 (d, *J* = 9.0 Hz, 2H), 5.29 (s, 1H), 5.10 (s, 2H), 4.67 (d, *J* = 18.0 Hz, 1H), 4.47 (d, *J* = 18.0 Hz, 1H), 3.32-3.27 (m, 2H), 3.14-2.98 (m, 2H), 2.92-2.85 (m, 1H), 1.98-1.88 (m, 2H), 1.78 (s, 3H), 1.70-1.60 (m, 2H); ^{13}C NMR (75MHz, DMSO-d_6): δ 161.38, 159.05, 151.39, 144.21, 135.31, 132.81, 129.76, 121.68, 121.56, 88.43, 79.73, 74.54, 52.10, 51.40, 46.00, 35.77, 35.54, 26.96, 21.31, 3.11; HR-MS (ESI) m/z: calculated for $C_{22}H_{24}ClN_7O_2$ $[M+H]^+$: 454.1753, found:454.1750.

(*R*)-6-(3-aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((1-(2,6-dichloro phenyl)-1H-1,2,3-triazol-4-yl)methyl)pyrimidine-2,4(1H,3H)-dione hydrochloride (A10)

Following a similar procedure for the preparation of **A01**, **A10** was prepared starting from 2,6-dichloroaniline. White solid (142 mg); mp: 100-104°C; IR (ν_{max} cm^{-1}): 3442, 1705, 1655, 1601, 1483, 1441, 1231, 795; ^{1}H NMR (300MHz, DMSO-d_6) δ: 8.38 (s, 1H), 8.26 (s, 3H), 7.79-7.76 (m, 2H), 7.70-7.64 (m, 1H), 5.30 (s, 1H), 5.12 (s, 2H), 4.66 (d, *J* = 18 Hz, 1H), 4.47 (d, *J* = 18 Hz, 1H), 3.32-3.27 (m, 2H), 3.13-3.00 (m, 2H), 2.92-2.85 (m, 1H), 1.96-1.86 (m, 2H), 1.78 (s, 3H), 1.69-1.59 (m, 2H); ^{13}C NMR (75MHz, DMSO-d_6): δ 161.33, 159.03, 151.36, 142.93, 132.81, 132.60, 132.54, 129.16, 125.88, 88.41, 79.70, 74.51, 52.12, 51.37, 45.98, 35.68, 35.51, 26.93, 21.27, 3.11; HR-MS (ESI) m/z: calculated for $C_{22}H_{23}Cl_2N_7O_2$ $[M+H]^+$:488.1363, found:488.1363.

(*R*)-6-(3-aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((1-(*o*-tolyl)-1H-1,2,3-triazol-4-yl)methyl)pyrimidine-2,4(1H,3H)-dione hydrochloride (A11)

Following a similar procedure for the preparation of **A01**, **A11** was prepared starting from 2-methylaniline. White solid (120 mg); mp: 81-85 °C; IR (ν_{max} cm^{-1}): 3439, 1705, 1651, 1441, 1230; ^{1}H NMR (300MHz, DMSO-d_6) δ 8.35-8.29 (m, 4H), 7.48-7.44 (m, 2H), 7.40-7.39 (m, 2H), 5.28 (s, 1H), 5.11 (s, 2H), 4.66 (d, *J* = 18.0 Hz, 1H), 4.47 (d, *J* = 18.0 Hz, 1H), 3.39-3.28 (m, 2H), 3.13-2.97 (m, 2H), 2.92-2.85 (m, 1H), 2.12 (s, 3H), 1.97-1.85 (m, 2H), 1.78 (s, 3H), 1.69-1.60 (m, 2H); ^{13}C NMR (75MHz, DMSO-d_6): δ 161.40, 159.00, 151.40, 142.84, 136.18, 132.92, 131.29, 129.67, 126.89, 125.87, 124.88, 88.43, 79.67, 74.56, 52.11, 51.38, 45.99, 35.74, 35.50, 26.94, 21.39, 17.37, 3.11; HR-MS (ESI) m/z: calculated for $C_{23}H_{27}N_7O_2$ $[M+H]^+$: 434.2299, found:434.2298.

(*R*)-6-(3-aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((1-(*p*-tolyl)-1H-1,2,3-triazol-4-yl)methyl)pyrimidine-2,4(1H,3H)-dione hydrochloride (A12)

Following a similar procedure for the preparation of **A01**, **A12** was prepared starting from 4-methylaniline. White solid (116 mg); mp: 81-86 °C; IR (ν_{max} cm^{-1}):3447, 2920, 1705, 1655, 1440, 818, 786; ^{1}H NMR (300MHz, DMSO-d_6) δ 8.57 (s, 1H), 8.30 (brs, 3H), 7.75 (d, *J* = 8.1 Hz, 2H), 7.37 (d, *J* = 8.1 Hz, 2H), 5.28 (s, 1H), 5.09 (s, 2H), 4.66 (d, *J* = 18.0 Hz, 1H), 4.47 (d, *J* = 18.0 Hz, 1H), 3.40-3.28 (m, 2H), 3.14-2.98 (m, 2H), 2.92-2.85 (m, 1H), 2.37 (s, 3H), 1.98-1.86 (m, 2H), 1.78 (s, 3H), 1.68-1.59 (m, 2H); ^{13}C NMR (75MHz, DMSO-d_6): δ 161.40, 159.03, 151.39, 143.89, 138.15, 134.31, 130.14, 121.30, 119.84, 88.40, 79.71, 74.56, 52.09, 51.40, 45.98, 35.80, 35.53, 26.95, 21.28, 20.51, 3.12; HR-MS (ESI) m/z: calculated for $C_{23}H_{27}N_7O_2$ $[M+H]^+$: 434.2299, found:434.2297.

(*R*)-6-(3-aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((1-(2,6-dimethyl phenyl)-1H-1,2,3-triazol-4-yl)methyl)pyrimidine-2,4(1H,3H)-dione hydrochloride (A13)

Following a similar procedure for the preparation of **A01**, **A13** was prepared starting from 2,6-dimethylaniline. White solid (122 mg); mp: 95-98°C; IR (ν_{max} cm^{-1}): 3438, 2953, 1705, 1654, 1440, 1230, 786; ^{1}H NMR (300MHz, DMSO-d_6) δ: 8.26 (brs, 3H), 8.20 (s, 1H), 7.41-7.35 (m, 1H), 7.28-7.26 (m, 2H), 5.29 (s, 1H), 5.11 (s, 2H), 4.66 (d, *J* = 15.0 Hz, 1H), 4.46 (d, *J* = 15.0 Hz, 1H), 3.34-3.25 (m, 2H), 3.13-2.98 (m, 2H), 2.91-2.84 (m, 1H), 1.97-1.91 (m, 2H), 1.89 (s, 6H), 1.77 (s, 3H), 1.69-1.61 (m, 2H); ^{13}C NMR (75MHz, DMSO-d_6): δ 161.38, 158.97, 151.34, 142.81, 135.75, 134.75, 129.83, 128.28, 125.16, 88.37, 79.61, 74.55, 52.07, 51.37, 45.94, 35.78, 35.47, 26.91, 21.19, 16.84, 3.10; HR-MS (ESI) m/z: calculated for $C_{24}H_{29}N_7O_2$ $[M+H]^+$:448.2455, found:448.2448.

(*R*)-6-(3-aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((1-(5-chloro-2-methylphenyl)-1H-1,2,3-triazol-4-yl)methyl)pyrimidine-2,4(1H,3H)-dione hydrochloride (A14)

Following a similar procedure for the preparation of **A01**, **A14** was prepared starting from 5-chloro-4-methylaniline. White solid (137 mg); mp: 94-96°C; IR (ν_{max} cm^{-1}):3448, 2922, 1705, 1654, 1604, 1498, 1440, 828, 809, 786; ^{1}H NMR (300MHz, DMSO-d$_6$) δ: 8.36 (s, 1H), 8.29 (brs, 3H), 7.57-7.48 (m, 3H), 5.29 (s, 1H), 5.11 (s, 2H), 4.66 (d, *J* = 18 Hz, 1H), 4.47 (d, *J* = 18 Hz, 1H), 3.34-3.28 (m, 2H), 3.12-2.97 (m, 2H), 2.92-2.85 (m, 1H), 2.12 (s, 3H), 1.97-1.87 (m, 2H), 1.78 (s, 3H), 1.69-1.61 (m, 2H); ^{13}C NMR (75MHz, DMSO-d_6): δ 161.38, 159.01, 151.38, 142.98, 137.03, 132.91, 132.07, 130.76, 129.49, 125.60, 125.00, 88.42, 79.69, 74.55, 52.10, 51.39, 45.98, 35.70, 35.51, 26.93, 21.26, 17.06, 3.11; HR-MS (ESI) m/z: calculated for $C_{23}H_{26}ClN_7O_2$ $[M+H]^+$: 468.1909, found: 468.1912.

(*R*)-6-(3-aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((1-(2-hydroxy-4-methylphenyl)-1H-1,2,3-triazol-4-yl)methyl)pyrimidine-2,4(1H,3H)-dione hydrochloride (A15)

Following a similar procedure for the preparation of **A01**, **A15** was prepared starting from 2-hydroxy-4-methylaniline. White solid (117 mg); mp: 107-110°C; IR (ν_{max} cm^{-1}):3442, 2950, 1703, 1649, 1441, 806; ^{1}H NMR (300MHz, DMSO-d_6) δ: 10.51 (s, 1H), 8.28 (brs, 3H), 8.24 (s, 1H), 7.44 (d, *J* = 8.1 Hz, 1H), 6.94 (s, 1H), 6.77 (d, *J* = 8.1 Hz, 1H), 5.28 (s, 1H), 5.09 (s, 2H), 4.65 (d, *J* = 18.0 Hz, 1H), 4.47 (d, *J* = 18.0 Hz, 1H), 3.40-3.27 (m, 2H), 3.11-2.98 (m, 2H), 2.91-2.85 (m, 1H), 2.29 (s, 3H), 1.96-1.86 (m, 2H), 1.78 (s, 3H), 1.68-1.62 (m, 2H); ^{13}C NMR (75MHz, DMSO-d_6): δ 161.42, 158.99, 151.36, 149.17, 142.41, 139.74,

124.74, 124.62, 122.09, 120.11, 117.26, 88.37, 79.71, 74.55, 52.06, 51.35, 45.93, 35.71, 35.50, 26.91, 21.23, 20.80, 3.12; HR-MS (ESI) m/z: calculated for $C_{23}H_{27}N_7O_3$ $[M+H]^+$: 450.2248, found: 450.2241.

(*R*)-6-(3-aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((1-(4-(tert-butyl) phenyl)-1H-1,2,3-triazol-4-yl)methyl)pyrimidine-2,4(1H,3H)-dione hydrochloride (A16)

Following a similar procedure for the preparation of **A01**, **A16** was prepared starting from 4-(tert-buthyl)aniline. White solid (132 mg); mp: 101-103 °C; IR (ν_{max} cm^{-1}):3435, 2959, 2867, 1705, 1655, 1440, 839; ^{1}H NMR (300MHz, DMSO-d_6) δ: 8.58 (s, 1H), 8.31 (brs, 3H), 7.77 (d, *J* = 8.7 Hz, 2H), 7.58 (d, *J* = 8.7 Hz, 2H), 5.28 (s, 1H), 5.09 (s, 2H), 4.66 (d, *J* = 16.8 Hz, 1H), 4.47 (d, *J* = 16.8 Hz, 1H), 3.39-3.28 (m, 2H), 3.12-2.93 (m, 2H), 2.92-2.85 (m, 1H), 1.99-1.83 (m, 2H), 1.78 (s, 3H), 1.67-1.63 (m, 2H), 1.32 (s, 9H); ^{13}C NMR (75MHz, DMSO-d_6): δ 161.41, 159.03, 151.39, 151.18, 143.83, 134.22, 126.51, 121.42, 119.73, 88.34, 79.71, 74.57, 52.04, 51.40, 45.94, 35.79, 35.54, 34.44, 30.95, 26.89, 21.20, 3.14; HR-MS (ESI) m/z: calculated for $C_{26}H_{33}N_7O_2$ $[M+H]^+$: 476.2768, found: 476.2768.

(*R*)-6-(3-aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((1-(2-methoxy phenyl)-1H-1,2,3-triazol-4-yl)methyl)pyrimidine-2,4(1H,3H)-dione hydrochloride (A17)

Following a similar procedure for the preparation of **A01**, **A17** was prepared starting from 2-methoxyaniline. White solid (123 mg); mp: 80-84 °C; IR (ν_{max} cm^{-1}):3435, 2949, 1705, 1654, 1602, 1507, 1475, 1441, 1232, 806; ^{1}H NMR (300MHz, DMSO-d_6) δ 8.58 (s, 1H), 8.31 (brs, 3H), 7.77 (d, *J* = 8.7 Hz, 2H), 7.58 (d, *J* = 8.7 Hz, 2H), 5.28 (s, 1H), 5.09 (s, 2H), 4.66 (d, *J* = 15.0 Hz, 1H), 4.47 (d, *J* = 15.0 Hz, 1H), 3.58 (s, 3H), 3.38-3.28 (m, 2H), 3.13-2.99 (m, 2H), 2.92-2.85 (m, 1H), 1.97-1.88 (m, 2H), 1.78 (s, 3H), 1.69-1.61 (m, 2H); ^{13}C NMR (75MHz, DMSO-d_6): δ 161.42, 159.01, 151.39, 142.49, 130.67, 125.71, 125.57, 125.26, 120.82, 112.95, 100.78, 88.37, 79.69, 74.57, 56.07, 52.07, 51.36, 45.94, 35.65, 35.50, 26.90, 21.17, 3.11; HR-MS (ESI) m/z: calculated for $C_{23}H_{27}N_7O_3$ $[M+H]^+$:450.2248, found:450.2245.

(*R*)-6-(3-aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((1-(3-methoxy phenyl)-1H-1,2,3-triazol-4-yl)methyl)pyrimidine-2,4(1H,3H)-dione hydrochloride (A18)

Following a similar procedure for the preparation of **A01**, **A18** was prepared starting from 3-methoxyaniline. White solid (114 mg); mp: 81-85 °C; IR (ν_{max} cm^{-1}):3434, 2945, 1706, 1651, 1609, 1440, 782; ^{1}H NMR (300MHz, DMSO-d_6) δ: 8.66 (s, 1H), 8.32 (brs, 3H), 7.51-7.44 (m, 3H), 7.05-7.02 (m, 1H), 5.28 (s, 1H), 5.09 (s, 2H), 4.67 (d, *J* = 18.0 Hz, 1H), 4.47 (d, *J* = 18.0 Hz, 1H), 3.84 (s, 3H), 3.39-3.29 (m, 2H), 3.13-2.97 (m, 2H), 2.93-2.86 (m, 1H), 1.97-1.86 (m, 2H), 1.78 (s, 3H), 1.70-1.61 (m, 2H); ^{13}C NMR (75MHz, DMSO-d_6): δ 161.41, 160.12, 159.04, 151.39, 143.95, 137.58, 130.75, 121.57, 114.29, 111.99, 105.62, 88.40, 79.73, 74.54, 55.59, 52.08, 51.40, 45.99, 35.79, 35.53, 26.94, 21.27, 3.11; HR-MS (ESI) m/z: calculated for $C_{23}H_{27}N_7O_3$ $[M+H]^+$: 450.2248, found: 450.2244.

(*R*)-6-(3-aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((1-(4-methoxy phenyl)-1H-1,2,3-triazol-4-yl)methyl)pyrimidine-2,4(1H,3H)-dione hydrochloride (A19)

Following a similar procedure for the preparation of **A01**, **A19** was prepared starting from 4-methoxyaniline. White solid (108 mg); mp: 94-97 °C; IR (ν_{max} cm^{-1}): 3441, 2949, 1705, 1655, 1605, 1441, 1230, 836; ^{1}H NMR (300MHz, DMSO-d_6) δ: 8.51 (s, 1H), 8.29 (brs, 3H), 7.77 (d, *J* = 9.0 Hz, 2H), 7.11 (d, *J* = 9.0 Hz, 2H), 5.28 (s, 1H), 5.08 (s, 2H), 4.67 (d, *J* = 18.0 Hz, 1H), 4.47 (d, *J* = 18.0 Hz, 1H), 3.82 (s, 3H), 3.39-3.27 (m, 2H), 3.14-2.96 (m, 2H), 2.92-2.85 (m, 1H), 1.99-1.87 (m, 2H), 1.78 (s, 3H), 1.69-1.58 (m, 2H); ^{13}C NMR (75MHz, DMSO-d_6): δ 161.40, 159.16, 159.02, 151.39, 143.75, 129.98, 121.65, 121.42, 114.79, 88.41, 79.71, 74.56, 55.52, 52.08, 51.40, 45.98, 35.80, 35.52, 26.95, 21.28, 3.12; HR-MS (ESI) m/z: calculated for $C_{23}H_{27}N_7O_3$ $[M+H]^+$: 450.2248, found: 450.2245

ethyl (*R*)-2-(4-((4-(3-aminopiperidin-1-yl)-3-(but-2-yn-1-yl)-2,6-dioxo-3,6-dihydropyrim idin-1(2H)-yl)methyl)-1H-1,2,3-triazol-1-yl)benzoate hydrochloride (A20)

Following a similar procedure for the preparation of **A01**, **A20** was prepared starting from ethyl 2-aminobenzoate. White solid (97 mg); mp: 171-174 °C; IR (ν_{max} cm^{-1}): 3434, 2949, 1707, 1654, 1604, 1440, 1295, 1131, 766; ^{1}H NMR (300MHz, DMSO-d_6) δ: 8.35-8.32 (m, 4H), 7.91-7.88 (m, 1H), 7.81-7.76 (m, 1H), 7.71-7.63 (m, 2H), 5.29 (s, 1H), 5.10 (s, 2H), 4.66 (d, *J* = 16.5 Hz, 1H), 4.46 (d, *J* = 16.5 Hz, 1H), 4.00 (q, *J* = 7.2 Hz, 2H), 3.39-3.27 (m, 2H), 3.13-2.96 (m, 2H), 2.91-2.84 (m, 1H), 1.98-1.87 (m, 2H), 1.77 (s, 3H), 1.63-1.59 (m, 2H), 0.91 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75MHz, DMSO-d_6): δ 165.34, 161.33, 159.00, 151.33, 143.02, 135.09, 132.80, 130.40, 129.96, 127.66, 126.21, 124.82, 88.40, 79.68, 74.54, 61.13, 52.08, 51.38, 45.93, 35.46, 26.91, 21.21, 13.39, 3.10; HR-MS (ESI) m/z: calculated for $C_{25}H_{29}N_7O_4$ $[M+H]^+$:492.2354, found:492.2353.

ethyl (*R*)-3-(4-((4-(3-aminopiperidin-1-yl)-3-(but-2-yn-1-yl)-2,6-dioxo-3,6- dihydropyrimidin-1(2H)-yl)methyl)-1H-1,2,3-triazol-1-yl)benzoate hydrochloride (A21)

Following a similar procedure for the preparation of **A01**, **A21** was prepared starting from ethyl 3-aminobenzoate. Yellow solid (87 mg); mp: 86-89 °C; IR (ν_{max} cm^{-1}): 3434, 2952, 1708, 1654, 1441, 757; ^{1}H NMR (300MHz, DMSO-d_6) δ: 8.79 (s, 1H), 8.38 (brs, 4H), 8.18 (d, *J* = 9.0 Hz, 1H), 8.03 (d, *J* = 9.0 Hz, 1H), 7.74 (t, *J* = 9.0 Hz, 1H), 5.28 (s, 1H), 5.11 (s, 2H), 4.68 (d, *J* = 15.6 Hz, 1H), 4.48 (d, *J* = 15.6 Hz, 1H), 4.37 (q, *J* = 6.0 Hz, 2H), 3.40-3.30 (m, 2H), 3.14- 2.99 (m, 2H), 2.93-2.86 (m, 1H), 1.98-1.89 (m, 2H), 1.78 (s, 3H), 1.69-1.61 (m, 2H), 1.35 (t, *J* = 6.0 Hz, 3H); ^{13}C NMR (75MHz, DMSO-d_6): δ 164.74, 161.40, 159.06, 151.40, 144.33, 136.76, 131.44, 130.51, 128.93, 124.45, 121.69, 120.09, 88.31, 79.72, 74.57, 61.29, 52.03, 51.42, 45.92, 35.81, 35.57, 26.91, 21.16, 14.08, 3.14; HR-MS (ESI) m/z: calculated for $C_{25}H_{29}N_7O_4$ $[M+H]^+$: 492.2354, found:492.2360.

ethyl (*R*)-4-(4-((4-(3-aminopiperidin-1-yl)-3-(but-2-yn-1-yl)-2,6-dioxo-3,6- dihydropyrim idin-1(2H)-yl)methyl)-1H-1,2,3-triazol-1-yl)benzoate (A22)

Following a similar procedure for the preparation of **A01**, **A22** was prepared starting from ethyl 4-aminobenzoate. Yellow solid (94 mg); mp: 95-99 °C; IR (ν_{max} cm^{-1}):3435, 1713, 1651, 1608, 1519, 1442, 1232, 770; ^{1}H NMR (300MHz, DMSO-d_6) δ: 8.79 (s, 1H), 8.35 (brs, 3H), 8.13 (d, *J* =9.0 Hz, 2H), 8.07 (d, *J* = 9.0 Hz, 2H), 5.29 (s, 1H), 5.11 (s, 2H), 4.67 (d, *J* = 18.0 Hz, 1H), 4.47 (d, *J* = 18.0 Hz, 1H), 4.34 (q, *J* = 7.2 Hz, 2H), 3.41-3.29 (m, 2H), 3.18-2.99 (m, 2H), 2.92-2.85 (m, 1H), 1.97-1.85 (m, 2H), 1.78 (s, 3H), 1.69-1.59 (m, 2H), 1.34 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75MHz, DMSO-d_6): δ 164.79, 161.39, 159.06, 151.38,

144.47, 139.63, 130.87, 129.46, 121.60, 119.75, 88.31, 79.73, 74.55, 61.06, 52.03, 51.42, 45.93, 44.15, 35.78, 35.58, 26.93, 21.20, 14.11, 3.14; HR-MS (ESI) m/z: calculated for $C_{25}H_{29}N_7O_4$ $[M+H]^+$: 492.2354, found:492.2347.

(*R*)-2-(4-((4-(3-aminopiperidin-1-yl)-3-(but-2-yn-1-yl)-2,6-dioxo-3,6-dihydropyrimidin-1(2H)-yl)methyl)-1H-1,2,3-triazol-1-yl)benzoic acid hydrochloride (A23)

To the solution of Boc precursor of **A20** (160 mg, 0.27 mmol) in methanol (5 mL), was added NaOH (1M, 2 mL). the resulting mixture was stirred at r.t. for 2 h. After evaporation of solvent, the residue was dissolved in water (3 mL) and acidified with HCl (1M, 2 mL) to adjust the pH value to 3. The precipitate was collected by filtration, purified by flash chromatography (dichloromethane/methanol, 100:1~ 30:1) to give Boc precursor of **A23,** which was dissolved in EtOAc (5 mL) and ether (30 mL), and bubbled with freshly prepared HCl gas at °C. After TLC indicated consumption of starting materials, the precipitate was collected by filtration, dried in vacuo to to give compound **A23** as a white solid (70 mg, yield 62%). mp:111-116 °C; IR (ν_{max} cm^{-1}):3448, 2921, 1705, 1651, 1442, 800,767; ^1H NMR (300MHz, DMSO-d_6) δ: 13.21 (brs, 1H), 8.38 (brs, 3H), 8.32 (s, 1H), 7.89 (d, J = 7.8 Hz, 1H), 7.73 (t, J = 7.5 Hz, 1H), 7.65 (t, J = 7.5 Hz, 1H), 7.57 (d, J = 7.8 Hz, 1H), 5.27 (s, 1H), 5.09 (s, 2H), 4.67 (d, J = 18.0 Hz, 1H), 4.47 (d, J = 18.0 Hz, 1H), 3.40- 3.28 (m, 2H), 3.13- 2.98 (m, 2H), 2.93-2.86 (m, 1H), 1.97-1.85 (m, 2H), 1.78 (s, 3H), 1.69- 1.60 (m, 2H); ^{13}C NMR (75MHz, DMSO-d_6): δ 166.57, 161.36, 159.00, 151.39, 142.82, 135.06, 132.29, 130.28, 129.76, 128.54, 126.25, 124.64, 88.32, 79.73, 74.58, 52.04, 51.39, 45.92, 35.72, 35.56, 26.90, 21.23, 3.14; HR-MS (ESI) m/z: calculated for $C_{23}H_{25}N_7O_4$ $[M+H]^+$:464.2041, found:464.2044.

(*R*)-3-(4-((4-(3-aminopiperidin-1-yl)-3-(but-2-yn-1-yl)-2,6-dioxo-3,6-dihydropyrimidin-1(2H)-yl)methyl)-1H-1,2,3-triazol-1-yl)benzoic acid hydrochloride (A24)

Following a similar procedure for the preparation of **A23**, **A24** was prepared starting from Boc precursor of **A21**. White solid (65 mg); mp: 115-119 °C; IR (ν_{max} cm^{-1}): 3448, 1706, 1643, 1443, 1232, 760; ^1H NMR (300MHz, DMSO-d_6) δ: 8.78 (s, 1H), 8.36 (s, 1H), 8.32 (brs, 3H), 8.15 (d, J = 7.8 Hz, 1H), 8.02 (d, J = 7.8 Hz, 1H), 7.72 (t, J = 7.8 Hz, 1H), 5.29 (s, 1H), 5.10 (s, 2H), 4.67 (d, J = 17 Hz, 1H), 4.47 (d, J = 17 Hz, 1H), 3.35- 3.28 (m, 2H), 3.14-3.01 (m, 2H), 2.93-2.85 (m, 1H), 1.95-1.85 (m, 2H), 1.78 (s, 3H), 1.70-1.59 (m, 2H); ^{13}C NMR (75MHz, DMSO-d_6): δ 166.29, 161.41, 159.07, 151.41, 144.33, 136.68, 132.43, 130.35, 129.10, 124.07, 121.57, 120.24, 88.31, 79.71, 74.58, 52.02, 51.42, 45.92, 35.86, 35.58, 26.90, 21.19, 3.14; HR-MS (ESI) m/z: calculated for $C_{23}H_{25}N_7O_4$ $[M+H]^+$: 464.2041, found:464.2046.

(*R*)-4-(4-((4-(3-aminopiperidin-1-yl)-3-(but-2-yn-1-yl)-2,6-dioxo-3,6-dihydropyrimidin-1(2H)-yl)methyl)-1H-1,2,3-triazol-1-yl)benzoic acid hydrochloride (A25)

Following a similar procedure for the preparation of **A23**, **A25** was prepared starting from Boc precursor of **A22**. White solid (86 mg); mp: 122-126 °C; IR (ν_{max} cm^{-1}):3457, 2950, 1705, 1647, 1608, 1443, 799, 773; ^1H NMR (300MHz, DMSO-d_6) δ: 13.22 (brs, 1H), 8.77 (s, 1H), 8.25 (brs, 3H), 8.12 (d, J = 8.1 Hz, 2H), 8.04 (d, J = 8.1 Hz, 2H), 5.30 (s, 1H), 5.11 (s, 2H), 4.66 (d, J = 15.0 Hz, 1H), 4.47 (d, J = 15.0 Hz, 1H), 3.28-3.19 (m, 2H), 3.12- 3.01 (m, 2H), 2.91-2.84 (m, 1H), 1.96- 1.85 (m, 2H), 1.78 (s, 3H), 1.68- 1.59 (m, 2H); ^{13}C NMR (75MHz, DMSO-d_6): δ 166.35, 161.39, 159.05, 151.38, 144.41, 139.42, 131.02, 130.47, 121.58, 119.66, 88.33, 79.73, 74.56, 52.05, 51.41, 45.94, 35.77, 35.57, 26.92, 21.18, 3.14; HR-MS (ESI) m/z: calculated for $C_{23}H_{25}N_7O_4$ $[M+H]^+$: 464.2041, found:464.2047.

ethyl 2-azidoacetate (J01)

To the solution of ethyl bromoacetate (10 g, 60 mmol) in acetone (50 mL), was added dropwise the solution of NaN_3 (9.73 g, 150 mmol) in water (40 mL) at 0–5 °C. The mixture was stirred at 60 °C for 4 h. After cooling to r.t., the solvent was evaporated. Water was added, and extracted with with dichloromethane (4 × 40 mL), washed with saturated $NaHCO_3$ (3 × 20 mL) and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo to give **J01** as colourless oil (7 g, 90%), MS (ESI) m/z: 130.1 $[M+H]^+$; ^1H NMR (300 MHz, $CDCl_3$): δ 4.25 (q, J = 7.2 Hz, 2H), 3.83 (s, 2H), 1.27 (t, J = 7.2 Hz, 3H).

ethyl (*R*)-2-(4-((3-(but-2-yn-1-yl)-4-(3-((tert-butoxycarbonyl)amino) piperidin-1-yl)-2,6-dioxo-3,6-dihydropyrimidin-1(2H)-yl)methyl)-1H-1,2,3-triazol-1-yl)acetate (K01)

Following a similar procedure for the preparation of **H01**, **J01** was prepared starting from compound **D** (5 g, 12.5 mmol) and compoud **I01** (1.93 mg, 15.0 mmol). The crude product was purified by flash chromatography (EtOAc/ether 100:1~30:1) to give **K01** as a white solid (4.3 g, 65%). mp: 78-80 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 7.71 (s, 1H), 5.32 (s, 2H), 5.28 (s, 1H), 5.02 (s, 2H), 4.83-4.74 (m, 1H), 4.67 (d, J = 15.3 Hz, 1H), 4.48 (d, J = 15.3 Hz, 1H), 4.08 (q, J = 7.2 Hz, 2H), 3.87-3.78 (m, 1H), 3.34-3.21 (m, 1H), 3.15-3.02 (m, 1H), 2.86-2.80 (m, 1H), 2.72-2.59 (m, 1H), 1.96-1.87 (m, 1H), 1.82 (s, 3H), 1.74-1.67 (m, 2H), 1.45 (s, 9H), 1.26 (t, J = 7.2 Hz, 3H). HR-MS (ESI) m/z: calculated for $C_{25}H_{36}N_7O_6$ $[M+H]^+$: 529.4512, found: 529.4516.

(*R*)-2-(4-((4-(3-aminopiperidin-1-yl)-3-(but-2-yn-1-yl)-2,6-dioxo-3,6-dihydropyrimidin-1(2H)-yl)methyl)-1H-1,2,3-triazol-1-yl)acetic acid hydrochloride (B01)

Following a similar procedure for the preparation of **A23**, **B01** was prepared starting from **K01** (3.5 g, 6.6 mmol). White solid (86 mg, 65%); mp: 114-117 °C; IR (ν_{max} cm^{-1}): 3448, 2978, 1743, 1704, 1650, 1442, 1233, 1053, 815; ^1H NMR (300MHz, DMSO-d_6) δ: 13.37 (brs, 1H), 8.18 (brs, 3H), 7.93 (s, 1H), 5.28 (s, 1H), 5.22 (s, 2H), 5.01 (s, 2H), 4.64 (d, J = 18.0 Hz, 1H), 4.45 (d, J = 18.0 Hz, 1H), 3.31-3.23 (m, 2H), 3.10-3.00 (m, 2H), 2.90-2.83 (m, 1H), 1.95-1.87 (m, 2H), 1.79 (s, 3H), 1.69-1.61 (m, 2H); ^{13}C NMR (75MHz, DMSO-d_6): δ 168.51, 161.34, 158.98, 151.29, 142.55, 124.86, 88.36, 79.74, 74.50, 64.87, 59.71, 52.07, 51.34, 50.34, 45.93, 35.73, 35.47, 34.08, 26.90, 21.25, 20.72, 15.12, 14.03, 3.13; HR-MS (ESI) m/z: calculated for $C_{18}H_{23}N_7O_4$ $[M+H]^+$: 402.1884, found: 402.1881.

ethyl (R)-2-(4-((4-(3-aminopiperidin-1-yl)-3-(but-2-yn-1-yl)-2,6-dioxo-3,6-dihydr- opyrimidin-1(2H)-yl)methyl)-1H-1,2,3-triazol-1-yl)acetate hydrochloride (B02)

Following a similar procedure for the preparation of **A23**, **B02** was prepared starting from **K01.** White solid (120 mg, yield: 78%); mp: 71-73 °C; IR (ν_{max} cm^{-1}): 3435, 2926, 1751, 1651, 1439, 750; ^1H NMR (300MHz, DMSO-d_6): δ 8.36 (brs, 3H), 7.96 (s, 1H), 5.33 (s, 2H), 5.26 (s, 1H), 5.02 (s, 2H), 4.65 (d, J = 18.0 Hz, 1H), 4.45 (d, J = 18.0 Hz, 1H),

4.16 (q, *J* = 6.6 Hz, 2H), 3.40-3.27 (m, 2H), 3.12-2.97 (m, 2H), 2.91-2.84 (m, 1H), 1.96-1.85 (m, 2H), 1.78 (s, 3H), 1.68-1.57 (m, 2H), 1.20 (t, *J* = 6.6 Hz, 3H); ^{13}C NMR (75MHz, DMSO-d_6): δ 167.21, 161.35, 159.00, 151.29, 142.68, 124.96, 88.36, 79.74, 74.50, 61.40, 52.07, 51.36, 50.21, 45.93, 35.72, 35.48, 26.88, 21.18, 13.93, 3.14; HR-MS (ESI) m/z: calculated for $C_{20}H_{27}N_7O_4$ $[M+H]^+$:430.2197, found:430.2198.

(*R*)-3-(4-((4-(3-aminopiperidin-1-yl)-3-(but-2-yn-1-yl)-2,6-dioxo-3,6-dihydropyrimidin-1(2H)-yl)methyl)-1H-1,2,3-triazol-1-yl)propanoic acid hydrochloride(**B03**)

Following a similar procedure for the preparation of **B01. B03** was prepared starting from methyl 3-bromopropionate. White solid (78 mg), mp: 74-76°C; IR (ν_{max} cm^{-1}): 3447, 2956, 1704, 1647, 1443, 1231, 807; ^{1}H NMR (300MHz, DMSO-d_6) δ: 8.45 (brs, 3H), 7.92 (s, 1H), 5.24 (s, 1H), 4.98 (s, 2H), 4.70-4.61 (m, 1H), 4.51-4.41 (m, 3H), 3.38-3.30 (m, 2H), 3.11-2.96 (m, 2H), 2.88-2.83 (m, 3H), 1.93-1.85 (m, 2H), 1.79 (s, 3H), 1.66-1.62 (m, 2H); ^{13}C NMR (75MHz, DMSO-d_6): δ 171.74, 161.34, 158.96, 151.28, 142.57, 123.57, 88.35, 79.72, 74.53, 52.07, 51.36, 45.92, 45.23, 35.76, 35.45, 33.93, 26.87, 21.26, 3.13. HR-MS (ESI) m/z: calculated for $C_{19}H_{25}N_7O_4$ $[M+H]^+$:416.2041, found:416.2041.

methyl (R)-3-(4-((4-(3-aminopiperidin-1-yl)-3-(but-2-yn-1-yl)-2,6-dioxo-3,6-dihy dropyri midin-1(2H)-yl)methyl)-1H-1,2,3-triazol-1-yl)propanoate hydrochloride (**B04**)

Following a similar procedure for the preparation of **B02. B04** was prepared starting from methyl 3-bromopropionate. White solid (113 mg); mp: 73-76°C; IR (ν_{max} cm^{-1}): 3445, 2936, 1705, 1643, 1447, 1236, 806; ^{1}H NMR (300MHz, DMSO-d_6): δ 8.45 (brs, 3H), 7.93 (s, 1H), 5.24 (s, 1H), 4.98 (s, 2H), 4.66 (d, *J* = 18.0 Hz, 1H), 4.53 (t, *J* = 6.6 Hz, 2H), 4.45 (d, *J* = 18.0 Hz, 1H), 3.59 (s, 3H), 3.38-3.30 (m, 2H), 3.12-3.03 (m, 2H), 2.95 (t, *J* = 6.6 Hz, 2H), 2.89-2.83 (m, 1H), 1.98-1.87 (m, 2H), 1.79 (s, 3H), 1.69-1.62 (m, 2H); ^{13}C NMR (75MHz, DMSO-d_6): δ 170.78, 161.34, 158.97, 151.28, 142.60, 123.64, 88.35, 79.72, 74.53, 52.06, 51.60, 51.37, 45.93, 45.05, 35.74, 35.46, 33.62, 26.88, 21.18, 3.14; HR-MS (ESI) m/z: calculated for $C_{20}H_{27}N_7O_4$ $[M+H]^+$:430.2197, found:430.2196.

(*R*)-4-(4-((4-(3-aminopiperidin-1-yl)-3-(but-2-yn-1-yl)-2,6-dioxo-3,6-dihydropyrimidin-1(2H)-yl)methyl)-1H-1,2,3-triazol-1-yl)butanoic acid hydrochloride (**B05**)

Following a similar procedure for the preparation of **B01. B05** was prepared starting from ethyl 4-bromobutyrate. White solid (68 mg); mp: 160-163 °C; IR (ν_{max} cm^{-1}): 3448, 2952, 1706, 1651, 1442, 805; ^{1}H NMR (300MHz, DMSO-d_6) δ: 8.33 (s, 3H), 7.94 (s, 1H), 5.25 (s, 1H), 4.99 (s, 2H), 4.65 (d, *J* = 18 Hz, 1H), 4.45 (d, *J* = 18 Hz, 1H), 4.32 (t, *J* = 7.0 Hz, 2H), 3.43-3.33 (m, 2H), 3.13-2.95 (m, 2H), 2.92-2.83 (m, 1H), 2.21 (t, *J* = 7.1 Hz, 2H), 2.01-1.90 (m, 4H), 1.79 (s, 3H), 1.70-1.58 (m, 2H); ^{13}C NMR (75MHz, DMSO-d_6): δ 173.53, 161.36, 158.96, 151.30, 142.72, 123.33, 88.37, 79.69, 74.54, 52.07, 51.36, 48.50, 45.94, 35.80, 35.43, 30.34, 26.89, 25.20, 21.22, 3.12; HR-MS (ESI) m/z: calculated for $C_{20}H_{27}N_7O_4$ $[M+H]^+$: 430.2197, found: 430.2193.

2-(4-((4-((*R*)-3-aminopiperidin-1-yl)-3-(but-2-yn-1-yl)-2,6-dioxo-3,6-dihydropyrimidin-1(2H)-yl)methyl)-1H-1,2,3-triazol-1-yl) propanoic acid hydrochloride (**B06**)

Following a similar procedure for the preparation of **B01. B05** was prepared starting from ethyl 2-bromopropionate. White solid (103 g); mp: 178-181 °C; IR (ν_{max} cm^{-1}): 3447, 2948, 1704, 1650, 1231, 805; ^{1}H NMR (300MHz, DMSO-d_6): δ 8.41 (brs, 3H), 8.00 (s, 1H), 5.47-5.45 (m, 1H), 5.25 (s, 1H), 5.01 (s, 2H), 4.66 (d, *J* = 18.0 Hz, 1H), 4.46 (d, *J* = 18.0 Hz, 1H), 3.34-3.20 (m, 2H), 3.07-2.97 (m, 2H), 2.92-2.89 (m, 1H), 1.96-1.92 (m, 2H), 1.79 (s, 3H), 1.79-1.68 (m, 5H); ^{13}C NMR (75MHz, DMSO-d_6): δ 170.81, 161.36, 158.97, 151.32, 142.41, 123.14, 88.39, 79.73, 74.53, 57.41, 52.08, 51.38, 45.92, 35.82, 35.47, 26.91, 21.11, 17.07, 3.13; HR-MS (ESI) m/z: calculated for $C_{19}H_{25}N_7O_4$ $[M+H]^+$:416.2041, found:416.2032.

ethyl 2-(4-((4-((R)-3-aminopiperidin-1-yl)-3-(but-2-yn-1-yl)-2,6-dioxo-3,6-dihy- dropyrimidin-1(2H)-yl)methyl)-1H-1,2,3-triazol-1-yl)propanoate hydrochloride (**B07**)

Following a similar procedure for the preparation of **B02. B07** was prepared starting from ethyl 2-bromopropionate. White solid (126 mg); mp: 146-149 °C; IR (ν_{max} cm^{-1}):3434, 2947, 2360, 1746, 1705, 1633, 1441, 856; ^{1}H NMR (300MHz, DMSO-d_6): δ 8.24 (brs, 3H), 8.03 (s, 1H), 5.58 (q, *J* = 7.2 Hz, 1H), 5.27 (s, 1H), 5.04 (s, 2H), 4.64 (d, *J* = 16.2 Hz, 1H), 4.46 (d, *J* = 16.2 Hz, 1H), 4.14 (q, *J* = 7.2 Hz, 2H), 3.34-3.22 (m, 2H), 3.12-2.96 (m, 2H), 2.91-2.82 (m, 1H), 1.99-1.85 (m, 2H), 1.79 (s, 3H), 1.71 (d, *J* = 7.2 Hz, 3H), 1.65-1.55 (m, 2H), 1.17 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75MHz, DMSO-d_6): δ: 183.31, 169.25, 161.35, 158.98, 151.31, 142.57, 123.25, 88.36, 79.72, 74.52, 61.55, 57.30, 52.07, 51.38, 45.93, 35.80, 35.49, 26.91, 21.21, 17.04, 13.83, 3.13; HR-MS (ESI) m/z: calculated for $C_{21}H_{29}N_7O_4$ $[M+H]^+$:444.2354, found:444.2344.

(*R*,*E*)-4-(4-((4-(3-aminopiperidin-1-yl)-3-(but-2-yn-1-yl)-2,6-dioxo-3,6-dihydropyrimidin-1(2H)-yl)methyl)-1H-1,2,3-triazol-1-yl)but-2-enoic acid hydrochloride (**B08**)

Following a similar procedure for the preparation of **B01. B08** was prepared starting from ethyl 4-bromocrotonate. White solid (113 mg); mp: 210-213 °C; IR (ν_{max} cm^{-1}): 3435, 2950, 1705, 1650, 1442, 806; ^{1}H NMR (300MHz, DMSO-d_6): δ 8.44 (s, 3H), 8.35 (s, 0.5 H), 7.98 (s, 0.5 H), 7.40 (d, *J* = 14.4 Hz, 0.5 H), 6.88 (dt, *J* = 15.3, 5.1 Hz, 0.5 H), 6.47-6.32 (m, 0.5 H), 5.66 (d, *J* = 14.4 Hz, 1H), 5.25 (s, 1H), 5.18 (d, *J* = 5.1 Hz, 1H), 5.01 (s, 2H), 4.66 (d, *J* = 17.7 Hz, 1H), 4.45 (d, *J* = 17.7 Hz, 1H), 3.38-3.29 (m, 2H), 3.23 (d, *J* = 7.4 Hz, 1H), 3.06-3.00 (m, 2H), 2.95-2.80 (m, 1H), 1.94-1.89 (m, 2H), 1.78 (s, 3H), 1.71-1.55 (m, 2H); ^{13}C NMR (75MHz, DMSO-d_6): δ 172.02, 171.81, 161.35, 159.02, 151.28, 143.46, 143.00, 142.57, 141.45, 126.15, 124.37, 123.95, 123.85, 120.61, 119.21, 115.49, 88.35, 79.69, 75.89, 74.51, 56.84, 52.06, 51.35, 49.60, 45.93, 36.72, 35.75, 35.52, 35.45, 34.13, 26.88, 21.20, 14.04, 3.12. HR-MS (ESI) m/z: calculated for $C_{20}H_{25}N_7O_4$ $[M+H]^+$: 428.2041, found: 428.2038.

ethyl (*R*,*E*)-4-(4-((4-(3-aminopiperidin-1-yl)-3-(but-2-yn-1-yl)-2,6-dioxo-3,6-di hydropyrimidin-1(2H)-yl)methyl)-1H-1,2,3-triazol-1-yl)but-2-enoate hydrochloride (**B09**)

Following a similar procedure for the preparation of **B02. B09** was prepared starting from ethyl ethyl 4-bromocrotonate. White solid (134 mg, yield 76%); mp: 177-180 °C; IR (ν_{max} cm^{-1}): 3435, 2954, 1708, 1651, 1442, 805; ^{1}H NMR (300MHz, DMSO-d_6): δ 8.29 (s, 3H), 8.00 (s, 1H), 6.96 (dt, *J* = 15.0, 6.0 Hz, 1H), 5.73 (d, *J* = 15.0 Hz, 1H), 5.26 (s, 1H), 5.20 (d, *J* = 6.0 Hz, 2H), 5.01 (s, 2H), 4.64 (d, *J* = 15.0 Hz, 1H), 4.45

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(d, *J* = 15.0 Hz, 1H), 4.11 (q, *J* = 7.2 Hz, 2H), 3.36-3.25 (m, 2H), 3.12-2.97 (m, 2H), 2.91-2.82 (m, 1H), 1.96-1.85 (m, 2H), 1.78 (s, 3H), 1.69-1.61 (m, 2H), 1.17 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75MHz, DMSO-d_6): δ 164.83, 161.36, 158.98, 151.30, 143.02, 142.16, 123.90, 122.86, 88.35, 79.69, 74.52, 60.22, 52.06, 51.37, 49.61, 45.93, 35.77, 35.47, 26.90, 21.21, 14.00, 3.13; HR-MS (ESI) m/z: calculated for $C_{22}H_{29}N_7O_4$ $[M+H]^+$:456.2354, found:456.2354.

In vitro assay for inhibition of DPP-4, DPP-8 and DPP-9

The DPP-4 Drug Discovery Kit (Enzo Life Sciences International, Inc.) was used for the assay of inhibition of DPP-4 activity. The assay is based on the cleavage of 7-amino-4-methylcoumarin (AMC) moiety from the C-terminus of the peptide substrate (H-Gly-Pro-AMC), which increases its fluorescence intensity at 460 nm. The DPP-4 inhibitor P32/98 was selected as a control. The substrate and DPP-4 enzyme were diluted 1/50 with assay buffer (50mM Tris, pH=7.5). 25 µL of assay buffer, 15µL enzyme solution and 10 µL of appropriately diluted solutions of the test compounds were added sequently to 96-well microtiter plates. After incubation at 37 °C for 10 min, 50 µL of diluted substrate solution was added. Fluorescence was measured using an excitation wavelength of 380 nm and an emission wavelength of 460 nm by a Synergy H1 MultMode Reader (BioTek, USA). The inhibitory rate relative to the control without inhibitor was calculated and IC_{50} value was determined by nonlinear regression fitted by GraphPad Prism 5. The assays for inhibition of DPP-8 and DPP-9 activity were performed in similar procedure by using DPP4-Glo™ Assay kit (Promega, Cat.No.G8531), DPP-8 and DPP-9 enzymes (BPS, Cat. NO. 80080 and 80080).

Molecular modeling

Docking studies were carried out using Glide 5.9 in Schrödinger 2013 suite. The DPP-4 protein was extracted from RCSB Protein Data Bank (PDB ID: 2RGU). Protein structures were prepared using Maestro protein preparation wizard applying the default parameters. Ligands were built using Maestro build panel and prepared by LigPrep application using default parameters. A docking grid was constructed by using the centroid of the bound ligand and a maximum size of 10 Å. Molecular docking of all molecules into the generated grid was performed by using the standard precision (SP) docking mode.

In vivo study

Animals

Male ICR mice aged 10 weeks (18–22 g) and male C57BL/6 mice (20 ± 2 g) were purchased from Comparative Medicine Centre of Yangzhou University, and were kept under conventional conditions of controlled temperature, humidity, and lighting for 1 week before the experimental period. All animal procedures were done in accordance with the applicable institutional and governmental regulations concerning the ethical use of animals.

In vivo oral glucose tolerance test (OGTT) in ICR mice

The male ICR mice were fasted overnight (12 h), weighted, bled via tail tip, and randomized into groups (n = 8). Mice were dosed orally with single doses of vehicle (ultrapure water), alogliptin (suspended in vehicle; 3 mg/kg) or tested compounds (suspended in vehicle; 3 or 10 mg/kg), 30 min prior to oral glucose load (20% aqueous glucose solution, 2.5 g/kg). The blood glucose measured for the grouping of the animals was used as the data before administration of vehicle, alogliptin or compounds (time -30). Blood samples were collected time 0, 15, 30, 45, 60 and 120 min. The blood glucose was measured by blood glucose test strips (SanNuo ChangSha, ChangSha, China).

In vivo oral glucose tolerance test (OGTT) in C57BL/6 mice

Male C57BL/6 mice left to acclimatize for 1 week were fed with high-fat diet (MD 12032, rodent diet with 45 kcal% fat, from Mediscience Ltd., Yangzhou, China) for 12 weeks to induce insulin resistance. The mice with fasting blood glucose level 10 mmol/L or higher were considered as type 2 diabetic model and selected for acute oral glucose tolerance test. Type 2 diabetic C57BL/6 mice were fasted for 12 h, weighted, randomized into groups (n = 6). Mice were dosed orally with single doses of vehicle (ultrapure water), test compounds (suspended in vehicle; 10 mL/kg; 1-10 mg/kg), 30 min prior to oral glucose load (10% aqueous glucose solution, 1 g/kg). The blood glucose levels were measured by blood glucose test strips (SanNuo GA-3 type, ChangSha, China) before administration of vehicle, alogliptin or test compounds (–30 min). After glucose oral adminstration, blood glucose were measured at 0, 15, 30, 60 and 120 min. The blood glucose was measured by blood glucose test strips (SanNuo ChangSha, ChangSha, China).

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