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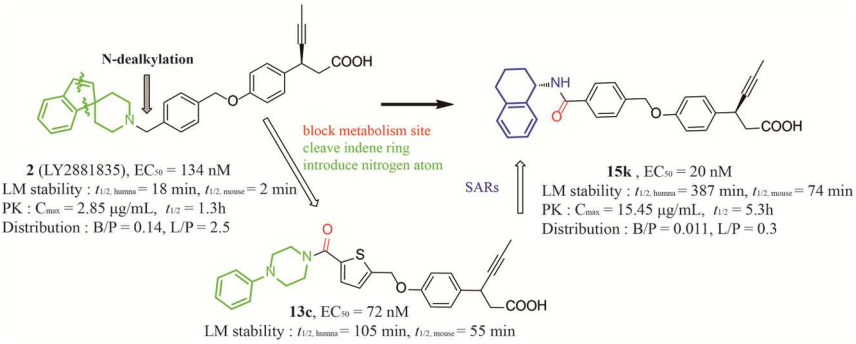

2 (LY2881835), EC_{50} = 134 nM
LM stability : $t_{1/2,\ humna}$ = 18 min, $t_{1/2,\ mouse}$ = 2 min
PK : C_{max} = 2.85 μg/mL, $t_{1/2}$ = 1.3h
Distribution : B/P = 0.14, L/P = 2.5

15k , EC_{50} = 20 nM
LM stability : $t_{1/2,\ humna}$ = 387 min, $t_{1/2,\ mouse}$ = 74 min
PK : C_{max} = 15.45 μg/mL, $t_{1/2}$ = 5.3h
Distribution : B/P = 0.011, L/P = 0.3

13c, EC_{50} = 72 nM
LM stability : $t_{1/2,\ humna}$ = 105 min, $t_{1/2,\ mouse}$ = 55 min

Design, Synthesis and Structure−activity Relationship Studies of GPR40 Agonists Containing Amide Linker

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ABSTRACT: Free fatty acid receptor 1 (FFAR1/GPR40) attracted significant attention as a potential target for developing novel antidiabetic drugs because of its unique mechanism in glucose homeostasis. Several reports have expressed concerns about central nervous system (CNS) penetration of GPR40 agonists, which is possibly attributed to their high lipophilicity and low total polar surface area. Herein, we report our efforts to improve the physicochemical properties and pharmacokinetic profiles of LY2881835, a GPR40 agonist that had undergone Phase I clinical trial, through a series of structural optimizations. We identified an orally efficacious compound, **15k**, which possessed increased plasma exposure, prolonged half-life and reduced CNS exposure and liver to plasma distribution ratio compared with LY2881835. **15k** is a potentially valuable lead compound in the development of safe and efficacious GPR40-targeted drugs to treat type 2 diabetes mellitus.

Keywords: GPR40, FFAR1, insulin secretion, type 2 diabetes mellitus, CNS

1. Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disorder disease characterized by both defective insulin secretion and insulin action in maintaining glucose homeostasis [1]. T2DM often leads to multiple macrovascular and microvascular complications, including cardiovascular diseases, nephropathy, neuropathy and retinopathy. [2-4]. The incidence of T2DM is increasing at an alarming rate. By 2017, the number of individuals affected worldwide was about 425 million and this figure may reach 629 million in 2045 [5]. This severe situation, along with the undesirable side effects caused by current hypoglycemic agents, such as high risk of hypoglycemia, body weight gain and gastric symptoms [6-8], stimulate urgent needs for novel drugs with improved safety and glycemic control.

Free fatty acid receptor 1 (FFAR1, also known as GPR40) belongs to the family of G protein-coupled receptors (GPCRs) and was de-orphanized in 2003 as a receptor for medium- to long-chain free fatty acids (FFAs) [9, 10]. Mainly expressed in beta cells of pancreatic islets, GPR40 can be activated by the endogenous FFAs, which subsequently elicit amplified glucose-dependent insulin secretion without high risk of hypoglycemia [9, 11-15]. GPR40 is also expressed in the incretin-producing enteroendocrine L cells of the intestinal tract and its activation results in secretion of incretins such as glucagon like peptide 1 (GLP-1) [16, 17]. Therefore, the ability of GPR40 to regulate glucose homeostasis by two mechanisms, glucose-stimulated insulin secretion and incretin secretion, makes it an excellent target for T2DM drug

development [18, 19].

Reports described a range of synthetic GPR40 agonists with desirable safety and efficacy profiles, compared with existing antidiabetic therapies such as insulin and sulfonylureas [20-28]. Among them, several small molecules were tested in the clinical trial such as TAK-875 [20, 29], AMG 837 [21, 30], LY2922083 and LY2881835 [23, 31] (Figure 1). The most advanced compound TAK-875 (Fasiglifam, **1**) from Takeda, while providing proof of concept, was withdrawn from phase III trial due to liver toxicity [32]. The exact reason for its hepatotoxicity is currently unclear, but some reports indicated that it may be linked with high distribution of TAK-875 and TAK-875 conjugations (TAK-875 acyl glucuronide, TAK-875 glucuronide and TAK-875 taurine conjugate) in liver, as well as their ability to alter the bile acid homeostasis [33-35]. Moreover, GPR40 is highly expressed in the brain and can be activated by polyunsaturated FFAs [9]. The exact function of GPR40 in the brain remains unclear, but it might have correlation with antinociception, adult neurogenesis and neurovascular degeneration [36-41]. Therefore, to balance the blood glucose levels, it would be more preferable to directly target the GPR40 in the pancreatic beta cells and enteroendocrine L cells rather than to elicit the undesired effects in the central nervous system (CNS) or liver.

In general, properties such as total polar surface area (tPSA), LogP, molecular weight, hydrogen bond, p*K*a and rigidity of the molecule are closely linked to blood-brain barrier (BBB) penetration [42]. Most reported GPR40 agonists possess

relatively high lipophilicity [43-45]. Amgen described an analogue of AMG 837 (LogP = 6.8, tPSA = 47) with significant CNS exposure (total brain-to-plasma drug distribution ratio, B/P = 0.6). Incorporation of polar groups, as in AMG-3189 (B/P = 0.04) and AMG-4668 (B/P = 0.02) (Figure 1), substantially decreased BBB penetration [46, 47]. LY2881835 (**2**), which finished Phase I clinical trial in 2011, possessed high *in vitro* potency and selectivity, as well as significant insulin and GLP-1 secretion in both *in vitro* and *in vivo* assays [23, 31]. However, **2** (LogP = 6.5, tPSA = 50), like AMG-837, had a certain amount of CNS exposure (B/P = 0.14). In addition, **2** displayed high clearance *in vitro* ($t_{1/2}$ = 18 min in human liver microsomes, $t_{1/2}$ = 2 min in mouse liver microsomes) and short half-life *in vivo* ($t_{1/2}$ = 1.3 h). We sought to modify the pharmacokinetic (PK) and physicochemical properties of **2** by introducing polar atoms, blocking the metabolically susceptible position and cleaving the indene ring. After a systematic structure-activity relationship (SAR) study and further optimization, we identified an orally active compound **15k**, which exhibited improved metabolic stability and high plasma exposure, along with lowered CNS exposure and liver to plasma distribution ratio (L/P).

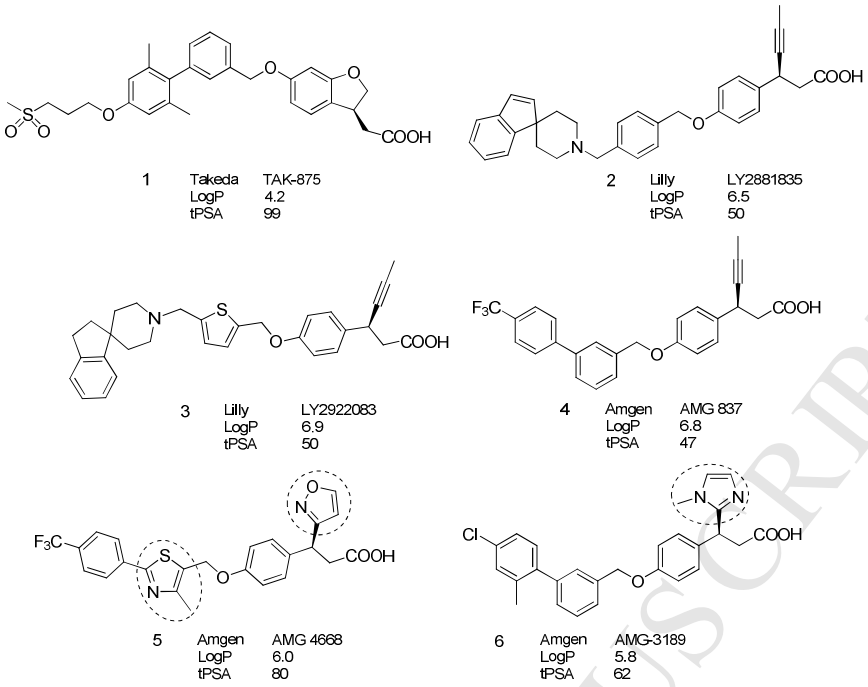


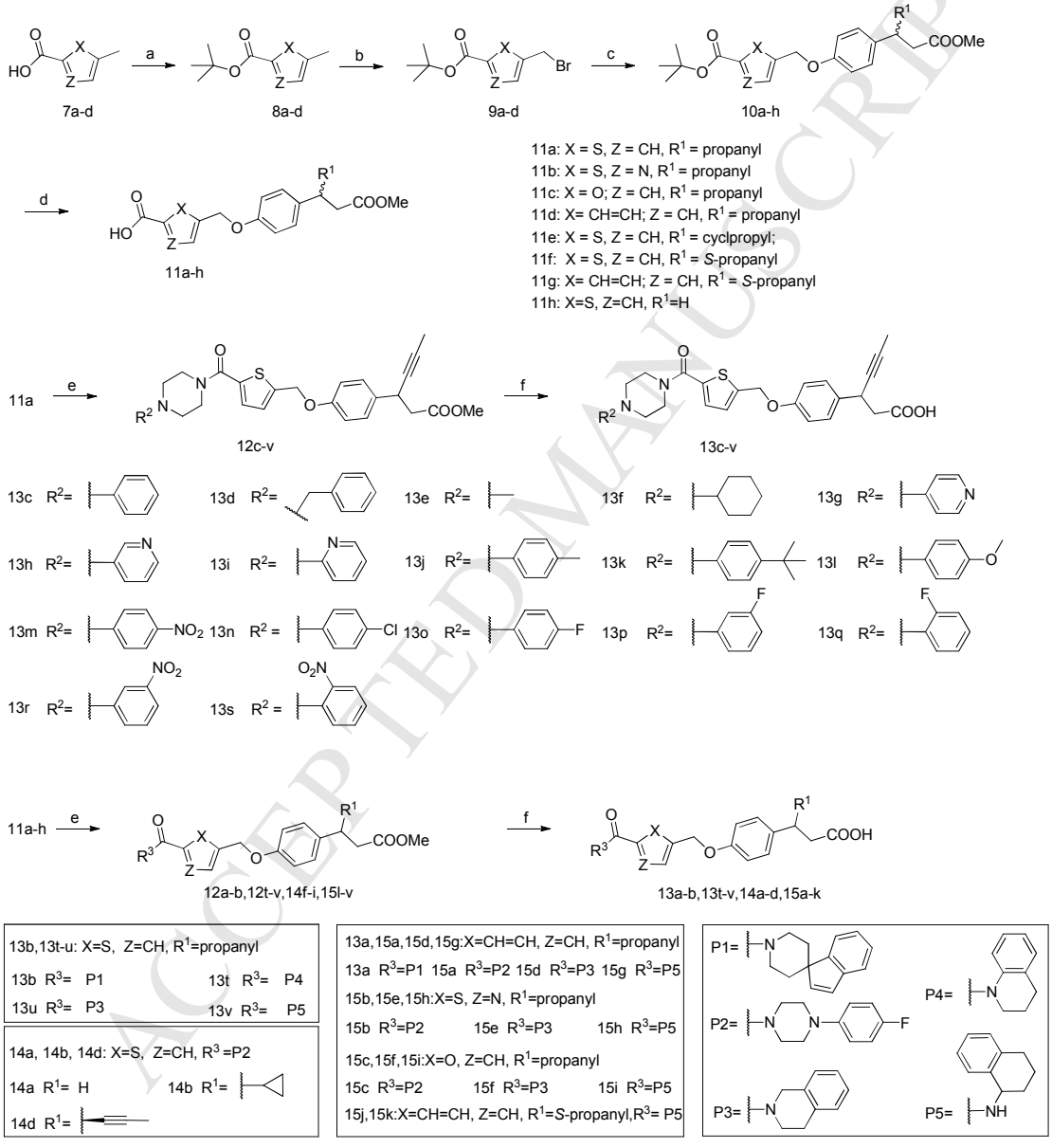
Figure 1. [a] Selected examples of reported GPR40 agonists. [a]tPSA and LogP values were calculated from ChemBioDraw Ultra 12.0.

2. Chemistry

The general synthetic approach for amide analogues is summarized in scheme 1. The carboxylic acids of **7a-d** were protected by tert-butyl group, yielding intermediates **8a-d**, and subsequent bromination of **8a-d** gave intermediates **9a-d**. A nucleophilic substitution reaction between **9a-b** and methyl 3-(4-hydroxyphenyl)hex-4-ynoic acid (**16**), (S)-methyl 3-(4-hydroxyphenyl)hex-4-ynoate (**17**), methyl 3-cyclopropyl-3-(4-hydroxyphenyl)propanoate (**18**), methyl 2-(6-hydroxy-2,3-dihydrobenzofuran-3-yl)acetate (**19**) or methyl 3-(4-hydroxyphenyl)propanoate (**20**) in the presence of K_2CO_3 produced intermediates **10a-g**. Deprotection of the tert-butyl group with CF_3COOH yielded

acids **11a-g**. **11a-g** were then condensed with corresponding amines using HATU, and the resultant amide **12a-v**, **14f-i** and **15l-v** were hydrolyzed to afforded **13a-v**, **14a-d** and **15a-k**.

Scheme 1. Synthesis of **13a-v**, **14a-d** and **15a-k**.



Reagents and conditions: (a) $(BOC)_2O$, DMAP, DIPEA, DCM, reflux, 24h; (b) NBS, CCl_4, 75°C, 4h; (c) K_2CO_3, DMF, rt, overnight; (d) CF_3COOH, DCM, rt, overnight; (e) HATU, DIPEA, DMF, amine, rt, 4-8h; (f) MeOH/H_2O, LiOH, rt, 0.5-12h.

3. Results and Discussion

A diverse set of compounds were synthesized to improve the PK and physicochemical properties of **2**. Compounds' agonist effects on hGPR40 were assessed by calcium mobilization assay using hGPR40-HEK293 cell line which stably expressed human GPR40.

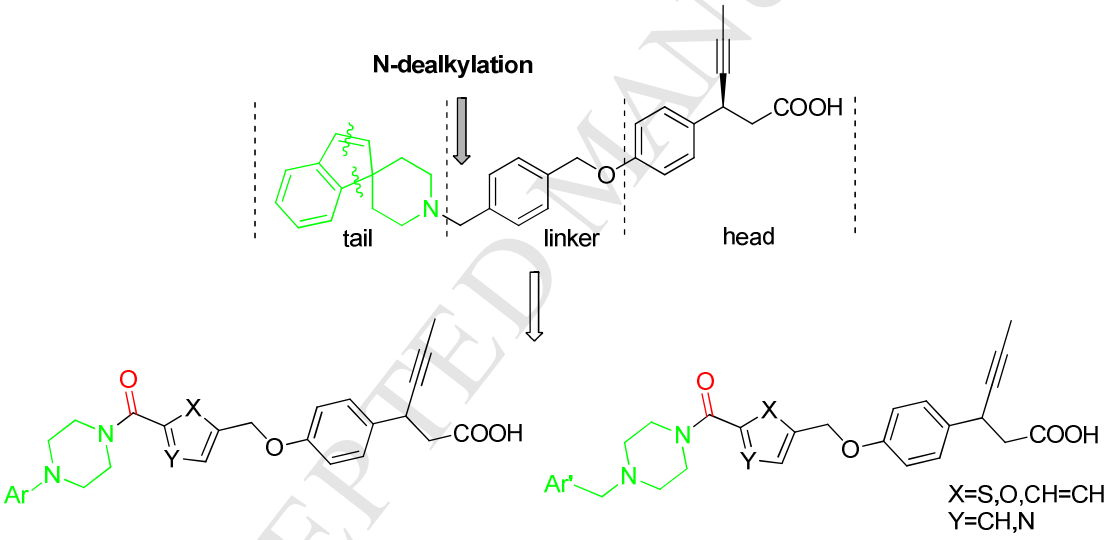


Figure 2. Design of new GPR40 agonists based on the LY2881835 scaffold

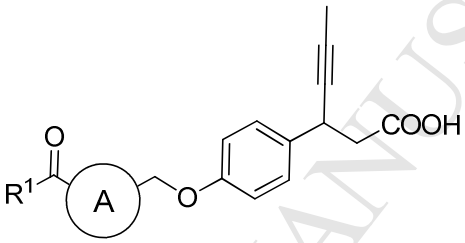
Preliminary explorations focused on the linker of **2** (Table 1). Lilly reported that the methylene between the phenyl and the piperidyl group was metabolically susceptible to N-dealkylation, so we replaced the methylene with a carbonyl moiety to block the metabolic site [23, 48] (Figure 2). In addition, the carbonyl replacement increased

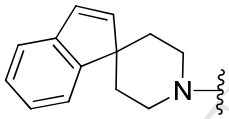
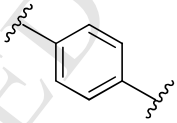
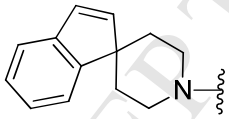
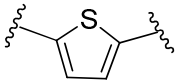
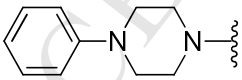
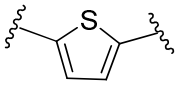
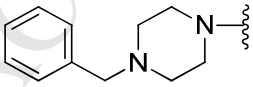
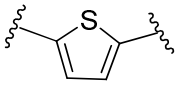
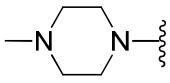
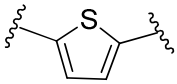
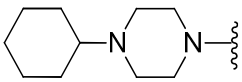
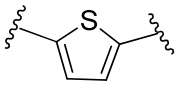
tPSA and reduced LogP, which would be expected to reduce the CNS penetration. Direct replacement with the carbonyl in the linker gave **13a**. **13a** was less potent but possessed lower LogP (5.9) and higher tPSA (67) than **2**. Based on the structure of another compound from Lilly, LY2922083, we produced **13b** with a thiophene ring in the linker. **13b** displayed comparable activity to that of **13a**. Further liver microsomal stability and Caco-2 permeability assays (Table 2) showed that, **13a** and **13b** retained the permeability of **2**. Introduction of the carbonyl group gave a slight increase in metabolic stability in mouse liver microsomes. As **13b** was more preferred for better metabolic stability on both human and mouse liver microsomes than 2 and 13a, 5-carbonylthiophene moiety was chosen as linker for the subsequent compounds.

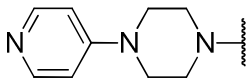
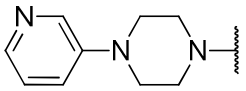
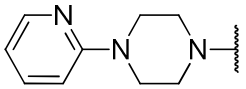
In the optimization of both the stability and activity, we modified the indene ring in the tail to improve stability because the nonaromatic double bond of indene could be susceptible to oxidative metabolism in liver microsomes [49]. First, we opened the five-member ring of indene in two ways (Figure 2) and introduced a nitrogen atom to the piperidyl group, affording **13c** and **13d** with 4-phenylpiperazine and 4-benzylpiperazine as the tail, respectively. 4-phenylpiperazine (**13c**) was more favorable and displayed a 2.2-fold increase in potency than **13d**. As expected, **13c** revealed promoted stability in human and mouse liver microsomes. Furthermore, conversion of the benzene ring into a methyl of cyclohexyl group gave **13e** and **13f** respectively. These two compounds had either markedly decreased potency or complete loss of activity, suggesting that the aromatic ring was critical for maintaining the agonistic activity. Also, we replaced the benzene ring with pyridyl

rings (**13g**, **13h**, **13i**), giving lower LogP and higher tPSA. Alteration of the benzene ring into 2-pyridyl (**13i**) was well tolerated, while 3-pyridyl (**13h**) and 4-pyridyl (**13g**) replacement resulted in more than 16-fold and 70-fold loss in potency compared with **13c** respectively, demonstrating that moving the nitrogen atom to different positions greatly impacted the activity. In view of these outcomes, **13c** was selected for further exploration.

Table 1. Variations of R^1 (the tail) and ring A (the linker)



Compd	R^1	Ring A	hGPR40 EC_{50}(nM)[a]	tPSA[b]	LogP[b]
13a			179	67	5.9
13b			172	67	5.9
13c			72	70	5.4
13d			155	70	5.1
13e			>5000	70	3.3
13f			820	70	4.9

13g		>5000	82	4.8
13h		1136	82	4.0
13i		138	82	4.1
2		134	50	6.5

[a]EC_{50} was the average of at least 3 determination, [b]tPSA and LogP values were Calculated from ChemBioDraw Ultra 12.0.

Table 2. Metabolic stability and Caco-2 permeability of **2**, **13a** and **13b**

Compd	Liver microsomal stability ($t_{1/2}$, min)		Caco-2 permeability[a]	
	human	mouse	A to B (10^{-6}cm/s)	Efflux Ratio
2	18	2	15.0	0.4
13a	9	6	10.9	0.3
13b	25	7	19.3	0.3
13c	105	55	ND[b]	ND[b]

[a]'A to B' means from apical to basolateral, [b]ND means not determined.

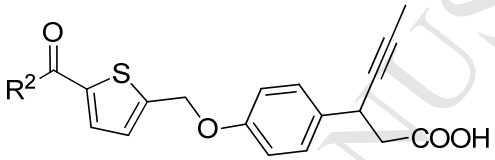
The SAR of **13c** was then investigated according to three parts of the molecule, the 4-phenylpiperazine tail, the central linker, and the β-propynyl substituted 3-benzenepropanoic acid head. We first investigated the influence of various substituents *para* to the phenyl of the tail considering that the *para*-position was potentially the major site of oxidative metabolism for the phenylpiperazine moiety [50] (Table 3).

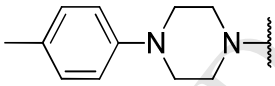
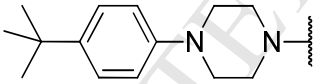
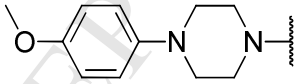
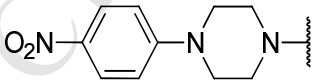
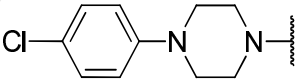
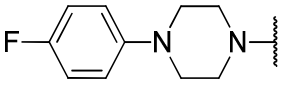
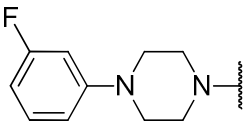
Among these substituents, **13k** with a *tert*-butyl group showed the greatest loss of potency (5.8-fold), while the smaller methyl group (**13j**) had a 3-fold decrease in potency only. This indicated that a bulky substituent at the *para* position of the phenyl group was deleterious to activity. Neither an electron-donating methoxy group (**13l**) nor a strong electron-withdrawing nitro group (**13m**) was beneficial for activity. A comparison of **13l** and **13m** with **13k** suggested that the size of R^2 contributed to the major loss of potency. Similar outcomes were observed with **13n** and **13o**, that the compound with the bulkier chlorine group (**13n**) was less potent than that with the smaller fluorine group (**13o**), and that **13o** had higher potency than **13c**. These SAR findings led to our speculation that small substituents at the *para* position of the phenyl would be favorable.

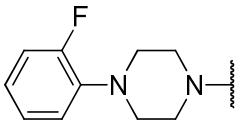
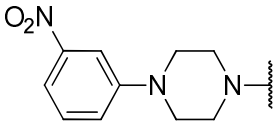
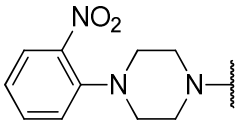
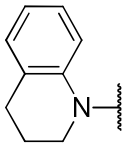
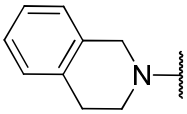
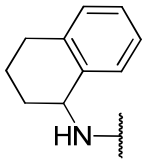
Further explorations at the *ortho*- and *meta*- postions of the phenyl in the tail proceeded on the basis of the preliminary results. First, we investigated the fluorine group at the *meta*- (**13p**) and *othro*- positions (**13q**) of the phenyl group, with both compounds being slightly less potent than **13o**. Surprisingly, conversion of fluorine into the strong electron withdrawing nitro group at the *ortho*- position (**13s**) displayed a potency similar to that of **13q**, while **13r** (nitro group at the *meta*- position) was less active than **13s** but more potent than **13m**. We further examined chlorine and methoxy substitutions *ortho* and *meta* to the phenyl, and a similar trend of potency to that of **13r** and **13s** (data not shown). These results imply that the *ortho*- position is well tolerated with electron-withdrawing/donating and sterically hindered substituents, while the length and size of the substituents at the *meta*- position may impact activity.

In addition, we assessed the viability of 1,2,3,4-tetrahydroquinoline (**13t**) and 1,2,3,4-tetrahydroisoquinoline (**13u**) as the tail on account of our previous SAR results. To our delight, **13t** and **13u** each had comparable activity to that of **13c**, with **13u** being slightly more potent than **13c**. Further structural expansion of **13t** led to **13v,** a compound with slightly decreased potency. These result indicated that a linear fused-ring tail was more favorable.

Table 3. Variations of the terminal carboxamide group



Compd	R^2	hGPR40 EC_{50}(nM)[a]	tPSA[b]	LogP[b]
13j		211	70	5.9
13k		417	70	6.8
13l		289	79	5.3
13m		231	121	--[c]
13n		133	70	6.0
13o		54	70	5.6
13p		97	70	5.6

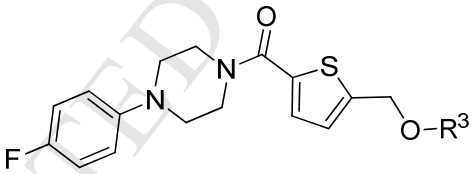
13q		113	70	5.6
13r		187	122	-- [c]
13s		94	122	-- [c]
13t		80	67	5.6
13u		57	67	5.3
13v		109	76	5.9

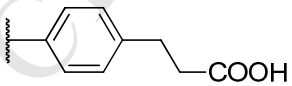
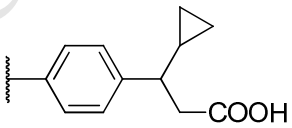
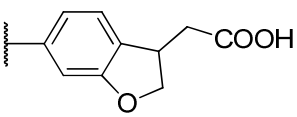
[a]EC_{50} was the average of at least 3 determination, the EC_{50} of **13j** was the average of 2 determinations. [b]tPSA and LogP was calculated from ChemBioDraw Ultra 12.0. [c]tPSA can not be calculated by ChemBioDraw Ultra 12.0.

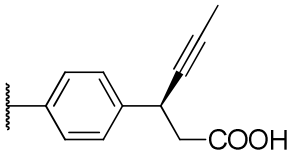
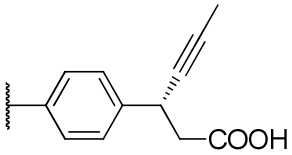
Altogether, these SAR results revealed that the potency was strongly impacted by the size of the substituents and steric hindrance around the phenyl ring in the tail. The incorporation of a small fluorine group at *para*-position of the tail gave the most potent compound **13o**. Further exploration of the tail led to identification of another potent compound, **13u**, with a shorter fused-ring as the tail. These results were followed by continued structural modifications to the head and linker of the chemical structure.

Modifications to the β-substituted benzenepropanoic acid in the head of **13o** (Table 4) did not yield any improvement in potency. For example, replacement of the 1-propynyl group with a cyclopropyl group (**14b**), direct removal of the propynyl group (**14a**) and further alteration of the head moiety into 3-((2,3-dihydrobenzofuran-3-yl))acetate acid (**14c**) all resulted in markedly eroded potency. Next, the stereochemistry at the β-position was examined on account of previous reports that the *S*-isomer was more favorable [23]. The *S*-isomer (**14d)** indeed had a slightly improved potency over **13o** (racemate), while the *R*-isomer (**14e)** had markedly lower potency. These results supported that (*S*)-β-propynyl substituted 3-benzenepropanoic acid was an optimal head for the amide structure.

Table 4. Variations of the β-substituents of benzenepropanoic acid in the head



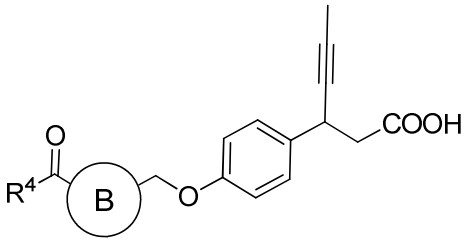
Compd	R^3	hGPR40 EC_{50}(nM)[a]	tPSA[b]	LogP[b]
14a		610	70	5.0
14b		>1667	70	5.5
14c		>1667	79	4.3

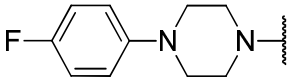
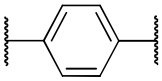
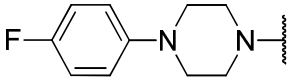
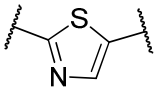
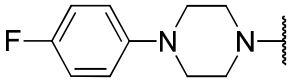
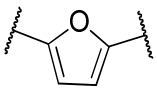
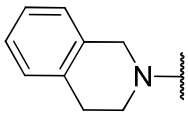
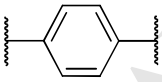
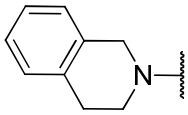
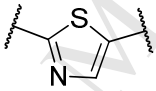
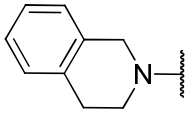
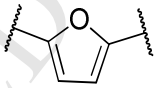
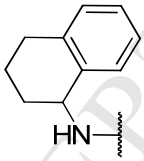
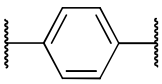
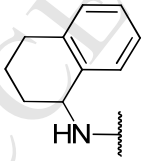
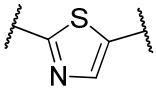
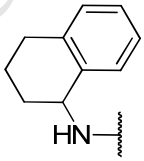
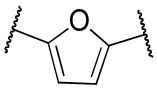
14d		47	70	5.6
14e		>5000	70	5.6

[a]EC_{50} was the average of at least 3 determination, [b]tPSA and LogP was calculated from ChemBioDraw Ultra 12.0.

The SAR exploration of the linker aimed to replace the thiophene ring with bioisosteres. Some moieties found to be favorable for potency in our previous SAR findings were combined with the linker in an effort to produce the most potent compound(s). (*S*)-β-Propynyl substituted 3-benzenepropanoic acid was chosen as the head and, for the tail, 4-fluorine phenylpiperazine, 1,2,3,4-tetrahydroisoquinoline and 1,2,3,4-tetrahydro-1-naphthylamine were selected, with focus on both potency and structural diversity. Thiazole, benzene and furan rings were introduced to replace the thiophene ring of the linker (Table 5). The thiazole ring was deleterious for potency with three types of tails (**15b**, **15e**, **15h**). The combinations of a 1,2,3,4-tetrahydroisoquinoline and a furan linker (**15f**) or 1,2,3,4-tetrahydro-1-naphthylamine and a benzene linker (**15g**) afforded high potency, with **15g** exhibiting the best activity.

Table 5. SAR analysis of ring B in the linker



Compd	R^4	Ring B	hGPR40 EC_{50}(nM)[a]	tPSA[b]	LogP[b]
15a			114	70	5.5
15b			203	82	5.0
15c			308	79	4.2
15d			106	67	5.2
15e			531	79	4.7
15f			50	76	3.9
15g			42	76	5.8
15h			144	88	5.3
15i			133	85	4.5

[a]EC_{50} was the average of at least 3 determination, [b]tPSA and LogP was calculated from ChemBioDraw Ultra 12.0.

15g, which was the most active agonist for hGPR40 among the analogues we synthesized, has two chiral centers and hence exists as four diastereomers. Considering the more favorable *S*-configuration at the β-position of the head, the impact of the absolute configuration at the tail was evaluated (Figure 3). The *S*,*R*-isomers (**15k**) had 2-fold promotion in potency over the *S*,*S*-isomers (**15j**) and the racemate (**15g**). Furthermore, **15k**, **15j** and **14d** exhibited good agonistic activities for both rat and mouse GPR40, indicating low species-dependent differences (Table 6).

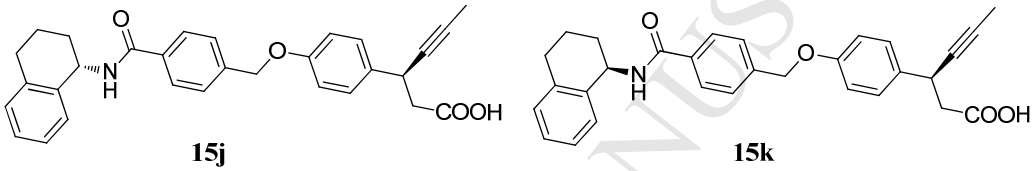


Figure 3. Effect of the stereochemistry of the 1,2,3,4-tetrahydro-1-naphthylamine moiety of **15g** on the hGPR40 activity

Table 6. Agonistic activities for GPR40 from different species

Compd	hGPR40 EC_{50}(nM)[a]	rGPR40 EC_{50}(nM)[a]	mGPR40 EC_{50}(nM)[a]
14d	47	25	31
15j	39	28	42
15k	20	14	23

[a]EC_{50} was the average of at least 3 determination.

15k, **15j** and **14d**, were then submitted to metabolic stability studies in human and mouse liver microsomes and permeability test with Caco-2 cells (Table 7). **15k**, **15j** and **14d** all retained excellent permeability (P_{app} value greater than 15.2×10^{-6} cm/s)

and displayed modestly improved metabolic stability, compared with **2**. **14d** with 4-fluorine phenylpiperazine as tail demonstrated the best stability in mouse liver microsomes. When the tail and linker were replaced with (*S/R*)-1,2,3,4-tetrahydro-1-naphthylamine and a benzene ring (**15k**) there was a markedly improved stability in human liver microsomes. The *S,R*-isomeres (**15k**) was more stable than the *S,S*-isomeres (**15j**) in both human and mouse liver microsomes, indicating that the stereochemistry of the tail had little effect on potency but great impact on metabolic stability.

Table 7. *In vitro* metabolic stability and permeability of **15k**, **15j** and **14d**

Compd	Liver microsomal stability ($t_{1/2}$, min)		Caco-2 permeability[a]	
	human	mouse	A to B (10^{-6}cm/s)	Efflux Ratio
14d	64	120	20.6	0.8
15j	214	27	15.2	1.2
15k	387	74	18.8	0.9

[a] 'A to B' means from apical to basolateral

The PK and the tissue distribution profiles of **15k**, **15j**, **14d** and **2** (Table 8) in male ICR mice were assessed. **15k**, **15j**, **14d** all exhibited prolonged half-lives and improved plasma exposure. **15k** achieved the highest concentration (15.45μg/mL) in plasma among four compounds. The observation that **15j** (*S,S*-isomers) and **15k** (*S,R*-isomers) differed substantially in peak times and peak plasma concentrations indicated that the stereochemistry of the tail significantly affected absorption.

Compared with **2**, **15k** had 5.4-fold, 4.1-fold and 15.5-fold improvement in plasma exposure, half-life time and AUC (area under the curve), respectively.

Table 8. *In vivo* PK and distribution parameters of selected compounds in ICR Mice[a]

compd	AUC_{0-8} (h*μg/mL)[a]	$T_{1/2}$ (h)[a]	T_{max} (h)[a]	C_{max} (μg/mL)[a]	B/P[b]	L/P[b]
2	5.15	1.3	0.7	2.85	0.14	2.5
14d	28.45	3.8	0.8	13.31	0.018	15.1
15j	3.76	3.0	0.25	3.77	0.006	0.9
15k	79.98	5.3	1.2	15.45	0.011	0.3

[a]Dose orally to male ICR mice at 30mg/kg (n = 3), vehicle was 0.1% Tween-80 in 1% hydropropylcellulose sodium. [b]Dose orally to male ICR mice at 30mg/kg (n = 3). All mice was sacrificed at the time point 0.75h (**15j**, **2**), 1h (**14d**, **15k**), respectively. B/P means total brain-to-plasma drug distribution ratio, L/P means the liver-to-plasma drug distribution ratio.

Tissue distribution assays showed that **14d**, **15j** and **15k** had much lower CNS exposure (B/P = 0.018, 0.006 and 0.011 respectively) than **2**. We also examined the distribution of compounds to the liver, based on the report that TAK-875 had 3 times higher distribution to the liver than to plasma [35]. **15j** and **15k** displayed lower L/P compared with **2**. However, **14d** was not studied any further because of safety concerns associated with its 15-times higher distribution to the liver than to plasma. **15k**, with promising PK and safety profiles, was progressed to in *vivo* efficacy studies, where it demonstrated a significant decrease ($P < 0.001$) in blood glucose level

following a 2.5 g/kg oral glucose load in the oral glucose tolerated test (OGTT) (Figure 4).

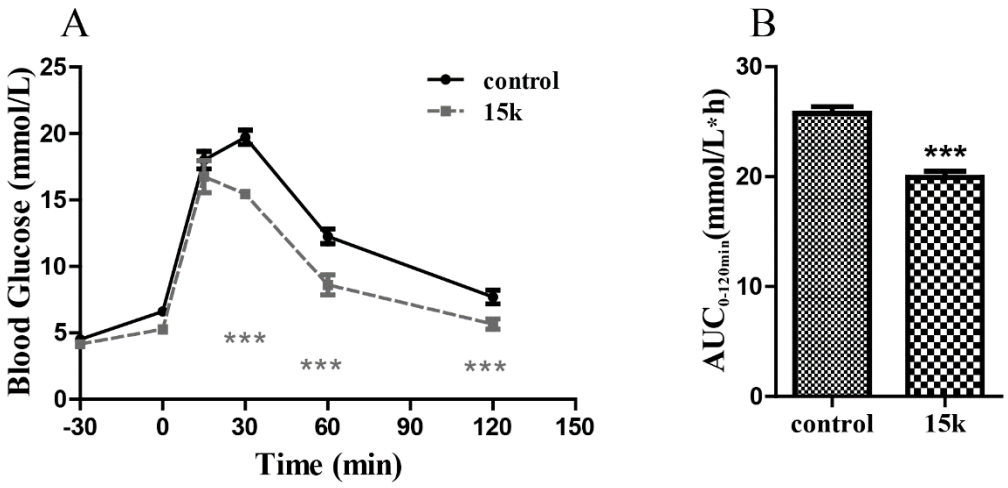


Figure 4. OGTT assay of **15k** (100mg/kg) in ICR mice. (**A**) blood glucose concentration of **15k** during OGTT; (**B**) blood glucose $AUC_{0\text{-}120\ min}$ after administraion of **15k**. Compound **15k** was administered orally to ICR mice (n=8) at 30min prior to a oral glucose load (2.5 g/kg). Blood glucose levels were measured berefore and after glucose load. ** $p<0.01$, *** $p<0.001$ versus control were analyzed by Student's t-test. Error bar indicateds SEM.

4. Conclusion

We developed a series of amide derivatives driven by the moderate CNS exposure, high *in vitro* clearance and limited oral exposure of **2**. By inserting a carbonyl at the position prone to *N*-dealkylation metabolism and opening up the indene, as well as introducing a nitrogen atom to reduce LogP and elevate tPSA, we obtained **13c** with improved physicochemical properties and liver microsomal stability. Further SAR

research and optimization yielded **15k**, which exhibited the best *in vitro* activity, excellent permeability and markedly improved *in vitro* stability. Furthermore, studies demonstrated that **15k** possessed superior PK properties *in vivo*, lower distribution to brain and liver than to plasma compared with LY2881835, supporting that **15k** is more likely to avoid the undesired effects in the CNS and liver. In conclusion, our results disclosed an orally efficacious compound **15k**, which represents a promising lead compound for developing a safe antidiabetic drug that acts via activation of GPR40.

5. Experimental section

5.1 Chemistry

All reagents were purchased from commercial suppliers and used without further purification. Column chromatography was carried out on silica gel (200-300 mesh) or with pre-packed silica cartridges (4-40 g) from Bonna-Agela Technologies Inc. (Tianjin, China) and eluted with a CombiFlash$^{@}$ Rf 200 from Teledyne Isco. Prep-HPLC separation was carried out in Unimicro Easysep-1010 series LC (UV 254 nM, 25 °C, flow rate = 10 mL min^{-1}) with the column of Agilent Prep-C18 (10 μm, 21.2×250mm) or CHIRALPAK IG (5μm, 10×250mm), while the mobile phase was 40-90% MeOH /H_2O or 80% MeOH /DCM. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian-Mercury Plus-300 or a Bruker Avance III 400 or a Bruker Avance III 500 NMR spectrometer using tetramethylsilane or solvent signals as an internal reference. High-resolution mass spectra (ESI) were obtained on a Q-TOF or

Thermo Orbitrap Elite. The purity of tested compounds was determined by HPLC [Agilent LC1260, Agilent ChemStation, ZORBAX SB-C18 (5 μm, 4.6 × 150 mm, UV 254 nM, 20 °C, flow rate = 1.0 mL min^{-1})]. All of the assayed compounds possess >95% purity. The enantiomeric excess and diastereoisomeric excess were defined by HPLC [Agilent LC1260, Agilent ChemStation, CHIRALPAK IG (5 μm, 4.6 mm × 150 mm) or CHIRALPAK IA (5 μm, 4.6 × 250 mm), UV 254 nM, flow rate = 1.0 mL min^{-1})]. The optical rotation of tested compounds was measured by Rudoph Autopol VI automatic polarimeter.

*5.1.1. General procedure A for preparation of intermediates **8a-d***

To a solution of **7a-d** (1 equiv) in DCM was added DMAP (0.1 equiv) and DIPEA (1.5 equiv) at 0°C, then added *di*-tert-butyl dicarbonate (2 equiv) dropwise over 15min, and the mixture was stirred at 40°C for 24h. The mixture was washed with brine, dried over MgSO4, and evaporated in vacuo. The residue was purified with column chromatography (petroleum ether/ethyl acetate = 20:1) to yield intermediate **8a-d**.

*5.1.1.1. tert-butyl 5-methylthiophene-2-carboxylate (**8a**)*

Intermediate **8a** was prepared with general procedure A using 5-methylthiophene-2-carboxylic acid to a colorless oil in 74.6%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, *J* = 3.7 Hz, 1H), 6.75 (d, *J* = 3.6 Hz, 1H), 2.52 (s, 3H), 1.58 (s, 9H).

*5.1.1.2. tert-butyl 5-methylthiazole-2-carboxylate (**8b**)*

Intermediate **8b** was prepared with general procedure A using 5-methylthiazole-2-carboxylic acid to afford a light-yellow oil in 64.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H), 2.71 (s, 3H), 1.55 (s, 9H).

*5.1.1.3. tert-butyl 5-methylfuran-2-carboxylate (**8c**)*

Intermediate **8c** was prepared with general procedure A using 5-methylfuran-2-carboxylic acid to afford a light-yellow oil in 67.2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.98 (d, *J* = 3.3 Hz, 1H), 6.08 (d, *J* = 3.3 Hz, 1H), 2.37 (s, 3H), 1.58 (s, 9H).

*5.1.1.4. tert-butyl 4-methylbenzoate (**8d**)*

Intermediate **8d** was prepared with general procedure A using 4-methylbenzoic acid to afford a colorless oil in 69.3%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, *J* = 8.2 Hz, 2H), 7.22 (d, *J* = 8.0 Hz, 2H), 2.40 (s, 3H), 1.60 (s, 9H).

*5.1.2. General procedure B for preparation of intermediates **9a-d***

To a solution of **8a-d** (1 equiv) in CCl_4 was added N-bromobutanimide (0.9 equiv) and benzoyl peroxide (0.1 equiv), then the mixture was refluxed at 75°C for 4h. The mixture was washed with brine, dried over MgSO4, filtered, and concentrated. The residue obtained was purified with column chromatography (petroleum ether/ethyl acetate = 20:1) to yield intermediate **9a-d**.

*5.1.2.1. tert-butyl 5-(bromomethyl)thiophene-2-carboxylate (**9a**)*

Intermediate **9a** was prepared with general procedure B using **8a** to afford a colorless oil in 85.8%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, *J* = 3.4Hz, 1H), 7.22 (d, *J* = 3.4 Hz, 1H), 4.96 (s, 2H), 1.42 (s, 9H).

*5.1.2.2. tert-butyl 5-(bromomethyl)thiazole-2-carboxylate (**9b**)*

Intermediate **9b** was prepared with general procedure B using **8b** to afford a light-yellow oil in 71.6%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H), 5.30 (s, 2H), 1.42 (s, 9H).

*5.1.2.3. tert-butyl 5-(bromomethyl)furan-2-carboxylate (**9c**)*

Intermediate **9c** was prepared with general procedure B using **8c** to afford a light-yellow oil in 86.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.00 (d, *J* = 3.2 Hz, 1H), 6.47 (d, *J* = 3.1 Hz, 1H), 4.49 (s, 2H), 1.58 (s, 9H).

*5.1.2.4. tert-butyl 4-(bromomethyl)benzoat**e** (**9d**)*

Intermediate **9d** was prepared with general procedure B using **8d** to afford a colorless oil in 84.5%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, *J* = 8.3 Hz, 2H), 7.44 (d, *J* = 8.3 Hz, 2H), 4.50 (s, 2H), 1.60 (s, 9H).

*5.1.3. General procedure C for preparation of intermediates **10a-i***

To a solution of **16**-**20** (1 equiv) in DMF was added K_2CO_3 (2 equiv), stirred for 5min then add the intermediates **9a-d** (1.1 equiv), then the mixture was stirred at room temperature overnight. Water was added to the mixture and extracted with ethyl acetate, then washed with brine, dried over MgSO4, filtered, and concentrated. The

residue obtained was purified with column chromatography (petroleum ether/ethyl acetate = 8:1) to yield intermediate ***10a-i***.

5.1.3.1. *tert-butyl 5-((4-(1-methoxy-1-oxohex-4-yn-3-yl)phenoxy)methyl)thiophene-2-carboxylate (**10a**)*

Intermediate **10a** was prepared with general procedure C using **9a** and methyl 3-(4-hydroxyphenyl)hex-4-ynoate (**16**) to afford a yellow oil in 78.3%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, *J* = 3.7 Hz, 1H), 7.31 (d, *J* = 8.6 Hz, 2H), 7.05 (d, *J* = 3.8 Hz, 1H), 6.93 (d, *J* = 8.7 Hz, 2H), 5.20 (s, 2H), 4.11 – 4.05 (m, 1H), 3.68 (s, 3H), 2.81 – 2.63 (m, 2H), 1.85 (d, *J* = 2.4 Hz, 3H), 1.59 (s, 9H).

5.1.3.2. *tert-butyl 5-((4-(1-methoxy-1-oxohex-4-yn-3-yl)phenoxy)methyl)thiazole-2-carboxylate (**10b**)*

Intermediate **10b** was prepared with general procedure C using **9b** and methyl 3-(4-hydroxyphenyl)hex-4-ynoate (**16**) to afford a yellow oil in 82.1%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (s, 1H), 7.33 (d, *J* = 8.6 Hz, 2H), 6.96 (d, *J* = 8.6 Hz, 2H), 5.35 (s, 2H), 4.12 – 4.06 (m, 1H), 3.68 (s, 3H), 2.78 (dd, *J* = 15.3, 8.3 Hz, 1H), 2.67 (dd, *J* = 15.3, 7.0 Hz, 1H), 1.85 (d, J = 2.4 Hz, 3H), 1.59 (s, 9H).

5.1.3.3. *tert-butyl 5-((4-(1-methoxy-1-oxohex-4-yn-3-yl)phenoxy)methyl)furan-2-carboxylate (**10c**)*

Intermediate **10c** was prepared with general procedure C using **9c** and methyl 3-(4-hydroxyphenyl)hex-4-ynoate (**16**) to afford a yellow oil in 75.2%. ^{1}H NMR (400

MHz, $CDCl_3$) δ 7.31 (d, *J* = 8.6 Hz, 2H), 7.06 (d, *J* = 3.4 Hz, 1H), 6.93 (d, *J* = 8.6 Hz, 2H), 6.49 (d, *J* = 3.4 Hz, 1H), 5.05 (s, 2H), 4.13-4.05 (m, 1H), 3.68 (s, 3H), 2.78 (dd, *J* = 15.3, 8.3 Hz, 1H), 2.67 (dd, *J* = 15.2, 7.0 Hz, 1H), 1.85 (d, *J* = 2.3 Hz, 3H), 1.59 (s, 9H).

*5.1.3.4. tert-butyl 4-((4-(1-methoxy-1-oxohex-4-yn-3-yl)phenoxy)methyl)benzoate (**10d**)*

Intermediate **10d** was prepared with general procedure C using **9d** and methyl 3-(4-hydroxyphenyl)hex-4-ynoate (**16**) to afford a colorless oil in 63.5%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, *J* = 8.3 Hz, 2H), 7.48 (d, *J* = 8.3 Hz, 2H), 7.30 (d, *J* = 8.6 Hz, 2H), 6.92 (d, *J* = 8.7 Hz, 2H), 5.12 (s, 2H), 4.10 – 4.05 (m, 1H), 3.68 (s, 3H), 2.78 (dd, *J* = 15.3, 8.3 Hz, 1H), 2.67 (dd, *J* = 15.3, 7.0 Hz, 1H), 1.84 (d, *J* = 2.4 Hz, 3H), 1.62 (s, 9H).

*5.1.3.5. tert-butyl 5-((4-(1-cyclopropyl-3-methoxy-3-oxopropyl)phenoxy)methyl)thiophene-2-carboxylate (**10e**)*

Intermediate **10e** was prepared by general procedure C using **9a** and methyl 3-cyclopropyl-3-(4-hydroxyphenyl)propanoate (**17**) to afford a brown oil in 67.7%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, *J* = 3.7 Hz, 1H), 7.39 (d, *J* = 15.0 Hz, 2H), 7.04 (d, *J* = 3.7 Hz, 1H), 6.86 (d, *J* = 14.9 Hz, 2H), 5.19 (s, 2H), 3.60 (s, 3H), 2.79 – 2.67 (m, 2H), 2.34 (dd, *J* = 17.3, 7.6 Hz, 1H), 1.57 (s, 9H), 1.05 – 0.95 (m, 1H), 0.62

– 0.53 (m, 1H), 0.46 – 0.38 (m, 1H), 0.25 (td, *J* = 9.6, 4.9 Hz, 1H), 0.14 (td, *J* = 9.8, 5.1 Hz, 1H).

*5.1.3.6. (S)-tert-butyl 5-((4-(1-methoxy-1-oxohex-4-yn-3-yl)phenoxy)methyl)thiophene-2-carboxylate (**10f**)*

Intermediate **10f** was prepared by general procedure C using **9a** and (S)-methyl 3-(4-hydroxyphenyl)hex-4-ynoate (**18**) to afford a light-yellow oil in 78.8%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, *J* = 3.7 Hz, 1H), 7.30 (d, *J* = 8.6 Hz, 2H), 7.04 (d, *J* = 3.7 Hz, 1H), 6.91 (d, *J* = 8.7 Hz, 2H), 5.19 (s, 2H), 4.09 – 4.04 (m, 1H), 3.67 (s, 3H), 2.76 (dd, *J* = 15.3, 8.3 Hz, 1H), 2.66 (dd, *J* = 15.3, 7.0 Hz, 1H), 1.84 (d, *J* = 2.4 Hz, 3H), 1.57 (s, 9H).

*5.1.3.7. (S)-tert-butyl 4-((4-(1-methoxy-1-oxohex-4-yn-3-yl)phenoxy)methyl)benzoate (**10g**)*

Intermediate **10g** was prepared by general procedure C using **9d** and (S)-methyl 3-(4-hydroxyphenyl)hex-4-ynoate (**18**) to afford a light-yellow oil in 75.9.2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, *J* = 8.4 Hz, 2H), 7.47 (d, *J* = 8.4 Hz, 2H), 7.29 (d, *J* = 8.6 Hz, 2H), 6.91 (d, *J* = 8.7 Hz, 2H), 5.11 (s, 2H), 4.09 – 4.03 (m, 1H), 3.67 (s, 3H), 2.76 (dd, *J* = 15.3, 8.4 Hz, 1H), 2.66 (dd, *J* = 15.3, 6.9 Hz, 1H), 1.83 (d, *J* = 2.4 Hz, 3H), 1.60 (s, 9H).

*5.1.3.8. tert-butyl 5-((4-(3-methoxy-3-oxopropyl)phenoxy)methyl)thiophene-2-carboxylate (**10h**)*

Intermediate **10h** was prepared by general procedure C using **9a** and methyl 3-(4-hydroxyphenyl)propanoate (**19**) to afford a light-yellow oil in 79.4%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, *J* = 3.7 Hz, 1H), 7.13 (d, *J* = 8.6 Hz, 2H), 7.04 (d, *J* = 3.7 Hz, 1H), 6.89 (d, *J* = 8.6 Hz, 2H), 5.18 (s, 2H), 3.67 (s, 3H), 2.90 (t, *J* = 7.8 Hz, 2H), 2.61 (t, *J* = 7.8 Hz, 2H), 1.57 (s, 9H).

5.1.3.9. tert-butyl 5-(((3-(2-methoxy-2-oxoethyl)-2,3-dihydrobenzofuran-6-yl)oxy)methyl)thiophene-2-carboxylate ***(10i)***

Intermediate **10i** was prepared by general procedure C using **9a** and methyl 2-(6-hydroxy-2,3-dihydrobenzofuran-3-yl)acetate (**20**) to afford a light-yellow oil in 74.3%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, *J* = 3.7 Hz, 1H), 7.04 (d, *J* = 4.0 Hz, 1H), 7.03 (s, 1H), 6.47 (dd, *J* = 8.1, 2.3 Hz, 1H), 6.45 (d, *J* = 2.2 Hz, 1H), 5.16 (s, 2H), 4.77 (t, *J* = 9.0 Hz, 1H), 4.28 (dd, *J* = 9.2, 6.1 Hz, 1H), 3.81 (ddd, *J* = 14.9, 9.1, 5.9 Hz, 1H), 3.72 (s, 3H), 2.76 (dd, *J* = 16.5, 5.5 Hz, 1H), 2.57 (dd, *J* = 16.5, 9.3 Hz, 1H), 1.57 (s, 9H).

*5.1.4. General procedure D preparation of intermediates **11a-i***

To a solution of **10a-i** (1 equiv) in DCM was added CF_3COOH (4 equiv), and the mixture was stirred at room temperature overnight. The solvent was evaporated under reduced pressure without further purification to yield intermediate **11a-i**.

*5.1.4.1. 5-((4-(1-methoxy-1-oxohex-4-yn-3-yl)phenoxy)methyl)thiophene-2-carboxylic acid **(11a)***

Intermediate **11a** was prepared by general procedure D using **10a** to afford a light-brown solid in 96.5%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, *J* = 3.8 Hz, 1H), 7.31 (d, *J* = 8.6 Hz, 2H), 7.11 (d, *J* = 3.8 Hz, 1H), 6.92 (d, *J* = 8.7 Hz, 2H), 5.23 (s, 2H), 4.11 – 4.04 (m, 1H), 3.67 (s, 3H), 2.77 (dd, *J* = 15.3, 8.3 Hz, 1H), 2.66 (dd, *J* = 15.3, 7.0 Hz, 1H), 1.84 (d, *J* = 2.4 Hz, 3H).

*5.1.4.2. 5-((4-(1-methoxy-1-oxohex-4-yn-3-yl)phenoxy)methyl)thiazole-2-carboxylic acid (**11b**)*

Intermediate **11b** was prepared by general procedure D using **10b** to afford a light-brown solid in 94.2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (s, 1H), 7.33 (d, *J* = 10.1 Hz, 2H), 6.92 (d, *J* = 8.7 Hz, 2H), 5.30 (s, 2H), 4.08 (td, *J* = 7.8, 2.3 Hz, 1H), 3.68 (s, 3H), 2.78 (dd, *J* = 15.3, 8.2 Hz, 1H), 2.67 (dd, *J* = 15.3, 7.0 Hz, 1H), 1.84 (d, *J* = 2.4 Hz, 3H).

*5.1.4.3. 5-((4-(1-methoxy-1-oxohex-4-yn-3-yl)phenoxy)methyl)furan-2-carboxylic acid (**11c**)*

Intermediate **11c** was prepared by general procedure D using **10c** to afford a yellow solid in 95.7%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.28 (m, 3H), 6.91 (d, *J* = 8.7 Hz, 2H), 6.57 (d, *J* = 3.5 Hz, 1H), 5.07 (s, 2H), 4.10 – 4.03 (m, 1H), 3.68 (s, 3H), 2.77 (dd, *J* = 15.2, 8.4 Hz, 1H), 2.67 (dd, *J* = 15.2, 6.9 Hz, 1H), 1.83 (d, *J* = 2.4 Hz, 3H).

*5.1.4.4. 4-((4-(1-methoxy-1-oxohex-4-yn-3-yl)phenoxy)methyl)benzoic acid (**11d**)*

Intermediate **11d** was prepared by general procedure D using **10d** to afford a white solid in 92.8%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (d, *J* = 8.2 Hz, 2H), 7.54 (d, *J* = 8.3 Hz, 2H), 7.31 (d, *J* = 8.6 Hz, 2H), 6.92 (d, *J* = 8.7 Hz, 2H), 5.14 (s, 2H), 4.11 – 4.04 (m, 1H), 3.67 (s, 3H), 2.77 (dd, *J* = 15.3, 8.3 Hz, 1H), 2.66 (dd, *J* = 15.3, 7.0 Hz, 1H), 1.84 (d, *J* = 2.4 Hz, 3H).

5.1.4.5.

*5-((4-(1-cyclopropyl-3-methoxy-3-oxopropyl)phenoxy)methyl)thiophene-2-carboxylic acid (**11e**)*

Intermediate **11e** was prepared by general procedure D using **10e** to afford a yellow solid in 93.1%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, *J* = 3.8 Hz, 1H), 7.18 (d, *J* = 8.6 Hz, 2H), 7.12 (d, *J* = 3.7 Hz, 1H), 6.92 (d, *J* = 8.6 Hz, 2H), 5.23 (s, 2H), 3.61 (s, 3H), 2.77 (dd, *J* = 14.7, 7.2 Hz, 1H), 2.70 (dd, *J* = 14.8, 8.0 Hz, 1H), 2.34 (dd, *J* = 17.0, 7.8 Hz, 1H), 1.05 – 0.95 (m, 1H), 0.63 – 0.54 (m, 1H), 0.47 – 0.38 (m, 1H), 0.25 (td, *J* = 9.5, 4.9 Hz, 1H), 0.13 (td, *J* = 9.8, 5.1 Hz, 1H).

5.1.4.6.

*(S)-5-((4-(1-methoxy-1-oxohex-4-yn-3-yl)phenoxy)methyl)thiophene-2-carboxylic acid (**11f**)*

Intermediate **11f** was prepared by general procedure D using **10f** to afford a white solid in 92.6%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, *J* = 2.6 Hz, 1H), 7.33 (d, *J* = 8.0 Hz, 2H), 7.12 (d, *J* = 1.8 Hz, 1H), 6.94 (d, *J* = 7.9 Hz, 2H), 5.25 (s, 2H), 4.12 –

4.04 (m, 1H), 3.69 (s, 3H), 2.78 (dd, *J* = 14.9, 8.1 Hz, 1H), 2.68 (dd, *J* = 15.2, 6.5 Hz, 1H), 1.85 (s, 3H).

*5.1.4.7. (S)-4-((4-(1-methoxy-1-oxohex-4-yn-3-yl)phenoxy)methyl)benzoic acid (**11g**)*

Intermediate **11g** was prepared by general procedure D using **10g** to afford a white solid in 91.5%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, *J* = 8.2 Hz, 2H), 7.54 (d, *J* = 8.1 Hz, 2H), 7.31 (d, *J* = 8.6 Hz, 2H), 6.92 (d, *J* = 8.6 Hz, 2H), 5.14 (s, 2H), 4.11 – 4.03 (m, 1H), 3.68 (s, 3H), 2.77 (dd, *J* = 15.2, 8.3 Hz, 1H), 2.67 (dd, *J* = 15.2, 7.0 Hz, 1H), 1.83 (d, *J* = 2.2 Hz, 3H).

*5.1.4.8. 5-((4-(3-methoxy-3-oxopropyl)phenoxy)methyl)thiophene-2-carboxylic acid (**11h**)*

Intermediate **11h** was prepared by general procedure D using **10h** to afford a yellow solid in 94.8%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, *J* = 3.8 Hz, 1H), 7.15 (d, *J* = 8.6 Hz, 2H), 7.12 (d, *J* = 3.8 Hz, 1H), 6.92 (d, *J* = 8.6 Hz, 2H), 5.24 (s, 2H), 3.69 (s, 3H), 2.92 (t, *J* = 7.8 Hz, 2H), 2.63 (t, *J* = 7.8 Hz, 2H).

5.1.4.9.

*5-(((3-(2-methoxy-2-oxoethyl)-2,3-dihydrobenzofuran-6-yl)oxy)methyl)thiophene-2-carboxylic acid (**11i**)*

Intermediate **11i** was prepared by general procedure D using **10i** to afford a light-yellow solid in 96.1%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, *J* = 3.6 Hz, 1H), 7.11 (d, *J* = 3.4 Hz, 1H), 7.05 (d, *J* = 8.1 Hz, 1H), 6.48 (d, *J* = 8.3 Hz, 1H), 6.46 (s,

1H), 5.21 (s, 2H), 4.77 (t, *J* = 9.0 Hz, 1H), 4.29 (dd, *J* = 9.0, 6.1 Hz, 1H), 3.82 (ddd, *J* = 14.6, 8.9, 5.7 Hz, 1H), 3.73 (s, 3H), 2.76 (dd, *J* = 16.5, 5.3 Hz, 1H), 2.58 (dd, *J* = 16.4, 9.2 Hz, 1H).

*5.1.5. General procedure E for preparation of **12a-v**, **14f-i** and **15l-v***

To a solution of **11a-i** (1equiv) in DMF was added DIPEA (2equiv) and HATU (2equiv), stirred for 5min, then added the amines (1.1equiv). The mixture was stirred at room temperature for 4-8h. Then water was added to the mixture and was extracted with ethyl acetate, washed with brine, dried over MgSO4, filtered, and concentrated, and the residue obtained was purified with column chromatography to yield intermediate **12a-v**, **14f-i** and **15l-v**.

*5.1.5.1. methyl 3-(4-((4-(spiro[indene-1,4'-piperidin]-1'-ylcarbonyl)benzyl)oxy)phenyl)hex-4-ynoate **(12a)***

Intermediate **12a** was prepared by general procedure E using **11d** and spiro[indene-1,4'-piperidine] to afford a light-yellow solid in 56.8%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 – 7.50 (m, 4H), 7.37 (dd, *J* = 7.1, 5.0 Hz, 2H), 7.33 – 7.29 (m, 3H), 7.28 – 7.23 (m, 1H), 6.94 (d, *J* = 8.7 Hz, 2H), 6.91 (d, *J* = 5.7 Hz, 1H), 6.85 (d, *J* = 5.7 Hz, 1H), 5.10 (s, 2H), 4.83 (brs, 1H), 4.12 – 4.05 (m, 1H), 3.94 (brs, 1H), 3.69 (s, 3H), 3.43 (brs, 1H), 3.26 (brs, 1H), 2.81 – 2.74 (m, 1H), 2.68 (dd, *J* = 15.3, 7.0 Hz, 1H), 2.17 (brs, 1H), 2.03 (brs, 1H), 1.85 (d, *J* = 2.4 Hz, 3H), 1.66 (brs, 1H), 1.52 (brs, 1H).

*5.1.5.2. methyl 3-(4-((5-(spiro[indene-1,4'-piperidin]-1'-ylcarbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoate (**12b**)*

Intermediate **12b** was prepared by general procedure E using **11a** and spiro[indene-1,4'-piperidine] to afford a yellow solid in 56.1%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (d, *J* = 7.9 Hz, 2H), 7.32 (d, *J* = 8.6 Hz, 2H), 7.30 – 7.27 (m, 2H), 7.25 (d, *J* = 6.0 Hz, 1H), 7.06 (d, *J* = 3.6 Hz, 1H), 6.95 (d, *J* = 8.7 Hz, 2H), 6.91 (d, *J* = 5.7 Hz, 1H), 6.85 (d, *J* = 5.7 Hz, 1H), 5.22 (s, 2H), 4.56 (brs, 2H), 4.10 (dd, *J* = 10.9, 4.3 Hz, 1H), 3.68 (s, 3H), 3.41 (brs, 2H), 2.79 (dd, *J* = 15.3, 8.3 Hz, 1H), 2.68 (dd, *J* = 15.3, 7.0 Hz, 1H), 2.18 – 2.08 (m, 2H), 1.85 (d, *J* = 2.3 Hz, 3H), 1.46 (d, *J* = 13.7 Hz, 2H).

*5.1.5.3. methyl 3-(4-((5-(4-phenylpiperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoate (**12c**)*

Intermediate **12c** was prepared by general procedure E using **11a** and 1-phenylpiperazine to afford a yellow solid in 58.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (t, *J* = 8.1 Hz, 4H), 7.24 (d, *J* = 3.7 Hz, 1H), 7.05 (d, *J* = 3.7 Hz, 1H), 6.97 – 6.89 (m, 5H), 5.20 (s, 2H), 4.11 – 4.05 (m, 1H), 3.95 – 3.88 (m, 4H), 3.67 (s, 3H), 3.27 – 3.19 (m, 4H), 2.81 – 2.75 (m, 1H), 2.70 – 2.65 (m, 1H), 1.84 (d, *J* = 2.4 Hz, 3H).

5.1.5.4. *methyl 3-(4-((5-(4-benzylpiperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoate* ***(12d)***

Intermediate **12d** was prepared by general procedure E using **11a** and 1-benzylpiperazine to afford a yellow solid in 52.6%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.30 (m, 4H), 7.30 – 7.24 (m, 3H), 7.16 (d, *J* = 3.6 Hz, 1H), 7.00 (d, *J* = 3.6 Hz, 1H), 6.90 (d, *J* = 8.6 Hz, 2H), 5.16 (s, 2H), 4.09 – 4.03 (m, 1H), 3.77 – 3.72 (m, 4H), 3.65 (s, 3H), 3.54 (s, 2H), 2.76 (dd, *J* = 15.3, 8.3 Hz, 1H), 2.65 (dd, *J* = 15.3, 7.0 Hz, 1H), 2.51 – 2.45 (m, 4H), 1.82 (d, *J* = 2.3 Hz, 3H).

5.1.5.5. *methyl 3-(4-((5-(4-methylpiperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoate* ***(12e)***

Intermediate **12e** was prepared by general procedure E using **11a** and 1-methylpiperazine to afford a light-yellow oil in 49.8%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (d, *J* = 8.6 Hz, 2H), 7.18 (d, *J* = 3.6 Hz, 1H), 7.01 (d, *J* = 3.6 Hz, 1H), 6.89 (d, *J* = 8.6 Hz, 2H), 5.16 (s, 2H), 4.07 – 4.00 (m, 1H), 3.82 – 3.75 (m, 4H), 3.64 (s, 3H), 2.78 (s, 3H), 2.74 (dd, *J* = 15.3, 8.3 Hz, 1H), 2.67 – 2.62 (m, 1H), 2.61 – 2.56 (m, 4H), 1.80 (d, *J* = 2.3 Hz, 3H).

5.1.5.6. *methyl 3-(4-((5-(4-cyclohexylpiperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoate* ***(12f)***

Intermediate **12f** was prepared by general procedure E using **11a** and 1-cyclohexylpiperazine to afford a yellow solid in 61.2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (d, *J* = 8.3 Hz, 2H), 7.22 (d, *J* = 3.5 Hz, 1H), 7.02 (d, *J* = 3.5 Hz, 1H), 6.89 (d, *J* = 8.4 Hz, 2H), 5.17 (s, 2H), 4.07-4.00 (m, 1H), 3.85 (s, 4H), 3.65 (s, 3H), 2.87 (brs, 4H), 2.74 (dd, *J* = 15.3, 8.3 Hz, 1H), 2.64 (dd, *J* = 15.2, 6.9 Hz, 2H), 1.90 (brs, 2H), 1.81 (d, *J* = 1.5 Hz, 3H), 1.62 (d, *J* = 12.5 Hz, 1H), 1.33 (d, *J* = 6.9 Hz, 1H), 1.29-1.21 (m, 5H), 1.15 – 1.04 (m, 1H).

*5.1.5.7. methyl 3-(4-((5-(4-(pyridin-4-yl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoate (**12g**)*

Intermediate **12g** was prepared by general procedure E using **11a** and 1-(pyridin-4-yl)piperazine to afford a light-brown solid in 52.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (d, *J* = 6.4 Hz, 2H), 7.29 (d, *J* = 8.6 Hz, 2H), 7.25 (d, *J* = 3.7 Hz, 1H), 7.04 (d, *J* = 3.7 Hz, 1H), 6.91 (d, *J* = 8.7 Hz, 2H), 6.72 (d, *J* = 6.6 Hz, 2H), 5.19 (s, 2H), 4.08 – 4.03 (m, 1H), 3.94 – 3.89 (m, 4H), 3.65 (s, 3H), 3.51 – 3.47 (m, 4H), 2.75 (dd, *J* = 15.3, 8.3 Hz, 1H), 2.64 (dd, *J* = 15.3, 7.0 Hz, 1H), 1.82 (d, *J* = 2.4 Hz, 3H).

*5.1.5.8. methyl 3-(4-((5-(4-(pyridin-3-yl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoate (**12h**)*

Intermediate **12h** was prepared by general procedure E using **11a** and 1-(pyridin-3-yl)piperazine to afford a light-brown solid in 54.2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (s, 1H), 8.18 – 8.13 (m, 1H), 7.30 (d, *J* = 8.7 Hz, 2H), 7.24 (d, *J* = 3.7 Hz, 1H), 7.22 – 7.20 (m, 2H), 7.05 (d, *J* = 3.7 Hz, 1H), 6.92 (d, *J* = 8.7 Hz, 2H), 5.20 (s, 2H), 4.09 – 4.03 (m, 1H), 3.95 – 3.91 (m, 4H), 3.66 (s, 3H), 3.29 – 3.25 (m, 4H), 2.76 (dd, *J* = 15.3, 8.3 Hz, 1H), 2.66 (dd, *J* = 15.3, 7.0 Hz, 1H), 1.83 (d, *J* = 2.4 Hz, 3H).

*5.1.5.9. methyl 3-(4-((5-(4-(pyridin-2-yl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoate (**12i**)*

Intermediate **12i** was prepared by general procedure E using **11a** and 1-(pyridin-2-yl)piperazine to afford a light-yellow solid in 51.6%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (d, *J* = 4.7 Hz, 1H), 7.55 – 7.49 (m, 1H), 7.31 (d, *J* = 8.6 Hz, 2H), 7.24 (d, *J* = 3.6 Hz, 1H), 7.05 (d, *J* = 3.6 Hz, 1H), 6.93 (d, *J* = 8.6 Hz, 2H), 6.68 (dd, *J* = 10.4, 4.2 Hz, 2H), 5.20 (s, 2H), 4.11 – 4.03 (m, 1H), 3.91 – 3.84 (m, 4H), 3.67 (s, 3H), 3.65 – 3.60 (m, 4H), 2.77 (dd, *J* = 15.3, 8.3 Hz, 1H), 2.66 (dd, *J* = 15.3, 7.0 Hz, 1H), 1.83 (d, *J* = 2.2 Hz, 3H).

*5.1.5.10. methyl 3-(4-((5-(4-(p-tolyl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoate (**12j**)*

Intermediate **12j** was prepared by general procedure E using **11a** and 1-(p-tolyl)piperazine to afford a yellow solid in 62.1%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (d, *J* = 8.6 Hz, 2H), 7.23 (d, *J* = 3.6 Hz, 1H), 7.11 (d, *J* = 8.3 Hz, 2H), 7.04 (d, *J* = 3.6 Hz, 1H), 6.93 (d, *J* = 8.7 Hz, 2H), 6.87 (d, *J* = 8.5 Hz, 2H), 5.20 (s, 2H), 4.10 – 4.05 (m, 1H), 3.93 – 3.89 (m, 4H), 3.67 (s, 3H), 3.26 – 3.22 (m, 1H), 3.20 – 3.16 (m, 3H), 2.77 (dd, *J* = 15.3, 8.3 Hz, 1H), 2.66 (dd, *J* = 15.3, 7.0 Hz, 1H), 2.29 (s, 3H), 1.84 (d, *J* = 2.4 Hz, 3H).

*5.1.5.11. methyl 3-(4-((5-(4-(4-(tert-butyl)phenyl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoate (**12k**)*

Intermediate **12k** was prepared by general procedure E using **11a** and 1-(4-(tert-butyl)phenyl)piperazine to afford a yellow solid in 53.6%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (dd, *J* = 8.6, 5.2 Hz, 4H), 7.24 (d, *J* = 3.6 Hz, 1H), 7.06 (d, *J* = 3.7 Hz, 1H), 6.93 (dd, *J* = 12.5, 8.7 Hz, 4H), 5.22 (s, 2H), 4.12-4.05 (m, 1H), 3.96 – 3.88 (m, 4H), 3.69 (s, 3H), 3.26 – 3.19 (m, 4H), 2.78 (dd, *J* = 15.3, 8.3 Hz, 1H), 2.68 (dd, *J* = 15.3, 7.0 Hz, 1H), 1.85 (d, *J* = 2.3 Hz, 3H), 1.32 (s, 9H).

*5.1.5.12. methyl 3-(4-((5-(4-(4-methoxyphenyl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)h ex-4-ynoate (**12l**)*

Intermediate **12l** was prepared by general procedure E using **11a** and 1-(4-methoxyphenyl)piperazine to afford a light-yellow solid in 58.3%. ^{1}H NMR (400

MHz, $CDCl_3$) δ 7.31 (d, *J* = 8.7 Hz, 2H), 7.23 (d, *J* = 3.7 Hz, 1H), 7.04 (d, *J* = 3.7 Hz, 1H), 6.92 (d, *J* = 8.9, 4H), 6.86 (d, *J* = 9.1 Hz, 2H), 5.20 (s, 2H), 4.10 – 4.04 (m, 1H), 3.93 – 3.89 (m, 4H), 3.78 (s, 3H), 3.67 (s, 3H), 3.13 – 3.09 (m, 4H), 2.77 (dd, *J* = 15.3, 8.3 Hz, 1H), 2.66 (dd, *J* = 15.3, 7.0 Hz, 1H), 1.83 (d, *J* = 2.4 Hz, 3H).

*5.1.5.13. methyl 3-(4-((5-(4-(4-nitrophenyl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoate (**12m**)*

Intermediate **12m** was prepared by general procedure E using **11a** and 1-(4-nitrophenyl)piperazine to afford a brown solid in 57.3%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (d, *J* = 9.3 Hz, 2H), 7.30 (d, *J* = 8.7 Hz, 2H), 7.26 (d, *J* = 3.7 Hz, 1H), 7.05 (d, *J* = 3.7 Hz, 1H), 6.91 (d, *J* = 8.7 Hz, 2H), 6.82 (d, *J* = 9.4 Hz, 2H), 5.19 (s, 2H), 4.09 – 4.03 (m, 1H), 3.97 – 3.91 (m, 4H), 3.66 (s, 3H), 3.55 – 3.48 (m, 4H), 2.76 (dd, *J* = 15.3, 8.2 Hz, 1H), 2.65 (dd, *J* = 15.3, 7.0 Hz, 1H), 1.82 (d, *J* = 2.4 Hz, 3H).

*5.1.5.14. methyl 3-(4-((5-(4-(4-chlorophenyl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoate (**12n**)*

Intermediate **12n** was prepared by general procedure E using **11a** and 1-(4-chlorophenyl)piperazine to afford a light-yellow solid in 63.4%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (d, *J* = 8.6 Hz, 2H), 7.27 – 7.22 (m, 3H), 7.06 (d, *J* = 3.6 Hz, 1H), 6.94 (d, *J* = 8.6 Hz, 2H), 6.87 (d, *J* = 8.8 Hz, 2H), 5.21 (s, 2H), 4.12 – 4.05 (m,

1H), 3.96 – 3.88 (m, 4H), 3.68 (s, 3H), 3.25 – 3.18 (m, 4H), 2.78 (dd, *J* = 15.3, 8.3 Hz, 1H), 2.68 (dd, *J* = 15.3, 7.0 Hz, 1H), 1.85 (d, *J* = 2.2 Hz, 3H).

*5.1.5.15. methyl 3-(4-((5-(4-(4-fluorophenyl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoate (**12o**)*

Intermediate **12o** was prepared by general procedure E using **11a** and 1-(4-fluorophenyl)piperazine to afford a yellow solid in 61.5%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (d, *J* = 8.6 Hz, 2H), 7.23 (d, *J* = 3.7 Hz, 1H), 7.04 (d, *J* = 3.7 Hz, 1H), 6.99 (t, *J* = 8.7 Hz, 2H), 6.94 – 6.88 (m, 4H), 5.19 (s, 2H), 4.10 – 4.04 (m, 1H), 3.94 – 3.89 (m, 4H), 3.66 (s, 3H), 3.17 – 3.12 (m, 4H), 2.77 (dd, *J* = 15.3, 8.3 Hz, 1H), 2.66 (dd, *J* = 15.3, 7.0 Hz, 1H), 1.83 (d, *J* = 2.4 Hz, 3H).

*5.1.5.16. methyl 3-(4-((5-(4-(3-fluorophenyl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoate (**12p**)*

Intermediate **12p** was prepared by general procedure E using **11a** and 1-(3-fluorophenyl)piperazine to afford a light-yellow solid in 53.8%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (d, *J* = 8.6 Hz, 2H), 7.27 – 7.20 (m, 2H), 7.06 (d, *J* = 3.6 Hz, 1H), 6.94 (d, *J* = 8.7 Hz, 2H), 6.73 – 6.67 (m, 1H), 6.65 – 6.57 (m, 2H), 5.22 (s, 2H), 4.12 – 4.05 (m, 1H), 3.96 – 3.89 (m, 4H), 3.68 (s, 3H), 3.31 – 3.23 (m, 4H), 2.78 (dd, *J* = 15.3, 8.3 Hz, 1H), 2.68 (dd, *J* = 15.3, 7.0 Hz, 1H), 1.85 (d, *J* = 2.3 Hz, 3H).

5.1.5.17. *methyl 3-(4-((5-(4-(2-fluorophenyl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoate (**12q**)*

Intermediate **12q** was prepared by general procedure E using **11a** 1-(2-fluorophenyl)piperazine to afford a light-yellow solid in 56.4%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (d, *J* = 8.6 Hz, 2H), 7.23 (d, *J* = 3.7 Hz, 1H), 7.11 – 7.06 (m, 2H), 7.04 (d, *J* = 3.8 Hz, 1H), 7.01 – 6.94 (m, 2H), 6.93 (d, *J* = 8.7 Hz, 2H), 5.20 (s, 2H), 4.11 – 4.04 (m, 1H), 3.96 – 3.91 (m, 4H), 3.67 (s, 3H), 3.16 – 3.10 (m, 4H), 2.77 (dd, *J* = 15.3, 8.3 Hz, 1H), 2.66 (dd, *J* = 15.3, 7.0 Hz, 1H), 1.83 (d, *J* = 2.4 Hz, 3H).

5.1.5.18. *methyl 3-(4-((5-(4-(3-nitrophenyl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoate (**12r**)*

Intermediate **12r** was prepared by general procedure E using **11a** and 1-(3-nitrophenyl)piperazine to afford a light-brown solid in 74.5%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 – 7.69 (m, 2H), 7.41 (t, *J* = 8.0 Hz, 1H), 7.30 (d, *J* = 8.6 Hz, 2H), 7.25 (d, *J* = 3.7 Hz, 1H), 7.21 (dd, *J* = 8.2, 1.8 Hz, 1H), 7.06 (d, *J* = 3.7 Hz, 1H), 6.92 (d, *J* = 8.7 Hz, 2H), 5.20 (s, 2H), 4.10 – 4.04 (m, 1H), 3.97 – 3.92 (m, 4H), 3.66 (s, 3H), 3.38 – 3.32 (m, 4H), 2.76 (dd, *J* = 15.3, 8.3 Hz, 1H), 2.66 (dd, *J* = 15.3, 7.0 Hz, 1H), 1.83 (d, *J* = 2.4 Hz, 3H).

5.1.5.19. *methyl 3-(4-((5-(4-(2-nitrophenyl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoate (**12s**)*

Intermediate **12s** was prepared by general procedure E using **11a** and 1-(2-nitrophenyl)piperazine to afford a light-brown solid in 68.7%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.55 – 7.50 (m, 1H), 7.30 (d, *J* = 8.6 Hz, 2H), 7.22 (d, *J* = 3.7 Hz, 1H), 7.17 (d, *J* = 8.4 Hz, 1H), 7.13 (d, *J* = 8.3 Hz, 1H), 7.04 (d, *J* = 3.7 Hz, 1H), 6.92 (d, *J* = 8.7 Hz, 2H), 5.19 (s, 2H), 4.09 – 4.03 (m, 1H), 3.95 – 3.90 (m, 4H), 3.66 (s, 3H), 3.14 – 3.08 (m, 4H), 2.76 (dd, *J* = 15.3, 8.3 Hz, 1H), 2.66 (dd, *J* = 15.3, 7.0 Hz, 1H), 1.83 (d, *J* = 2.4 Hz, 3H).

5.1.5.20. *methyl 3-(4-((5-(1,2,3,4-tetrahydroquinoline-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoate (**12t**)*

Intermediate **12t** was prepared by general procedure E using **11a** and 1,2,3,4-tetrahydroquinline to afford a light-yellow solid in 58.1%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (d, *J* = 8.5 Hz, 2H), 7.20 (d, *J* = 7.5 Hz, 1H), 7.13 – 7.07 (m, 1H), 7.01 (d, *J* = 5.7 Hz, 2H), 6.91 – 6.87 (m, 3H), 6.86 (d, *J* = 3.8 Hz, 1H), 5.13 (s, 2H), 4.10 – 4.05 (m, 1H), 3.93 (t, *J* = 6.7 Hz, 2H), 3.67 (s, 3H), 2.83 – 2.74 (m, 3H), 2.66 (dd, *J* = 15.3, 6.9 Hz, 1H), 2.07 – 2.02 (m, 2H), 1.84 (d, *J* = 2.4 Hz, 3H).

5.1.5.21. *methyl 3-(4-((5-(1,2,3,4-tetrahydroisoquinoline-2-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoate (**12u**)*

Intermediate **12u** was prepared by general procedure E using **11a** and 1,2,3,4-tetrahydroisoquinline to afford a light-yellow solid in 63.2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (t, *J* = 6.2 Hz, 3H), 7.23-7.17 (m, 3H), 7.14 (brs, 1H), 7.06 (d, *J* = 3.7 Hz, 1H), 6.93 (d, *J* = 8.6 Hz, 2H), 5.21 (s, 2H), 4.88 (s, 2H), 4.10 – 4.03 (m, 1H), 3.95 (t, *J* = 5.9 Hz, 2H), 3.67 (s, 3H), 2.97 (t, *J* = 5.9 Hz, 2H), 2.77 (dd, *J* = 15.3, 8.3 Hz, 1H), 2.66 (dd, *J* = 15.3, 7.0 Hz, 1H), 1.83 (d, *J* = 2.3 Hz, 3H).

5.1.5.22. *methyl 3-(4-((5-((1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoate (**12v**)*

Intermediate **12v** was prepared by general procedure E using **11a** and 1,2,3,4-tetrahydronaphthalen-1-amine to afford a light-yellow solid in 61.8%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (d, *J* = 3.7 Hz, 1H), 7.34 – 7.30 (m, 1H), 7.29 (d, *J* = 8.6 Hz, 2H), 7.19 (td, *J* = 6.6, 1.7 Hz, 2H), 7.12 (dd, *J* = 7.0, 1.5 Hz, 1H), 7.03 (d, *J* = 3.7 Hz, 1H), 6.90 (d, *J* = 8.7 Hz, 2H), 6.18 (d, *J* = 8.5 Hz, 1H), 5.34 (dd, *J* = 13.4, 5.8 Hz, 1H), 5.18 (s, 2H), 4.09 – 4.02 (m, 1H), 3.66 (s, 3H), 2.86 – 2.81 (m, 1H), 2.75 (dd, *J* = 15.3, 8.3 Hz, 2H), 2.64 (dd, *J* = 15.3, 7.0 Hz, 1H), 2.18 – 2.08 (m, 1H), 1.96 – 1.85 (m, 2H), 1.82 (d, *J* = 2.4 Hz, 3H), 1.70 – 1.63 (m, 1H).

5.1.5.23. *methyl 3-(4-((5-(4-(4-fluorophenyl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)propanoate (**14f**)*

Intermediate **14f** was prepared by general procedure E using **11h** and 1-(4-fluorophenyl)piperazine to afford a light-yellow solid in 68.1%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (d, *J* = 3.7 Hz, 1H), 7.13 (d, *J* = 8.6 Hz, 2H), 7.05 (d, *J* = 3.7 Hz, 1H), 6.99 (t, *J* = 8.7 Hz, 2H), 6.90 (dd, *J* = 8.8, 3.6 Hz, 4H), 5.19 (s, 2H), 3.94 – 3.88 (m, 4H), 3.67 (s, 3H), 3.17 – 3.12 (m, 4H), 2.91 (t, *J* = 7.7 Hz, 2H), 2.61 (t, *J* = 7.7 Hz, 2H).

5.1.5.24. *methyl 3-cyclopropyl-3-(4-((5-(4-(4-fluorophenyl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)propanoate (**14g**)*

Intermediate **14g** was prepared by general procedure E using **11e** and 1-(4-fluorophenyl)piperazine to afford a yellow solid in 51.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 – 7.20 (m, 2H), 7.05 (d, *J* = 3.6 Hz, 1H), 6.98 (t, *J* = 8.7 Hz, 2H), 6.90 (d, *J* = 4.6 Hz, 1H), 6.88 (dd, *J* = 8.3, 4.0 Hz, 3H), 6.82 (dd, *J* = 8.0, 1.7 Hz, 1H), 5.20 (s, 2H), 3.93 – 3.87 (m, 4H), 3.60 (s, 3H), 3.16 – 3.10 (m, 4H), 2.80 – 2.67 (m, 2H), 2.35 (dd, *J* = 17.2, 7.6 Hz, 1H), 1.06 – 0.96 (m, 1H), 0.61 – 0.53 (m, 1H), 0.47 – 0.38 (m, 1H), 0.26 (td, *J* = 9.6, 4.9 Hz, 1H), 0.14 (td, *J* = 9.8, 5.0 Hz, 1H).

5.1.5.25. *methyl 2-(6-((5-(4-(4-fluorophenyl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)-2,3-dihydrobenzofuran-3-yl)acetate (**14h**)*

Intermediate **14h** was prepared by general procedure E using **11i** and 1-(4-fluorophenyl)piperazine to afford a light-yellow solid in 48.6%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (d, *J* = 3.7 Hz, 1H), 7.06 (d, *J* = 4.3 Hz, 1H), 7.06 – 6.98 (m, 3H), 6.95 – 6.89 (m, 2H), 6.50 (dd, *J* = 8.1, 2.3 Hz, 1H), 6.47 (d, *J* = 2.2 Hz, 1H), 5.19 (s, 2H), 4.78 (t, *J* = 9.0 Hz, 1H), 4.29 (dd, *J* = 9.2, 6.1 Hz, 1H), 3.96 – 3.89 (m, 4H), 3.86 – 3.80 (m, 1H), 3.74 (s, 3H), 3.15 (d, *J* = 5.0 Hz, 4H), 2.77 (dd, *J* = 16.5, 5.5 Hz, 1H), 2.58 (dd, *J* = 16.4, 9.2 Hz, 1H), 1.63 (s, 3H).

*5.1.5.26. (S)-methyl 3-(4-((5-(4-(4-fluorophenyl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex -4-ynoate (**14i**)*

Intermediate **14i** was prepared by general procedure E using **11f** and 1-(4-fluorophenyl)piperazine to afford a light-yellow solid in 49.1%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 – 7.45 (m, 4H), 7.31 (d, *J* = 8.6 Hz, 2H), 6.99 (t, *J* = 8.6 Hz, 2H), 6.93 (d, *J* = 8.7 Hz, 2H), 6.90 (dd, *J* = 9.0, 4.6 Hz, 2H), 5.09 (s, 2H), 4.11 – 4.04 (m, 1H), 3.93 (brs, 2H), 3.67 (s, 3H), 3.65 – 3.44 (m, 2H), 3.24 – 2.98 (m, 4H), 2.78 (dd, *J* = 15.3, 8.3 Hz, 1H), 2.67 (dd, *J* = 15.3, 7.0 Hz, 1H), 1.84 (d, *J* = 2.3 Hz, 3H).

*5.1.5.27. methyl 3-(4-((4-(4-(4-fluorophenyl)piperazine-1-carbonyl)benzyl)oxy)phenyl)hex-4-ynoate (**15l**)*

Intermediate **15l** was prepared by general procedure E using **11d** and 1-(4-fluorophenyl)piperazine to afford a light-yellow solid in 52.6%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (q, *J* = 8.3 Hz, 4H), 7.31 (d, *J* = 8.6 Hz, 2H), 6.99 (t, *J* = 8.6 Hz,

2H), 6.93 (d, *J* = 8.7 Hz, 2H), 6.90 (dd, *J* = 9.0, 4.6 Hz, 2H), 5.09 (s, 2H), 4.11 – 4.04 (m, 1H), 3.93 (brs, 2H), 3.67 (s, 3H), 3.65-3.45 (m, 2H), 2.98-3.24 (m, 4H), 2.78 (dd, *J* = 15.3, 8.3 Hz, 1H), 2.67 (dd, *J* = 15.3, 7.0 Hz, 1H), 1.84 (d, *J* = 2.3 Hz, 3H).

*5.1.5.28. methyl 3-(4-((2-(4-(4-fluorophenyl)piperazine-1-carbonyl)thiazol-5-yl)methoxy)phenyl)hex-4-ynoate (**15m**)*

Intermediate **15m** was prepared by general procedure E using **11b** and 1-(4-fluorophenyl)piperazine to afford a yellow solid in 43.7%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (s, 1H), 7.32 (d, *J* = 8.6 Hz, 2H), 7.02 – 6.94 (m, 4H), 6.90 (dd, *J* = 9.1, 4.6 Hz, 2H), 5.34 (s, 2H), 4.07 (td, *J* = 8.5, 2.3 Hz, 1H), 3.92 – 3.86 (m, 4H), 3.66 (s, 3H), 3.19 – 3.12 (m, 4H), 2.77 (dd, *J* = 15.3, 8.2 Hz, 1H), 2.66 (dd, *J* = 15.3, 7.0 Hz, 1H), 1.83 (d, *J* = 2.2 Hz, 3H).

*5.1.5.29. methyl 3-(4-((5-(4-(4-fluorophenyl)piperazine-1-carbonyl)furan-2-yl)methoxy)phenyl)hex-4-ynoate (**15n**)*

Intermediate **15n** was prepared by general procedure E using **11c** and 1-(4-fluorophenyl)piperazine to afford a yellow solid in 45.3%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (d, *J* = 8.6 Hz, 2H), 7.02 (d, *J* = 3.4 Hz, 1H), 6.98 (d, *J* = 8.4 Hz, 2H), 6.94 – 6.88 (m, 4H), 6.52 (d, *J* = 3.4 Hz, 1H), 5.04 (s, 2H), 4.10 – 4.04 (m, 1H), 3.93 (s, 4H), 3.67 (s, 3H), 3.16 – 3.10 (m, 4H), 2.77 (dd, *J* = 15.3, 8.2 Hz, 1H), 2.69 – 2.63 (m, 1H), 1.82 (d, *J* = 2.3 Hz, 3H).

5.1.5.30. *methyl 3-(4-((4-(1,2,3,4-tetrahydroisoquinoline-2-carbonyl)benzyl)oxy)phenyl)hex-4-ynoate* ***(15o)***

Intermediate **15o** was prepared by general procedure E using **11d** and 1,2,3,4-tetrahydroisoquinoline to afford a light-yellow solid in 53.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, *J* = 8.5 Hz, 4H), 7.31 (d, *J* = 8.6 Hz, 2H), 7.20 (dd, *J* = 12.4, 6.5 Hz, 4H), 6.93 (d, *J* = 8.7 Hz, 2H), 5.09 (s, 2H), 4.91 (brs, 1H), 4.61 (brs, 1H), 4.10-4.05 (m, 1H), 4.00 (brs, 1H), 3.67 (s, 3H), 3.64 (brs, 1H), 2.99 (brs, 1H), 2.89 (brs, 1H), 2.78 (dd, *J* = 15.3, 8.3 Hz, 1H), 2.67 (dd, *J* = 15.3, 7.0 Hz, 1H), 1.84 (d, *J* = 2.4 Hz, 3H).

5.1.5.31. *methyl 3-(4-((2-(1,2,3,4-tetrahydroisoquinoline-2-carbonyl)thiazol-5-yl)methoxy)phenyl)hex-4-ynoate* ***(15p)***

Intermediate **15p** was prepared by general procedure E using **11b** and 1,2,3,4-tetrahydroisoquinoline to afford a light-yellow solid in 48.4%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (s, 1H), 7.34 (d, *J* = 8.7 Hz, 2H), 7.23 (ddd, *J* = 13.2, 7.8, 4.2 Hz, 4H), 6.98 (d, *J* = 8.7 Hz, 2H), 5.37 (s, 2H), 4.89 (s, 2H), 4.12 – 4.06 (m, 1H), 3.96 (t, *J* = 4.9 Hz, 2H), 3.69 (s, 3H), 3.00 (t, *J* = 5.9 Hz, 2H), 2.79 (dd, *J* = 15.3, 8.3 Hz, 1H), 2.68 (dd, *J* = 15.3, 7.0 Hz, 1H), 1.85 (d, *J* = 2.4 Hz, 3H).

5.1.5.32. *methyl 3-(4-((5-(1,2,3,4-tetrahydroisoquinoline-2-carbonyl)furan-2-yl)methoxy)phenyl)hex-4-ynoate* ***(15q)***

Intermediate **15q** was prepared by general procedure E using **11c** and 1,2,3,4-tetrahydroisoquinoline to afford a light-yellow solid in 57.3%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (d, *J* = 8.6 Hz, 2H), 7.23 – 7.10 (m, 4H), 7.01 (d, *J* = 3.4 Hz, 1H), 6.93 (d, *J* = 8.7 Hz, 2H), 6.51 (d, *J* = 3.4 Hz, 1H), 5.05 (s, 2H), 4.87 (s, 2H), 4.10 – 4.03 (m, 1H), 3.95 (t, *J* = 5.8 Hz, 2H), 3.66 (s, 3H), 2.96 (d, *J* = 4.4 Hz, 2H), 2.76 (dd, *J* = 15.3, 8.3 Hz, 1H), 2.65 (dd, *J* = 15.3, 7.0 Hz, 1H), 1.82 (d, *J* = 2.4 Hz, 3H).

*5.1.5.33. methyl 3-(4-((4-((1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)benzyl)oxy)phenyl)hex-4-ynoate (**15r**)*

Intermediate **15r** was prepared by general procedure E using **11d** and 1,2,3,4-tetrahydronaphthalen-1-amine to afford a light-yellow solid in 51.7%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, *J* = 8.2 Hz, 2H), 7.48 (d, *J* = 8.1 Hz, 2H), 7.37 – 7.32 (m, 1H), 7.29 (d, *J* = 8.7 Hz, 2H), 7.22 – 7.18 (m, 2H), 7.17 – 7.12 (m, 1H), 6.90 (d, *J* = 8.6 Hz, 2H), 6.37 (d, *J* = 8.3 Hz, 1H), 5.40 (dd, *J* = 13.6, 5.9 Hz, 1H), 5.10 (s, 2H), 4.09 – 4.02 (m, 1H), 3.67 (s, 3H), 2.86 (tt, *J* = 12.4, 6.1 Hz, 2H), 2.76 (dd, *J* = 15.3, 8.3 Hz, 1H), 2.65 (dd, *J* = 15.3, 7.0 Hz, 1H), 2.22 – 2.11 (m, 1H), 1.97 (dd, *J* = 11.7, 5.8 Hz, 1H), 1.93 – 1.86 (m, 2H), 1.83 (d, *J* = 2.3 Hz, 3H).

*5.1.5.34. methyl 3-(4-((2-((1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)thiazol-5-yl)methoxy)phenyl)hex-4-ynoate (**15s**)*

Intermediate **15s** was prepared by general procedure E using **11b** and 1,2,3,4-tetrahydronaphthalen-1-amine to afford a light-yellow solid in 46.8%. ^{1}H

NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 7.32 (d, *J* = 8.5 Hz, 3H), 7.23 – 7.18 (m, 2H), 7.16-7.12 (m, 1H), 6.94 (d, *J* = 8.6 Hz, 2H), 6.32 (t, *J* = 7.8 Hz, 1H), 5.39-5.34 (m, 1H), 5.33 (s, 2H), 4.11 – 4.05 (m, 1H), 3.67 (s, 3H), 2.85 (dd, *J* = 16.5, 10.2 Hz, 2H), 2.80 – 2.73 (m, 1H), 2.66 (dd, *J* = 15.3, 7.0 Hz, 1H), 2.15 (dt, *J* = 11.8, 6.1 Hz, 1H), 2.00 – 1.87 (m, 3H), 1.85 (d, *J* = 2.4 Hz, 3H).

*5.1.5.35. methyl 3-(4-((5-((1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)furan-2-yl)methoxy)phenyl)hex-4-ynoate (**15t**)*

Intermediate **15t** was prepared by general procedure E using **11c** and 1,2,3,4-tetrahydronaphthalen-1-amine to afford a light-yellow solid in 57.4%. ^1H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.33 (m, 1H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.24 – 7.19 (m, 2H), 7.15 (d, *J* = 3.4 Hz, 2H), 6.90 (d, *J* = 8.7 Hz, 2H), 6.64 (d, *J* = 8.6 Hz, 1H), 6.54 (d, *J* = 3.4 Hz, 1H), 5.41 – 5.35 (m, 1H), 4.97 (s, 2H), 4.07 (t, *J* = 5.6 Hz, 1H), 3.68 (s, 3H), 2.93 – 2.81 (m, 2H), 2.77 (dd, *J* = 15.3, 8.3 Hz, 1H), 2.65 (dd, *J* = 15.3, 7.0 Hz, 1H), 2.19 – 2.09 (m, 1H), 2.00 – 1.88 (m, 3H), 1.84 (d, *J* = 2.4 Hz, 3H).

*5.1.5.36. (S)-methyl 3-(4-((4-(((S)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)benzyl)oxy)phenyl)hex-4-ynoate (**15u**)*

Intermediate **15u** was prepared by general procedure E using **11g** and (*S*)-1,2,3,4-tetrahydronaphthalen-1-amine to afford a light-yellow solid in 53.6%. ^1H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, *J* = 8.3 Hz, 2H), 7.47 (d, *J* = 8.2 Hz, 2H), 7.33 (d, *J* = 8.4 Hz, 1H), 7.28 (d, *J* = 8.6 Hz, 2H), 7.19 (td, *J* = 6.5, 1.7 Hz, 2H), 7.14 (t, *J* =

7.3 Hz, 1H), 6.90 (d, *J* = 8.7 Hz, 2H), 6.47 (d, *J* = 8.4 Hz, 1H), 5.39 (dd, *J* = 13.6, 5.9 Hz, 1H), 5.09 (s, 2H), 4.08 – 4.02 (m, 1H), 3.66 (s, 3H), 2.83 (dd, *J* = 14.5, 6.5 Hz, 2H), 2.75 (dd, *J* = 15.3, 8.4 Hz, 1H), 2.64 (dd, *J* = 15.3, 7.0 Hz, 1H), 2.14 (dt, *J* = 10.7, 4.2 Hz, 1H), 1.97 – 1.88 (m, 3H), 1.83 (d, *J* = 2.4 Hz, 3H).

*5.1.5.37. (S)-methyl 3-(4-((4-(((R)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)benzyl)oxy)phenyl)hex-4-ynoate (**15v**)*

Intermediate **15v** was prepared by general procedure E using **11g** and (*R*)-1,2,3,4-tetrahydronaphthalen-1-amine to afford a light-yellow solid in 54.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, *J* = 8.1 Hz, 2H), 7.47 (d, *J* = 8.0 Hz, 2H), 7.33 (d, *J* = 6.7 Hz, 1H), 7.28 (d, *J* = 8.6 Hz, 2H), 7.19 (t, *J* = 5.7 Hz, 2H), 7.13 (d, *J* = 7.2 Hz, 1H), 6.89 (d, *J* = 8.6 Hz, 2H), 6.42 (d, *J* = 8.3 Hz, 1H), 5.43 – 5.35 (m, 1H), 5.09 (s, 2H), 4.09 – 4.02 (m, 1H), 3.66 (s, 3H), 2.90-2.80 (m, 2H), 2.75 (dd, *J* = 15.3, 8.3 Hz, 1H), 2.65 (dd, *J* = 15.3, 6.9 Hz, 1H), 2.19-2.11 (m, 1H), 1.98-1.87 (m, 3H), 1.83 (d, *J* = 2.2 Hz, 3H).

*5.1.6. General Procedure F for Preparation of Compound **13a-v**, **14a-e** and **15a-k***

To a solution of **12a-v**, **14f-i** and **15l-v** (1 equiv) in menthol/THF/H_2O (V/V/V = 2:1:1) was added LiOH (2 equiv). The resulting mixture was stirred at room temperature for 0.5 to 12h. The solution was concentrated under vacuum and water was added to the residue. Adjusted the pH to 2~3 with 1 M HCl (aq), and the crude was collected after filtration. The crude was further purified by preparative HPLC to afford **13a-z**, **14a-e** and **15a-k**.

5.1.6.1.

*3-(4-((4-(spiro[indene-1,4'-piperidin]-1'-ylcarbonyl)benzyl)oxy)phenyl)hex-4-ynoic acid(**13a**)*

13a was prepared by general procedure F using **12a** to afford a light-brown solid in 87.5%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.54 (dd, *J* = 8.0, 4.1 Hz, 5H), 7.35 (d, *J* = 6.8 Hz, 1H), 7.29 (d, *J* = 8.7 Hz, 2H), 7.26 – 7.17 (m, 2H), 7.14 (d, *J* = 5.7 Hz, 1H), 6.97 (d, *J* = 8.7 Hz, 2H), 6.85 (d, *J* = 5.6 Hz, 1H), 5.14 (s, 2H), 4.58 (brs, 1H), 3.95 (dt, *J* = 7.4, 3.7 Hz, 1H), 3.69 (brs, 1H), 3.46 (brs, 1H), 3.24 (brs, 1H), 2.60 (d, *J* = 7.6 Hz, 2H), 2.06 (brs, 2H), 1.78 (d, *J* = 2.3 Hz, 3H), 1.30 (brs, 1H), 1.16 (brs, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.90 (s), 169.08 (s), 157.19 (s), 151.43 (s), 142.47 (s), 141.42 (s), 138.51 (s), 135.87 (s), 133.63 (s), 129.94 (s), 128.43 (s), 127.59 (s), 127.01 (s), 125.33 (s), 122.03 (s), 121.32 (s), 114.75 (s), 80.66 (s), 78.27 (s), 68.86 (s), 52.20 (s), 42.84 (s), 40.10 (s), 39.94 (s), 32.73 (s), 3.25 (s). HRMS (ESI) m/z calcd for $C_{33}H_{32}NO_4$ ($[M + H]^+$), 506.2331; found 506.2326.

5.1.6.2.

*3-(4-((5-(spiro[indene-1,4'-piperidin]-1'-ylcarbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoic acid (**13b**)*

13b was prepared by general procedure F using **12b** to afford a light-yellow solid in 86.6%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (d, *J* = 7.0 Hz, 2H), 7.32 (d, *J* = 8.4 Hz, 2H), 7.29-7.22 (m, 3H), 7.05 (d, *J* = 2.7 Hz, 1H), 6.93 (d, *J* = 8.2 Hz, 2H), 6.90 (d, *J* = 5.7 Hz, 1H), 6.84 (d, *J* = 5.4 Hz, 1H), 5.21 (s, 2H), 4.57 (brs, 2H), 4.07 (brs, 1H), 3.41 (brs, 2H), 2.81 (dd, *J* = 15.5, 8.2 Hz, 1H), 2.71 (dd, *J* = 15.5, 6.8 Hz, 1H), 2.17 – 2.05

(td, *J* =12.0, 4.2 Hz, 2H), 1.83 (s, 3H), 1.45 (d, *J* = 13.0 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.35 (s), 163.42 (s), 156.82 (s), 150.74 (s), 142.63 (s), 142.39 (s), 139.55 (s), 137.08 (s), 133.74 (s), 130.52 (s), 128.43 (s), 128.22 (s), 126.96 (s), 125.72 (s), 125.29 (s), 121.42 (s), 121.34 (s), 114.95 (s), 79.14 (s), 78.76 (s), 64.76 (s), 51.87 (s), 42.89 (s), 33.54 (s), 32.93 (s), 29.39 (s), 3.35 (s). HRMS (ESI) m/z calcd for $C_{31}H_{30}NO_4S$ ($[M + H]^+$), 512.1896; found 512.1892.

5.1.6.3.

*3-(4-((5-(4-phenylpiperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoic acid (**13c**)*

13c was prepared by general procedure F using **12c** to afford a light-yellow solid in 89.4%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 – 7.30 (m, 3H), 7.28 (d, *J* = 5.0 Hz, 1H), 7.24 (d, *J* = 3.6 Hz, 1H), 7.04 (d, *J* = 3.6 Hz, 1H), 6.94 (t, *J* = 9.2 Hz, 5H), 5.20 (s, 2H), 4.10-4.03 (m, 1H), 3.95 – 3.88 (m, 4H), 3.27 – 3.20 (m, 4H), 2.81 (dd, *J* = 15.6, 8.2 Hz, 1H), 2.70 (dd, *J* = 15.6, 6.9 Hz, 1H), 1.84 (d, *J* = 2.2 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.78 (s), 163.56 (s), 157.21 (s), 150.92 (s), 143.36 (s), 137.08 (s), 134.17 (s), 129.41 (s), 129.16 (s), 128.65 (s), 126.12 (s), 120.86 (s), 116.87 (s), 115.31 (s), 79.57 (s), 79.17 (s), 65.12 (s), 49.84 (s), 43.33 (s), 33.34 (s), 3.79 (s). HRMS (ESI) m/z calcd for $C_{28}H_{29}N_2O_4S$ ($[M + H]^+$), 489.1848; found 489.1845.

5.1.6.4.

*3-(4-((5-(4-benzylpiperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoic acid (**13d**)*

13d was prepared by general procedure F using **12d** to afford a light-yellow solid in 69.5%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (dd, *J* = 10.3, 6.5 Hz, 7H), 7.12 (d, *J* = 3.7 Hz, 1H), 6.96 (d, *J* = 3.6 Hz, 1H), 6.90 (d, *J* = 8.6 Hz, 2H), 5.14 (s, 2H), 4.08-4.02 (m, 1H), 3.71 (brs, 4H), 3.61 (s, 2H), 2.81 (dd, *J* = 15.3, 7.5 Hz, 1H), 2.70 (dd, *J* = 15.3, 7.8 Hz, 1H), 2.55 (brs, 4H), 1.82 (d, *J* = 2.2 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.83 (s), 163.17 (s), 156.94 (s), 143.20 (s), 136.84 (s), 135.31 (s), 134.37 (s), 129.71 (s), 128.89 (s), 128.55 (s), 128.47 (s), 127.84 (s), 125.89 (s), 115.03 (s), 79.96 (s), 78.60 (s), 64.92 (s), 62.19 (s), 52.31 (s), 43.61 (s), 33.46 (s), 30.29 (s), 29.66 (s), 3.65 (s). HRMS (ESI) m/z calcd for $C_{29}H_{31}N_2O_4S$ ($[M + H]^+$), 503.2005; found 503.2000.

5.1.6.5.

*3-(4-((5-(4-methylpiperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoic acid (**13e**)*

13e was prepared by general procedure F using **12e** to afford a white solid in 78.3%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (d, *J* = 8.2 Hz, 2H), 7.16 (d, *J* = 3.2 Hz, 1H), 6.99 (d, *J* = 2.9 Hz, 1H), 6.89 (d, *J* = 8.2 Hz, 2H), 5.17 (s, 2H), 4.04 (brs, 1H), 3.88 (s, 4H), 2.91 – 2.77 (m, 5H), 2.69 (dd, *J* = 15.2, 8.5 Hz, 1H), 2.56 (s, 3H), 1.83 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.76 (s), 163.30 (s), 156.98 (s), 143.58 (s), 136.94 (s), 134.75 (s), 129.03 (s), 128.88 (s), 126.07 (s), 115.04 (s), 80.47 (s), 78.46 (s), 65.06 (s), 45.04 (s), 33.78 (s), 29.84 (s), 29.79 (s), 29.47 (s), 29.37 (s), 3.85 (s). HRMS (ESI) m/z calcd for $C_{23}H_{27}N_2O_4S$ ($[M + H]^+$), 427.1692; found 427.1687.

5.1.6.6.

*3-(4-((5-(4-cyclohexylpiperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-yno ic acid (**13f**)*

13f was prepared by general procedure F using **12f** to afford a light-yellow solid in 82.1%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (d, *J* = 8.1 Hz, 2H), 7.14 (d, *J* = 2.9 Hz, 1H), 6.96 (d, *J* = 2.3 Hz, 1H), 6.86 (d, *J* = 8.2 Hz, 2H), 5.14 (s, 2H), 4.03 (brs, 1H), 3.94 (brs, 4H), 2.99 (brs, 4H), 2.88 (dd, *J* = 15.6, 7.2 Hz, 1H), 2.79 (dd, *J* = 15.2, 6.4 Hz, 1H), 2.68 (dd, *J* = 14.9, 8.8 Hz, 1H), 2.08 (d, *J* = 9.4 Hz, 2H), 1.88 (d, *J* = 11.7 Hz, 2H), 1.80 (s, 3H), 1.68 (d, *J* = 13.4 Hz, 1H), 1.44 – 1.27 (m, 4H), 1.17 – 1.04 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.01 (s), 163.30 (s), 156.80 (s), 144.30 (s), 135.81 (s), 134.88 (s), 129.66 (s), 128.76 (s), 126.15 (s), 115.05 (s), 80.53 (s), 78.37 (s), 65.30 (s), 64.95 (s), 48.12 (s), 48.10 (s), 44.09 (s), 33.68 (s), 26.97 (s), 25.33 (s), 25.25 (s), 3.78 (s). HRMS (ESI) m/z calcd for $C_{28}H_{35}N_2O_4S$ ($[M + H]^+$), 495.2318; found 495.2322.

5.1.6.7.

*3-(4-((5-(4-(pyridin-4-yl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoic acid (**13g**)*

13g was prepared by general procedure F using **12g** to afford a light-yellow solid in 82.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.29 (d, *J* = 7.3 Hz, 2H), 7.41 (d, *J* = 3.7 Hz, 1H), 7.28 (d, *J* = 8.6 Hz, 2H), 7.22 (d, *J* = 3.7 Hz, 1H), 7.14 (d, *J* = 7.4 Hz, 2H), 6.97 (d, *J* = 8.6 Hz, 2H), 5.30 (s, 2H), 3.96-3.91 (m, 1H), 3.83 (d, *J* = 9.3 Hz, 8H), 2.60 (s, 1H), 2.58 (s, 1H), 1.76 (d, *J* = 2.2 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ

172.28 (s), 162.76 (s), 156.99 (s), 144.18 (s), 140.29 (s), 137.68 (s), 134.36 (s), 129.84 (s), 128.85 (s), 127.40 (s), 115.24 (s), 107.84 (s), 81.04 (s), 78.67 (s), 64.62 (s), 45.53 (s), 43.20 (s), 40.49 (s), 33.09 (s), 3.68 (s). HRMS (ESI) m/z calcd for $C_{27}H_{28}N_3O_4S$ ($[M + H]^+$), 490.1801; 490.1803.

5.1.6.8.

*3-(4-((5-(4-(pyridin-3-yl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoic acid (**13h**)*

13h was prepared by general procedure F using **12h** to afford a light-yellow solid in 86.3%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, *J* = 6.8 Hz, 2H), 7.42 (t, *J* = 8.2 Hz, 1H), 7.32 (d, *J* = 8.6 Hz, 2H), 7.25 (d, *J* = 3.7 Hz, 1H), 7.23 – 7.19 (m, 1H), 7.06 (d, *J* = 3.7 Hz, 1H), 6.93 (d, *J* = 8.6 Hz, 2H), 5.21 (s, 2H), 4.09 – 4.03 (m, 1H), 3.99 – 3.92 (m, 4H), 3.39 – 3.32 (m, 4H), 2.81 (dd, *J* = 15.6, 8.1 Hz, 1H), 2.70 (dd, *J* = 15.6, 7.0 Hz, 1H), 1.83 (d, *J* = 2.2 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 176.01 (s), 163.69 (s), 157.16 (s), 151.41 (s), 149.34 (s), 143.75 (s), 136.64 (s), 134.19 (s), 130.04 (s), 129.40 (s), 128.64 (s), 126.14 (s), 121.71 (s), 115.23 (s), 114.67 (s), 110.27 (s), 79.56 (s), 79.16 (s), 65.07 (s), 48.76 (s), 43.36 (s), 33.31 (s), 29.78 (s), 3.76 (s). HRMS (ESI) m/z calcd for $C_{27}H_{28}N_3O_4S$ ($[M + H]^+$), 490.1801; found 490.1799.

5.1.6.9.

*3-(4-((5-(4-(pyridin-2-yl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoic acid (**13i**)*

13i was prepared by general procedure F using **12i** to afford a light-yellow solid in 83.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (d, *J* = 4.9 Hz, 1H), 7.69 (t, *J* = 7.8 Hz,

1H), 7.32 (d, *J* = 8.6 Hz, 2H), 7.25 (d, *J* = 3.6 Hz, 1H), 7.05 (d, *J* = 3.5 Hz, 1H), 6.92 (d, *J* = 8.6 Hz, 2H), 6.84 – 6.77 (m, 2H), 5.25 (s, 2H), 4.09 – 4.04 (m, 1H), 3.92 – 3.87 (m, 4H), 3.74 (brs, 4H), 2.84 (dd, *J* = 15.6, 7.3 Hz, 1H), 2.71 (dd, *J* = 15.6, 8.0 Hz, 1H), 1.84 (d, *J* = 2.3 Hz, 3H). ^{13}C NMR (151 MHz, MeOD) δ 174.74 (s), 165.44 (s), 158.46 (s), 158.22 (s), 146.01 (s), 144.76 (s), 141.45 (s), 137.63 (s), 135.68 (s), 130.78 (s), 129.53 (s), 127.40 (s), 116.08 (s), 114.86 (s), 110.88 (s), 80.76 (s), 79.53 (s), 65.91 (s), 49.43 (s), 46.44 (s), 44.55 (s), 34.62 (s), 3.15 (s). HRMS (ESI) m/z calcd for $C_{27}H_{28}N_3O_4S$ ($[M + H]^+$), 490.1801; found 490.1800.

5.1.6.10.

*3-(4-((5-(4-(p-tolyl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoic acid (**13j**)*

13j was prepared by general procedure F using **12j** to afford a light-yellow solid in 84.2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (d, *J* = 8.6 Hz, 2H), 7.22 (d, *J* = 3.6 Hz, 1H), 7.10 (d, *J* = 8.3 Hz, 2H), 7.03 (d, *J* = 3.6 Hz, 1H), 6.92 (d, *J* = 8.6 Hz, 2H), 6.86 (d, *J* = 8.4 Hz, 2H), 5.18 (s, 2H), 4.09-4.02 (m, 1H), 3.93 – 3.87 (m, 4H), 3.20 – 3.12 (m, 4H), 2.80 (dd, *J* = 15.6, 8.2 Hz, 1H), 2.69 (dd, *J* = 15.6, 6.9 Hz, 1H), 2.28 (s, 3H), 1.83 (d, *J* = 2.2 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.72 (s), 163.53 (s), 157.22 (s), 148.76 (s), 143.33 (s), 137.08 (s), 134.19 (s), 130.57 (s), 129.92 (s), 129.14 (s), 128.63 (s), 126.09 (s), 117.28 (s), 115.29 (s), 79.60 (s), 79.14 (s), 65.12 (s), 50.45 (s), 43.36 (s), 33.35 (s), 20.58 (s), 3.77 (s). HRMS (ESI) m/z calcd for $C_{29}H_{31}N_2O_4S$ ($[M + H]^+$), 503.2005; found 503.2002.

5.1.6.11.

*3-(4-((5-(4-(4-(tert-butyl)phenyl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoic acid (**13k**)*

13k was prepared by general procedure F using **12k** to afford a light-yellow solid in 83.1%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (d, *J* = 8.3 Hz, 2H), 7.33 (d, *J* = 8.4 Hz, 2H), 7.25 (d, *J* = 2.6 Hz, 1H), 7.11 – 6.99 (m, 3H), 6.94 (d, *J* = 8.4 Hz, 2H), 5.22 (s, 2H), 4.11 – 3.95 (m, 5H), 3.26 (brs, 4H), 2.83 (dd, *J* = 15.6, 8.1 Hz, 1H), 2.72 (dd, *J* = 15.6, 7.0 Hz, 1H), 1.85 (d, *J* = 1.8 Hz, 3H), 1.32 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.71 (s), 163.71 (s), 157.15 (s), 143.77 (s), 136.54 (s), 134.21 (s), 129.47 (s), 128.66 (s), 126.76 (s), 126.61 (s), 126.20 (s), 115.37 (s), 115.21 (s), 79.58 (s), 79.17 (s), 65.34 (s), 65.13 (s), 43.24 (s), 34.40 (s), 33.32 (s), 31.45 (s), 29.81 (s), 3.77 (s). HRMS (ESI) m/z calcd for $C_{32}H_{37}N_2O_4S$ ($[M + H]^+$), 545.2474; found 545.2466.

5.1.6.12.

*3-(4-((5-(4-(4-methoxyphenyl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)h ex-4-ynoic acid (**13l**)*

13l was prepared by general procedure F using **12l** to afford a light-yellow solid in 81.4%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.35 (d, *J* = 3.5 Hz, 1H), 7.29 (d, *J* = 8.4 Hz, 2H), 7.19 (d, *J* = 3.4 Hz, 1H), 6.97 (d, *J* = 8.4 Hz, 2H), 6.92 (d, *J* = 9.0 Hz, 2H), 6.84 (d, *J* = 8.9 Hz, 2H), 5.29 (s, 2H), 3.96 (brs, 1H), 3.77 (brs, 4H), 3.68 (s, 3H), 3.05 (brs, 4H), 2.58 (d, *J* = 6.3 Hz, 2H), 1.77 (d, *J* = 1.5 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 172.16 (s), 162.04 (s), 156.56 (s), 153.40 (s), 145.06 (s), 143.10 (s),

137.33 (s), 134.13 (s), 128.93 (s), 128.40 (s), 126.81 (s), 118.10 (s), 114.82 (s), 114.34 (s), 80.81 (s), 78.13 (s), 64.23 (s), 55.21 (s), 50.09 (s), 43.16 (s), 32.77 (s), 3.24 (s). HRMS (ESI) m/z calcd for $C_{29}H_{31}N_2O_5S$ ($[M + H]^+$), 519.1954; found 519.1950.

5.1.6.13.

*3-(4-((5-(4-(4-nitrophenyl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoic acid (**13m**)*

13m was prepared by general procedure F using **12m** to afford a light-yellow solid in 82.7%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, *J* = 9.2 Hz, 2H), 7.45 (d, *J* = 3.5 Hz, 1H), 7.34 (d, *J* = 8.4 Hz, 2H), 7.26 (d, *J* = 3.3 Hz, 1H), 7.03 (t, *J* = 8.0 Hz, 4H), 5.35 (s, 2H), 4.00 (brs, 1H), 3.87 (brs, 4H), 3.65 (brs, 4H), 2.64 (d, *J* = 7.3 Hz, 2H), 1.82 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.98 (s), 162.23 (s), 156.56 (s), 154.25 (s), 143.48 (s), 137.32 (s), 137.00 (s), 134.03 (s), 129.21 (s), 128.40 (s), 126.88 (s), 125.78 (s), 114.82 (s), 112.40 (s), 80.69 (s), 78.18 (s), 64.23 (s), 45.83 (s), 42.96 (s), 39.94 (s), 39.77 (s), 32.72 (s), 3.23 (s). HRMS (ESI) m/z calcd for $C_{28}H_{28}N_3O_6S$ ($[M + H]^+$), 534.1699; found 534.1690.

5.1.6.14.

*3-(4-((5-(4-(4-chlorophenyl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoic acid (**13n**)*

13n was prepared by general procedure F using **12n** to afford a light-yellow solid in 83.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (d, *J* = 7.9 Hz, 2H), 7.24 (d, *J* = 4.9 Hz,

3H), 7.05 (brs, 1H), 6.93 (d, *J* = 7.7 Hz, 2H), 6.86 (d, *J* = 8.2 Hz, 2H), 5.20 (s, 2H), 4.06 (brs, 1H), 3.91 (brs, 4H), 3.19 (brs, 4H), 2.81 (dd, *J* = 15.2, 8.2 Hz, 1H), 2.71 (dd, *J* = 15.3, 6.6 Hz, 1H), 1.84 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 176.07 (s), 163.62 (s), 157.20 (s), 149.49 (s), 143.52 (s), 136.83 (s), 134.15 (s), 129.29 (s), 129.25 (s), 128.63 (s), 126.11 (s), 125.74 (s), 118.04 (s), 115.26 (s), 79.53 (s), 79.19 (s), 65.10 (s), 49.77 (s), 43.33 (s), 33.31 (s), 29.79 (s), 3.76 (s). HRMS (ESI) m/z calcd for $C_{28}H_{28}ClN_2O_4S$ ($[M + H]^+$), 523.1458; found 523.1454.

5.1.6.15.

*3-(4-((5-(4-(4-fluorophenyl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoic acid (**13o**)*

13o was prepared by general procedure F using **12o** to afford a light-yellow solid in 84.5%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (d, *J* = 8.5 Hz, 2H), 7.22 (d, *J* = 3.6 Hz, 1H), 7.03 (d, *J* = 3.7 Hz, 1H), 7.02 – 6.97 (m, 2H), 6.90 (dd, *J* = 8.3, 5.6 Hz, 4H), 5.19 (s, 2H), 4.10 – 4.02 (m, 1H), 3.93 – 3.87 (m, 4H), 3.17 – 3.11 (m, 4H), 2.79 (dd, *J* = 15.4, 8.0 Hz, 1H), 2.68 (dd, *J* = 15.5, 7.0 Hz, 1H), 1.82 (d, *J* = 2.0 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 162.02 (s), 156.82 (d, *J* = 1237.07 Hz), 156.52 (s), 147.62 (s), 143.14 (s), 137.29 (s), 134.13 (s), 128.96 (s), 128.40 (s), 126.84 (s), 117.78 (s), 117.73 (s), 115.47 (s), 115.33 (s), 114.77 (s), 80.84 (s), 78.07 (s), 64.17 (s), 49.33 (s), 40.06 (s), 32.75 (s), 3.26 (s). HRMS (ESI) m/z calcd for $C_{28}H_{28}FN_2O_4S$ ($[M + H]^+$), 507.1754; found 507.1749.

5.1.6.16.

*3-(4-((5-(4-(3-fluorophenyl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoic acid (**13p**)*

13p was prepared by general procedure F using **12p** to afford a light-yellow solid in 83.6%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (d, *J* = 8.5 Hz, 2H), 7.22 (d, *J* = 3.6 Hz, 1H), 7.08 (d, *J* = 7.7 Hz, 1H), 7.04 (d, *J* = 3.8 Hz, 1H), 7.03 – 7.00 (m, 1H), 6.99 (s, 1H), 6.98 – 6.94 (m, 1H), 6.93 (d, *J* = 8.6 Hz, 2H), 5.21 (s, 2H), 4.10 – 4.03 (m, 1H), 3.96 – 3.91 (m, 4H), 3.16 – 3.10 (m, 4H), 2.81 (dd, *J* = 15.6, 8.2 Hz, 1H), 2.70 (dd, *J* = 15.5, 7.1 Hz, 1H), 1.84 (d, *J* = 2.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.74 (s), 163.49 (d, *J* = 238.14 Hz), 163.51 (d, *J* = 244.44Hz), 163.14 (s), 156.77 (s), 143.07 (s), 136.57 (s), 133.80 (s), 129.99 (s), 128.75 (s), 128.23 (s), 125.71 (s), 114.98 (s), 111.32 (s), 106.58 (s), 106.41 (s), 102.90 (s), 79.15 (s), 78.76 (s), 64.74 (s), 48.76 (s), 42.83 (s), 32.96 (s), 30.02 (s), 29.39 (s), 3.35 (s). HRMS (ESI) m/z calcd for $C_{28}H_{28}FN_2O_4S$ ($[M + H]^+$), 507.1754; found 507. 1757.

5.1.6.17.

*3-(4-((5-(4-(2-fluorophenyl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoic acid (**13q**)*

13q was prepared by general procedure F using **12q** to afford a light-yellow solid in 83.2%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.33 (d, *J* = 8.5 Hz, 2H), 7.23 (d, *J* = 3.7 Hz, 1H), 7.13 – 7.06 (m, 2H), 7.05 (d, *J* = 3.6 Hz, 1H), 7.04 – 6.99 (m, 1H), 6.99 – 6.94 (m, 1H), 6.92 (d, *J* = 8.6 Hz, 2H), 5.20 (s, 2H), 4.07 (brs, 1H), 3.97 – 3.92 (m,

4H), 3.17 – 3.11 (m, 4H), 2.77 (dd, *J* = 15.0, 8.3 Hz, 1H), 2.67 (dd, *J* = 15.0, 6.7 Hz, 1H), 1.83 (d, *J* = 1.9 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.53 , 157.11 (s), 156.93 (d, *J* = 246.76 Hz), 143.39 (s), 139.63 (d, *J* = 8.6 Hz), 137.23 (s), 134.78 (s), 129.03 (s), 128.69 (s), 126.08 (s), 124.72 (s), 123.36 (s), 119.43 (s), 116.36 (s), 115.27 (s), 80.22 (s), 78.71 (s), 65.17 (s), 50.92 (s), 44.29 (s), 33.73 , 29.84 (s), 3.83 (s). HRMS (ESI) m/z calcd for $C_{28}H_{28}FN_2O_4S$ ($[M + H]^+$), 507.1754; found 507.1752.

5.1.6.18.

*3-(4-((5-(4-(3-nitrophenyl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoic acid (**13r**)*

13r was prepared by general procedure F using **12r** to afford a light-yellow solid in 85.3%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, *J* = 6.8 Hz, 2H), 7.42 (t, *J* = 8.2 Hz, 1H), 7.32 (d, *J* = 8.6 Hz, 2H), 7.25 (d, *J* = 3.7 Hz, 1H), 7.23 – 7.19 (m, 1H), 7.06 (d, *J* = 3.7 Hz, 1H), 6.93 (d, *J* = 8.6 Hz, 2H), 5.21 (s, 2H), 4.09 – 4.03 (m, 1H), 3.99 – 3.92 (m, 4H), 3.39 – 3.32 (m, 4H), 2.81 (dd, *J* = 15.6, 8.1 Hz, 1H), 2.70 (dd, *J* = 15.6, 7.0 Hz, 1H), 1.83 (d, *J* = 2.2 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 176.01 (s), 163.69 (s), 157.16 (s), 151.41 (s), 149.34 (s), 143.75 (s), 136.64 (s), 134.19 (s), 130.04 (s), 129.40 (s), 128.64 (s), 126.14 (s), 121.71 (s), 115.23 (s), 114.67 (s), 110.27 (s), 79.56 (s), 79.16 (s), 65.07 (s), 48.76 (s), 43.36 (s), 33.31 (s), 29.78 (s), 3.76 (s). HRMS (ESI) m/z calcd for $C_{28}H_{28}N_3O_6S$ ($[M + H]^+$), 534.1699; found 534.1693.

5.1.6.19.

*3-(4-((5-(4-(2-nitrophenyl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoic acid (**13s**)*

13s was prepared by general procedure F using **12s** to afford a light-yellow solid in 87.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (dd, *J* = 8.1, 1.2 Hz, 1H), 7.56 – 7.50 (m, 1H), 7.31 (d, *J* = 8.5 Hz, 2H), 7.22 (d, *J* = 3.7 Hz, 1H), 7.15 (dd, *J* = 16.1, 8.0 Hz, 2H), 7.04 (d, *J* = 3.6 Hz, 1H), 6.92 (d, *J* = 8.6 Hz, 2H), 5.20 (s, 2H), 4.09-4.02 (m, 1H), 3.95 – 3.89 (m, 4H), 3.14 – 3.08 (m, 4H), 2.80 (dd, *J* = 15.6, 8.2 Hz, 1H), 2.70 (dd, *J* = 15.6, 6.9 Hz, 1H), 1.83 (d, *J* = 2.1 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 175.78 (s), 163.66 (s), 157.18 (s), 145.64 (s), 144.25 (s), 143.44 (s), 137.03 (s), 134.16 (s), 133.81 (s), 129.17 (s), 128.65 (s), 126.15 (s), 126.00 (s), 123.24 (s), 121.73 (s), 115.30 (s), 79.57 (s), 79.17 (s), 65.10 (s), 52.25 (s), 43.33 (s), 33.33 (s), 30.43 (s), 29.82 (s), 3.81 (s). HRMS (ESI) m/z calcd for $C_{28}H_{28}N_3O_6S$ ($[M + H]^+$), 534.1699; found 534.1692.

5.1.6.20.

*3-(4-((5-(1,2,3,4-tetrahydroquinoline-1-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoic acid (**13t**)*

13t was prepared by general procedure F using **12t** to afford a light-yellow solid in 81.5%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (d, *J* = 8.5 Hz, 2H), 7.21 (d, *J* = 7.5 Hz, 1H), 7.14 – 7.08 (m, 1H), 7.01 (d, *J* = 3.1 Hz, 2H), 6.89 (d, *J* = 9.5 Hz, 3H), 6.85 (d, *J* = 3.6 Hz, 1H), 5.14 (s, 2H), 4.10-4.03 (m, 1H), 3.93 (t, *J* = 6.7 Hz, 2H), 2.85-2.77 (m,

3H), 2.71 (dd, *J* = 15.6, 6.7 Hz, 1H), 2.10 – 2.01 (m, 2H), 1.85 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.63 (s), 163.74 (s), 157.11 (s), 143.28 (s), 137.60 (s), 134.09 (s), 132.84 (s), 128.96 (s), 128.67 (s), 128.52 (s), 126.87 (s), 126.60 (s), 126.41 (s), 126.04 (s), 115.24 (s), 79.50 (s), 79.03 (s), 65.07 (s), 43.27 (s), 33.25 (s), 29.71 (s), 29.13 (s), 3.68 (s). HRMS (ESI) m/z calcd for $C_{27}H_{26}NO_4S$ ($[M + H]^+$), 460.1583; found 460.1577.

5.1.6.21.

*3-(4-((5-(1,2,3,4-tetrahydroisoquinoline-2-carbonyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoic acid (**13u**)*

13u was prepared by general procedure F using **12u** to afford a light-yellow solid in 83.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (d, *J* = 8.7 Hz, 2H), 7.29 (d, *J* = 3.7 Hz, 1H), 7.20 (ddd, *J* = 16.2, 7.8, 4.1 Hz, 3H), 7.13 (brs, 1H), 7.06 (d, *J* = 3.7 Hz, 1H), 6.93 (d, *J* = 8.7 Hz, 2H), 5.20 (s, 2H), 4.88 (s, 2H), 4.10 – 4.03 (m, 1H), 3.95 (t, *J* = 5.9 Hz, 2H), 2.97 (t, *J* = 5.9 Hz, 2H), 2.81 (dd, *J* = 15.6, 8.3 Hz, 1H), 2.70 (dd, *J* = 15.6, 6.9 Hz, 1H), 1.84 (d, *J* = 2.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.77 (s), 163.84 (s), 157.23 (s), 143.39 (s), 137.71 (s), 134.50 (s), 134.17 (s), 132.96 (s), 129.08 (s), 128.78 (s), 128.63 (s), 126.98 (s), 126.71 (s), 126.55 (s), 126.15 (s), 115.34 (s), 79.58 (s), 79.16 (s), 65.18 (s), 43.34 , 33.35 (s), 30.45 (s), 29.82 (s), 29.56 (s), 3.79 (s). HRMS (ESI) m/z calcd for $C_{27}H_{26}NO_4S$ ($[M + H]^+$), 460.1583; found 460.1578.

5.1.6.22.

*3-(4-((5-((1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)thiophen-2-yl)methoxy)phenyl)hex-4-ynoic acid (**13v**)*

13v was prepared by general procedure F using **12v** to afford a light-yellow solid in 82.5%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (d, *J* = 3.7 Hz, 1H), 7.32 (t, *J* = 7.6 Hz, 3H), 7.24 – 7.16 (m, 2H), 7.14 (d, *J* = 7.6 Hz, 1H), 7.04 (d, *J* = 3.6 Hz, 1H), 6.91 (d, *J* = 8.5 Hz, 2H), 6.16 (d, *J* = 8.6 Hz, 1H), 5.35 (dd, *J* = 13.2, 5.6 Hz, 1H), 5.20 (s, 2H), 4.09 – 4.02 (m, 1H), 2.91 – 2.76 (m, 3H), 2.70 (dd, *J* = 15.6, 6.9 Hz, 1H), 2.17 – 1.97 (m, 2H), 1.88 (dd, *J* = 10.3, 4.7 Hz, 2H), 1.84 (d, *J* = 1.8 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.18 (s), 157.18 (s), 144.58 (s), 139.16 (s), 137.90 (s), 136.49 (s), 134.25 (s), 129.43 (s), 128.96 (s), 128.69 (s), 128.00 (s), 127.64 (s), 126.79 (s), 126.57 (s), 115.49 (s), 79.59 (s), 79.22 (s), 65.37 (s), 48.22 (s), 43.19 (s), 33.43 (s), 30.35 (s), 29.39 (s), 20.18 (s), 3.80 (s). HRMS (ESI) m/z calcd for $C_{28}H_{28}NO_4S$ ($[M + H]^+$), 474.1739; found 474.1732.

5.1.6.23.

*3-(4-((5-(4-(4-fluorophenyl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)propanoic acid (**14a**)*

14a was prepared by general procedure F using **14f** to afford a light-yellow solid in 89.2%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.36 (d, *J* = 3.5 Hz, 1H), 7.19 (d, *J* = 3.4 Hz, 1H), 7.15 (d, *J* = 8.3 Hz, 2H), 7.08 (t, *J* = 8.7 Hz, 2H), 6.99 (d, *J* = 4.5 Hz, 1H), 6.97 (d, *J* = 4.8 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 2H), 5.28 (s, 2H), 3.78 (brs, 4H), 3.14

(brs, 4H), 2.75 (t, *J* = 7.5 Hz, 2H), 2.48 (d, *J* = 7.8 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.96 (s), 162.23 (s), 156.18 (s), 155.58 (d, *J* = 236.88 Hz), 143.42 (s), 137.32 (s), 133.67 (s), 129.43 (s), 129.12 (s), 126.90 (s), 118.05 (s), 117.99 (s), 115.65 (s), 115.47 (s), 114.92 (s), 64.33 (s), 49.56 (s), 40.02 (s), 39.86 (s), 35.65 (s), 29.65 (s). HRMS (ESI) m/z calcd for $C_{25}H_{26}FN_2O_4S$ ($[M + H]^+$), 469.1597; found 469.1595.

5.1.6.24.

*3-cyclopropyl-3-(4-((5-(4-(4-fluorophenyl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl)propanoic acid (**14b**)*

14b was prepared by general procedure F using **14g** to afford a light-yellow solid in 76.3%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (t, *J* = 7.9 Hz, 1H), 7.19 (d, *J* = 3.7 Hz, 1H), 7.02 – 6.96 (m, 3H), 6.94 – 6.88 (m, 3H), 6.85 (dd, *J* = 8.1, 2.1 Hz, 1H), 6.80 (s, 1H), 5.23 (s, 2H), 3.93 – 3.87 (m, 4H), 3.16 – 3.11 (m, 4H), 2.78 (dd, *J* = 15.0, 6.0 Hz, 1H), 2.68 (dd, *J* = 14.9, 9.0 Hz, 1H), 2.36 – 2.29 (m, 1H), 1.07 – 0.98 (m, 1H), 0.62 – 0.54 (m, 1H), 0.47 – 0.40 (m, 1H), 0.28 (td, *J* = 9.5, 4.8 Hz, 1H), 0.16 (dt, *J* = 13.9, 4.8 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 176.06 (s), 163.76 (s), 157.76 (d, *J* = 240.66 Hz), 157.70 (s), 147.48 (d, *J* = 2.0 Hz), 146.09 (s), 143.53 (s), 136.57 (s), 129.50 (s), 128.96 (s), 126.02 (s), 120.81 (s), 118.73 (s), 118.67 (s), 115.85 (s), 115.67 (s), 115.56 (s), 113.50 (s), 65.32 (s), 50.72 (s), 47.21 (s), 41.79 (s), 29.71 (s), 17.23 (s), 5.23 (s), 4.31 (s). HRMS (ESI) m/z calcd for $C_{28}H_{30}FN_2O_4S$ ($[M + H]^+$), 509.1910; found 509.1903.

5.1.6.25.

*2-(6-((5-(4-(4-fluorophenyl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)-2,3-dihydr obenzofuran-3-yl)acetic acid (**14c**)*

14c was prepared by general procedure F using **14h** to afford a light-yellow solid in 81.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.36 (d, *J* = 3.6 Hz, 1H), 7.19 (d, *J* = 3.5 Hz, 1H), 7.12 (d, *J* = 8.8 Hz, 1H), 7.08 (t, *J* = 8.8 Hz, 2H), 6.98 (dd, *J* = 9.0, 4.6 Hz, 2H), 6.49 (d, *J* = 5.4 Hz, 2H), 5.26 (s, 2H), 4.69 (t, *J* = 9.0 Hz, 1H), 4.19 (dd, *J* = 8.5, 7.4 Hz, 1H), 3.78 (brs, 4H), 3.74-3.70 (m, 1H), 3.13 (brs, 4H), 2.66 (dd, *J* = 16.4, 5.4 Hz, 1H), 2.44 (dd, *J* = 16.5, 9.2 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.43 (s), 162.11 (s), 160.76 (s), 158.49 (s), 156.37 (d, *J* = 236.88 Hz), 147.64 (s), 143.20 (s), 137.25 (s), 128.99 (s), 126.83 (s), 124.73 (s), 122.74 (s), 117.83 (s), 117.77 (s), 115.51 (s), 115.34 (s), 106.95 (s), 96.93 (s), 77.37 (s), 64.44 (s), 49.37 (s), 39.92 (s), 39.77 (s), 37.32 (s). HRMS (ESI) m/z calcd for $C_{26}H_{26}FN_2O_5S$ ($[M + H]^+$), 497.1546; found 497.1547. $[\alpha]_D$ = +7.00° (c 0.50, CH_3OH).

5.1.6.26.

*(S)-3-(4-((5-(4-(4-fluorophenyl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl) hex-4-ynoic acid (**14d**)*

14d was prepared by general procedure F using **14i** to afford a light-yellow solid in 83.6%. **14d** (retention time 4.06 min) was obtained with a 99.3% ee [column, CHIRALPAK IG (5 μm, 4.6 mm × 150 mm); mobile phase, MeOH/DCM/HAc = 80/20/0.1 (V/V/V) by isocratic elution; flow rate, 1.0 mL/min; detection, UV 254 nm; temperature, 35 °C)]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (brs, 2H), 7.24 (s, 1H), 7.06

(s, 1H), 7.01 (t, *J* = 8.5 Hz, 2H), 6.96 – 6.89 (m, 4H), 5.21 (s, 2H), 4.08 (brs, 1H), 3.93 (brs, 4H), 3.15 (brs, 4H), 2.82 (brs, 1H), 2.74 (brs, 1H), 1.85 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 162.47 (s), 156.92 (s), 156.77 (d, *J* = 251.72 Hz),148.06 (s), 143.60 (s), 137.73 (s), 134.78 (s), 129.41 (s), 128.84 (s), 127.26 (s), 118.22 (s), 118.17 (s), 115.91 (s), 115.77 (s), 115.19 (s), 81.49 (s), 78.38 (s), 64.62 (s), 49.77 (s), 47.16 (s), 44.03 (s), 40.49 (s), 33.32 (s), 3.70 (s). HRMS (ESI) m/z calcd for $C_{28}H_{28}FN2O_4S$ ($[M + H]^+$), 507.1754; found 507.1751. $[\alpha]_D$ = +5.25° (c 0.40, CH_3OH).

5.1.6.27.

*(R)-3-(4-((5-(4-(4-fluorophenyl)piperazine-1-carbonyl)thiophen-2-yl)methoxy)phenyl) hex-4-ynoic acid (**14e**)*

14e was was optically resolved using normal phase preparative HPLC [column, CHIRALPAK IG (5 μm, 10 mm × 250 mm); mobile phase, MeOH/DCM/HAc = 80/20/0.1 (V/V/V) by isocratic elution; flow rate, 10 mL/min; detection, UV 254 nm; temperature, 35 °C)] from **13o**. **14e** (retention time 3.51 min) was obtained with a 98.6% ee (column, CHIRALPAK IG 4.6 mm i.d. × 150 mmL; mobile phase, MeOH/DCM/HAc = 80/20/0.1 (V/V/V) by isocratic elution; flow rate, 1.0 mL/min; detection, UV 254 nm; temperature, 35 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (d, *J* = 7.2 Hz, 2H), 7.22 (d, *J* = 3.5 Hz, 1H), 7.03 (d, *J* = 3.4 Hz, 1H), 6.99 (t, *J* = 8.6 Hz, 2H), 6.93 – 6.87 (m, 4H), 5.18 (s, 2H), 4.06 (brs, 1H), 3.93 – 3.87 (m, 4H), 3.16 – 3.11 (m, 4H), 2.75 (brs, 1H), 2.69 (brs, 1H), 1.82 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.56 (s), 157.85 (d, *J* = 201.60 Hz), 157.09 (s), 147.62 (d, *J* = 2.0 Hz), 143.48 (s),

137.06 (s), 134.70 (s), 129.15 (s), 128.72 (s), 126.14 (s), 118.85 (s), 118.80 (s), 115.97 (s), 115.83 (s), 115.22 (s), 80.14 (s), 78.83 (s), 65.11 (s), 50.88 (s), 44.80 (s), 33.68 (s), 29.85 (s), 3.87 (s). HRMS (ESI) m/z calcd for $C_{28}H_{28}FN2O_4S$ ($[M + H]^+$), 507.1754; found 507.1752. $[\alpha]_D$ = -7.00° (c 0.50, CH_3OH).

5.1.6.28.

*3-(4-((4-(4-(4-fluorophenyl)piperazine-1-carbonyl)benzyl)oxy)phenyl)hex-4-ynoic acid (**15a**)*

15a was prepared by general procedure F using **15l** to afford a light-yellow solid in 80.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 – 7.42 (m, 4H), 7.30 (d, *J* = 8.4 Hz, 2H), 7.03 (m, 4H), 6.91 (d, *J* = 8.5 Hz, 2H), 5.08 (s, 2H), 4.09 – 3.89 (m, 3H), 3.70 (brs, 2H), 3.17 (brs, 4H), 2.80 (dd, *J* = 15.6, 8.1 Hz, 1H), 2.69 (dd, *J* = 15.6, 7.0 Hz, 1H), 1.83 (d, *J* = 2.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.54 (s), 174.75 (s), 170.28 (s), 157.54 (s), 139.29 (s), 134.60 (s), 133.70 (s), 133.65 (s), 128.50 (s), 127.54 (s), 116.24 (s), 116.06 (s), 115.08 (s), 115.01 (s), 79.46 (s), 79.01 (s), 69.51 (s), 69.48 (s), 43.18 (s), 42.97 (s), 33.19 (s), 29.71 (s), 3.66 (s). HRMS (ESI) m/z calcd for $C_{30}H_{30}FN_2O_4S$ ($[M + H]^+$), 501.2190; found 501.2189.

5.1.6.29.

*3-(4-((2-(4-(4-fluorophenyl)piperazine-1-carbonyl)thiazol-5-yl)methoxy)phenyl)hex-4-ynoic acid (**15b**)*

15b was prepared by general procedure F using **15m** to afford a light-yellow solid in 81.8%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (s, 1H), 7.33 (d, *J* = 8.5 Hz, 2H), 7.00 (t, *J* = 8.7 Hz, 2H), 6.94 – 6.89 (m, 4H), 5.27 (s, 2H), 4.56 (brs, 2H), 4.10 – 4.02 (m,

1H), 3.96 (brs, 2H), 3.19 (brs, 4H), 2.81 (dd, *J* = 15.4, 8.2 Hz, 1H), 2.70 (dd, *J* = 15.6, 6.9 Hz, 1H), 1.84 (d, *J* = 2.0 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 175.26 (s), 165.38 (s), 159.20 (s), 158.24(d, *J* = 241.6 Hz) 156.88 (s), 146.87 (s), 141.93 (s), 139.65 (s), 134.49 (s), 128.77 (s), 119.18 (s), 119.13 (s), 116.11 (s), 115.96 (s), 115.37 (s), 79.47 (s), 79.28 (s), 62.77 (s), 51.56 (s), 51.08 (s), 46.20 (s), 43.18 (s), 33.34 (s), 29.84 (s), 3.81 (s). HRMS (ESI) m/z calcd for $C_{27}H_{27}FN_3O_4S$ ($[M + H]^+$), 508.1706; found 508.1702.

5.1.6.30.

*3-(4-((5-(4-(4-fluorophenyl)piperazine-1-carbonyl)furan-2-yl)methoxy)phenyl)hex-4-ynoic acid (**15c**)*

15c was prepared by general procedure F using **15n** to afford a light-yellow solid in 82.7%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (d, *J* = 8.3 Hz, 2H), 7.11 – 6.98 (m, 5H), 6.92 (dd, *J* = 14.3, 8.5 Hz, 2H), 6.53 (dd, *J* = 9.2, 3.3 Hz, 1H), 5.08 (s, 2H), 4.05 (brs, 1H), 3.94 (brs, 2H), 3.55 (brs, 2H), 3.17 (brs, 4H), 2.84 (dd, *J* = 15.7, 7.1 Hz, 1H), 2.71 (dd, *J* = 14.8, 8.5 Hz, 1H), 1.81 (d, *J* = 2.1 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 175.49 (s), 159.04 (s), 157.90(d, *J* = 220.1 Hz), 157.21 (s), 152.15 (s), 147.88 (s), 147.50 (d, *J* = 2.1 Hz), 134.21 (s), 128.66 (s), 118.92 (s), 118.87 (s), 117.81 (s), 115.94 (s), 115.80 (s), 115.19 (s), 111.34 (s), 79.56 (s), 79.18 (s), 62.44 (s), 50.97 (s), 43.24 (s), 33.30 (s), 3.77 (s). HRMS (ESI) m/z calcd for $C_{28}H_{28}FN_2O_5$ ($[M + H]^+$), 491.1982; found 491.1977.

5.1.6.31.

*3-(4-((4-(1,2,3,4-tetrahydroisoquinoline-2-carbonyl)benzyl)oxy)phenyl)hex-4-ynoic acid (**15d**)*

15d was prepared by general procedure F using **15o** to afford a light-yellow solid in 81.6%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (s, 4H), 7.32 (d, *J* = 8.6 Hz, 2H), 7.20 (dd, *J* = 13.4, 7.2 Hz, 4H), 6.93 (d, *J* = 8.6 Hz, 2H), 5.09 (s, 2H), 4.91 (brs, 1H), 4.61 (brs, 1H), 4.07 (brs, 1H), 4.00 (brs, 1H), 3.66 (brs, 1H), 2.99 (brs, 1H), 2.88 (brs, 1H), 2.80 (dd, *J* = 15.6, 8.2 Hz, 1H), 2.69 (dd, *J* = 15.6, 6.8 Hz, 1H), 1.83 (d, *J* = 2.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.34 (s), 170.93 (s), 157.59 (s), 139.03 (s), 135.41 (s), 133.81 , 132.82 (s), 129.03 (s), 128.67 (s), 128.50 (s), 127.41 (s), 127.27 (s), 126.93 (s), 126.67 (s), 126.40 (s), 125.89 (s), 114.97 (s), 79.66 (s), 78.89 (s), 69.56 (s), 67.93 (s), 43.34 (s), 33.24 (s), 25.60 (s), 3.69 (s). HRMS (ESI) m/z calcd for $C_{29}H_{28}NO_4$ ($[M + H]^+$), 454.2018; found 454.2012.

5.1.6.32.

*3-(4-((2-(1,2,3,4-tetrahydroisoquinoline-2-carbonyl)thiazol-5-yl)methoxy)phenyl)hex-4-ynoic acid (**15e**)*

15e was prepared by general procedure F using **15p** to afford a light-yellow solid in 84.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, *J* = 8.3 Hz, 1H), 7.33 (d, *J* = 8.2 Hz, 2H), 7.17 (ddd, *J* = 12.8, 9.1, 5.3 Hz, 4H), 6.92 (d, *J* = 7.6 Hz, 2H), 5.50 (s, 1H), 5.27 (s, 2H), 4.90 (s, 1H), 4.51 (t, *J* = 5.8 Hz, 1H), 4.09-4.03 (m, 1H), 4.00 (t, *J* = 6.0 Hz, 1H), 3.00 (dd, *J* = 12.3, 6.2 Hz, 2H), 2.81 (dd, *J* = 15.7, 8.3 Hz, 1H), 2.71 (dd, *J* = 15.6, 6.9 Hz, 1H), 1.84 (d, *J* = 2.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.68 (s),

165.72 (s), 159.88 (s), 156.96 (s), 142.01 (s), 134.48 (s), 128.92 (s), 128.75 (s), 128.67 (s), 126.98 (s), 126.79 (s), 126.59 (s), 126.36 (s), 125.65 (s), 115.44 (s), 79.49 (s), 79.28 (s), 62.87 (s), 48.51 (s), 43.27 (s), 42.11 (s), 33.37 (s), 30.47 (s), 3.80 (s). HRMS (ESI) m/z calcd for $C_{26}H_{25}N_2O_4S$ ($[M + H]^+$), 461.1535; found 461.1531.

5.1.6.33.

*3-(4-((5-(1,2,3,4-tetrahydroisoquinoline-2-carbonyl)furan-2-yl)methoxy)phenyl)hex-4-ynoic acid (**15f**)*

15f was prepared by general procedure F using **15q** to afford a light-yellow solid in 85.6%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (d, *J* = 8.5 Hz, 2H), 7.20 (d, *J* = 7.5 Hz, 4H), 7.03 (d, *J* = 3.3 Hz, 1H), 6.96 (d, *J* = 8.5 Hz, 2H), 6.53 (d, *J* = 3.3 Hz, 1H), 5.07 (s, 2H), 4.90 (s, 2H), 4.12 – 4.05 (m, 1H), 4.00 – 3.94 (m, 2H), 2.97 (t, *J* = 5.6 Hz, 2H), 2.82 (dd, *J* = 15.6, 8.3 Hz, 1H), 2.71 (dd, *J* = 15.6, 6.8 Hz, 1H), 1.85 (d, *J* = 2.0 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 175.86 (s), 157.24 (s), 152.20 (s), 148.00 (s), 134.17 (s), 132.99 (s), 128.64 (s), 126.92 (s), 126.64 (s), 117.39 (s), 115.15 (s), 111.24 (s), 79.55 (s), 79.17 (s), 62.57 (s), 43.35 (s), 33.29 (s), 3.79 (s). HRMS (ESI) m/z calcd for $C_{27}H_{26}NO_5$ ($[M + H]^+$), 444.1811; found 444.1807.

5.1.6.34.

*3-(4-((4-((1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)benzyl)oxy)phenyl)hex-4-ynoic acid (**15g**)*

15g was prepared by general procedure F using **15r** to afford a light-yellow solid in 83.9%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.82 (d, *J* = 8.6 Hz, 1H), 7.97 (d, *J* = 8.2 Hz, 2H), 7.56 (d, *J* = 8.2 Hz, 2H), 7.32 (d, *J* = 8.6 Hz, 2H), 7.25 – 7.21 (m, 1H), 7.17

(ddd, *J* = 8.6, 6.4, 1.7 Hz, 3H), 7.00 (d, *J* = 8.6 Hz, 2H), 5.30 (dt, *J* = 12.8, 6.4 Hz, 1H), 5.20 (s, 2H), 3.99 (td, *J* = 7.4, 2.3 Hz, 1H), 2.87 – 2.76 (m, 2H), 2.63 (d, *J* = 6.5 Hz, 2H), 1.99 (dd, *J* = 19.5, 5.9 Hz, 2H), 1.92 – 1.82 (m, 2H), 1.81 (d, *J* = 2.2 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 165.68 (s), 156.83 (s), 140.40 (s), 137.70 (s), 137.20 (s), 134.32 (s), 133.93 (s), 128.75 (s), 128.35 (s), 127.80 (s), 127.63 (s), 127.11 (s), 126.64 (s), 125.87 (s), 114.65 (s), 81.41 (s), 77.66 (s), 68.64 (s), 47.22 (s), 44.14 (s), 32.95 (s), 29.89 (s), 28.93 (s), 20.54 (s), 3.29 (s). HRMS (ESI) m/z calcd for $C_{30}H_{30}NO_4$ ($[M + H]^+$), 468.2175; found 468.2177.

5.1.6.35.

*3-(4-((2-((1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)thiazol-5-yl)methoxy)phenyl) hex-4-ynoic acid (**15h**)*

15h was prepared by general procedure F using **15s** to afford a light-yellow solid in 82.1%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (s, 1H), 7.33 (d, *J* = 8.3 Hz, 3H), 7.24 – 7.10 (m, 3H), 6.93 (d, *J* = 8.3 Hz, 2H), 6.41 (d, *J* = 8.0 Hz, 1H), 5.35 (t, *J* = 7.2 Hz, 1H), 5.31 (s, 2H), 4.10-4.02 (m, 1H), 2.91 – 2.75 (m, 3H), 2.69 (dd, *J* = 15.6, 7.0 Hz, 1H), 2.18 – 2.08 (m, 1H), 1.99-1.93 (m, 1H), 1.92-1.86 (m, 2H), 1.84 (d, *J* = 1.2 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.49 (s), 159.81 (s), 156.65 (s), 142.92 (s), 137.91 (s), 136.08 (s), 135.36 (s), 134.87 (s), 129.47 (s), 128.93 (s), 128.80 (s), 127.74 (s), 126.59 (s), 115.13 (s), 79.75 (s), 79.16 (s), 67.46 (s), 48.39 (s), 43.63 (s), 33.43 (s), 30.20 (s), 29.32 (s), 20.08 (s), 3.77 (s). HRMS (ESI) m/z calcd for $C_{27}H_{27}N_2O_4S$ ($[M + H]^+$), 475.1692; found 475.1691.

5.1.6.36.

*3-(4-((5-((1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)furan-2-yl)methoxy)phenyl)hex-4-ynoic acid (**15i**)*

15i was prepared by general procedure F using **15t** to afford a light-yellow solid in 81.5%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (d, *J* = 6.7 Hz, 1H), 7.29 (d, *J* = 8.4 Hz, 2H), 7.20 (s, 2H), 7.16 – 7.11 (m, 2H), 6.87 (d, *J* = 8.3 Hz, 2H), 6.62 (d, *J* = 9.7 Hz, 1H), 6.52 (d, *J* = 3.1 Hz, 1H), 5.39 – 5.33 (m, 1H), 4.95 (s, 2H), 4.06-4.02 (m, 1H), 2.87 – 2.80 (m, 2H), 2.75 (dd, *J* = 15.3, 8.5 Hz, 1H), 2.65 (dd, *J* = 15.1, 6.7 Hz, 1H), 2.17 – 2.09 (m, 1H), 1.96-1.87 (m, 3H), 1.82 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.97 (s), 157.73 (s), 157.24 (s), 152.06 (s), 148.24 (s), 137.87 (s), 136.44 (s), 134.24 (s), 129.40 (s), 129.09 (s), 128.64 (s), 127.61 (s), 126.51 (s), 115.35 (s), 115.13 (s), 112.25 (s), 79.49 (s), 79.27 (s), 62.54 (s), 47.39 (s), 43.15 (s), 33.34 (s), 30.36 (s), 29.38 (s), 20.14 (s), 3.78 (s). HRMS (ESI) m/z calcd for $C_{28}H_{28}NO_5$ ($[M + H]^+$), 458.1967; found 458.1963.

5.1.6.37.

*(S)-3-(4-((4-(((S)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)benzyl)oxy)phenyl)hex-4-ynoic acid (**15j**)*

15j was prepared by general procedure F using **15u** to afford a light-yellow solid in 85.2%. **15j** (retention time 6.59 min) was obtained with a 99.1% de [column, CHIRALPAK IA (5 μm, 4.6 × 250 mm); mobile phase, n-hexane/IPA/TFA = 85/15/0.1 (V/V/V) by isocratic elution; flow rate, 1.0 mL/min; detection, UV 254 nm; temperature, 20 °C]. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.82 (d, *J* = 8.6 Hz, 1H), 7.97

(d, *J* = 8.2 Hz, 2H), 7.56 (d, *J* = 8.2 Hz, 2H), 7.32 (d, *J* = 8.6 Hz, 2H), 7.25 – 7.21 (m, 1H), 7.17 (ddd, *J* = 8.6, 6.4, 1.7 Hz, 3H), 7.00 (d, *J* = 8.6 Hz, 2H), 5.30 (dt, *J* = 12.8, 6.4 Hz, 1H), 5.20 (s, 2H), 3.99 (td, *J* = 7.4, 2.3 Hz, 1H), 2.87 – 2.76 (m, 2H), 2.63 (d, *J* = 6.5 Hz, 2H), 1.99 (dd, *J* = 19.5, 5.9 Hz, 2H), 1.92 – 1.82 (m, 2H), 1.81 (d, *J* = 2.2 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 172.06 (s), 165.85 (s), 157.07 (s), 140.46 (s), 137.73 (s), 137.30 (s), 134.01 (s), 133.74 (s), 128.84 (s), 128.44 (s), 127.84 (s), 127.71 (s), 127.20 (s), 126.73 (s), 125.94 (s), 114.85 (s), 80.76 (s), 78.26 (s), 68.76 (s), 47.35 (s), 43.00 (s), 32.79 (s), 29.95 (s), 28.99 (s), 20.61 (s), 3.29 (s). HRMS (ESI) m/z calcd for $C_{30}H_{30}NO_4$ ($[M + H]^+$), 468.2175; found 468.2170. $[\alpha]_D$ = -5.80° (c 0.50, CH_3OH).

5.1.6.38.

*(S)-3-(4-((4-(((R)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)benzyl)oxy)phenyl)hex-4-ynoic acid (**15k**)*

15k was prepared by general procedure F using **15v** to afford a light-yellow solid in 84.3%. **15k** (retention time 5.03 min) was obtained with a 99.1% de [column, CHIRALPAK IA (5 μm, 4.6 × 250 mm); mobile phase, n-hexane/IPA/TFA = 85/15/0.1 (V/V/V) by isocratic elution; flow rate, 1.0 mL/min; detection, UV 254 nm; temperature, 20 °C]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, *J* = 8.1 Hz, 2H), 7.48 (d, *J* = 8.1 Hz, 2H), 7.35 (d, *J* = 6.8 Hz, 1H), 7.31 (d, *J* = 8.5 Hz, 2H), 7.21 (td, *J* = 6.4, 1.4 Hz, 2H), 7.16 (t, *J* = 6.4 Hz, 1H), 6.91 (d, *J* = 8.6 Hz, 2H), 6.42 (d, *J* = 8.3 Hz, 1H), 5.41 (dd, *J* = 13.3, 5.9 Hz, 1H), 5.10 (s, 2H), 4.07 (s, 1H), 2.90 – 2.77 (m, 3H), 2.71 (dd, *J* = 15.6, 6.7 Hz, 1H), 2.21 – 2.12 (m, 1H), 2.01 – 1.95 (m, 1H), 1.91 (dd, *J*

= 11.2, 5.3 Hz, 2H), 1.84 (d, *J* = 2.1 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 175.84 (s), 166.53 (s), 157.50 (s), 140.78 (s), 137.77 (s), 136.50 (s), 134.05 (s), 133.60 (s), 129.30 (s), 128.79 (s), 128.46 (s), 127.45 (s), 127.29 (s), 127.28 (s), 126.40 (s), 114.96 (s), 79.44 (s), 79.04 (s), 69.33 (s), 48.06 (s), 43.19 (s), 33.16 (s), 30.16 (s), 29.28 (s), 20.05 (s), 3.68 (s). HRMS (ESI) m/z calcd for $C_{30}H_{30}NO_4$ ($[M + H]^+$), 468.2175; found 468.2174. $[\alpha]_D$ = +20.60° (c 0.50, CH_3OH).

5.2. Calcium mobilization assay in human GPR40-HEK293 cells

HEK293 cells stably expressing human GPR40 (hGPR40) were plated into 96- well plates at a density of 25 K cells/well in dulbecco's modified eagle medium supplemented with 10% fetal bovine serum for 24 hours. After removal of the culture media, cells were incubated with 100 μL/well of Hank's Balanced Salt Solution (containing 2μM Fluo-8, 2 mM Probenecid, 1.5mM Tartrazine and 4mM Acid Red 1) at 37 °C for 1 hour. Compounds diluted to desired concentration with HBSS (Hank's Balanced Salt Solution) were added to cells by Flexstation III instrument (Molecular Devices, CA) and fluorescent values were detected. EC_{50} value of compounds was estimated by Prism 5 software (GraphPad).

5.2. In Vitro Liver Microsomal Stability Assay

The test compound was dissolved in DMSO and diluted to the desired concentration with DMSO and 0.1% aqueous BSA. Liver microsomes were incubated in a 96-well plate containing 0.1M Tris buffer (pH 7.4), 0.33 mg/mL microsomes protein, 0.1 mM test compound, 5.0 mM $MgCl_2$, 0.005% BSA, and 1.0 mM NADPH. Incubations

were conducted at 37 °C. An aliquot was removed at each time point and the enzymatic reaction was stopped by protein precipitation in methanol. The loss of the test compound was determined by liquid chromatography-tandem mass spectrometry.

Half-live of the compound in liver microsomes was calculated using the following equation:

$$T_{1/2} = 0.693/k$$

(-k) was the slope of the linear regression from log [substrate] versus time plot.

5.3. Caco-2 Permeability Assay.

Caco-2 cells were obtained from the American Type Culture Collection (Cat#HTB-37) and maintained in Dulbecco's modified Eagle's Medium containing 10% fetal bovine serum, 1% glutamine, 1% nonessential amino acids, 100 μg/mL streptomycin, and 100 U/mL penicillin. Caco-2 cells were cultured at 37 °C in a 5% CO_2 and 90% relative humidity environment. Caco-2 cells were passaged every 7 days at a ratio of 1:10. Cells were used between passages 30 and 40. After 21 days of culture, the integrity of the cell monolayer was verified by measuring the transepithelial electrical resistance. Drug transport from the apical side to the basolateral side (A-B) and from the basolateral side to the apical side (B–A) was measured simultaneously under the same condition. Propranolol and atenolol were used as the hypertonic and hypotonic control, respectively. Digoxin was used as the positive control for Pgp-mediated drug efflux. In brief, the method was as follows. After washing the monolayer with HBSS three times, the compounds were diluted

and added to the appropriate well (pH 6.8 for apical side and pH 7.4 for basolateral side). The plate was incubated at 37 °C for 95 min. Samples were collected from the donor side at 5 and 95 min, and from the receiver side at 35 and 95 min post-incubation. The concentration of samples was measured by liquid chromatography-tandem mass spectrometry. The P_{app} was calculated from the following equation:

$$P_{app} = (V_A/(SA \times T)) \times ([drug]_{acceptor} / [durg]_{initial\ donor})$$

Where V_A is the volume of the acceptor well, SA is the surface area of the membrane, T is the total transport time, $[drug]_{acceptor}$ is the drug level at the acceptor side, and [drug]initial donor is the drug level at the donor side at $T = 0$.

5.4. Animals

Male ICR mice were purchased from the Shanghai SLAC Laboratory Animal Co. Ltd. (Shanghai, China). Animal experiments were approved by the Animal Care and Use Committee (IACUC), Shanghai Institute of Materia Medica, Chinese Academy of Sciences (IACUC approval no.:2017-02-YY-05).

5.5. PK studies

Test compounds were subjected to PK studies on male ICR mice with three animals in each group. Compounds for oral administration (30 mg/kg) were dispersed in 0.1% Tween-80 and 1% hydroxypropyl methylcellulose sodium. Blood samples were collected at predose, 0.25, 0.5, 0.75, 1, 2, 4, 8 and 24h following oral administration.

The concentration of compounds in the plasma samples was determined with a liquid chromatography-mass spectrometry. PK parameters were calculated from the mean serum concentration by non-compartmental analyses using DAS software 2.1.1.

5.6. Tissue Distribution Study

Compounds **2**, **14d**, **15j**, **15k** was dosed orally (30 mg/kg) to overnight-fasted ICR mice with three animals in each group. Blood samples were collected at 0.75 (**15j**, **2**) and 1h (**14d**, **15k**), respectively. Blood samples were collected and frozen at -20 °C. Then brains and livers were perfused with saline, and liver and brain tissues were excised and frozen at -20 °C. The brain and liver tissues were then thawed, added solution of methanol/acetonitrile (V/V = 1:1), sonicated and centrifuged 15,000 RPM for 5 min. The supernatant fraction was collected and mixed with water for further analysis. The concentration of compounds in the plasma samples, brain and liver tissues was determined with a liquid chromatography-mass spectrometry.

5.7. Oral Glucose Tolerated Test in ICR mice

100 mg/kg of **15k** was administered orally to overnight-fasted ICR mice (n = 8) 30 min prior to the oral glucose load of 2.5 g/kg. Blood glucose levels were measured via blood drops obtained by clipping the tail of the mice using an Accu-Chek Advantage II Glucose Monitor (Roche, Indianapolis, IN, USA) at -30, 0, 15, 30, 60, and 120 min. The area under the concentration−time curve from 0 to 120 min ($AUC_{0-120\ min}$, Glu) of blood glucose after the glucose load was calculated by the trapezoidal rule.

5.8. Statistical Analysis

All data were expressed as the mean or mean ± SEM. The statistical analysis was performed by using the Student's t-test. $P < 0.05$ was considered to be statistically significant.

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T.-T.C. and M.-M.N. designed and performed experiments, T.-T.C. prepared the figures and wrote the manuscript. M.-M.N., Y.-L.Y. and K.W. reviewed the manuscript. J.-H.S. and Y.L. designed experiments and reviewed the manuscript. All authors have given approval to the final version of the manuscript. †These authors contributed equally.

Declaration of interest

The authors declare no competing financial interest.

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Abbreviations

AUC, area under curve; BBB, brain-blood-barrier; $(BOC)_2O$, di-tert-butyl-dicarbonate; B/P, total brain-to-plasma drug distribution ratio; CCl_4, carbon tetrachloride; CL, clearance; CNS, central nervous system; C_{max}, maximum plasma concentration; DCM, dichloromethane; DIPEA, N-diisopropylethylamine; DMAP, 4-dimethylaminopyridine; DMF, *N*,*N*-dimethylformadide; DMSO, dimethylsulfoxide; FFAR1, free fatty acid receptor 1; FFAs, free fatty acids; FLIPR, Fluorometric Imaging Plate Reader; GLP-1, glucagon like peptide 1; GPR40, G-protein coupled receptor 40; HATU, 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate; HPLC, High Prformance Liquid Chromatography; ICR mice, Institute of Cancer Research mice; L/P, liver-to-plasma drug distribution ratio; NADPH, Nicotinamide Adenine Dinucleotide Phosphate; NBS, Nbromosuccinimide; PK, pharmacokinetic; SAR, structure-activity relationship; $T_{1/2}$, half-life time; T2DM, type 2 diabetes mellitus; tPSA, total polar surface area.

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Highlights：

- A series of amide analogues has been synthesized.
- Compound **15k** had promoted liver microsomal stability and potency *in vitro*.
- Compound **15k** displayed improved plasma concentration and prolonged half-time in ICR mice.
- Compound **15k** possessed minimal central nervous system exposure and live/plasma distribution ratio in ICR mice.