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Article

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Discovery and Characterization of 1*H*-1,2,3-Triazole Derivatives as Novel Prostanoid EP4 Receptor Antagonists for Cancer Immunotherapy

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ABSTRACT

The prostanoid EP4 receptor is one of the key receptors associated with inflammatory mediator PGE_2-elicited immunosuppression in the tumor microenvironment. Blockade of EP4 signaling to enhance immunity-mediated tumor elimination has recently

emerged as a promising strategy for cancer immunotherapy. In our efforts to discover novel subtype-selective EP4 antagonists, we designed and synthesized a class of 1*H*-1,2,3-triazole-based ligands that display low nanomolar antagonism activity towards the human EP4 receptor and excellent subtype selectivity. The most promising compound **59** exhibits single-digit nanomolar potency in the EP4 calcium flux and CRE reporter assays, and effectively suppresses the expression of multiple immunosuppression-related genes in macrophage cells. On the basis of its favorable ADMET properties, compound **59** was chosen for further in vivo biological evaluation. Oral administration of compound **59** significantly inhibited tumor growth in the mouse CT26 colon carcinoma model accompanied by enhanced infiltration of cytotoxic T lymphocytes in tumor tissue.

INTRODUCTION

Prostanoids are a family of endogenous chemical mediators expressed in various human tissues and fluids, which are biosynthesized from arachidonic acid through the activity of cyclooxygenases (COX1 and COX2),[1] peroxidases, and downstream tissue-specific synthases. These bioactive lipids generally contain twenty carbons including a five-membered carbon ring and play essential roles in maintaining body homeostasis. Among them, prostaglandin E_2 (PGE_2) is the most abundant prostaglandin within the human body and has been widely implicated in numerous physiological and pathophysiological processes, including inflammation, pain, atherosclerosis, renal disease, osteoporosis, and cancer. Recently, a growing body of evidence supports chronic inflammation as a major factor promoting cancer development.[2] As an important pro-inflammatory mediator, PGE_2 is heavily involved in modulating tumor progression through multiple signaling pathways regulating angiogenesis and immunosuppression, and is often correlated with a poor prognosis in cancer patients.[3, 4] In contrast, 15-hydroxyprostaglandin dehydrogenase (15-PGDH), a primary PGE_2-degrading enzyme highly expressed in normal tissues, is found to be ubiquitously downregulated in various

human cancers.[5] Upregulation of COX2 expression in tumor cells and myeloid cells can result in elevated levels of PGE_2, which diffuses into the tumor microenvironment and subsequently binds to a cluster of four G protein-coupled receptors (GPCRs), referred to as EP receptors (EP1-4). Among the four receptors, the EP4 receptor is mainly expressed in myeloid cells in the tumor microenvironment, and is the major contributor to the PGE_2-elicited immunosuppressive activity that promotes tumor development and progression.[6-8] Upon ligand binding, G_S-coupled EP4 receptor stimulates the intracellular production of cyclic AMP (cAMP) and the consequent activation of protein kinase A (PKA).[9] The cAMP pathway is thought to be the primary signal for EP4 receptor mediated immunosuppression.[6] Previous studies have demonstrated that selective deletion of EP4 receptor in myeloid cells markedly inhibited the development and progression of colorectal cancer in *Apc*$^{Min/+}$ mice.[10] Meanwhile, pharmacologically blocking the EP4 receptor could induce effective anti-tumor immune responses in vivo by reducing intratumoral immunosuppressive myeloid cells.[11] So far, numerous preclinical studies and clinical trials have indicated that long-term administration of traditional nonsteroidal anti-inflammatory drugs (NSAIDs) or selective COX2 inhibitors is beneficial for cancer

chemoprevention,[12] however, these agents may cause severe or even life-threatening gastrointestinal (GI) bleeding and ulcers in some patients, and increase the risk of heart attacks, strokes, and heart-related deaths especially for long-term medication.[13] Besides, under physiological conditions, PGE_2 is responsible for a wide range of homeostasis in mammals, and NSAIDs used to decrease the production of PGE_2 may be associated with a diverse range of pathological conditions. A growing body of studies have indicated that a selective EP4 antagonist could be an effective alternative with better GI tolerability compared to NSAIDs.[14] Furthermore, a preferable cardiovascular safety profile can be envisaged with the proper use of selective EP4 antagonists because they do not interfere with the biosynthesis of other prostanoids such as PGI_2.[15] Given these advantages of EP4 antagonists and the recent demonstration of the crystal structure of human EP4 receptor in complex with an antagonist,[16] selective inhibition of PGE_2/EP4 signaling pathway represents an attractive strategy for cancer immunotherapy.

To date, a number of structurally diverse EP4 antagonists have been identified and developed for clinical studies in multiple diseases, such as pain, inflammation disorders, and cancer (Figure 1). Compound **2** (Grapiprant),[17] the first and only EP4 antagonist on

the market from Aratana Therapeutics, was approved by the FDA in 2016 for the management of pain and inflammation associated with osteoarthritis in dogs.[18] Another compound, BGC20-1531 (**3**), discovered by Pharmagene using a virtual screening method, was advanced to Phase II clinical development for alleviating the symptoms of migraine headache.[19] In 2015, Eisai unveiled its EP4 antagonist E7046 (**4**), which was capable of preventing the differentiation of monocytic myeloid lineage cells into a pro-tumorigenic phenotype in the tumor microenvironment and was advanced to phase I clinical trial in patients with diverse cancer types.[20] Despite their chemical structures being not available yet, some EP4 antagonists such as ONO-4578[21] and LY3127760[22] are now under development at different stages of clinical studies including advanced or metastatic solid tumors and rheumatoid arthritis. In addition, some novel preclinical EP4 antagonists, as exemplified by CJ-042794 (**5**)[23], MF-2894 (**6**)[24], MF-766 (**7**)[25] and BAY 1316957 (**8**)[26] can also serve as chemical tools in studying the EP4 receptor and its role in tumors.

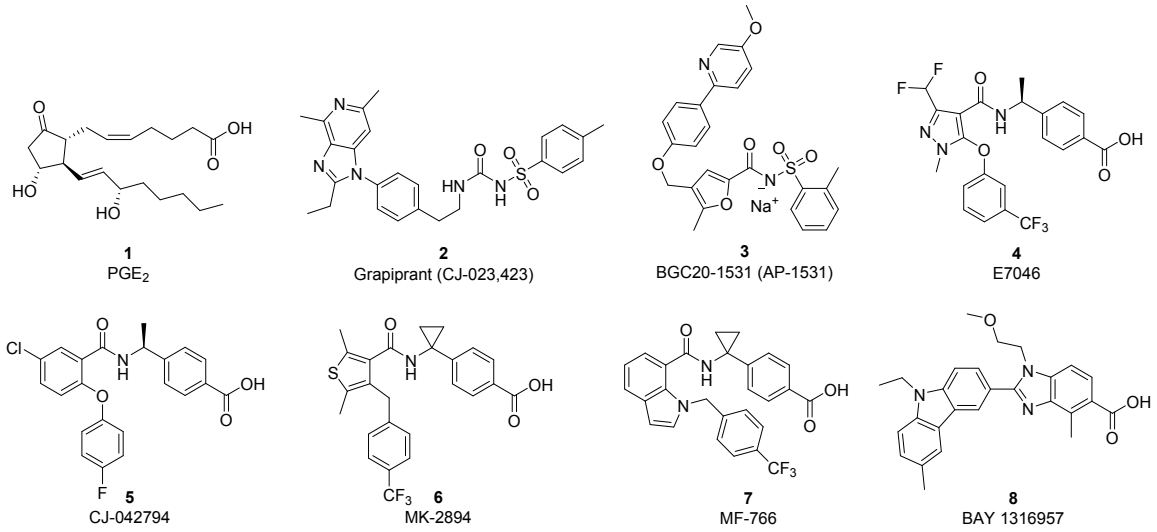


Figure 1. The Chemical Structure of PGE_2 and Selected EP4 Antagonists.

On the basis of chemical structure features, the present EP4 antagonists can be roughly grouped into two broad categories: the first-generation EP4 antagonists containing sulfonyl urea/acylsulfonamide moieties, and the second-generation ligands bearing carboxamido-benzoic acid and related scaffolds.[27, 28] With the exception of compound **2**, the development of the first-generation EP4 antagonists for clinical use was unsuccessful primarily due to their poor pharmacokinetic profiles such as species-dependent metabolic liability and low blood-brain barrier permeability.[24, 25, 29] Therefore, we turned our attention to the second-generation EP4 antagonists reported in literature which exhibited better pharmacokinetic and metabolism profiles while maintaining high

potency and selectivity towards the EP4 receptor. Through structure-activity relationship (SAR) analysis, it was found that these second-generation EP4 antagonists generally included three essential structural requirements: a core skeleton, a benzoic-acid-containing side chain, and a hydrophobic side chain (Figure 2). The chemical structure of the benzoic-acid-containing side chain was relatively conserved, and it was located adjacent to the hydrophobic side chain in the core skeleton. To date, multiple structurally distinct scaffolds have been introduced into the core skeleton such as phenyl ring, thiophene, indole, and pyrazole as outlined in Figure 1. It is noteworthy that most of the second-generation EP4 antagonists reported in the literature have relatively limited structural modifications on their core scaffolds, such as compound **4**[30], **5**[31], **6** [24], and **7**[25]. In the process of our efforts to identify novel EP4 antagonist, 1,2,3-triazole drew our attention. The 1,2,3-trizole ring is present in many biologically active compounds including several marketed drugs (Tazobactam, Rufinamide, Suvorexant, Cefatrizine), and is one of the of privileged scaffolds in medicinal chemistry possessing unique structural features.[32] The unique structural characteristics of 1,2,3-triazole like strong dipole moments, multiple hydrogen bond receptors and marked stability under metabolic

conditions make it an ideal bioisostere for various functional groups to improve molecule drug-like properties, including bioavailability and water solubility.[33] In addition, the synthetic routes of triazole derivatives are feasible and reliable, which could facilitate the follow-up lead optimization process. We assume that incorporation of a 1,2,3-triazole ring as core skeleton would be a reasonable strategy to develop novel EP4 antagonists. Some of the compounds reported in this work exhibited potent EP4 receptor antagonistic activity and good selectivity over other EP receptor subtypes. Cytotoxicity and preliminary ADMET studies were further carried out to evaluate their drug-like property. At last, immunological effects in vitro and anti-tumor efficiency were also assessed for the best compound **59** in this series.

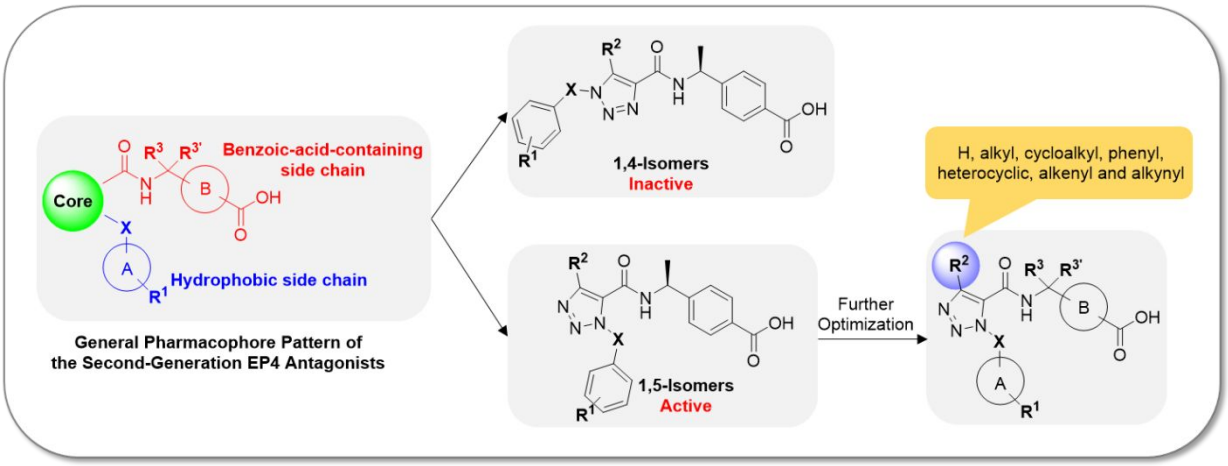


Figure 2. An overview of the design and optimization of triazole derivatives reported in this study.

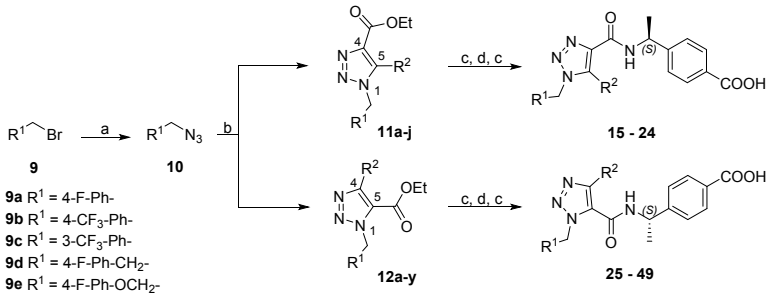
RESULTS AND DISCUSSION

Chemistry. The syntheses of triazole analogs **15 - 49** is described in Scheme 1. The bromide starting material **9** was treated with NaN_3 to afford the corresponding azide **10**, which underwent a traditional 1,3-dipolar cycloaddition reaction with different alkynoates to simultaneously yield a mixture of two triazole regioisomers.[34] After chromatographic separation, the structures of isomers **11** and **12** were determined by X-ray crystallography or 2D NMR spectroscopy (see Supporting Information for more details). It was interesting to note that the 1,4-regioisomer **11** had a lower R_f value than its 1,5-counterpart **12** when monitored by thin-layer chromatography (TLC) analysis and this phenomenon was applicable to all of the triazole intermediates synthesized in this work. After hydrolysis of the ester group, compound **11** was subjected to HATU coupling reaction with methyl-4-[(1*S*)-1-aminoethyl]benzoate and subsequent benzoate hydrolysis to obtain the desired

products **15 - 24**. Compounds **25 - 49** were prepared from **12** by the same sequence of steps.

The synthetic routes of compounds **50 - 65** were outlined in Scheme 2. 1-(Bromomethyl)-4-(trifluoromethyl)benzene **9b** was initially converted to the azide and then reacted with ethyl 3-bromopropiolate to form 1,5-regioisomer **13**. Subsequent Suzuki cross-coupling with substituted boric acids or Stille cross-coupling with organostannane yielded esters **14c-j** and **14k-l**, respectively. In addition, esters **14a-b** were prepared via Pd/C-catalyzed hydrogenation of cycloalkenyl analogs **14c-d**. Compounds **50 - 65** were synthesized from **14a-j** in the same sequence of steps as compounds **25 - 49**.

Scheme 1. Synthesis of Triazole Analogs **15** - **49**[a]



9a R^1 = 4-F-Ph-
9b R^1 = 4-CF_3-Ph-
9c R^1 = 3-CF_3-Ph-
9d R^1 = 4-F-Ph-CH_2-
9e R^1 = 4-F-Ph-OCH_2-
9f R^1 = Ph-
9g R^1 = 3,4-diF-Ph-
9h R^1 = 4-MeO-Ph-
9i R^1 = 3-MeO-Ph-
9j R^1 = 3-F-4-MeO-Ph-
9k R^1 = 3,4-diMeO-Ph-
9l R^1 = 4-CF_3-Ph-OCH_2-
9m R^1 = 3-CF_3-Ph-OCH_2-
9n R^1 = tetrahydro-2*H*-pyran-4-yl
9o R^1 = cyclohexyl

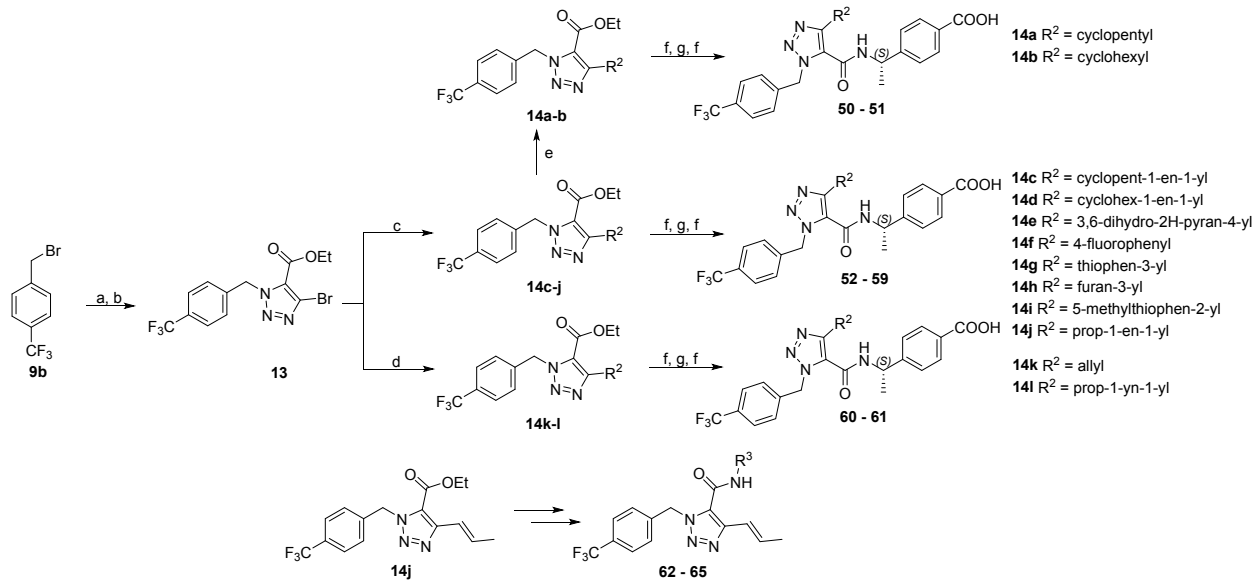
11a-12a R^1 = 4-F-Ph-, R^2 = CF_3
11b-12b R^1 = 4-CF_3-Ph-, R^2 = CF_3
11c-12c R^1 = 3-CF_3-Ph-, R^2 = CF_3
11d-12d R^1 = 4-F-Ph-CH_2-, R^2 = CF_3
11e-12e R^1 = 4-F-Ph-OCH_2-, R^2 = CF_3
11f-12f R^1 = 4-CF_3-Ph-, R^2 = H
11g-12g R^1 = 4-CF_3-Ph-, R^2 = Me

11h-12h R^1 = 4-CF_3-Ph-, R^2 = Et
11i-12i R^1 = 4-CF_3-Ph-, R^2 = n-Pr
11j-12j R^1 = 4-CF_3-Ph-, R^2 = Ph
12k R^1 = Ph-, R^2 = Ph
12l R^1 = 4-F-Ph-, R^2 = Ph
12m R^1 = 3,4-diF-Ph-, R^2 = Ph
12n R^1 = 3-CF_3-Ph-, R^2 = Ph

12o R^1 = 4-MeO-Ph-, R^2 = Ph
12p R^1 = 3-MeO-Ph-, R^2 = Ph
12q R^1 = 3-F-4-MeO-Ph-, R^2 = Ph
12r R^1 = 3,4-diMeO-Ph-, R^2 = Ph
12s R^1 = 4-F-Ph-OCH_2-, R^2 = Ph
12t R^1 = 4-CF_3-Ph-OCH_2-, R^2 = Ph
12u R^1 = 3-CF_3-Ph-OCH_2-, R^2 = Ph
12v R^1 = tetrahydro-2*H*-pyran-4-yl, R^2 = Ph
12w R^1 = cyclohexyl, R^2 = Ph
12x R^1 = 4-CF_3-Ph-, R^2 = cyclopropyl
12y R^1 = 4-CF_3-Ph-, R^2 = cyclobutyl

[a]Reagents and conditions: (a) NaN_3, dimethyl sulfoxide (DMSO), room temperature (rt), overnight, 90-100%; (b) substituted or unsubstituted ethyl propiolates, toluene, reflux, overnight, **11a-j**, 29-42%, **12a-y**, 20-45%; (c) $LiOH{\cdot}H_2O$, MeOH/THF/H_2O = 2:2:1, reflux, 2 h, 92-100%; (d) methyl-4-[(1*S*)-1-aminoethyl]benzoate, 2-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU), N,N-diisopropylethylamine (DIPEA), rt, overnight, 65-80%.

Scheme 2. Synthesis of Triazole Analogs **50** - **65**[a]



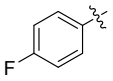
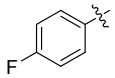
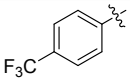
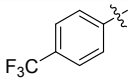
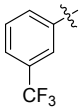
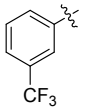
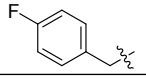
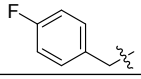
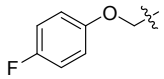
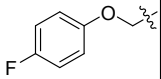
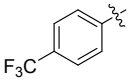
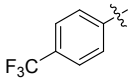
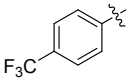
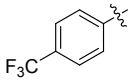
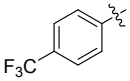
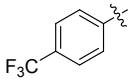
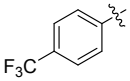
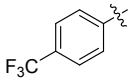
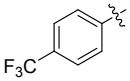
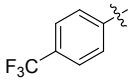
[a]Reagents and conditions: (a) NaN_3, DMSO, rt, overnight, 99%; (b) ethyl 3-bromopropiolate, toluene, reflux, overnight, 28%; (c) R^2-$B(OH)_2$, $Pd(PPh_3)_4$, toluene/EtOH/1N Na_2CO_3 = 2/1/2, 70 °C, 10 h, 32-64%; (d) allyltributylstannane or tributyl(prop-1-yn-1-yl)stannane, 10 mol% $Pd(OAc)_2$, PPh_3, DMF, 95 °C, 12 h, 60-64%; (e) Pd/C, H_2, EtOH, rt, 12 h, 100%; (f) $LiOH{\cdot}H_2O$, MeOH/THF/H_2O = 2:2:1, reflux, 2 h, 90-100%; (g) methyl-4-[(1*S*)-1-aminoethyl]benzoate, HATU, DIPEA, rt, overnight, 65-83%.

In Vitro Functional Studies. The calcium flux assay is an in vitro cell-based functional assay for rapidly screening GPCR ligands.[35, 36] To assess the antagonistic activity of these triazole analogs at the EP4 receptor, we established a calcium flux assay by co-

expressing $G\alpha_{16}$ and human EP4 receptor in CHO cells. All synthetic compounds were initially evaluated for their inhibitory activity at 10 μM in the presence of 10 nM PGE_2. Subsequently, those compounds exhibiting more than 50% inhibition were further evaluated for their IC_{50} values. Compound **4** was simultaneously tested as an active comparator and its antagonism activity (human EP4: IC_{50} = 7.5 ± 0.6 nM, mouse EP4: IC_{50} = 181.1 ± 14.6 nM) was consistent with previously reported data (human EP4: IC_{50} = 13.5 nM, mouse EP4: K_i = 371 nM).[37] Initial exploration of the structure-activity relationships (SARs) between the 1,4- and 1,5-regioisomers began with a brief examination of R^2 groups. As shown in Table 1, the antagonism activity of 1,5-regioisomer triazoles (compounds **30**, **31**, **26**, and **32 - 34**) improved as the size of R^2 substituent increased, suggesting that large steric hindrance was preferred in this position. On the other hand, the IC_{50} values of their counterparts (compounds **20**, **21**, **16**, and **22 - 24**) were more than 10 μM, indicating that the 1,5-regioisomer triazole was favorable for maintaining potency. It was noteworthy that the antagonism activity could be influenced dramatically by the substitution pattern of the benzyl group in compound **26**. Shifting the trifluoromethyl group from the para-position to meta-position (compound **27**) or replacing

the trifluoromethyl group with a fluorine atom (compound **25**) caused a 10-fold decrease in antagonistic activity at the EP4 receptor when compared to compound **26**. The insertion of an extra carbon atom between the benzyl group and the triazole core in compound **25** led to complete loss of antagonistic activity at the EP4 receptor (compound **28**) while compound **29** with one more oxygen atom maintained the activity. Since compound **34** was the best ligand so far with single-digit nanomolar potency, further optimization efforts around its R^1 substituent were then carried out. As shown in Table 2, a phenyl ring was essential to maintain the potency as tetrahydropyran **46** and cyclohexane **47** completely lost their activity against the EP4 receptor. The introduction of various substituents into the phenyl ring increased the potency of triazole analogs to some extent, especially those ligands possessing a trifluoromethyl or methoxyl group at the para-position (**34** and **39**). Although extending the distance between the phenyl ring and the triazole core (**43 - 45**) was beneficial for the potency, compound **45** was readily degraded in human/mouse liver microsome stability assays indicating that these ligands did not deserve further consideration.

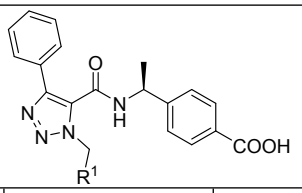
Table 1. Antagonistic Effect of Compounds **15 - 34** against the Human EP4 Receptor[a]

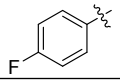
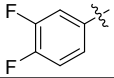
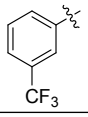
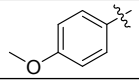
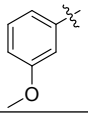
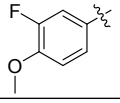
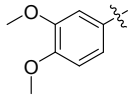
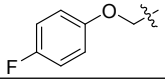
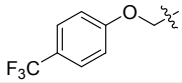
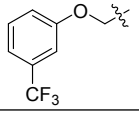
1,4-regioisomer				1,5-regioisomer			
Compd.	**R^1**	**R^2**	**IC_{50} (nM)[b]**	**Compd.**	**R^1**	**R^2**	**IC_{50} (nM)[b]**
15		CF_3	>10^4	**25**		CF_3	2315.7 ± 253.8
16		CF_3	>10^4	**26**		CF_3	224.8 ± 2.9
17		CF_3	>10^4	**27**		CF_3	1970.0 ± 163.2
18		CF_3	>10^4	**28**		CF_3	>10^4
19		CF_3	>10^4	**29**		CF_3	275.5 ± 1.3
20		H	>10^4	**30**		H	>10^4
21		Me	>10^4	**31**		Me	>10^4
22		Et	>10^4	**32**		Et	>10^4
23		n-Pr	>10^4	**33**		n-Pr	68.8 ± 3.0
24		Ph	>10^4	**34**		Ph	8.5 ± 3.0

[a]See Experimental Section. Data represent mean values ± SEM of at least three independent calcium flux experiments using CHO-human EP4-Gα_{16}. [b]The IC_{50} values represent the antagonistic activity at the human EP4 receptor. Compound **4** was used as an active comparator with IC_{50} value of 7.5 ± 0.6 nM against the human EP4 receptor.

In an attempt to further improve its EP4 potency, the R^2 substituent of compound **34** was again interrogated. Replacing the phenyl ring in compound **34** with a series of cycloalkyl groups yielded triazole analogs (**48** - **54**) that were approximately 3- to 20-fold less potent than parent compound **34**. In contrast, heterocyclic analogs (**55** - **58**) maintained the EP4 activity, especially compound **58** which was equipotent to **34**. Interestingly, incorporation of a less lipophilic allylic or propargyl group produced compounds **59** and **61** that maintained the EP4 potency relative to compound **34**, while terminal double bond analog **60** demonstrated a significant loss in potency, suggesting that a conjugated system composed of an unsaturated bond linked to the triazole ring was generally beneficial for EP4 activity. Along with above-mentioned SAR results, the R^2 substituents in the triazole core had an important impact on the synthesized compounds' activity. At last, some modifications of the (*S*)-4-(1-aminoethyl)benzoic acid group in compound **59** were found to be tolerated and resulted in analogs with similar potency compared to the parent compound, with the exception of compound **63** bearing a (*R*)-methyl group opposite to that of compound **59**.

Table 2. Antagonistic Effect of Compounds **35 - 47** against the Human EP4 Receptor[a]

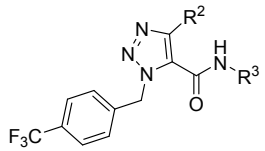


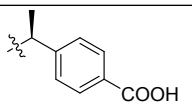
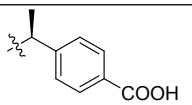
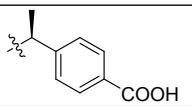
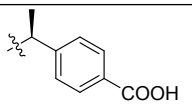
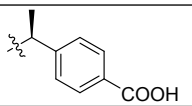
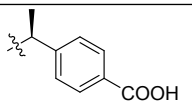
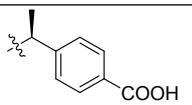
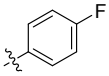
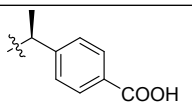
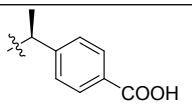
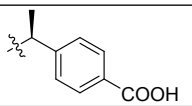
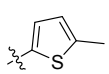
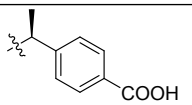
Compd.	R^1	IC_{50} (nM)[b]
35		646.6 ± 207.0
36		330.2 ± 106.2
37		147.5 ± 46.9
38		23.0 ± 7.5
39		9.3 ± 2.9
40		96.8 ± 30.7
41		29.8 ± 10.7
42		119.4 ± 40.3
43		3.1 ± 0.1
44		49.1 ± 6.4
45		1.3 ± 0.1
46		>10^4
47		>10^4

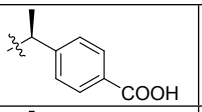
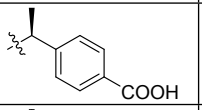
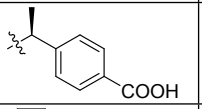
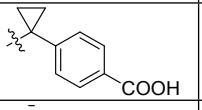
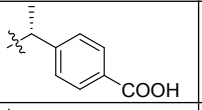
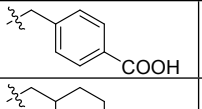
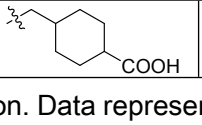
[a]See Experimental Section. Data represent mean values ± SEM of at least three independent calcium flux experiments using CHO-human EP4-Gα_{16}. [b]The IC_{50} values represent the antagonistic activity at

the human EP4 receptor. Compound **4** was used as an active comparator with IC_{50} value of 7.5 ± 0.6 nM against the human EP4 receptor.

Table 3. Antagonistic Effect of Compounds **48 - 65** against the Human EP4 Receptor[a]



Compd.	R^2	R^3	IC_{50} (nM)[b]
48			52.1 ± 4.1
49			75.3 ± 4.2
50			65.1 ± 4.7
51			161.3 ± 10.5
52			27.9 ± 2.7
53			183.0 ± 16.0
54			118.8 ± 19.9
55			57.9 ± 5.8
56			12.9 ± 1.3
57			45.6 ± 4.7
58			4.6 ± 0.1

59			6.1 ± 0.2
60			>10^3
61			32.0 ± 1.5
62			13.9 ± 1.4
63			>10^3
64			10.5 ± 0.5
65			13.9 ± 1.1

[a]See Experimental Section. Data represent mean values ± SEM of at least three independent calcium flux experiments using CHO-human EP4-Gα_{16}. [b]The IC_{50} values represent the antagonistic activity at the human EP4 receptor. Compound **4** was used as an active comparator with IC_{50} value of 7.5 ± 0.6 nM against the human EP4 receptor.

Next, selected triazole analogs with the IC_{50} value below 50 nM at the human EP4 receptor were further tested for their inhibitory potency at the mouse EP4 receptor and selectivity over human EP1-3 receptors (Table 4). As shown in Table 4, most of these compounds exhibited moderate to potent antagonistic activity at the mouse EP4 receptor. In general, compounds bearing an unsaturated alkyl chain or a heterocyclic ring in the 4-position of the 1*H*-1,2,3-triazole core showed better activity than cycloalkane and phenyl

analogs, especially compound **59** exhibiting an IC_{50} value below 20 nM. As for the selectivity, most of the tested compounds had no significant interactions with human EP1-3 receptors at concentrations up to 10 μM, with the exception of compound **61** which had very weak inhibitory activity at the human EP3 receptor.

Table 4. Antagonistic Activity and Subtype Selectivity of Selected Compounds[a]

Compd.	Human EP4 (IC_{50}, nM) [b]	Mouse EP4 (IC_{50}, nM)	Selectivity		
			Human EP1 (IC_{50}, nM)	Human EP2 (IC_{50}, nM)	Human EP3 (IC_{50}, nM)
4	7.5 ± 0.6	181.1 ±14.6	>10^4	>10^4	>10^4
34	8.5 ± 3.0	906.3 ± 23.2	>10^4	>10^4	>10^4
43	3.1 ± 0.1	132.3 ± 2.7	>10^4	>10^4	>10^4
45	1.3 ± 0.1	135.2 ± 1.8	>10^4	>10^4	>10^4
52	27.9 ± 2.7	400.1 ± 23.6	>10^4	>10^4	>10^4
56	12.9 ± 1.3	27.5 ± 1.9	>10^4	>10^4	>10^4
57	45.6 ± 4.7	57.6 ± 3.0	>10^4	>10^4	>10^4
58	4.6 ± 0.1	42.1 ± 2.9	>10^4	>10^4	>10^4
59	6.1 ± 0.2	16.2 ± 1.7	>10^4	>10^4	>10^4
61	32.0 ± 1.5	70.9 ± 6.3	>10^4	>10^4	2922.3 ± 276.4
62	13.9 ± 1.4	33.7 ± 5.0	ND[c]	ND	ND
64	10.5 ± 0.5	130.0 ± 10.9	ND	ND	ND
65	13.9 ± 1.1	219.7 ± 23.3	ND	ND	ND

[a] See Experimental Section. Data represent mean values ± SEM of at least three independent experiments determinations via calcium flux assay using CHO-EP1-4-Gα_{16}. [b] The IC_{50} values represent the antagonistic activity at EP1-4 receptor. [c] ND: not determined.

Preliminary ADME evaluation. Encouraged by the favorable biological data in vitro, we submitted selected triazole analogs for preliminary ADME tests. When incubated with human or pooled CD1 mouse liver microsomes,[38] at least 65% of tested compounds remained unchanged after 1h incubation at 10 μM, with the exception of the short half-time of compound **45** ($t_{1/2}$ < 40 min).

Table 5. Human or Mouse Liver Microsome Stability Data for Selected Compounds

Compd.	HLM			MLM		
	$t_{1/2}$[a] (min)	$CL_{int(mic)}$[b] (μL/min/mg)	Remaining (T=60 min)	$t_{1/2}$ (min)	$CL_{int(mic)}$ (μL/min/mg)	Remaining (T = 60 min)
34	>145	<9.6	84.9%	ND[c]	ND	ND
45	31.4	44.2	25.2%	38.4	36.1	28.5%
52	>145	<9.6	90.2%	ND	ND	ND
56	>145	<9.6	105.8%	>145	<9.6	75.5%
57	>145	<9.6	87.9%	ND	ND	ND
59	>145	<9.6	100.2%	>145	<9.6	82.0%
61	>145	<9.6	89.8%	ND	ND	ND
testosterone	19.4	71.3	0.0%	2.9	478.8	0.0%
diclofenac	10.6	130.6	1.9%	53.6	25.8	44.1%

propafenone	6.3	219.6	0.2%	1.2	1145.8	0.1%

[a]$t_{1/2}$ is half-life. [b]$CL_{int(mic)}$ is the intrinsic clearance; $CL_{int(mic)}$ = 0.693 /half-life /mg microsome protein per mL. [c]ND: not determined.

The inhibitory effect of compounds **45**, **56**, **59**, **61**, and **4** on in vitro cytochrome P450 (CYP) activity in human liver microsome was screened using a high-throughput multiple CYP assay for CYP1A2, CYP2B6, CYP2C9, CYP2D6, CYP2E1, and CYP3A4. At a concentration of 20 μM, tested compounds moderately inhibited CYP2C9 and CYP2E1, but had only weak (< 40%) inhibition of CYP2D6 and CYP3A4, two major CYP isomers involved in the metabolism of about 40% of marketed drugs.[39] Overall, compounds **56** and **59** displayed the best profiles in assays for CYP inhibition.

Table 6. CYP Inhibition Data for Selected Compounds (20 μM)

Compd.	CYP1A2	CYP2B6	CYP2C9	CYP2D6	CYP2E1	CYP3A4
45	30%	51%	67%	30%	67%	NA[a]
56	21%	28%	48%	25%	63%	NA
59	27%	26%	33%	23%	66%	NA
61	82%	34%	69%	38%	76%	NA
4	41%	23%	67%	35%	79%	NA

[a]NA: not active, defined as no inhibitory activity.

The pharmacokinetics (PK) profiles of selected triazole ligands were further characterized in BALB/c mice (Table 7).[40] When intravenously administered at a dose of 1 mg/kg, compounds **56** and **59** demonstrated moderate clearance (**56**: CL = 3.0 L/h/kg, **59**: CL = 1.7 L/h/kg) in mice with a corresponding favorable half-life of 3.3 h and 4.1 h, respectively. Both of them exhibited reasonable oral exposures (**56**: $AUC_{0-\infty}$ = 654.2 ng·h/mL, **59**: $AUC_{0-\infty}$ = 1243.5 ng·h/mL) and good bioavailability (**56**: F = 40.9%, **59**: F = 48.0%) when dosed orally (5 mg/kg). In general, the PK parameters of compound **59** featuring an allylic moiety was superior to that of thiophene analog **56**.

Table 7. Pharmacokinetic Parameters of Compounds **56** and **59** in BALB/c Mice

Compd.	Route	Dose (mg/kg)	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng·h/mL)	V_{ss} (L/kg)	V_z (L/kg)	CL (L/h/kg)	$t_{1/2}$ (h)	F (%)
56	iv	1	808.1	331.6	4.0	14.5	3.0	3.3	
	po	5	365.0	654.2				4.6	40.9
59	iv	1	1195.8	574.8	4.4	10.2	1.7	4.1	
	po	5	194.4	1243.5				4.7	48.0

Compound 59 inhibited EP4-mediated cAMP signaling pathway activation. Given its good potency and selectivity against the human EP4 receptor as well as its favorable ADME properties, compound **59** was selected for further profiling. A number of studies have reported that EP4 receptor stimulation can raise intracellular cAMP levels through the activation of the $G\alpha_S$ pathway.[9, 28] The Glosensor™ cAMP assay is an extremely sensitive and easy-to-use luciferase biosensor-based assay enabling facile measurements of intracellular cAMP accumulation in living cells.[41] To evaluate the effect of compound **59** on intracellular cAMP accumulation, we co-expressed EP4 and pGloSensor™-22F cAMP plasmids in HEK293 cells. As shown in Figure 3A, compound **59** dose-dependently inhibited PGE_2-stimulated cAMP accumulation in HEK293-EP4 cells, with an IC_{50} value of 18.7 ± 0.6 nM. The cAMP-response element (CRE) binding reporter assay is another commonly used method for GPCR ligand evaluation.[42, 43] We found that compound **59** dose-dependently inhibited the activity of the CRE reporter in HEK293 cells co-transfected CRE-luc plasmids, with an IC_{50} value of 5.2 ± 0.4 nM (Figure 3C). Compound **4** served as an active comparator in the Glosensor and CRE reporter assays (Figure 3B, 3D). Activation of EP4 receptor can also stimulate G protein-independent β-arrestin

signaling pathway[7, 44]. We next sought to investigate whether compound **59** was able to affect β-arrestin-dependent signaling by using a NanoBiT® β-arrestin recruitment assay.[45] The results shown that compound **59** dose-dependently inhibited PGE_2-stimulated β-arrestin recruitment in HEK293-EP4 cells, with an IC_{50} value of 0.4 ± 0.1 nM, which was about 5-fold more potent than that of compound **4** (Figure S2). Additionally, considerable evidence has recently indicated that the extracellular signal-regulated kinase (ERK) was one of the key cellular effectors activated by GPCRs.[46] To assess the effect of compound **59** on intracellular ERK activation, CHO-EP4 cells were pretreated with indicated concertation of compound **59** and then subjected to PGE_2 (30 nM) stimulation. Notably, compound **59** reversed PGE_2-induced ERK phosphorylation in a concentration-dependent manner (Figure 3E). Furthermore, it was found that increasing the concentration of compound **59** shifted the PGE_2 response curve rightwards without changing the maximal response of cAMP in Glosensor cAMP assay (Figure 3F). The pA_2 value calculated from the Schild plot analysis was 8.25 with a slope of 1.00 (Figure 3G). Compound **59** had a more than 1000-fold selectivity over EP1, EP2, and EP3 receptors

(Figure 3H), suggesting that compound **59** was a highly potent and selective competitive EP4 antagonist.

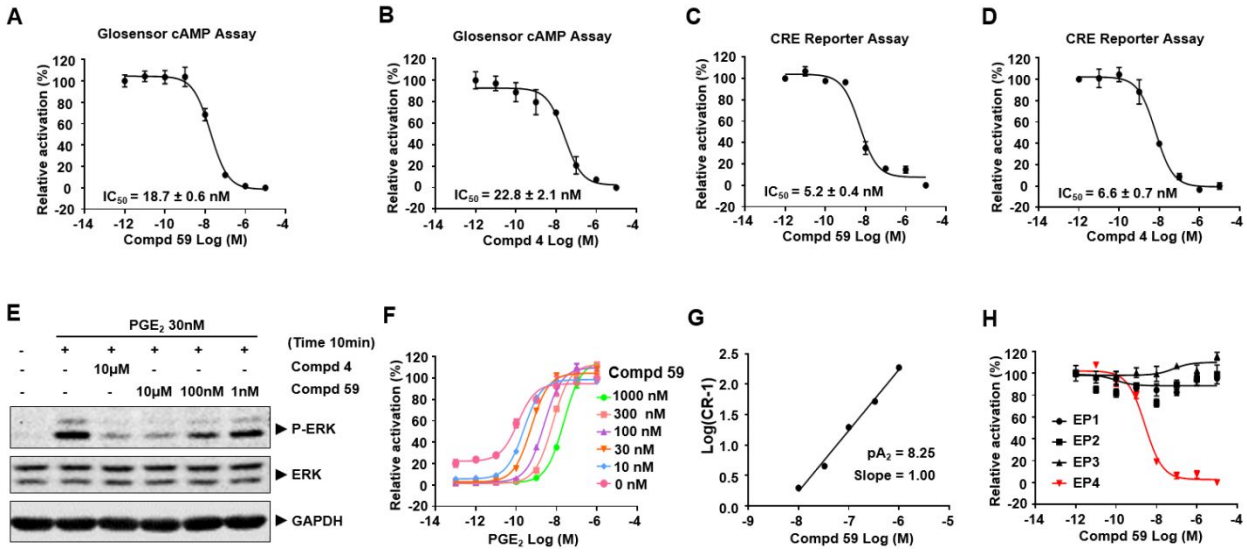


Figure 3. The antagonistic effects of compound **59** on human EP4. (A-B) Effect of compound **59** and **4** on PGE_2-induced intracellular cAMP accumulation determined by the Glosensor cAMP assay. (C-D) Dose-response curve of compound **59** and **4** in CRE reporter assay. (E) Western blot analysis of phosphorylated ERK1/2 in CHO-EP4 cells. CHO-EP4 cells were pretreated with indicated compounds for 20 min and then subjected to 30 nM PGE_2 simulation for 10 min. Compound **4** (10 μM) was used as an active comparator. (F) Schild plot analyses of compound **59** by Glosensor cAMP assay. (G) The pA_2 and slope of this Schild plot (F) were 8.25 and 1.00, respectively. (H) Dose-response curve of compound **59** against human EP1-4 receptors in the calcium flux assay. Data were normalized as

percentage of maximum response, and presented as mean ± SEM of three independent experiments. n = 3 per group.

Compound 59 reduced tumor immunosuppression-related gene expression in Raw264.7 cells. Previous studies have demonstrated that PGE_2/EP4 pathway activation could reprogram the tumor microenvironment through regulating the expression of a series of immuno-related genes, which consequently suppressed cytotoxic T cell-mediated anti-tumor immunity.[47, 48] Inflammatory factors such as *IL-1β*, *IL-4*, and *IL-6* can promote the expansion of immature immunosuppressive myeloid cells.[49, 50] Metabolic enzymes (*ARG-1*, *iNOS* and *COX2*), and cytokines (*IL-10* and *CXCL-1*) in the tumor microenvironment could block T cells-mediated tumor killing.[51-53] We then sought to determine the effects of compound **59** on the expression of tumor immunosuppression-related genes in RAW 264.7, a monocyte/macrophage-like cell line. As shown in Figure 4, stimulating RAW 264.7 cells with GM-CSF/IL-4 plus 10 nM PGE_2 significantly induced the expression of multiple immuno-related genes including *IL-1β* (65.7-fold, $P < 0.001$), *IL-4Rα*□(2.1-fold, $P < 0.01$), *IL-6* (2.5-fold, $P < 0.001$), *ARG1* (73.0-fold, $P < 0.001$), *iNOS*, *COX2* (4.3-fold,

P < 0.01), *IL-10* (2.3-fold, *P* < 0.05) and *CXCL1* (1.9-fold, *P* < 0.001). Notably, compound **59** dose-dependently inhibited the expression of these immuno-related genes. Collectively, these findings revealed that the EP4 blockade by compound **59** remarkably suppressed the expression of tumor immunosuppression-related genes.

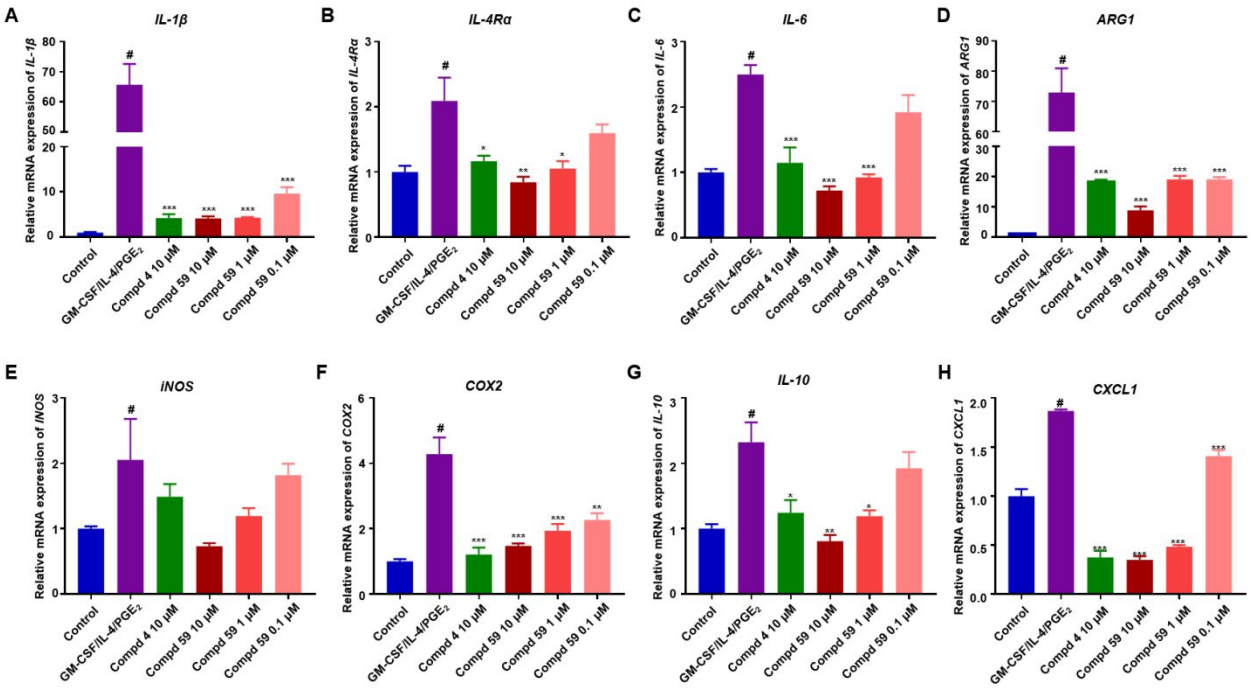


Figure 4. Compound **59** repressed the expression of immuno-related genes in Raw 264.7 cells. Raw 264.7 cells were treated with GM-CSF/ IL-4/ PGE_2 ± **4** (10 μM) or **59** (0.1 μM, 1 μM, 10 μM) for 24 h. The mRNA expression of *IL-1β* (A), *IL-4Rα* (B), *IL-6* (C), *ARG1* (D), *iNOS* (E), *COX2* (F), *IL-10* (G)

and *CXCL1* (H) were detected by Q-PCR. β-Actin was used as vehicle for normalization. Data are presented as mean ± SEM of three independent experiments. One-way ANOVA followed by Tukey *post hoc* tests were performed, $^{\#}P < 0.05$ v.s. vehicle group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ v.s. GM-CSF/IL-4/PGE_2 group. n = 3 per group.

In vivo antitumor efficacy of compound 59. Clinical and epidemiologic studies have indicated that the PGE_2/EP4 pathway plays a critical role in the development and progression of colorectal cancer,[54] and blockade of EP4 receptor was a potential therapeutic approach for colorectal cancer.[10, 11, 55] The CT26 colon cancer model was used to evaluate the in vivo anti-tumor activity of compound **59**. Mice were orally treated with compound **59** (16, 50, and 150 mg/kg), or **4** (150 mg/kg) as an active comparator, once daily for two weeks. As shown in Figure 5A-B, treatment with compound **59** caused significant inhibition of tumor growth: tumor growth inhibition (TGI) was 24.6% at 16 mg/kg, 54.7% at 50 mg/kg, and 63.8% at 150 mg/kg. No significant body weight loss was found in any mouse cohorts (Figure 5C), suggesting that compound **59** was well tolerated in mice at the tested dosage. Of note, individual tumor growth curves showed that the

medium- and high-dose groups of compound **59** (50 mg/kg and 150 mg/kg) demonstrated better antitumor efficacy than that of compound **4** (TGI = 43.2% at 150 mg/kg) (Figure 5D-G).

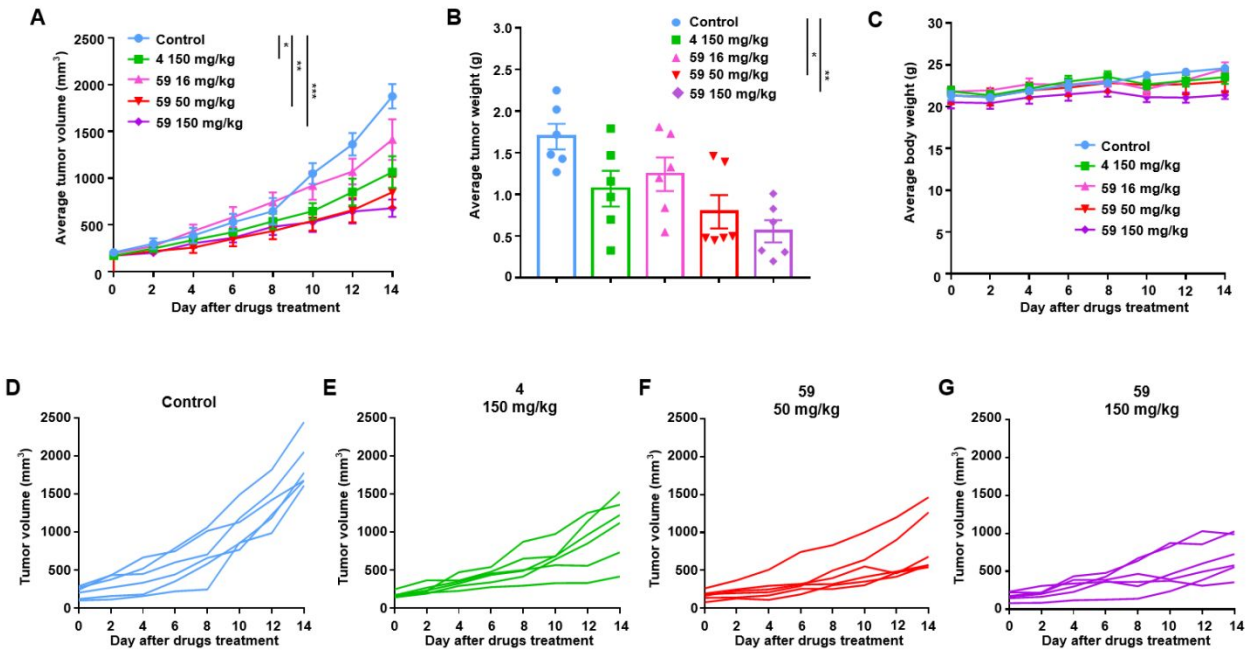


Figure 5. The anti-tumor activity of compound **59** in vivo. Mice bearing CT26 tumors were treated with vehicle, compound **4** (150 mg/kg; p.o.; daily) or compound **59** (16 mg/kg, 50 mg/kg, 150 mg/kg; p.o.; daily) for 14 days. Tumor sizes (A-B) and body weights (C) were measured every other day. Tumor weights were recorded on day 14. (D-G) Tumor growth curves of individual mice in vehicle treated with compound **4** (150 mg/kg) and compound **59** (50 and 150 mg/kg). Data were presented as mean

± SEM. One-way ANOVA followed by Tukey *post hoc* tests were performed; **P* < 0.05, ***P* < 0.01, ****P* < 0.001 v.s. vehicle group; n = 6 per group.

Compound 59 enhanced the infiltration of CD8⁺ T cells in the tumor microenvironment.

Previous studies have reported that blocking EP4 receptor can enhance an antitumor immune response.[56, 57] Next, we turned to investigate the effect of compound **59** on $CD8^+$ cytotoxic T lymphocytes in tumor tissue using flow cytometry and immunofluorescence analysis. As shown in Figure 6A-B, compound **59** (150 mg/kg) was able to enhance the accumulation of $CD45^+$ cells and $CD45^+CD8^+$ T cells when compared to vehicle-treated mice. Meanwhile, immunofluorescence analysis showed an increased infiltration of $CD8^+$ cytotoxic T cells following compound **59** treatment (Figure 6C). On the other hand, compound **59** and some selected triazole compounds had no direct cytotoxic activity against multiple cancer cells or normal HEK293 cells at concentrations up to 100 μM (Table S1). Taken together, our results indicated that the ability of compound **59** to suppress tumor growth was correlated with its effects on $CD8^+$ T cell-mediated anti-tumor immunity.

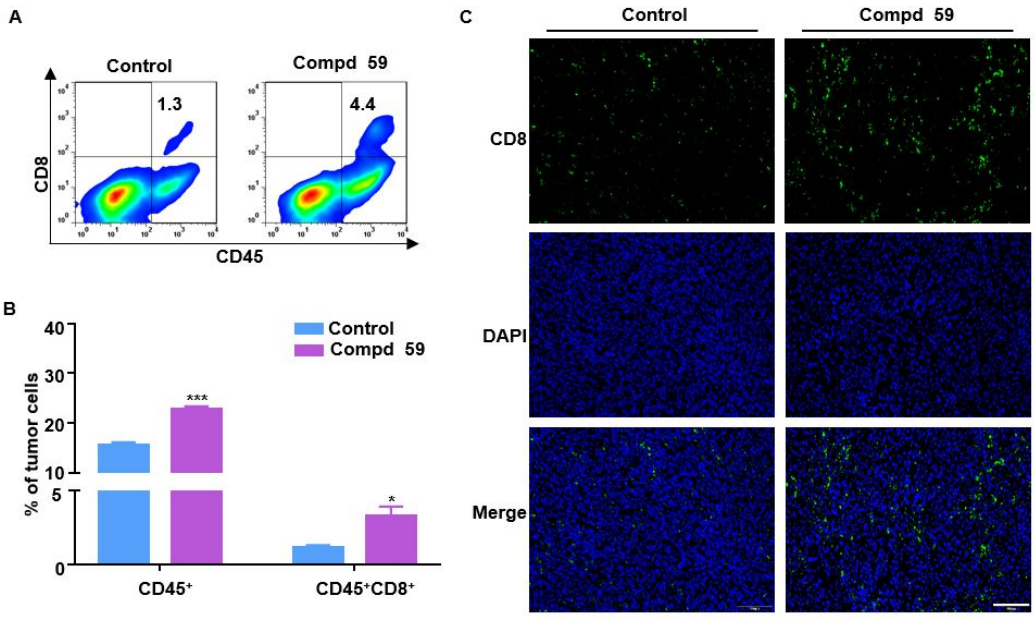


Figure 6. Compound 59 enhanced the infiltration of CD8⁺ T cells in the tumor microenvironment. Mice bearing CT26 tumors were treated with vehicle or 150 mg/kg compound **59** (p.o.; daily) for 14 days. (A, B) Mice were euthanized and single-cell suspension of tumor tissues were analyzed on day 14 post-drug treatment. $CD45^+$ cells and $CD45^+CD8^+$ T cells were analyzed by flow cytometry. Data are presented as mean ± SEM. Student's *t*-tests were performed; $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ v.s. vehicle group. n = 6 per group. (C) Tumors were examined by immunofluorescence for the presence of $CD8^+$ T cells. Scale bars = 100 μm.

CONCLUSIONS

In this work, we describe the discovery of a series of 1*H*-1,2,3-triazole ligands that represent a new class of potent and selective EP4 antagonists. Systematic SAR investigation, especially structural optimization around the triazole core, resulted in the most promising lead compound **59** that acted as a competitive EP4 antagonist with low nanomolar antagonistic activity in calcium flux, cAMP accumulation, and β-arrestin recruitment assays, together with more than 1600-fold selectivity for EP4 over other EP receptors. In addition, compound **59** was devoid of cytotoxicity versus a set of cancer or normal cells, and could effectively suppress the expression of tumor immunosuppression-related genes. On the basis of its favorable in vitro and in vivo ADME profiles, compound **59** was further evaluated in a mouse CT26 colon cancer model and shown to have better antitumor potency than compound **4**. The ability of compound **59** to inhibit tumor growth was correlated with $CD8^+$ T cell-mediated anti-tumor immunity. Taken together, these results indicated that the 1*H*-1,2,3-triazole-based EP4 antagonists reported in this article, as exemplified by compound **59**, were a potential therapeutic approach for cancer immunotherapy and might be suitable for further development in a clinical setting.

EXPERIMENTAL SECTION

Chemistry. *General*. All chemicals were purchased from Adamas-beta Ltd., J&K Scientific Ltd., Sigma-Aldrich Inc., or Aladdin-Reagents Inc., and solvents were used as received from Tansoole or Sigma-Aldrich Inc. without further purification unless otherwise stated. All reactions were executed with standard procedures under an inert atmosphere (Ar or N_2). All reaction vessels were oven-dried. The progress of each reaction was monitored by TLC on thin layer plates. ^{1}H NMR, ^{13}C NMR and HMBC spectra were generated on a Bruker 400 or 500 MHz instrument and obtained as $CDCl_3$ or DMSO-d_6 solutions. NMR chemical shifts were reported in δ (ppm) using the δ 7.26 signal of $CDCl_3$ (^{1}H NMR), δ 2.50 signal of DMSO-d_6 (^{1}H NMR), and the δ 77.23 signal of $CDCl_3$ (^{13}C NMR), δ 39.50 signal of DMSO-d_6 (^{13}C NMR) as the reference standards. Low-resolution mass spectra (LRMS) were obtained on an Agilent 1290 HPLC system (Agilent Technologies, USA) coupled with a 6460 triple-quadrupole mass spectrometer in electrospray ionization (ESI) mode. High-resolution mass spectra (HRMS) were obtained on a Bruker Micro TOF-Q II LC-MS instrument operating in ESI mode. The purity of all

final compounds (≥95%) was established by analytical HPLC, which was carried out on an Agilent 1200 HPLC system with an ACE Excel 5 C18 column (5 μm, 4.6 × 250 mm), column temperature 40 °C; with detection at 254 or 280 nm on a variable wavelength detector G1314B; flow rate = 1.0 mL/min; gradient of 10−90% MeOH in water (both containing 0.1 vol% of CF_3COOH) in 15 min. X-ray structures of isomer for compound **13** and intermediates for compounds **15**, **21** were deposited in the Cambridge Crystallographic Data Centre under deposition numbers 1938539 (**13**), 1928537 (**15**) and 1938538 (**21**). Authors will release the atomic coordinates and experimental data upon article publication.

General Procedure A for Synthesis of Azides (10). A solution of bromide **9** (1 mmol) and sodium azide (1.1 mmol) in anhydrous DMSO (3 mL) was stirred at rt overnight and then water (20 mL) was added. The mixture was extracted with Et_2O (2 × 20 mL) and the combined organic phases were washed with saturated aqueous sodium chloride, dried over anhydrous Na_2SO_4, and then concentrated under reduced pressure to afford azide **10**, which was directly used for next step without further purification.

General Procedure B for Huisgen Azide-Alkyne 1,3-Dipolar Cycloaddition. A solution of azide **10** (1 mmol) and alkynoate (1.1 mmol) in anhydrous toluene (3 mL) was stirred at 100 °C overnight. Then the reaction was cooled to rt and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel eluting with petroleum ether/ EtOAc (20:1 to 10:1, v/v) to afford 1,5-isomers **12** or **13** and eluting with petroleum ether/ EtOAc (5:1 to 2: 1, v/v) to afford 1,4-isomers **11**. The range of yield ratios for 1, 5-isomer and 1,4-isomer range from 0.4 to 1.2.

General Procedure C for Hydrolysis of Esters. A solution of ester **(**1 mmol) and lithium hydroxide monohydrate (5 mmol) in CH_3OH/H_2O/THF (2 mL/1 mL/2 mL) was stirred at 70 °C for 4 h. Then the reaction was cooled to rt and concentrated under reduced pressure. The reaction mixture was acidified to pH 4-5 with 1M aqueous HCl and extracted with EtOAc (2 × 20 mL) and the combined organic phases were washed with saturated aqueous sodium chloride, dried over anhydrous Na_2SO_4, and then concentrated under reduced pressure to afford the desired product.

General Procedure D for HATU coupling. The carboxylic acid (1 mmol), amine (1.1 mmol), HATU (1.2 mmol) and DIPEA (3 mmol) in an anhydrous DMF (3 mL) was stirred

at rt for 12 h and then water was added (10 mL). The mixture was extracted with EtOAc (2 × 20 mL) and the combined organic phases were washed with saturated aqueous sodium chloride, dried over anhydrous Na_2SO_4, and then concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel eluting with petroleum ether/EtOAc (5:1 to 2:1, v/v) to afford the desired product.

General Procedure E for Suzuki Cross-Coupling. Under an argon atmosphere, a solution of compound **13** (1 mmol), the appropriate boronic acid (1.5 mmol) and $Pd(PPh_3)_4$ (0.05 mmol) in toluene/EtOH/1N Na_2CO_3 (2 mL/1 mL/2 mL) was stirred at 70 °C for 12 h. Then, the reaction mixture was concentrated under reduced pressure and extracted with EtOAc (2 × 20 mL). The combined organic phases were washed with saturated aqueous sodium chloride, dried over anhydrous Na_2SO_4, and then concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel eluting with petroleum ether/EtOAc (20:1 to 15:1) to afford the desired product.

General Procedure F for Stille Cross-Coupling.[58] Under an argon atmosphere, a solution of compound **13** (1 mmol), palladium acetate (0.1 mmol) and triphenylphosphine (0.3 mmol) in anhydrous DMF (3 mL) was stirred at 95 °C, allyltributylstannane or tributyl(prop-

1-yn-1-yl)stannane (1.5 mmol) was added and the reaction was stirred for 10 h at 95 °C. When the solution was cooled to rt, water was added. The reaction mixture was extracted with EtOAc (2 × 20 mL) and the combined organic phases were washed with saturated aqueous sodium chloride, dried over anhydrous Na_2SO_4, and then concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel eluting with petroleum ether/EtOAc (15:1, v/v) to afford the desired product.

General Procedure G for Pd/C-catalyzed hydrogenation. Under a hydrogen atmosphere, a mixture of the appropriate ring olefin (1 mmol) and Pd/C (10 mg) in EtOH (5 mL) was stirred at rt until complete consumption of raw materials was observed by TLC. The solution was filtered on diatomaceous earth to give the filtrate, which was then concentrated under reduced pressure to furnish the desired products.

Ethyl 1-(4-fluorobenzyl)-5-(trifluoromethyl)-1*H*-1,2,3-triazole-4-carboxylate (11a). The titled compound was obtained from 4-fluorobenzyl bromide and ethyl 4,4,4-trifluorobut-2-ynoate employing general procedure A, and B. Yields 96 and 38%, respectively; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29 – 7.24 (m, 2H), 7.15 – 7.00 (m, 2H), 5.75 (s, 2H),

4.47 (q, J = 7.1 Hz, 2H), 1.43 (t, J = 7.1 Hz, 3H). LRMS (ESI): calcd for $C_{13}H_{12}F_4N_3O_2$ [M + H]$^+$, 318.1; found 318.

Ethyl 5-(trifluoromethyl)-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-4-carboxylate (11b). The titled compound was obtained from 4-(trifluoromethyl)benzyl bromide and ethyl 4,4,4-trifluorobut-2-ynoate employing general procedure A, and B. Yields 99 and 42%, respectively; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.64 (d, J = 8.0 Hz, 2H), 7.35 (d, J = 8.1 Hz, 2H), 5.84 (s, 2H), 4.50 – 4.44 (m, 2H), 1.42 (td, J = 7.1, 0.6 Hz, 3H). LRMS (ESI): calcd for $C_{14}H_{12}F_6N_3O_2$ [M + H]$^+$, 368.1; found 368.

Ethyl 5-(trifluoromethyl)-1-(3-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-4-carboxylate (11c). The titled compound was obtained from 3-(trifluoromethyl)benzyl bromide and ethyl 4,4,4-trifluorobut-2-ynoate employing general procedure A, and B. Yields 100 and 39%, respectively; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.63 (d, J = 7.8 Hz, 1H), 7.57 – 7.48 (m, 2H), 7.41 (d, J = 7.7 Hz, 1H), 5.84 (s, 2H), 4.45 (q, J = 7.1 Hz, 2H), 1.40 (t, J = 6.6 Hz, 3H). LRMS (ESI): calcd for $C_{14}H_{12}F_6N_3O_2$ [M + H]$^+$, 368.1; found 368.

Ethyl 1-(4-fluorophenethyl)-5-(trifluoromethyl)-1*H*-1,2,3-triazole-4-carboxylate (11d). The titled compound was obtained from 1-(2-bromoethyl)-4-fluorobenzene and ethyl

4,4,4-trifluorobut-2-ynoate employing general procedure A, and B. Yields 98 and 36%, respectively; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.14 – 7.08 (m, 2H), 7.00 (t, J = 8.4 Hz, 2H), 4.77 (t, J = 7.6 Hz, 2H), 4.52 – 4.41 (m, 2H), 3.24 (t, J = 7.6 Hz, 2H), 1.43 (t, J = 7.1 Hz, 3H). LRMS (ESI): calcd for $C_{14}H_{14}F_4N_3O_2$ $[M + H]^+$, 332.1; found 332.

Ethyl 1-(2-(4-fluorophenoxy)ethyl)-5-(trifluoromethyl)-1*H*-1,2,3-triazole-4-carboxylate (11e). The titled compound was obtained from 1-(2-bromoethoxy)-4-fluorobenzene and ethyl 4,4,4-trifluorobut-2-ynoate employing general procedure A, and B. Yields 95 and 33%, respectively; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.01 – 6.94 (m, 2H), 6.82 – 6.76 (m, 2H), 5.01 (t, J = 5.2 Hz, 2H), 4.49 (q, J = 7.1 Hz, 2H), 4.40 (t, J = 5.2 Hz, 2H), 1.44 (t, J = 7.1 Hz, 3H). LRMS (ESI): calcd for $C_{14}H_{14}F_4N_3O_3$ $[M + H]^+$, 348.1; found 348.

Ethyl 1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-4-carboxylate (11f). The titled compound was obtained from 4-(trifluoromethyl)benzyl bromide and ethyl propiolate employing general procedure A, and B. Yields 99 and 39%, respectively; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06 (s, 1H), 7.68 (d, J = 8.0 Hz, 2H), 7.42 (d, J = 8.0 Hz, 2H), 5.68 (s, 2H), 4.46 (q, J = 7.1 Hz, 2H), 1.40 (t, J = 6.6 Hz, 3H). LRMS (ESI): calcd for $C_{13}H_{13}F_3N_3O_2$ $[M + H]^+$, 300.1; found 300.

Ethyl 5-methyl-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-4-carboxylate (11g). The titled compound was obtained from 4-(trifluoromethyl)benzyl bromide and ethyl 2-butynoate employing general procedure A, and B. Yields 99 and 29%, respectively; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65 (d, J = 8.0 Hz, 2H), 7.31 (d, J = 8.5 Hz, 2H), 5.63 (s, 2H), 4.46 (q, J = 7.1 Hz, 2H), 2.50 (s, 3H), 1.45 (t, J = 7.1 Hz, 3H). LRMS (ESI): calcd for $C_{14}H_{15}F_3N_3O_2$ $[M + H]^+$, 314.1; found 314.

Ethyl 5-ethyl-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-4-carboxylate (11h). The titled compound was obtained from 4-(trifluoromethyl)benzyl bromide and ethyl pent-2-ynoate employing general procedure A, and B. Yields 99 and 38%, respectively; colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, J = 8.1 Hz, 2H), 7.27 (d, J = 8.1 Hz, 2H), 5.59 (s, 2H), 4.41 (q, J = 7.1 Hz, 2H), 2.89 (q, J = 7.5 Hz, 2H), 1.40 (t, J = 7.1 Hz, 3H), 1.02 (t, J = 7.5 Hz, 3H). LRMS (ESI): calcd for $C_{15}H_{17}F_3N_3O_2$ $[M + H]^+$, 328.1; found 328.

Ethyl 5-propyl-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-4-carboxylate (11i). The titled compound was obtained from 4-(trifluoromethyl)benzyl bromide and ethyl hex-2-ynoate employing general procedure A, and B. Yields 99 and 37%, respectively; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.64 (d, J = 8.2 Hz, 2H), 7.30 (d, J = 8.1 Hz, 2H), 5.62

(s, 2H), 4.45 (q, J = 7.1 Hz, 2H), 2.92 – 2.82 (m, 2H), 1.53 – 1.46 (m, 2H), 1.45 (t, J = 7.1 Hz, 3H), 0.92 (t, J = 7.4 Hz, 3H). LRMS (ESI): calcd for $C_{16}H_{19}F_3N_3O_2$ $[M + H]^+$, 342.1; found 342.

Ethyl 5-phenyl-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-4-carboxylate (11j). The titled compound was obtained from 4-(trifluoromethyl)benzyl bromide and ethyl 3-phenylpropiolate employing general procedure A, and B. Yields 99, 37%, respectively; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.56 – 7.49 (m, 3H), 7.48 – 7.42 (m, 2H), 7.22 – 7.17 (m, 2H), 7.11 (d, J = 8.1 Hz, 2H), 5.49 (s, 2H), 4.45 (q, J = 7.1 Hz, 2H), 1.41 (t, J = 6.6 Hz, 3H). LRMS (ESI): calcd for $C_{19}H_{17}F_3N_3O_2$ $[M + H]^+$, 376.1; found 376.

Ethyl 1-(4-fluorobenzyl)-4-(trifluoromethyl)-1*H*-1,2,3-triazole-5-carboxylate (12a). The titled compound was obtained from 4-fluorobenzyl bromide and ethyl 4,4,4-trifluorobut-2-ynoate employing general procedure A, and B. Yields 96 and 44%, respectively; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48 – 7.33 (m, 2H), 7.12 – 6.99 (m, 2H), 5.92 (s, 2H), 4.43 (q, J = 7.1 Hz, 2H), 1.39 (t, J = 7.1 Hz, 3H). LRMS (ESI): calcd for $C_{13}H_{12}F_4N_3O_2$ $[M + H]^+$, 318.1; found 318.

Ethyl 4-(trifluoromethyl)-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxylate (12b). The titled compound was obtained from 4-(trifluoromethyl)benzyl bromide and ethyl 4,4,4-trifluorobut-2-ynoate employing general procedure A, and B. Yields 99 and 45%, respectively; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.61 (d, J = 8.0 Hz, 2H), 7.47 (d, J = 8.0 Hz, 2H), 5.99 (s, 2H), 4.40 (q, J = 7.1 Hz, 2H), 1.35 (t, J = 7.4 Hz, 3H). LRMS (ESI): calcd for $C_{14}H_{12}F_6N_3O_2$ $[M + H]^+$, 368.1; found 368.

Ethyl 4-(trifluoromethyl)-1-(3-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxylate (12c). The titled compound was obtained from 3-(trifluoromethyl)benzyl bromide and ethyl 4,4,4-trifluorobut-2-ynoate employing general procedure A, and B. Yields 100 and 40%, respectively; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71 (d, J = 13.9 Hz, 1H), 7.63 (d, J = 7.6 Hz, 1H), 7.58 (d, J = 7.5 Hz, 1H), 7.51 (t, J = 7.7 Hz, 1H), 6.01 (s, 2H), 4.44 (qd, J = 7.1, 1.6 Hz, 2H), 1.39 (td, J = 7.1, 1.6 Hz, 3H). LRMS (ESI): calcd for $C_{14}H_{12}F_6N_3O_2$ $[M + H]^+$, 368.1; found 368.

Ethyl 1-(4-fluorophenethyl)-4-(trifluoromethyl)-1*H*-1,2,3-triazole-5-carboxylate (12d). The titled compound was obtained from 1-(2-bromoethyl)-4-fluorobenzene and ethyl 4,4,4-trifluorobut-2-ynoate employing general procedure A, and B. Yields 98 and 37%,

respectively; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.16 – 7.08 (m, 2H), 7.00 (t, J = 8.5 Hz, 2H), 5.04 – 4.93 (m, 2H), 4.40 (q, J = 7.1 Hz, 2H), 3.21 (t, J = 7.5 Hz, 2H), 1.40 (t, J = 7.1 Hz, 3H). LRMS (ESI): calcd for $C_{14}H_{14}F_4N_3O_2$ $[M + H]^+$, 332.1; found 332.

Ethyl 1-(2-(4-fluorophenoxy)ethyl)-4-(trifluoromethyl)-1H-1,2,3-triazole-5-carboxylate (12e). The titled compound was obtained from 1-(2-bromoethoxy)-4-fluorobenzene and ethyl 4,4,4-trifluorobut-2-ynoate employing general procedure A, and B. Yields 95 and 39%, respectively; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.02 – 6.93 (m, 2H), 6.82 – 6.75 (m, 2H), 5.18 (t, J = 5.4 Hz, 2H), 4.49 (q, J = 7.1 Hz, 2H), 4.40 (t, J = 5.4 Hz, 2H), 1.44 (t, J = 7.2 Hz, 3H). LRMS (ESI): calcd for $C_{14}H_{14}F_4N_3O_3$ $[M + H]^+$, 348.1; found 348.

Ethyl 1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxylate (12f). The titled compound was obtained from 4-(trifluoromethyl)benzyl bromide and ethyl propiolate employing general procedure A, and B. Yields 99 and 32%, respectively; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15 (s, 1H), 7.59 (d, J = 8.1 Hz, 2H), 7.44 (d, J = 8.1 Hz, 2H), 5.97 (s, 2H), 4.46 (q, J = 7.1 Hz, 2H), 1.38 (t, J = 6.6 Hz, 3H). LRMS (ESI): calcd for $C_{13}H_{13}F_3N_3O_2$ $[M + H]^+$, 300.1; found 300.

Ethyl 4-methyl-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxylate (12g). The titled compound was obtained from 4-(trifluoromethyl)benzyl bromide and ethyl 2-butynoate employing general procedure A, and B. Yields 99 and 40%, respectively; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.56 (d, J = 8.2 Hz, 2H), 7.39 (d, J = 8.1 Hz, 2H), 5.91 (s, 2H), 4.33 (q, J = 7.1 Hz, 2H), 2.53 (s, 3H), 1.34 (t, J = 7.1 Hz, 3H). LRMS (ESI): calcd for $C_{14}H_{15}F_3N_3O_2$ $[M + H]^+$, 314.1; found 314.

Ethyl 4-ethyl-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxylate (12h). The titled compound was obtained from 4-(trifluoromethyl)benzyl bromide and ethyl pent-2-ynoate employing general procedure A, and B. Yields 99 and 46%, respectively; colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (d, J = 8.2 Hz, 2H), 7.39 (d, J = 8.1 Hz, 2H), 5.92 (s, 2H), 4.33 (q, J = 7.1 Hz, 2H), 2.95 (q, J = 7.5 Hz, 2H), 1.32 (dt, J = 14.9, 7.3 Hz, 6H). LRMS (ESI): calcd for $C_{15}H_{17}F_3N_3O_2$ $[M + H]^+$, 328.1; found 328.

Ethyl 4-propyl-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxylate (12i). The titled compound was obtained from 4-(trifluoromethyl)benzyl bromide and ethyl hex-2-ynoate employing general procedure A, and B. Yields 99 and 44%, respectively; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 (d, J = 8.2 Hz, 2H), 7.41 (d, J = 8.1 Hz, 2H), 5.95

(s, 2H), 4.36 (q, *J* = 7.1 Hz, 2H), 2.99 – 2.89 (m, 2H), 1.81 – 1.71 (m, 2H), 1.39 – 1.36 (m, 3H), 1.00 (t, *J* = 7.4 Hz, 3H). LRMS (ESI): calcd for $C_{16}H_{19}F_3N_3O_2$ $[M + H]^+$, 342.1; found 342.

Ethyl 4-phenyl-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxylate (12j). The titled compound was obtained from 4-(trifluoromethyl)benzyl bromide and ethyl 3-phenylpropiolate employing general procedure A, and B. Yields 99, 33%, respectively; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 7.73 – 7.66 (m, 2H), 7.61 (d, *J* = 8.1 Hz, 2H), 7.50 – 7.39 (m, 5H), 5.99 (s, 2H), 4.41 (q, *J* = 7.1 Hz, 2H), 1.40 (t, *J* = 6.6 Hz, 3H). LRMS (ESI): calcd for $C_{19}H_{17}F_3N_3O_2$ $[M + H]^+$, 376.1; found 376.

Ethyl 1-benzyl-4-phenyl-1*H*-1,2,3-triazole-5-carboxylate (12k). The titled compound was obtained from (bromomethyl)benzene and ethyl 3-phenylpropiolate employing general procedure A, and B. Yields 99, 34%, respectively; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 – 7.70 (m, 2H), 7.49 – 7.40 (m, 3H), 7.40 – 7.31 (m, 5H), 5.96 (s, 2H), 4.27 (q, *J* = 7.1 Hz, 2H), 1.19 (t, *J* = 7.1 Hz, 3H). LRMS (ESI): calcd for $C_{18}H_{18}N_3O_2$ $[M + H]^+$, 308.1; found 308.

Ethyl 1-(4-fluorobenzyl)-4-phenyl-1*H*-1,2,3-triazole-5-carboxylate (12l). The titled compound was obtained from 1-(bromomethyl)-4-fluorobenzene and ethyl 3-phenylpropiolate employing general procedure A, and B. Yields 97, 32%, respectively; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 – 7.70 (m, 2H), 7.49 – 7.40 (m, 3H), 7.40 – 7.31 (m, 5H), 5.96 (s, 2H), 4.27 (q, J = 7.1 Hz, 2H), 1.19 (t, J = 7.1 Hz, 3H). LRMS (ESI): calcd for $C_{18}H_{17}FN_3O_2$ $[M + H]^+$, 326.1; found 326.

Ethyl 1-(3,4-difluorobenzyl)-4-phenyl-1*H*-1,2,3-triazole-5-carboxylate (12m). The titled compound was obtained from 4-(bromomethyl)-1,2-difluorobenzene and ethyl 3-phenylpropiolate employing general procedure A, and B. Yields 99, 33%, respectively; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74 – 7.68 (m, 2H), 7.47 – 7.42 (m, 3H), 7.27 – 7.21 (m, 1H), 7.19 – 7.12 (m, 2H), 5.91 (s, 2H), 4.30 (q, J = 7.1 Hz, 2H), 1.22 (t, J = 7.1 Hz, 3H). LRMS (ESI): calcd for $C_{18}H_{16}F_2N_3O_2$ $[M + H]^+$, 344.1; found 344.

Ethyl 4-phenyl-1-(3-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxylate (12n). The titled compound was obtained from 1-(bromomethyl)-3-(trifluoromethyl)benzene and ethyl 3-phenylpropiolate employing general procedure A, and B. Yields 92, 30%, respectively; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 – 7.70 (m, 2H), 7.68 (s, 1H), 7.63 – 7.57

(m, 1H), 7.58 – 7.53 (m, 1H), 7.53 – 7.41 (m, 4H), 6.00 (s, 2H), 4.28 (q, J = 7.1 Hz, 2H), 1.20 (t, J = 7.1 Hz, 3H). LRMS (ESI): calcd for $C_{19}H_{17}F_3N_3O_2$ $[M + H]^+$, 376.1; found 376.

Ethyl 1-(4-methoxybenzyl)-4-phenyl-1*H*-1,2,3-triazole-5-carboxylate (12o). The titled compound was obtained from 1-(bromomethyl)-4-methoxybenzene and ethyl 3-phenylpropiolate employing general procedure A, and B. Yields 97, 35%, respectively; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 – 7.69 (m, 2H), 7.50 – 7.40 (m, 3H), 7.33 (d, J = 8.4 Hz, 2H), 6.88 (d, J = 8.5 Hz, 2H), 5.88 (s, 2H), 4.28 (q, J = 7.1 Hz, 2H), 3.79 (s, 3H), 1.21 (t, J = 7.1 Hz, 3H). LRMS (ESI): calcd for $C_{19}H_{20}N_3O_3$ $[M + H]^+$, 338.1; found 338.

Ethyl 1-(3-methoxybenzyl)-4-phenyl-1*H*-1,2,3-triazole-5-carboxylate (12p). The titled compound was obtained from 1-(bromomethyl)-3-methoxybenzene and ethyl 3-phenylpropiolate employing general procedure A, and B. Yields 97, 34%, respectively; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 – 7.71 (m, 2H), 7.48 – 7.41 (m, 3H), 7.31 – 7.23 (m, 1H), 6.93 (d, J = 7.6 Hz, 1H), 6.90 – 6.84 (m, 2H), 5.93 (s, 2H), 4.28 (q, J = 7.1 Hz, 2H), 3.79 (s, 3H), 1.20 (t, J = 7.1 Hz, 3H). LRMS (ESI): calcd for $C_{19}H_{20}N_3O_3$ $[M + H]^+$, 338.1; found 338.

Ethyl 1-(3-fluoro-4-methoxybenzyl)-4-phenyl-1*H*-1,2,3-triazole-5-carboxylate (12q). The titled compound was obtained from 4-(bromomethyl)-2-fluoro-1-methoxybenzene and ethyl 3-phenylpropiolate employing general procedure A, and B. Yields 96, 31%, respectively; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 – 7.68 (m, 2H), 7.47 – 7.40 (m, 3H), 7.21 – 7.10 (m, 2H), 6.98 – 6.90 (m, 1H), 5.88 (s, 2H), 4.30 (q, J = 7.1 Hz, 2H), 3.89 (s, 3H), 1.22 (t, J = 7.1 Hz, 3H). LRMS (ESI): calcd for $C_{19}H_{19}FN_3O_3$ $[M + H]^+$, 356.1; found 356.

Ethyl 1-(3,4-dimethoxybenzyl)-4-phenyl-1*H*-1,2,3-triazole-5-carboxylate (12r). The titled compound was obtained from 4-(bromomethyl)-1,2-dimethoxybenzene and ethyl 3-phenylpropiolate employing general procedure A, and B. Yields 98, 34%, respectively; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 – 7.68 (m, 2H), 7.44 (d, J = 4.8 Hz, 3H), 6.97 (d, J = 8.7 Hz, 2H), 6.84 (d, J = 8.0 Hz, 1H), 5.89 (s, 2H), 4.29 (q, J = 7.1 Hz, 2H), 3.88 (d, J = 3.3 Hz, 6H), 1.22 (t, J = 7.1 Hz, 3H). LRMS (ESI): calcd for $C_{20}H_{22}N_3O_4$ $[M + H]^+$, 368.2; found 368.

Ethyl 1-(2-(4-fluorophenoxy)ethyl)-4-phenyl-1*H*-1,2,3-triazole-5-carboxylate (12s). The titled compound was obtained from 1-(2-bromoethoxy)-4-fluorobenzene and ethyl 3-

phenylpropiolate employing general procedure A, and B. Yields 98, 34%, respectively; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 – 7.71 (m, 2H), 7.51 – 7.39 (m, 3H), 7.02 – 6.91 (m, 2H), 6.87 – 6.76 (m, 2H), 5.22 – 5.12 (m, 2H), 4.45 – 4.39 (m, 2H), 4.39 – 4.33 (m, 2H), 1.28 – 1.26 (m, 3H). LRMS (ESI): calcd for $C_{19}H_{19}FN_3O_3$ $[M + H]^+$, 356.1; found 356.

Ethyl 4-phenyl-1-(2-(4-(trifluoromethyl)phenoxy)ethyl)-1*H*-1,2,3-triazole-5-carboxylate (12t). The titled compound was obtained from 1-(2-bromoethoxy)-4-(trifluoromethyl)benzene[59] and ethyl 3-phenylpropiolate employing general procedure A, and B. Yields 95, 37%, respectively; white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.59 – 7.50 (m, 5H), 7.46 (d, J = 7.6 Hz, 2H), 6.83 (d, J = 8.3 Hz, 2H), 4.66 (t, J = 5.2 Hz, 2H), 4.47 (t, J = 5.1 Hz, 2H), 4.33 (q, J = 7.1 Hz, 2H), 1.29 (t, J = 7.1 Hz, 3H). LRMS (ESI): calcd for $C_{20}H_{19}F_3N_3O_3$ $[M + H]^+$, 406.1; found 406.

Ethyl 4-phenyl-1-(2-(3-(trifluoromethyl)phenoxy)ethyl)-1*H*-1,2,3-triazole-5-carboxylate (12u). The titled compound was obtained from 1-(2-bromoethoxy)-3-(trifluoromethyl)benzene[59] and ethyl 3-phenylpropiolate employing general procedure A, and B. Yields 98, 35%, respectively; white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 – 7.70

(m, 2H), 7.49 – 7.43 (m, 3H), 7.43 – 7.36 (m, 1H), 7.24 (d, *J* = 7.7 Hz, 1H), 7.11 (s, 1H), 7.05 (d, *J* = 8.2 Hz, 1H), 5.24 – 5.16 (m, 2H), 4.56 – 4.48 (m, 2H), 4.44 – 4.34 (m, 2H), 1.31 – 1.28 (m, 3H). LRMS (ESI): calcd for $C_{20}H_{19}F_3N_3O_3$ $[M + H]^+$, 406.1; found 406.

Ethyl 4-phenyl-1-((tetrahydro-2*H*-pyran-4-yl)methyl)-1*H*-1,2,3-triazole-5-carboxylate (12v). The titled compound was obtained from 4-bromomethyltetrahydropyran and ethyl 3-phenylpropiolate employing general procedure A, and B. Yields 90, 39%, respectively; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74 – 7.68 (m, 2H), 7.49 – 7.40 (m, 3H), 4.69 – 4.51 (m, 2H), 4.32 (q, *J* = 7.1 Hz, 2H), 3.81 – 3.74 (m, 2H), 3.13 – 3.04 (m, 2H), 1.93 – 1.84 (m, 1H), 1,32 – 1.13 (m, 7H). LRMS (ESI): calcd for $C_{17}H_{22}N_3O_3$ $[M + H]^+$, 316.2; found 316.

Ethyl 1-(cyclohexylmethyl)-4-phenyl-1*H*-1,2,3-triazole-5-carboxylate (12w). The titled compound was obtained from (bromomethyl)cyclohexane and ethyl 3-phenylpropiolate employing general procedure A, and B. Yields 90, 20%, respectively; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 – 7.68 (m, 2H), 7.51 – 7.40 (m, 3H), 4.60 (d, *J* = 7.3 Hz, 2H), 4.35 (q, *J* = 7.1 Hz, 2H), 2.02 – 1.91 (m, 1H), 1.82 – 1.72 (m, 2H), 1.73 – 1.61 (m,

3H), 1.30 – 1.26 (m, 4H), 1.25 – 1.21 (m, 2H), 1.15 – 1.06 (m, 2H). LRMS (ESI): calcd for $C_{18}H_{24}N_3O_2$ $[M + H]^+$, 314.2; found 314.

Ethyl 4-cyclopropyl-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxylate (12x). The titled compound was obtained from 4-(trifluoromethyl)benzyl bromide and ethyl 3-cyclopropylpropiolate[60] employing general procedure A, and B. Yields 99, 38%, respectively; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 (d, J = 8.1 Hz, 2H), 7.40 (d, J = 8.1 Hz, 2H), 5.93 (s, 2H), 4.38 (q, J = 7.1 Hz, 2H), 2.47 – 2.38 (m, 1H), 1.37 (t, J = 7.0 Hz, 3H), 1.22 – 1.18 (m, 2H), 1.09 – 1.03 (m, 2H). LRMS (ESI): calcd for $C_{16}H_{17}F_3N_3O_2$ $[M + H]^+$, 340.1; found 340.

Ethyl 4-cyclobutyl-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxylate (12y). The titled compound was obtained from 4-(trifluoromethyl)benzyl bromide and ethyl 3-cyclobutylpropiolate[60] employing general procedure A, and B. Yields 99, 36%, respectively; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 (d, J = 8.0 Hz, 2H), 7.42 (d, J = 8.0 Hz, 2H), 5.94 (s, 2H), 4.35 (q, J = 7.1 Hz, 2H), 3.95 (p, J = 8.7 Hz, 1H), 2.58 – 2.46 (m, 2H), 2.36 (q, J = 8.8 Hz, 2H), 2.14 – 1.94 (m, 2H), 1.39 – 1.36 (m, 3H). LRMS (ESI): calcd for $C_{17}H_{19}F_3N_3O_2$ $[M + H]^+$, 354.1; found 354.

Ethyl 4-bromo-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxylate (13). Step 1: A solution of ethyl propiolate (9.8 g, 100 mmol), N-bromosuccinimide (19.58 g, 110 mmol) and $AgNO_3$ (1.7 g, 10 mmol) in acetone (200 mL) was stirred at rt for 10 h. The reaction mixture was filtered to get filtrate, which was then concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel eluting with petroleum ether to afford ethyl 3-bromopropiolate with a 95% yield, colorless oil.

Step 2: Ethyl 4-bromo-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxylate was obtained from ethyl 3-bromopropiolate and 1-(azidomethyl)-4-(trifluoromethyl)benzene employing general procedure B. Yield 28%; light yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.62 (d, J = 8.2 Hz, 2H), 7.44 (d, J = 9.0 Hz, 2H), 5.98 (s, 2H), 4.40 (q, J = 7.1 Hz, 2H), 1.40 (t, J = 7.1 Hz, 3H). LRMS (ESI): calcd for $C_{13}H_{12}BrF_3N_3O_2$ $[M + H]^+$, 378.0; found 378.

Ethyl 4-cyclopentyl-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxylate (14a). The titled compound was obtained from **14c** employing general procedure G. Yield 100%; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.59 (d, J = 7.9 Hz, 2H), 7.41 (d, J = 7.9 Hz, 2H), 5.93 (s, 2H), 4.35 (q, J = 7.1 Hz, 2H), 3.61 – 3.50 (m, 1H), 2.06 – 2.00 (m, 2H), 1.99

– 1.83 (m, 4H), 1.74 – 1.64 (m, 2H), 1.35 (t, J = 7.1 Hz, 3H). LRMS (ESI): calcd for $C_{17}H_{19}F_3N_3O_2$ $[M + H]^+$, 354.1; found 354. LRMS (ESI): calcd for $C_{18}H_{21}F_3N_3O_2$ $[M + H]^+$, 368.2; found 368.

Ethyl 4-cyclohexyl-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxylate (14b). The titled compound was obtained from **14d** employing general procedure G. Yield 100%; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 (d, J = 8.0 Hz, 2H), 7.40 (d, J = 8.0 Hz, 2H), 5.94 (s, 2H), 4.35 (q, J = 7.1 Hz, 2H), 3.15 (t, J = 11.7 Hz, 1H), 1.96 – 1.85 (m, 4H), 1.82 – 1.71 (m, 3H), 1.42 – 1.35 (m, 6H). LRMS (ESI): calcd for $C_{18}H_{21}F_3N_3O_2$ $[M + H]^+$, 382.2; found 382.

Ethyl 4-(cyclopent-1-en-1-yl)-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxylate (14c). The titled compound was obtained from **13** and cyclopent-1-en-1-ylboronic acid employing general procedure E. Yield 32%; white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 (d, J = 8.2 Hz, 2H), 7.40 (d, J = 8.1 Hz, 2H), 6.58 – 6.47 (m, 1H), 5.92 (s, 2H), 4.32 (q, J = 7.1 Hz, 2H), 2.95 – 2.78 (m, 2H), 2.65 – 2.49 (m, 2H), 2.07 – 1.95 (m, 2H), 1.30 (t, J = 7.1 Hz, 3H). LRMS (ESI): calcd for $C_{18}H_{19}F_3N_3O_2$ $[M + H]^+$, 366.1; found 366.

Ethyl 4-(cyclohex-1-en-1-yl)-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxylate (14d). The titled compound was obtained from **13** and cyclohex-1-en-1-ylboronic acid employing general procedure E. Yield 35%; white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 (d, J = 8.2 Hz, 2H), 7.40 (d, J = 8.1 Hz, 2H), 6.19 – 6.12 (m, 1H), 5.92 (s, 2H), 4.30 (q, J = 7.1 Hz, 2H), 2.46 – 2.38 (m, 2H), 2.26 – 2.19 (m, 2H), 1.82 – 1.75 (m, 2H), 1.74 – 1.66 (m, 2H), 1.32 – 1.29 (m, 3H). LRMS (ESI): calcd for $C_{19}H_{21}F_3N_3O_2$ [M + H]$^+$, 380.2; found 380.

Ethyl 4-(3,6-dihydro-2*H*-pyran-4-yl)-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxylate (14e). The titled compound was obtained from **13** and (3,6-dihydro-2*H*-pyran-4-yl)boronic acid employing general procedure E. Yield 52%; colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.61 (d, J = 8.1 Hz, 2H), 7.40 (d, J = 8.0 Hz, 2H), 6.32 (s, 1H), 5.93 (s, 2H), 4.38 – 4.30 (m, 4H), 3.93 (t, J = 5.4 Hz, 2H), 2.66 – 2.57 (m, 2H), 1.31 – 1.30 (m, 3H). LRMS (ESI): calcd for $C_{18}H_{19}F_3N_3O_3$ [M + H]$^+$, 382.1; found 382.

Ethyl 4-(4-fluorophenyl)-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxylate (14f). The titled compound was obtained from **13** and (4-fluorophenyl)boronic acid employing general procedure E. Yield 56%; light yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ

7.80 – 7.70 (m, 2H), 7.63 (d, *J* = 8.1 Hz, 2H), 7.47 (d, *J* = 8.0 Hz, 2H), 7.14 (t, *J* = 8.6 Hz, 2H), 6.01 (s, 2H), 4.28 (q, *J* = 7.1 Hz, 2H), 1.21 (t, *J* = 7.1 Hz, 3H). LRMS (ESI): calcd for $C_{19}H_{16}F_4N_3O_2$ $[M + H]^+$, 394.1; found 394.

Ethyl 4-(thiophen-3-yl)-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxylate (14g). The titled compound was obtained from **13** and thiophen-3-ylboronic acid employing general procedure E. Yield 64%; light yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86 (d, *J* = 3.6 Hz, 1H), 7.63 (d, *J* = 8.0 Hz, 2H), 7.47 (d, *J* = 5.1 Hz, 1H), 7.43 (d, *J* = 7.9 Hz, 2H), 7.14 (t, *J* = 4.4 Hz, 1H), 6.01 (s, 2H), 4.41 (q, *J* = 7.1 Hz, 2H), 1.37 – 1.35 (m, 3H). LRMS (ESI): calcd for $C_{17}H_{15}F_3N_3O_2S$ $[M + H]^+$, 382.1; found 382.

Ethyl 4-(furan-3-yl)-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxylate (14h). The titled compound was obtained from **13** and furan-3-ylboronic acid employing general procedure E. Yield 39%; light yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 (d, *J* = 3.6 Hz, 1H), 7.80 – 7.22 (m, 6H), 7.19 – 7.11 (d, *J* = 5.1 Hz, 1H), 6.01 (s, 2H), 4.41 (q, *J* = 7.1 Hz, 2H), 1.37 – 1.35 (m, 3H). LRMS (ESI): calcd for $C_{17}H_{15}F_3N_3O_3$ $[M + H]^+$, 366.1; found 366.

Ethyl 4-(5-methylthiophen-2-yl)-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxylate (14i). The titled compound was obtained from **13** and (5-methylthiophen-2-

yl)boronic acid employing general procedure E. Yield 58%; light yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.63 (d, J = 7.7 Hz, 2H), 7.47 (d, J = 4.6 Hz, 1H), 7.43 (d, J = 7.8 Hz, 2H), 7.15 (d, J = 5.5 Hz, 1H), 6.01 (s, 2H), 4.41 (dt, J = 7.1, 5.2 Hz, 2H), 2.45 (s, 3H), 1.36 – 1.33 (m, 3H). LRMS (ESI): calcd for $C_{18}H_{17}F_3N_3O_2S$ $[M + H]^+$, 396.1; found 396.

Ethyl (*E*)-4-(prop-1-en-1-yl)-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxylate (14j). The titled compound was obtained from **13** and (*E*)-prop-1-en-1-ylboronic acid employing general procedure E. Yield 60%; white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 (d, J = 8.1 Hz, 2H), 7.39 (d, J = 8.2 Hz, 2H), 7.05 – 6.97 (m, 1H), 6.74 (d, J = 15.8 Hz, 1H), 5.95 (s, 2H), 4.38 (q, J = 7.2 Hz, 2H), 1.97 (d, J = 6.0 Hz, 3H), 1.38 (t, J = 6.9 Hz, 3H). LRMS (ESI): calcd for $C_{16}H_{17}F_3N_3O_2$ $[M + H]^+$, 340.1; found 340.

Ethyl 4-allyl-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxylate (14k). The titled compound was obtained from **13** and allyltributylstannane employing general procedure F. Yield 60%; white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 (d, J = 8.2 Hz, 2H), 7.42 (d, J = 8.1 Hz, 2H), 6.08 – 5.99 (m, 1H), 5.96 (s, 2H), 5.17 – 5.11 (m, 2H), 4.36 (q, J = 7.1 Hz, 2H), 3.74 (d, J = 6.5 Hz, 2H), 1.38 – 1.36 (m, 3H). LRMS (ESI): calcd for $C_{16}H_{17}F_3N_3O_2$ $[M + H]^+$, 340.1; found 340.

Ethyl 4-(prop-1-yn-1-yl)-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxylate (14l). The titled compound was obtained from **13** and tributyl(prop-1-yn-1-yl)silane employing general procedure F. Yield 64%; white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74 (d, J = 8.2 Hz, 2H), 7.38 (d, J = 8.1 Hz, 2H), 5.98 (s, 2H), 4.35 (q, J = 7.1 Hz, 2H), 2.10 (d, J = 11.4 Hz, 3H), 1.35 (t, J = 6.9 Hz, 3H). LRMS (ESI): calcd for $C_{16}H_{15}F_3N_3O_2$ [M + H]$^+$, 338.1; found 338.

(*S*)-4-(1-(1-(4-Fluorobenzyl)-5-(trifluoromethyl)-1*H*-1,2,3-triazole-4-carboxamido)ethyl)benzoic acid (15). The titled compound was obtained from **11a** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 100, 72 and 95%, respectively; white solid. HPLC purity = 98.7%; t_R = 11.3 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.87 (s, 1H), 9.43 (d, J = 7.9 Hz, 1H), 7.91 (d, J = 6.8 Hz, 2H), 7.51 (d, J = 7.0 Hz, 2H), 7.36 – 7.14 (m, 4H), 5.88 (s, 2H), 5.22 – 5.14 (m, 1H), 1.49 (d, J = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.6, 163.4, 161.5, 158.0, 149.6, 143.4, 131.2 (d, J = 3.0 Hz), 130.1 (d, J = 8.5 Hz), 129.9 (2C), 129.9, 126.8 (2C), 126.0 (q, J = 42.0 Hz), 119.6 (q, J = 269.9 Hz), 116.3, 116.2, 53.9, 48.8, 22.1. HRMS (ESI): calcd for $C_{20}H_{16}F_4N_4NaO_3$ [M + Na]$^+$, 459.1051; found 459.1054.

(*S*)-4-(1-(5-(Trifluoromethyl)-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-4-carboxamido)ethyl)benzoic acid (16). The titled compound was obtained from **11b** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 98, 78 and 95%, respectively; white solid. HPLC purity = 97.6%; t_R = 11.9 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.87 (s, 1H), 9.45 (d, J = 8.0 Hz, 1H), 7.92 (d, J = 7.9 Hz, 2H), 7.78 (d, J = 8.0 Hz, 2H), 7.52 (d, J = 8.0 Hz, 2H), 7.41 (d, J = 7.9 Hz, 2H), 6.03 (s, 2H), 5.26 – 5.14 (m, 1H), 1.50 (d, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.5, 155.9, 148.3, 139.1, 134.7 (q, J = 38.5 Hz), 133.4 (d, J = 2.1 Hz), 130.1, 129.9 (2C), 129.4 (d, J = 31.9 Hz), 129.2 (2C), 126.7 (2C), 126.1 (q, J = 3.7 Hz, 2C), 124.4 (q, J = 272.2 Hz), 120.6 (q, J = 272.2 Hz), 52.5, 49.6, 21.9. HRMS (ESI): calcd for $C_{21}H_{16}F_6N_4NaO_3$ [M + Na]$^+$, 509.1019; found 509.1048.

(*S*)-4-(1-(5-(Trifluoromethyl)-1-(3-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-4-carboxamido)ethyl)benzoic acid (17). The titled compound was obtained from **11c** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 99, 73 and 97%, respectively; white solid. HPLC purity = 96.0%; t_R = 11.8 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.01 (s, 1H), 9.43 (d, J = 8.0 Hz, 1H), 7.91 (d, J = 8.2 Hz, 2H),

7.75 (d, J = 7.6 Hz, 1H), 7.68 (s, 1H), 7.67 – 7.59 (m, 1H), 7.50 (d, J = 8.2 Hz, 2H), 7.46 (d, J = 7.6 Hz, 1H), 6.02 (s, 2H), 5.28 – 5.11 (m, 1H), 1.49 (d, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.5, 156.0, 148.2, 136.0, 134.5 (q, J = 38.5 Hz), 133.5 (q, J = 2.1 Hz), 132.7, 130.5, 130.2, 130.0 (2C), 129.9 (q, J = 31.8 Hz), 126.6 (2C), 125.8 (q, J = 3.8 Hz), 125.3 (q, J = 3.8 Hz), 124.4 (q, J = 273.4 Hz), 120.7 (q, J = 269.4 Hz), 52.4, 49.6, 22.0. HRMS (ESI): calcd for $C_{21}H_{16}F_6N_4NaO_3$ $[M + Na]^+$, 509.1019; found 509.1012.

(*S*)-4-(1-(1-(4-Fluorophenethyl)-5-(trifluoromethyl)-1*H*-1,2,3-triazole-4-carboxamido)ethyl)benzoic acid (18). The titled compound was obtained from **11d** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 95, 72 and 96%, respectively; white solid. HPLC purity = 97.7%; t_R = 11.6 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.80 (s, 1H), 9.37 (d, J = 8.0 Hz, 1H), 7.91 (d, J = 7.2 Hz, 2H), 7.50 (d, J = 7.6 Hz, 2H), 7.23 – 7.17 (m, 2H), 7.14 – 7.07 (m, 2H), 5.23 – 5.13 (m, 1H), 4.83 (t, J = 7.0 Hz, 2H), 3.20 (t, J = 7.0 Hz, 2H), 1.49 (d, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.6, 161.7(d, J = 242.5 Hz), 158.0, 149.7, 142.8, 133.3 (d, J = 2.5 Hz), 131.1 (d, J = 8.1 Hz, 2C), 129.9 (2C), 129.8, 126.8 (2C), 126.3 (q, J = 42.8 Hz), 119.7

(q, J = 270.9 Hz), 115.8, 115.6, 52.6, 48.8, 35.0, 22.1. HRMS (ESI): calcd for $C_{21}H_{18}F_4N_4NaO_3$ $[M + Na]^+$, 473.1207; found 473.1241.

(*S*)-4-(1-(1-(2-(4-Fluorophenoxy)ethyl)-5-(trifluoromethyl)-1*H*-1,2,3-triazole-4-carboxamido)ethyl)benzoic acid (19). The titled compound was obtained from **11e** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 97, 76 and 96%, respectively; white solid. HPLC purity = 98.9%; t_R = 11.5 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.86 (s, 1H), 9.41 (d, J = 8.1 Hz, 1H), 7.91 (d, J = 8.1 Hz, 2H), 7.51 (d, J = 8.1 Hz, 2H), 7.16 – 7.05 (m, 2H), 6.92 – 6.86 (m, 2H), 5.24 – 5.14 (m, 1H), 5.00 (t, J = 4.7 Hz, 2H), 4.42 (t, J = 4.8 Hz, 2H), 1.49 (d, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.6, 158.0, 157.3 (d, J = 236.5 Hz), 154.4 (d, J = 1.8 Hz), 149.7, 142.8, 129.9 (2C), 129.8, 127.1 (q, J = 42.1 Hz), 126.7 (2C), 119.8 (q, J = 269.64 Hz), 116.5, 116.3, 116.2, 116.1, 66.8, 51.0, 48.8, 22.2. HRMS (ESI): calcd for $C_{21}H_{18}F_4N_4NaO_4$ $[M + Na]^+$, 489.1156; found 489.1169.

(*S*)-4-(1-(1-(4-(Trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-4-carboxamido)ethyl)benzoic acid (20). The titled compound was obtained from **11f** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 97, 72 and 99%,

respectively; white solid. HPLC purity = 97.1%; t_R = 11.7 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.79 (s, 1H), 9.05 (d, J = 8.3 Hz, 1H), 8.73 (s, 1H), 7.89 (d, J = 8.3 Hz, 2H), 7.76 (d, J = 8.2 Hz, 2H), 7.60 – 7.41 (m, 4H), 5.79 (s, 2H), 5.26 – 5.17 (m, 1H), 1.50 (d, J = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.6, 159.4, 150.1, 143.4, 140.8, 129.8 (2C), 129.7, 129.2 (3C), 127.6, 126.8 (2C), 126.2 (q, J = 3.8 Hz, 2C), 124.3 (q, J = 327.9 Hz), 52.8, 48.3, 22.2. HRMS (ESI): calcd for $C_{20}H_{17}F_3N_4NaO_3$ $[M + Na]^+$, 441.1145; found 441.1147.

(*S*)-4-(1-(5-Methyl-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-4-carboxamido)ethyl)benzoic acid (21). The titled compound was obtained from **11g** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 98, 76 and 95%, respectively; white solid. HPLC purity = 95.0%; t_R = 11.8 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.82 (s, 1H), 8.97 (d, J = 8.3 Hz, 1H), 7.90 (d, J = 8.0 Hz, 2H), 7.75 (d, J = 8.1 Hz, 2H), 7.53 (d, J = 8.1 Hz, 2H), 7.39 (d, J = 8.0 Hz, 2H), 5.77 (s, 2H), 5.23 – 5.17 (m, 1H), 2.43 (s, 3H), 1.51 (d, J = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.7, 160.6, 150.3, 140.5, 138.7, 136.9, 129.8 (2C), 129.7, 129.1, 128.5 (2C), 126.8

(2C), 126.3 (q, J = 3.8 Hz, 2C), 124.5 (q, J = 272.5 Hz), 50.4, 48.1, 22.2, 8.8. HRMS (ESI): calcd for $C_{21}H_{19}F_3N_4NaO_3$ $[M + Na]^+$, 455.1301; found 455.1307.

(*S*)-4-(1-(5-Ethyl-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-4-carboxamido)ethyl)benzoic acid (22). The titled compound was obtained from **11h** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 94, 78 and 96%, respectively; white solid. The structure of the intermediate ester **11h** was characterized by HMBC spectra shown in supporting information. HPLC purity = 98.2%; t_R = 12.4 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.77 (s, 1H), 8.98 (d, J = 8.3 Hz, 1H), 7.89 (d, J = 7.9 Hz, 2H), 7.75 (d, J = 8.1 Hz, 2H), 7.52 (d, J = 8.0 Hz, 2H), 7.40 (d, J = 8.0 Hz, 2H), 5.79 (s, 2H), 5.23 – 5.17 (m, 1H), 2.94 – 2.85 (m, 2H), 1.50 (d, J = 7.0 Hz, 3H), 0.89 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.6, 160.3, 150.3, 142.1, 141.0, 138.4, 129.8 (2C), 129.7, 129.0 (q, J = 32.1 Hz), 128.4 (2C), 126.8 (2C), 126.2 (q, J = 3.7 Hz, 2C), 124.5 (q, J = 272.1 Hz), 50.4, 48.1, 22.2, 16.2, 13.5. HRMS (ESI): calcd for $C_{22}H_{21}F_3N_4NaO_3$ $[M + Na]^+$, 469.1458; found 469.1461.

(*S*)-4-(1-(5-Propyl-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-4-carboxamido)ethyl)benzoic acid (23). The titled compound was obtained from **11i** and

methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 96, 73 and 96%, respectively; white solid. HPLC purity = 99.3%; t_R = 12.8 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.85 (s, 1H), 8.97 (d, J = 7.8 Hz, 1H), 7.90 (d, J = 6.8 Hz, 2H), 7.75 (d, J = 7.5 Hz, 2H), 7.52 (d, J = 7.0 Hz, 2H), 7.41 (d, J = 7.6 Hz, 2H), 5.79 (s, 2H), 5.31 – 5.13 (m, 1H), 2.95 – 2.80 (m, 2H), 1.51 (d, J = 6.4 Hz, 3H), 1.36 – 1.26 (m, 2H), 0.76 (t, J = 7.2, 6.1 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.6, 160.4, 150.3, 141.0, 140.7, 138.7, 129.8 (2C), 129.7, 129.0 (q, J = 31.9 Hz), 128.5 (2C), 126.8 (2C), 126.2 (q, J = 3.8 Hz, 2C), 124.5 (q, J = 272.0 Hz), 50.4, 48.2, 24.4, 22.2, 22.1, 13.9. HRMS (ESI): calcd for $C_{23}H_{23}F_3N_4NaO_3$ $[M + Na]^+$, 483.1614; found 483.1608.

(*S*)-4-(1-(5-Phenyl-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-4-carboxamido)ethyl)benzoic acid (24). The titled compound was obtained from **11j** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 94, 75 and 99%, respectively; white solid. The structure of the intermediate ester **11j** was characterized by HMBC spectra shown in supporting information. HPLC purity = 95.1%; t_R = 12.4 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.66 (s, 1H), 9.06 (d, J = 8.2 Hz, 1H), 7.88 (d, J = 8.3 Hz, 2H), 7.64 (d, J = 8.2 Hz, 2H), 7.48 (d, J = 8.3 Hz, 2H), 7.47 – 7.44 (m,

1H), 7.44 – 7.39 (m, 2H), 7.37 – 7.29 (m, 2H), 7.17 (d, J = 8.1 Hz, 2H), 5.65 (s, 2H), 5.17 – 5.06 (m, 1H), 1.49 (d, J = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.6, 159.6, 150.1, 140.5, 139.5, 139.3, 130.4 (2C), 130.1, 129.8 (2C), 129.7, 128.9 (q, J = 32.0 Hz), 128.7 (2C), 128.3 (2C), 126.8 (2C), 126.2, 126.0 (q, J = 3.8 Hz, 2C), 124.5 (q, J = 272.1 Hz), 51.2, 48.3, 22.2. HRMS (ESI): calcd for $C_{26}H_{21}F_3N_4NaO_3$ [M + Na]$^+$, 517.1458; found 517.1459.

(*S*)-4-(1-(1-(4-Fluorobenzyl)-4-(trifluoromethyl)-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (25). The titled compound was obtained from **12a** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 94, 73 and 96%, respectively; white solid. HPLC purity = 99.2%; t_R = 11.1 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.96 (s, 1H), 9.77 (d, J = 7.8 Hz, 1H), 7.91 (d, J = 8.2 Hz, 2H), 7.41 (d, J = 8.3 Hz, 2H), 7.27 – 7.21 (m, 2H), 7.20 – 7.06 (m, 2H), 5.72 – 5.58 (m, 2H), 5.17 – 5.08 (m, 1H), 1.40 (d, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.5, 162.5 (d, J = 245.0 Hz), 156.0, 148.3, 134.6 (q, J = 38.2 Hz), 133.2 (d, J = 1.9 Hz), 130.9, 130.9, 130.8, 130.2, 130.0 (2C), 126.7 (2C), 120.7 (q, J = 268.6 Hz), 116.2, 116.0, 52.4, 49.5, 22.0. HRMS (ESI): calcd for $C_{20}H_{16}F_4N_4NaO_3$ [M + Na]$^+$, 459.1051; found 459.1082.

(*S*)-4-(1-(4-(Trifluoromethyl)-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (26). The titled compound was obtained from **12b** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 97, 77 and 96%, respectively; white solid. HPLC purity = 97.1%; t_R = 12.1 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.90 (s, 1H), 9.69 (d, J = 7.7 Hz, 1H), 7.89 (d, J = 8.1 Hz, 2H), 7.68 (d, J = 8.1 Hz, 2H), 7.39 (d, J = 8.1 Hz, 4H), 5.85 – 5.72 (m, 2H), 5.17 – 5.07 (m, 1H), 1.37 (d, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.5, 155.9, 148.2, 139.2, 134.7 (q, J = 38.5 Hz), 133.4 (d, J = 2.0 Hz), 130.2, 129.9 (2C), 129.5 (q, J = 31.9 Hz), 129.3 (2C), 126.7 (2C), 126.1 (q, J = 3.8 Hz, 2C), 124.4 (q, J = 272.8 Hz), 120.6 (q, J = 268.8 Hz), 52.5, 49.5, 21.9. HRMS (ESI): calcd for $C_{21}H_{16}F_6N_4NaO_3$ [M + Na]$^+$, 509.1019; found 509.1006.

(*S*)-4-(1-(4-(Trifluoromethyl)-1-(3-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (27). The titled compound was obtained from **12c** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 98, 80 and 96%, respectively; white solid. HPLC purity = 99.0%; t_R = 11.9 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.94 (s, 1H), 9.69 (d, J = 7.6 Hz, 1H), 7.89 (d, J = 8.2 Hz, 2H),

7.73 (d, J = 7.7 Hz, 1H), 7.65 (s, 1H), 7.63 – 7.54 (m, 1H), 7.47 (d, J = 7.6 Hz, 1H), 7.39 (d, J = 8.2 Hz, 2H), 5.88 – 5.69 (m, 2H), 5.18 – 5.07 (m, 1H), 1.38 (d, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.6, 157.9, 149.6, 143.4, 136.4, 131.8, 130.6, 129.9 (q, J = 32.8 Hz), 129.9 (2C), 129.9, 126.8 (2C), 126.3 (q, J = 42.1 Hz), 125.7 (q, J = 3.4 Hz), 124.6 (q, J = 3.6 Hz), 124.4 (q, J = 272.2 Hz), 119.6 (q, J = 270.9 Hz), 53.9, 48.8, 22.2. HRMS (ESI): calcd for $C_{21}H_{16}F_6N_4NaO_3$ $[M + Na]^+$, 509.1019; found 509.1008.

(*S*)-4-(1-(1-(4-Fluorophenethyl)-4-(trifluoromethyl)-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (28). The titled compound was obtained from **12d** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 96, 72 and 97%, respectively; white solid. HPLC purity = 97.2%; t_R = 11.6 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.96 (s, 1H), 9.77 (d, J = 7.7 Hz, 1H), 7.94 (d, J = 8.1 Hz, 2H), 7.51 (d, J = 8.2 Hz, 2H), 7.13 – 6.97 (m, 4H), 5.26 – 5.16 (m, 1H), 4.65 – 4.51 (m, 2H), 3.09 – 2.96 (m, 2H), 1.48 (d, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.5, 162.6, 160.7, 156.2, 148.5, 134.3 (q, J = 76.5, 38.1 Hz), 133.3 (d, J = 2.8 Hz), 130.9 (d, J = 8.1 Hz, 2C), 130.3, 130.1 (2C), 126.8 (2C), 120.8 (q, J = 268.2 Hz), 115.7, 115.6,

51.0, 49.6, 34.7, 22.0. HRMS (ESI): calcd for $C_{21}H_{18}F_4N_4NaO_3$ [M + Na]$^+$, 473.1207; found 473.1204.

(*S*)-4-(1-(1-(2-(4-Fluorophenoxy)ethyl)-4-(trifluoromethyl)-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (29). The titled compound was obtained from **12e** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 97, 76 and 96%, respectively; white solid. HPLC purity = 95.1%; t_R = 11.3 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.87 (s, 1H), 9.41 (d, J = 8.0 Hz, 1H), 7.91 (d, J = 7.5 Hz, 2H), 7.51 (d, J = 7.8 Hz, 2H), 7.14 – 7.07 (m, 2H), 6.92 – 6.86 (m, 2H), 5.22 – 5.17 (m, 1H), 5.01 (t, J = 4.5 Hz, 2H), 4.42 (t, J = 4.6 Hz, 2H), 1.49 (d, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.5, 157.2 (d, J = 236.5 Hz), 156.1, 154.3, 148.4, 134.6 (q, J = 39.1 Hz), 134.0 (d, J = 2.1 Hz), 130.1, 130.0 (2C), 126.8 (2C), 120.7 (q, J = 268.4 Hz), 116.4, 116.3 (2C), 116.1, 66.5, 49.7, 49.6, 22.0. HRMS (ESI): calcd for $C_{21}H_{18}F_4N_4NaO_4$ [M + Na]$^+$, 489.1156; found 489.1170.

(*S*)-4-(1-(1-(4-(Trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (30). The titled compound was obtained from **12f** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 96, 72 and 96%,

respectively; white solid. HPLC purity = 98.2%; t_R = 11.4 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.85 (s, 1H), 9.24 (d, J = 7.8 Hz, 1H), 8.40 (s, 1H), 7.89 (d, J = 8.2 Hz, 2H), 7.66 (d, J = 8.2 Hz, 2H), 7.43 (d, J = 8.2 Hz, 2H), 7.34 (d, J = 8.1 Hz, 2H), 5.96 (s, 2H), 5.17 – 5.10 (m, 1H), 1.46 (d, J = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.5, 157.2, 149.3, 141.2, 135.0, 131.0, 129.9 (2C), 128.9 (q, J = 31.8 Hz), 128.7 (3C), 126.6 (2C), 126.0 (q, J = 3.7 Hz, 2C), 124.5 (q, J = 272.1 Hz), 52.1, 48.8, 22.1. HRMS (ESI): calcd for $C_{20}H_{17}F_3N_4NaO_3$ $[M + Na]^+$, 441.1145; found 441.1164.

(*S*)-4-(1-(4-Methyl-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (31). The titled compound was obtained from **12g** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 97, 77 and 95%, respectively; white solid. HPLC purity = 98.3%; t_R = 11.1 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.87 (s, 1H), 8.99 (d, J = 8.0 Hz, 1H), 7.87 (d, J = 8.0 Hz, 2H), 7.61 (d, J = 8.0 Hz, 2H), 7.37 (d, J = 8.0 Hz, 2H), 7.29 (d, J = 8.0 Hz, 2H), 5.74 (s, 2H), 5.22 – 4.94 (m, 1H), 2.37 (s, 3H), 1.40 (d, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.5, 158.5, 149.0, 142.8, 140.8, 129.9, 129.9 (2C), 129.5, 129.2, 128.9 (2C), 126.7

(2C), 126.0 (q, J = 3.8 Hz, 2C), 124.5 (q, J = 272.1 Hz), 51.9, 48.8, 21.9, 11.5. HRMS (ESI): calcd for $C_{21}H_{19}F_3N_4NaO_3$ [M + Na]$^+$, 455.1301; found 455.1286.

(*S*)-4-(1-(4-Ethyl-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (32). The titled compound was obtained from **12h** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 94, 78 and 96%, respectively; white solid. The structure of the intermediate ester **12h** was characterized by HMBC spectra shown in supporting information. HPLC purity = 98.2%; t_R = 11.4 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.80 (s, 1H), 9.06 (d, J = 8.0 Hz, 1H), 7.87 (d, J = 8.2 Hz, 2H), 7.60 (d, J = 7.5 Hz, 2H), 7.37 (d, J = 7.1 Hz, 2H), 7.27 (d, J = 7.6 Hz, 2H), 5.73 (s, 2H), 5.15 – 5.08 (m, 1H), 3.91 – 3.79 (m, 2H), 1.38 (d, J = 6.9 Hz, 3H), 1.17 (t, J = 7.5 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.6, 158.6, 148.8, 148.0, 140.7, 130.2, 129.8 (2C), 129.2, 129.2, 128.9 (2C), 126.7 (2C), 126.0 (q, J = 3.8 Hz, 2C), 124.5 (q, J = 272.1 Hz), 51.9, 48.8, 21.9, 18.9, 13.6.HRMS (ESI): calcd for $C_{22}H_{21}F_3N_4NaO_3$ [M + Na]$^+$, 469.1458; found 469.1472.

(*S*)-4-(1-(4-Propyl-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (33). The titled compound was obtained from **12i** and

methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 96, 74 and 96%, respectively; white solid. HPLC purity = 98.7%; t_R = 11.8 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.87 (s, 1H), 9.08 (d, J = 7.5 Hz, 1H), 7.87 (d, J = 7.5 Hz, 2H), 7.62 (d, J = 7.4 Hz, 2H), 7.47 -7.17 (m, 4H), 5.73 (s, 2H), 5.20 – 5.04 (m, 1H), 2.82 – 2.67 (m, 2H), 1.63 – 1.49 (m, 2H), 1.38 (d, J = 6.4 Hz, 3H), 0.82 (t, J = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.5, 158.6, 148.9, 146.8, 140.7, 130.0, 129.8 (2C), 129.6, 129.2, 129.0 (2C), 126.7 (2C), 126.0 (q, J = 3.5 Hz, 2C), 124.5 (q, J = 272.5 Hz), 51.9, 48.8, 27.5, 22.5, 21.8, 14.0. HRMS (ESI): calcd for $C_{23}H_{23}F_3N_4NaO_3$ [M + Na]$^+$, 483.1614; found 483.1642.

(*S*)-4-(1-(4-Phenyl-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (34). The titled compound was obtained from **12j** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 98, 79 and 96%, respectively; white solid. The structure of the intermediate ester **12j** was characterized by HMBC spectra shown in supporting information. HPLC purity = 99.3%; t_R = 12.0 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.75 (s, 1H), 9.41 (d, J = 7.9 Hz, 1H), 7.86 (d, J = 8.1 Hz, 2H), 7.69 - 7.62 (m, 4H), 7.42-7.36 (m, 5H), 7.33 (d, J = 8.1 Hz, 2H),

5.79 – 5.68 (m, 2H), 5.20 – 5.12 (m, 1H), 1.29 (d, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.5, 158.9, 148.5, 144.4, 140.2, 130.2, 130.1, 130.0, 129.8 (2C), 129.3, 129.1 (2C), 128.9 (3C), 127.1 (2C), 126.9 (2C), 126.0 (q, J = 3.4 Hz, 2C), 124.5 (q, J = 271.5 Hz), 52.0, 49.1, 21.5. HRMS (ESI): calcd for $C_{26}H_{21}F_3N_4NaO_3$ [M + Na]$^+$, 517.1458; found 517.1499.

(*S*)-4-(1-(1-Benzyl-4-phenyl-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (35). The titled compound was obtained from **12k** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 95, 78 and 99%, respectively; white solid. HPLC purity = 99.2%; t_R = 11.6 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.92 (s, 1H), 9.46 (d, J = 8.0 Hz, 1H), 7.87 (d, J = 8.2 Hz, 2H), 7.67 – 7.60 (m, 2H), 7.39 – 7.33 (m, 5H), 7.33 – 7.26 (m, 3H), 7.20 – 7.14 (m, 2H), 5.66 – 5.56 (m, 2H), 5.22 – 5.13 (m, 1H), 1.31 (d, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.6, 159.0, 148.6, 144.3, 135.6, 130.3, 130.0, 129.9(2C), 129.8, 129.1 (2C), 129.1, 128.9, 128.6, 128.3 (3C), 127.1 (2C), 126.9 (2C), 52.5, 49.1, 21.7. HRMS (ESI): calcd for $C_{25}H_{22}N_4NaO_3$ [M + Na]$^+$, 449.1584; found 449.1576.

(*S*)-4-(1-(1-(4-Fluorobenzyl)-4-phenyl-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (36). The titled compound was obtained from **12l** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 99, 72 and 99%, respectively; white solid. HPLC purity = 99.2%; t_R = 11.7 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.90 (s, 1H), 9.44 (d, J = 8.0 Hz, 1H), 7.88 (d, J = 8.2 Hz, 2H), 7.66 – 7.59 (m, 2H), 7.41 – 7.32 (m, 5H), 7.25 – 7.19 (m, 2H), 7.16 – 7.09 (m, 2H), 5.66 – 5.54 (m, 2H), 5.22 – 5.13 (m, 1H), 1.31 (d, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.6, 162.3 (d, J = 244.6 Hz), 159.0, 148.5, 144.3, 131.9 (d, J = 2.9 Hz), 130.6 (d, J = 8.5 Hz, 2C), 130.3, 130.0, 129.9 (2C), 129.8, 129.1 (2C), 128.9, 127.1 (2C), 126.9 (2C), 116.0, 115.9, 51.8, 49.1, 21.6. HRMS (ESI): calcd for $C_{25}H_{21}FN_4NaO_3$ $[M + Na]^+$, 467.1490; found 467.1484.

(*S*)-4-(1-(1-(3,4-Difluorobenzyl)-4-phenyl-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (37). The titled compound was obtained from **12m** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 93, 66 and 99%, respectively; white solid. HPLC purity = 96.5%; t_R = 11.9 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.94 (s, 1H), 9.54 (d, J = 7.5 Hz, 1H), 7.86 (d, J = 7.7 Hz, 2H),

7.64 (d, J = 3.1 Hz, 2H), 7.47 – 7.21 (m, 6H), 7.28 – 7.17 (m, 1H), 7.03 (s, 1H), 5.72 – 5.50 (m, 2H), 5.22 – 5.09 (m, 1H), 1.33 (d, J = 6.6 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.5, 158.9, 148.4, 144.3, 141.2, 130.2, 129.9, 129.8 (2C), 129.1 (2C), 128.9, 127.0 (2C), 126.8 (2C), 125.3 (d, J = 3.8 Hz), 125.2 (d, J = 3.1 Hz), 118.2 (d, J = 17.5 Hz, 2C), 117.4 (d, J = 17.3 Hz, 2C), 51.4, 49.2, 21.7. HRMS (ESI): calcd for $C_{25}H_{20}F_2N_4NaO_3$ [M + Na]$^+$, 485.1396; found 485.1388.

(*S*)-4-(1-(4-Phenyl-1-(3-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (38). The titled compound was obtained from **12n** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 95, 70 and 98%, respectively; white solid. HPLC purity = 99.2%; t_R = 12.3 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.89 (s, 1H), 9.49 (d, J = 8.0 Hz, 1H), 7.84 (d, J = 8.0 Hz, 2H), 7.71 (d, J = 7.7 Hz, 1H), 7.66 – 7.59 (m, 3H), 7.60 – 7.54 (m, 1H), 7.46 (d, J = 7.7 Hz, 1H), 7.37 (d, J = 6.2 Hz, 3H), 7.27 (d, J = 8.0 Hz, 2H), 5.84 – 5.65 (m, 2H), 5.20 – 5.10 (m, 1H), 1.30 (d, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.2, 158.9, 144.2, 137.0, 132.5 (2C), 130.3 (2C), 130.2, 130.1, 129.7 (3C), 129.1 (2C), 128.9 (2C), 127.0

(2C), 126.3 (2C), 124.9 (q, J = 3.9 Hz), 124.4 (q, J = 272.3 Hz), 51.9, 49.1, 21.7. HRMS (ESI): calcd for $C_{26}H_{21}F_3N_4NaO_3$ [M + Na]$^+$, 517.1458; found 517.1448.

(*S*)-4-(1-(1-(4-Methoxybenzyl)-4-phenyl-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (39). The titled compound was obtained from **12o** and methyl (S)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 96, 72 and 94%, respectively; white solid. HPLC purity = 95.3%; t_R = 11.6 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.87 (s, 1H), 9.41 (d, J = 7.3 Hz, 1H), 7.89 (d, J = 6.9 Hz, 2H), 7.63 (s, 2H), 7.49 – 7.25 (m, 5H), 7.11 (d, J = 7.2 Hz, 2H), 6.83 (d, J = 7.1 Hz, 2H), 5.62 – 5.45 (m, 2H), 5.25 – 5.13 (m, 1H), 3.72 (s, 3H), 1.34 (d, J = 5.8 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.6, 159.6, 159.1, 148.6, 144.3, 130.4, 130.0, 130.0 (2C), 129.9 (2C), 129.6, 129.1 (2C), 128.8, 127.6, 127.1 (2C), 127.0 (2C), 114.4 (2C), 55.6, 52.1, 49.1, 21.6. HRMS (ESI): calcd for $C_{26}H_{24}N_4NaO_4$ [M + Na]$^+$, 479.1690; found 479.1688.

(*S*)-4-(1-(1-(3-Methoxybenzyl)-4-phenyl-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (40). The titled compound was obtained from **12p** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 99, 74 and 95%, respectively; white solid. HPLC purity = 99.2%; t_R = 11.6 min. ^{1}H NMR (500 MHz,

DMSO-d_6) δ 12.93 (s, 1H), 9.52 (d, J = 7.9 Hz, 1H), 7.86 (d, J = 8.1 Hz, 2H), 7.70 – 7.60 (m, 2H), 7.45 – 7.31 (m, 5H), 7.26 – 7.20 (m, 1H), 6.93 – 6.86 (m, 1H), 6.77 (s, 1H), 6.72 (d, J = 7.6 Hz, 1H), 5.69 – 5.53 (m, 2H), 5.23 – 5.12 (m, 1H), 3.70 (s, 3H), 1.33 (d, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.5, 159.8, 159.0, 148.6, 144.2, 137.1, 130.3, 130.3, 130.0, 129.9, 129.8 (2C), 129.1 (2C), 128.8, 127.1 (2C), 126.8 (2C), 120.3, 113.9 (2C), 55.5, 52.5, 49.1, 21.7. HRMS (ESI): calcd for $C_{26}H_{24}N_4NaO_4$ $[M + Na]^+$, 479.1690; found 479.1688.

(*S*)-4-(1-(1-(3-Fluoro-4-methoxybenzyl)-4-phenyl-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (41). The titled compound was obtained from **12q** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 99, 74 and 95%, respectively; white solid. HPLC purity = 95.3%; t_R = 11.6 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.91 (s, 1H), 9.43 (d, J = 7.9 Hz, 1H), 7.88 (d, J = 8.1 Hz, 2H), 7.63 (d, J = 3.6 Hz, 2H), 7.50 – 7.27 (m, 5H), 7.12 – 6.93 (m, 3H), 5.63 – 5.48 (m, 2H), 5.24 – 5.14 (m, 1H), 3.81 (s, 3H), 1.34 (d, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.6, 159.0, 152.6, 148.6, 147.5, 144.3, 130.3, 130.0, 129.9 (2C), 129.7, 129.1 (2C), 128.9, 128.2, 127.1 (2C), 126.9 (2C), 125.0 (d, J = 3.5 Hz), 115.9 (d, J = 18.7 Hz), 114.3,

56.5, 51.6, 49.1, 21.7. HRMS (ESI): calcd for $C_{26}H_{23}FN_4NaO_4$ [M + Na]$^+$, 497.1596; found 497.1581.

(*S*)-4-(1-(1-(3,4-Dimethoxybenzyl)-4-phenyl-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (42). The titled compound was obtained from **12r** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 97, 74 and 98%, respectively; white solid. HPLC purity = 98.7%; t_R = 11.2 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.78 (s, 1H), 9.43 (d, J = 7.9 Hz, 1H), 7.89 (d, J = 8.0 Hz, 2H), 7.63 (d, J = 3.3 Hz, 2H), 7.48 – 7.28 (m, 5H), 6.89 – 6.77 (m, 2H), 6.68 (d, J = 8.0 Hz, 1H), 5.62 – 5.45 (m, 2H), 5.25 – 5.15 (m, 1H), 3.72 (s, 3H), 3.66 (s, 3H) 1.34 (d, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.6, 159.1, 149.2, 149.2, 148.6, 144.2, 130.4, 130.0, 129.9 (2C), 129.7, 129.1 (2C), 128.8, 127.8, 127.1 (2C), 126.9 (2C), 121.0, 112.1, 112.1, 55.9, 55.9, 52.5, 49.1, 21.7. HRMS (ESI): calcd for $C_{27}H_{26}N_4NaO_5$ [M + Na]$^+$, 509.1795; found 509.1802.

(*S*)-4-(1-(1-(2-(4-Fluorophenoxy)ethyl)-4-phenyl-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (43). The titled compound was obtained from **12s** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields

96, 72 and 94%, respectively; white solid. HPLC purity = 98.0%; t_R = 11.5 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.92 (s, 1H), 9.57 (d, J = 7.8 Hz, 1H), 7.89 (d, J = 8.0 Hz, 2H), 7.71 – 7.61 (m, 2H), 7.47 (d, J = 8.1 Hz, 2H), 7.44 – 7.34 (m, 3H), 7.14 – 7.01 (m, 2H), 6.93 – 6.77 (m, 2H), 5.29 – 5.20 (m, 1H), 4.80 (t, J = 4.8 Hz, 2H), 4.30 (t, J = 4.3 Hz, 2H), 1.42 (d, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.6, 159.2, 157.2 (d, J = 236.3 Hz), 154.5 (d, J = 1.7 Hz), 148.7, 144.1, 130.4 (d, J = 7.3 Hz, 2C), 130.0, 129.9 (2C), 129.1 (2C), 128.8, 127.1 (2C), 127.0 (2C), 116.4, 116.4, 116.3, 116.1, 66.9, 49.2, 48.9, 21.7. HRMS (ESI): calcd for $C_{26}H_{23}FN_4NaO_4$ $[M + Na]^+$, 497.1596; found 497.1629.

(*S*)-4-(1-(4-Phenyl-1-(2-(4-(trifluoromethyl)phenoxy)ethyl)-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (44). The titled compound was obtained from **12t** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 92, 75 and 95%, respectively; white solid. HPLC purity = 96.5%; t_R = 12.3 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.93 (s, 1H), 9.53 (d, J = 7.7 Hz, 1H), 7.90 (d, J = 8.0 Hz, 2H), 7.71 – 7.63 (m, 2H), 7.60 (d, J = 8.3 Hz, 2H), 7.48 (d, J = 7.7 Hz, 2H), 7.44 – 7.27 (m, 3H), 7.01 (d, J = 8.3 Hz, 2H), 5.32 – 5.20 (m, 1H), 4.94 – 4.76 (m, 2H), 4.54 – 4.36 (m, 2H), 1.42 (d, J = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.6, 161.0, 159.1, 148.6,

144.1, 130.5, 130.4, 130.2, 129.9 (2C), 129.1 (2C), 128.8, 127.3 (q, J = 3.7 Hz, 2C), 127.1 (2C), 127.0 (2C), 124.9 (q, J = 271.2 Hz), 122.1 (q, J = 32.0 Hz), 115.5 (2C), 66.7, 49.3, 48.8, 21.7. HRMS (ESI): calcd for $C_{27}H_{23}F_3N_4NaO_4$ [M + Na]$^+$, 547.1564; found 547.1570.

(*S*)-4-(1-(4-Phenyl-1-(2-(3-(trifluoromethyl)phenoxy)ethyl)-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (45). The titled compound was obtained from **12u** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 94, 70 and 95%, respectively; white solid. HPLC purity = 98.8%; t_R = 12.3 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.84 (s, 1H), 9.57 (d, J = 7.7 Hz, 1H), 7.88 (d, J = 8.1 Hz, 2H), 7.65 (d, J = 3.3 Hz, 2H), 7.52 – 7.44 (m, 3H), 7.39 (d, J = 2.4 Hz, 3H), 7.29 (d, J = 7.5 Hz, 1H), 7.17 (d, J = 7.1 Hz, 2H), 5.29 – 5.21 (m, 1H), 4.89 – 4.77 (m, 2H), 4.50 – 4.38 (m, 2H), 1.42 (d, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.5, 159.2, 158.4, 148.7, 144.1, 131.1, 130.6, 130.6, 130.4, 130.0, 129.9 (2C), 129.1 (2C), 128.8, 127.1 (2C), 126.9 (2C), 124.4 (q, J = 272.2 Hz), 119.0, 118.0 (q, J = 3.3 Hz), 111.9 (q, J = 4.1 Hz), 66.8, 49.3, 48.8, 21.7. HRMS (ESI): calcd for $C_{27}H_{23}F_3N_4NaO_4$ [M + Na]$^+$, 547.1564; found 547.1582.

(*S*)-4-(1-(4-Phenyl-1-((tetrahydro-2*H*-pyran-4-yl)methyl)-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (46). The titled compound was obtained from **12v** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 97, 68 and 97%, respectively; white solid. HPLC purity = 99.7%; t_R = 10.5 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.93 (s, 1H), 9.53 (d, J = 8.1 Hz, 1H), 7.93 (d, J = 8.2 Hz, 2H), 7.68 (d, J = 9.4 Hz, 2H), 7.48 (d, J = 8.3 Hz, 2H), 7.44 – 7.37 (m, 3H), 5.30 -5.26 (m, 1H), 4.29 – 4.20 (m, 2H), 3.80 – 3.73 (m, 2H), 3.14 – 3.06 (m, 2H), 1.92 – 1.84 (m, 1H), 1.42 (d, J = 7.0 Hz, 3H), 1.31 – 1.25 (m, 2H), 1.21 – 1.12 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.6, 159.3, 148.6, 143.8, 130.4, 130.2, 130.1, 129.9 (2C), 129.2 (2C), 128.8, 127.0 (2C), 126.9 (2C), 66.7 (2C), 54.3, 49.2, 35.8, 30.2 (2C), 21.6. HRMS (ESI): calcd for $C_{24}H_{26}N_4NaO_4$ $[M + Na]^+$, 457.1846; found 457.1825.

(*S*)-4-(1-(1-(Cyclohexylmethyl)-4-phenyl-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (47). The titled compound was obtained from **12w** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 94, 65 and 94%, respectively; white solid. HPLC purity = 98.0%; t_R = 12.2 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.87 (s, 1H), 9.54 (d, J = 8.0 Hz, 1H), 7.92 (d, J = 8.2 Hz, 2H),

7.73 – 7.59 (m, 2H), 7.47 (d, *J* = 8.2 Hz, 2H), 7.45 – 7.28 (m, 3H), 5.31 – 5.19 (m, 1H), 4.26 – 4.12 (m, 2H), 1.67 – 1.51 (m, 4H), 1.42 (d, *J* = 7.1 Hz, 3H), 1.41 – 1.36 (m, 2H), 1.10 – 0.96 (m, 3H), 0.89 – 0.79 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.5, 159.3, 148.7, 143.8, 130.5, 130.2, 130.1, 129.9 (2C), 129.1 (2C), 128.7, 127.0 (2C), 126.9 (2C), 54.9, 49.1, 38.2, 30.1 (2C), 26.0, 25.4 (2C), 21.6. HRMS (ESI): calcd for $C_{25}H_{28}N_4NaO_3$ $[M + Na]^+$, 455.2054; found 455.2056.

(*S*)-4-(1-(4-Cyclopropyl-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (48). The titled compound was obtained from **12x** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 96, 70 and 97%, respectively; white solid. HPLC purity = 97.4%; t_R = 11.6 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.86 (s, 1H), 9.18 (d, *J* = 7.9 Hz, 1H), 7.87 (d, *J* = 8.2 Hz, 2H), 7.61 (d, *J* = 8.2 Hz, 2H), 7.39 (d, *J* = 8.3 Hz, 2H), 7.27 (d, *J* = 8.1 Hz, 2H), 5.72 (s, 2H), 5.35 – 4.91 (m, 1H), 2.10 – 2.03 (m, 1H), 1.41 (d, *J* = 7.0 Hz, 3H), 1.02 – 0.94 (m, 2H), 0.90 – 0.83 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.6, 158.5, 149.0, 148.1, 140.7, 130.0, 129.8 (2C), 129.8, 129.1 (q, *J* = 32.1 Hz), 128.9 (2C), 126.7 (2C), 126.0 (q, *J* =

3.6 Hz, 2C), 124.5 (q, J = 272.0 Hz), 51.9, 49.0, 22.0, 8.4, 8.2, 6.9. HRMS (ESI): calcd for $C_{23}H_{21}F_3N_4NaO_3$ $[M + Na]^+$, 481.1458; found 481.1469.

(*S*)-4-(1-(4-Cyclobutyl-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (49). The titled compound was obtained from **12y** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 94, 78 and 95%, respectively; white solid. HPLC purity = 99.0%; t_R = 12.0 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.83 (s, 1H), 9.00 (d, J = 8.2 Hz, 1H), 7.91 (d, J = 8.1 Hz, 2H), 7.75 (d, J = 8.0 Hz, 2H), 7.54 (d, J = 8.0 Hz, 2H), 7.32 (d, J = 7.9 Hz, 2H), 5.81 (s, 2H), 5.42 – 5.08 (m, 1H), 3.85 – 3.75 (m, 1H), 2.58 – 2.39 (m, 2H), 2.00 – 1.90 (m, 2H), 1.88 – 1.70 (m, 2H), 1.51 (d, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.5, 158.6, 149.9, 148.9, 140.6, 130.0, 129.8 (2C), 129.2, 128.9 (2C), 128.6, 126.8 (2C), 126.0 (q, J = 3.8 Hz, 2C), 124.5 (q, J = 272.3 Hz), 51.8, 48.9, 31.0, 28.7, 28.4, 21.8, 18.7. HRMS (ESI): calcd for $C_{24}H_{23}F_3N_4NaO_3$ $[M + Na]^+$, 495.1614; found 495.1614.

(*S*)-4-(1-(4-Cyclopentyl-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (50). The titled compound was obtained from **14a** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields

98, 74 and 94%, respectively; white solid. HPLC purity = 96.8%; t_R = 12.3 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.83 (s, 1H), 9.12 (d, J = 8.0 Hz, 1H), 7.87 (d, J = 8.0 Hz, 2H), 7.62 (d, J = 8.0 Hz, 2H), 7.37 (d, J = 8.0 Hz, 2H), 7.29 (d, J = 8.0 Hz, 2H), 5.77 – 5.61 (m, 2H), 5.17 – 5.08 (m, 1H), 3.29 – 3.23 (m, 1H), 1.98 – 1.84 (m, 2H), 1.76 – 1.63 (m, 4H), 1.61 – 1.52 (m, 2H), 1.38 (d, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.5, 158.8, 150.1, 148.9, 140.6, 130.0, 129.8 (2C), 129.4, 129.2, 129.0 (2C), 126.7 (2C), 126.0 (q, J = 3.7 Hz, 2C), 124.5 (q, J = 272.2 Hz), 51.9, 48.8, 36.0, 32.8, 32.5, 25.4 (2C), 21.8. HRMS (ESI): calcd for $C_{25}H_{25}F_3N_4NaO_3$ $[M + Na]^+$, 509.1771; found 509.1766.

(*S*)-4-(1-(4-Cyclohexyl-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (51). The titled compound was obtained from **14b** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 99, 71 and 96%, respectively; white solid. HPLC purity = 97.2%; t_R = 12.5 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.87 (s, 1H), 9.13 (d, J = 8.1 Hz, 1H), 7.88 (d, J = 7.9 Hz, 2H), 7.64 (d, J = 8.0 Hz, 2H), 7.38 (d, J = 7.9 Hz, 2H), 7.31 (d, J = 7.9 Hz, 2H), 5.75 – 5.64 (m, 2H), 5.31 – 4.86 (m, 1H), 2.91 – 2.82 (m, 1H), 1.79 – 1.63 (m, 4H), 1.53 – 1.43 (m, 2H), 1.37 (d, J = 7.0 Hz, 3H), 1.24 – 1.15 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.8,

167.6, 158.9, 150.9, 148.9, 140.6, 130.1, 129.8 (2C), 129.1, 129.0 (2C), 126.7 (2C), 126.0 (q, J = 3.7 Hz, 2C), 124.5 (q, J = 272.2 Hz), 60.2, 51.8, 48.7, 34.8, 32.4, 26.3 (d, J = 12.3 Hz), 25.9, 21.8, 21.2. HRMS (ESI): calcd for $C_{26}H_{27}F_3N_4NaO_3$ [M + Na]$^+$, 523.1927; found 523.1950.

(*S*)-4-(1-(4-(Cyclopent-1-en-1-yl)-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (52). The titled compound was obtained from **14c** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 93, 76 and 94%, respectively; white solid. HPLC purity = 98.3%; t_R = 12.2 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.84 (s, 1H), 9.34 (d, J = 8.1 Hz, 1H), 7.86 (d, J = 8.0 Hz, 2H), 7.64 (d, J = 8.1 Hz, 2H), 7.35 (d, J = 8.0 Hz, 2H), 7.30 (d, J = 8.0 Hz, 2H), 6.05 (s, 1H), 5.72 – 5.58 (m, 2H), 5.16 – 5.08 (m, 1H), 2.70 – 2.55 (m, 2H), 2.42 – 2.36 (m, 2H), 1.91 – 1.84 (m, 2H), 1.34 (d, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.5, 158.8, 148.5, 142.1, 140.3, 132.8, 130.0, 129.8 (2C), 129.6 (d, J = 2.5 Hz, 2C), 129.1 (d, J = 32.0 Hz), 128.8 (2C), 126.9 (2C), 126.0 (q, J = 3.8 Hz, 2C), 124.5 (q, J = 272.3 Hz), 51.8, 49.0, 33.6, 33.1, 23.0, 21.6. HRMS (ESI): calcd for $C_{25}H_{23}F_3N_4NaO_3$ [M + Na]$^+$, 507.1614; found 507.1592.

(*S*)-4-(1-(4-(Cyclohex-1-en-1-yl)-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (53). The titled compound was obtained from **14d** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 99, 69 and 94%, respectively; white solid. HPLC purity = 95.7%; t_R = 12.5 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.45 (s, 1H), 9.22 (d, J = 8.1 Hz, 1H), 7.86 (d, J = 8.0 Hz, 2H), 7.65 (d, J = 8.0 Hz, 2H), 7.39 – 7.28 (m, 4H), 6.02 (s, 1H), 5.81 – 5.53 (m, 2H), 5.14 – 5.06 (m, 1H), 2.36 – 2.30 (m, 2H), 2.06 – 1.97 (m, 2H), 1.65 – 1.55 (m, 4H), 1.31 (d, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 175.1, 167.7, 159.1, 148.1, 146.4, 140.4, 129.8 (2C), 129.3 (d, J = 3.13 Hz), 129.0 (2C), 128.9, 127.8, 127.6, 126.7 (2C), 126.0 (q, J = 3.8 Hz, 2C), 124.5 (q, J = 272.1 Hz), 51.7, 48.8, 26.5, 25.3, 22.5, 21.9, 21.7. HRMS (ESI): calcd for $C_{26}H_{25}F_3N_4NaO_3$ $[M + Na]^+$, 521.1771; found 521.1737.

(*S*)-4-(1-(4-(3,6-Dihydro-2H-pyran-4-yl)-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (54). The titled compound was obtained from **14e** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 93, 76 and 96%, respectively; white solid. HPLC purity = 95.2%; t_R = 11.4 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.87 (s, 1H), 9.34 (d, J = 8.2 Hz, 1H), 7.87 (d, J = 7.9 Hz, 2H),

7.65 (d, J = 8.0 Hz, 2H), 7.43 – 7.27 (m, 4H), 6.07 (s, 1H), 5.83 – 5.50 (m, 2H), 5.16 – 5.08 (m, 1H), 4.13 – 4.01 (m, 2H), 3.78 – 3.64 (m, 2H), 2.42 (d, J = 21.5 Hz, 2H), 1.33 (d, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.5, 158.8, 148.4, 144.7, 140.3, 130.1, 129.9 (2C), 129.2, 129.1 (d, J = 32.0 Hz), 129.0 (2C), 126.9 (2C), 126.0 (q, J = 3.6 Hz, 2C), 125.8, 125.4, 124.5 (d, J = 272.1 Hz), 64.9, 63.7, 51.8, 48.9, 26.5, 21.5. HRMS (ESI): calcd for $C_{25}H_{23}F_3N_4NaO_4$ $[M + Na]^+$, 523.1564; found 523.1552.

(*S*)-4-(1-(4-(4-Fluorophenyl)-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (55). The titled compound was obtained from **14f** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 95, 73 and 95%, respectively; light yellow solid. HPLC purity = 98.3%; t_R = 12.1 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.91 (s, 1H), 9.53 (d, J = 7.8 Hz, 1H), 7.85 (d, J = 7.7 Hz, 2H), 7.74 – 7.54 (m, 4H), 7.42 – 7.29 (m, 4H), 7.28 – 7.18 (m, 2H), 5.81 – 5.68 (m, 2H), 5.18 – 5.09 (m, 1H), 1.31 (d, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.5, 162.5 (d, J = 245.6 Hz), 158.7, 148.5, 143.7, 140.2, 130.1, 129.8, 129.8 (2C), 129.3 (d, J = 8.3 Hz, 2C), 129.1 (d, J = 31.8 Hz), 128.9 (2C), 126.9 (2C), 126.8 (d, J = 3.0 Hz), 126.0

(q, J = 3.7 Hz, 2C), 124.5 (q, J = 272.3 Hz), 116.2, 116.0, 52.1, 49.2, 21.5. HRMS (ESI): calcd for $C_{26}H_{20}F_4N_4NaO_3$ $[M + Na]^+$, 535.1364; found 535.1358.

(*S*)-4-(1-(4-(Thiophen-3-yl)-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (56). The titled compound was obtained from **14g** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 98, 77 and 95%, respectively; white solid. HPLC purity = 98.7%; t_R = 11.9 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.89 (s, 1H), 9.44 (d, J = 7.9 Hz, 1H), 7.86 (d, J = 8.1 Hz, 2H), 7.70 (s, 1H), 7.65 (d, J = 8.1 Hz, 2H), 7.64 – 7.60 (m, 1H), 7.39 (d, J = 5.0 Hz, 1H), 7.37 – 7.30 (m, 4H), 5.77 – 5.69 (m, 2H), 5.19 – 5.13 (m, 1H), 1.35 (d, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.5, 158.6, 148.5, 141.1, 140.3, 131.0, 130.0, 129.9 (2C), 129.3, 129.1 (q, J = 31.8 Hz), 128.8 (2C), 127.5, 126.9 (2C), 126.7, 126.0 (q, J = 3.8 Hz, 2C), 124.5 (q, J = 272.0 Hz), 123.6, 52.0, 49.2, 21.6. HRMS (ESI): calcd for $C_{24}H_{19}F_3N_4NaO_3S$ $[M + Na]^+$, 523.1022; found 523.1026.

(*S*)-4-(1-(4-(Furan-3-yl)-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (57). The titled compound was obtained from **14h** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields

96, 83 and 93%, respectively; light yellow solid. HPLC purity = 96.9%; t_R = 11.6 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.81 (s, 1H), 9.39 (d, J = 7.9 Hz, 1H), 7.94 (s, 1H), 7.86 (d, J = 8.0 Hz, 2H), 7.77 (s, 1H), 7.63 (d, J = 8.0 Hz, 2H), 7.35 (d, J = 8.0 Hz, 2H), 7.30 (d, J = 8.0 Hz, 2H), 6.74 (s, 1H), 5.81 – 5.67 (m, 2H), 5.19 – 5.11 (m, 1H), 1.37 (d, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.5, 158.4, 148.6, 144.5, 141.0, 140.3, 138.4, 130.1, 129.9 (2C), 129.1, 129.1 (q, J = 32.1 Hz), 128.8 (2C), 126.8 (2C), 126.0 (q, J = 3.7 Hz, 2C), 124.5 (q, J = 272.4 Hz), 116.1, 109.7, 52.0, 49.2, 21.6. HRMS (ESI): calcd for $C_{24}H_{19}F_3N_4NaO_4$ $[M + Na]^+$, 507.1251; found 507.1258.

(*S*)-4-(1-(4-(5-Methylthiophen-2-yl)-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (58). The titled compound was obtained from **14i** and methyl (S)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 90, 74 and 93%, respectively; light yellow solid. HPLC purity = 96.4%; t_R = 12.3 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.76 (s, 1H), 9.50 (d, J = 8.1 Hz, 1H), 7.86 (d, J = 8.1 Hz, 2H), 7.65 (d, J = 8.1 Hz, 2H), 7.46 – 7.25 (m, 4H), 7.04 (d, J = 3.4 Hz, 1H), 6.75 (d, J = 2.2 Hz, 1H), 5.79 – 5.65 (m, 2H), 5.19 – 5.11 (m, 1H), 2.45 (s, 3H), 1.37 (d, J = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.5, 158.2, 148.5, 140.6, 140.2, 140.1, 129.8

(2C), 129.5, 129.1 (q, J = 32.1 Hz), 129.0, 128.8 (2C), 128.4, 127.0 (2C), 126.7, 126.5, 126.0 (q, J = 3.7 Hz, 2C), 124.5 (q, J = 272.3 Hz), 52.2, 49.3, 21.6, 15.3. HRMS (ESI): calcd for $C_{25}H_{21}F_3N_4NaO_3S$ [M + Na]$^+$, 537.1179; found 537.1159.

(*S*,*E*)-4-(1-(4-(Prop-1-en-1-yl)-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (59). The titled compound was obtained from **14j** and methyl (S)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 100, 75 and 90%, respectively; white solid. HPLC purity = 97.4%; t_R = 11.8 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.90 (s, 1H), 9.31 (d, J = 7.9 Hz, 1H), 7.87 (d, J = 7.0 Hz, 2H), 7.62 (d, J = 7.7 Hz, 2H), 7.37 (d, J = 7.2 Hz, 2H), 7.27 (d, J = 7.7 Hz, 2H), 6.59 – 6.47 (m, 1H), 6.38 (d, J = 15.8 Hz, 1H), 5.72 (s, 2H), 5.14 – 5.06 (m, 1H), 1.85 (d, J = 6.6 Hz, 3H), 1.39 (d, J = 6.7 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.6, 158.3, 148.9, 143.8, 140.6, 130.1, 129.9, 129.9 (2C), 129.1 (q, J = 31.8 Hz), 128.8 (2C), 128.3, 126.7 (2C), 126.0 (q, J = 3.6 Hz, 2C), 124.5 (q, J = 272.2 Hz), 119.1, 51.9, 49.0, 21.9, 18.9. HRMS (ESI): calcd for $C_{23}H_{21}F_3N_4NaO_3$ [M + Na]$^+$, 481.1458; found 481.1459.

(*S*)-4-(1-(4-Allyl-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (60). The titled compound was obtained from **14k** and

methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 99, 78 and 94%, respectively; white solid. HPLC purity = 96.7%; t_R = 11.6 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.77 (s, 1H), 9.08 (d, J = 7.9 Hz, 1H), 7.87 (d, J = 8.0 Hz, 2H), 7.62 (d, J = 8.0 Hz, 2H), 7.45 – 7.22 (m, 4H), 5.98 – 5.86 (m, 1H), 5.75 (s, 2H), 5.14 – 5.06 (m, 1H), 5.02 (d, J = 10.1 Hz, 1H), 4.97 (d, J = 17.1 Hz, 1H), 3.62 – 3.53 (m, 2H), 1.39 (d, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.6, 158.3, 148.7, 144.6, 140.7, 135.3, 130.2, 129.8 (3C), 129.1 (q, J = 32.0 Hz), 128.9 (2C), 126.7 (2C), 126.0 (q, J = 3.6 Hz, 2C), 124.5 (q, J = 272.1 Hz), 116.8, 52.0, 48.8, 30.1, 21.8. HRMS (ESI): calcd for $C_{23}H_{21}F_3N_4NaO_3$ $[M + Na]^+$, 481.1458; found 481.1475.

(*S*)-4-(1-(4-(Prop-1-yn-1-yl)-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (61). The titled compound was obtained from **14l** and methyl (*S*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 96, 73 and 96%, respectively; white solid. HPLC purity = 98.6%; t_R = 12.1 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.43 (s, 1H), 9.07 (d, J = 7.8 Hz, 1H), 7.88 (d, J = 8.0 Hz, 2H), 7.66 (d, J = 8.0 Hz, 2H), 7.44 (d, J = 8.1 Hz, 2H), 7.34 (d, J = 7.9 Hz, 2H), 5.86 – 5.76 (m, 2H), 5.15 – 5.08 (m, 1H), 2.11 (s, 3H), 1.39 (d, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz,

DMSO-d_6) δ 172.5, 167.5, 156.8, 148.9, 140.3, 133.6, 130.2 (d, J = 70.6 Hz), 129.9 (2C), 129.2 (q, J = 31.8 Hz), 128.9 (2C), 126.6 (2C), 126.0 (q, J = 3.7 Hz, 2C), 124.5 (q, J = 272.2 Hz), 94.0, 69.3, 52.3, 49.0, 21.5, 4.4. HRMS (ESI): calcd for $C_{23}H_{19}F_3N_4NaO_3$ [M + Na]$^+$, 479.1301; found 479.1330.

(*E*)-4-(1-(4-(Prop-1-en-1-yl)-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxamido)cyclopropyl)benzoic acid (62). The titled compound was obtained from **14j** and methyl 4-(1-aminocyclopropyl)benzoate employing general procedure C, D, and C. Yields 100, 80 and 96%; light yellow solid. HPLC purity = 95.2%; t_R = 11.7 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.71 (s, 1H), 9.45 (s, 1H), 7.76 (d, J = 8.4 Hz, 2H), 7.71 (d, J = 8.2 Hz, 2H), 7.30 (d, J = 8.1 Hz, 2H), 7.08 (d, J = 8.4 Hz, 2H), 6.62 – 6.55 (m, 1H), 6.44 – 6.39 (m, 1H), 5.80 (s, 2H), 1.90 (d, J = 6.7 Hz, 3H), 1.32 – 1.29 (m, 2H), 1.21 – 1.18 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.5, 159.7, 148.1, 144.1, 140.9, 130.3, 129.6 (2C), 128.9, 128.8, 128.5 (2C), 128.1, 126.0 (q, J = 3.7 Hz, 2C), 125.0 (2C), 124.5 (d, J = 272.3 Hz), 119.0, 52.0, 38.7, 35.0, 19.1, 18.9. HRMS (ESI): calcd for $C_{24}H_{21}F_3N_4NaO_3$ [M + Na]$^+$, 493.1458; found 493.1485.

(*R*,*E*)-4-(1-(4-(Prop-1-en-1-yl)-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxamido)ethyl)benzoic acid (63). The titled compound was obtained from **14j** and methyl (*R*)-4-(1-aminoethyl)benzoate employing general procedure C, D, and C. Yields 100, 74 and 96%, respectively; white solid. HPLC purity = 97.3%; t_R = 11.8 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.78 (s, 1H), 9.35 (d, J = 8.0 Hz, 1H), 7.87 (d, J = 8.3 Hz, 2H), 7.61 (d, J = 8.2 Hz, 2H), 7.37 (d, J = 8.3 Hz, 2H), 7.27 (d, J = 8.1 Hz, 2H), 6.56 – 6.47 (m, 1H), 6.38 (d, J = 15.8, 1.6 Hz, 1H), 5.73 (s, 2H), 5.13 – 5.06 (m, 1H), 1.84 (d, J = 6.7, 1.4 Hz, 3H), 1.39 (d, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.6, 158.3, 148.8, 143.8, 140.6, 130.4, 129.9, 129.8 (2C), 129.1 (q, J = 31.9 Hz), 128.8 (2C), 128.3, 126.7 (2C), 126.0 (q, J = 3.7 Hz, 2C), 124.5 (q, J = 272.1 Hz), 119.1, 51.9, 49.1, 21.9, 18.9. HRMS (ESI): calcd for $C_{23}H_{21}F_3N_4NaO_3$ $[M + Na]^+$, 481.1458; found 481.1475.

(*E*)-4-((4-(Prop-1-en-1-yl)-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxamido)methyl)benzoic acid (64). The titled compound was obtained from **14j** and methyl 4-(aminomethyl)benzoate employing general procedure C, D, and C. Yields 100, 81 and 96%, respectively; white solid. HPLC purity = 98.4%; t_R = 11.5 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.91 (s, 1H), 9.29 (s, 1H), 7.85 (d, J = 7.5 Hz, 2H), 7.66 (d, J = 7.5

Hz, 2H), 7.39 – 7.24 (m, 4H), 6.64 – 6.53 (m, 1H), 6.45 (d, J = 15.8 Hz, 1H), 5.80 (s, 2H), 4.48 (d, J = 4.9 Hz, 2H), 1.86 (d, J = 5.9 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 172.3, 163.8, 148.7, 148.6, 145.6, 134.9, 134.8, 134.6 (2C), 133.8 (q, J = 31.5 Hz), 133.4 (2C), 132.7, 132.6 (2C), 130.8 (q, J = 3.8 Hz, 2C), 124.8 (q, J = 264.4 Hz), 123.7, 56.8, 47.7, 23.6. HRMS (ESI): calcd for $C_{22}H_{19}F_3N_4NaO_3$ [M + Na]$^+$, 467.1301; found 467.1277.

(*E*)-4-((4-(Prop-1-en-1-yl)-1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-5-carboxamido)methyl)cyclohexane-1-carboxylic acid (65). The titled compound was obtained from **14j** and methyl 4-(aminomethyl)cyclohexane-1-carboxylate employing general procedure C, D, and C. Yields 100, 65 and 96%, respectively; white solid. HPLC purity = 96.5%; t_R = 11.7 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.00 (s, 1H), 8.66 (d, J = 5.3 Hz, 1H), 7.71 (d, J = 8.1 Hz, 2H), 7.35 (d, J = 8.1 Hz, 2H), 6.61 – 6.52 (m, 1H), 6.43 – 6.35 (m, 1H), 5.77 (s, 2H), 3.12 – 2.95 (m, 2H), 2.15 – 1.95 (m, 2H), 1.91 – 1.80 (m, 5H), 1.61 – 1.53 (m, 2H), 1.46 – 1.32 (m, 2H), 1.23 – 1.15 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 177.1, 162.1, 158.9, 143.5, 140.9, 129.7, 129.0 (q, J = 31.7 Hz), 128.7 (2C), 126.0 (q, J = 3.8 Hz, 2C), 124.5 (q, J = 272.2 Hz), 119.1, 51.9, 45.5, 42.9, 37.1, 29.8 (2C), 28.6 (2C), 18.9. HRMS (ESI): calcd for $C_{22}H_{25}F_3N_4NaO_3$ [M + Na]$^+$, 473.1771; found 473.1799.

Cell culture. HEK293 human embryonic kidney cells, CHO Chinese hamster ovary cells, and Panc02 mouse pancreatic cancer cells were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China); CT26 mouse colon cancer cells, LLC mouse lung cancer cells, EMT6 mouse mammary tumor cells and Raw 264.7 mouse monocyte cells were purchased from ATCC (USA). CHO was cultured in DMEM/F12 (Gibco, USA) and other cell lines were maintained in DMEM medium (Gibco, USA) containing 10% FBS (Gibco, South America) and 1% Penicillin-Streptomycin Solution (Gibco, USA) in a 37 °C incubator with a humidified atmosphere of 5% CO_2. CHO-Gα_{16} was established in our previous study and maintained in DMEM/F12 medium containing puromycin (4 μg/mL). [61, 62]

Calcium flux assay. CHO-Gα_{16} cells were transfected with 4 μg EP1-4 plasmid. Then, transfected cells (3×10^4 cells/well) were seeded into 96-well-black plates (Costar, USA) and cultured overnight. The plates were loaded with 100 μL/well Calcium-5 assay kit reagent (Molecular Devices, USA) for 45 min at 37 °C. After pretreating with 25 μL

compounds for 15 min at rt, cells were treated with 25 μL of PGE_2 by the Flexstation®3 Multi-Mode Microplate Reader (Molecular Devices, USA). Subsequently, the intracellular calcium flux was continuously recorded at an excitation wavelength of 485 nm and an emission wavelength of 525 nm for 2 min. The EC_{80} (80% of the maximal effect Concentration, ~10 nM) of PGE_2 was used in antagonist evaluation.

CYP inhibition. The potential inhibition of CYP1A2, CYP2B6, CYP2C9, CYP2D6, CYP2E1 and CYP3A4 was evaluated using human liver microsomes, as previously reported.[63]

Pharmacokinetic study. 24 BALB/c female mice, weighing between 18 - 22 g, were randomly divided into four groups of 6 mice for intravenous injection and oral administration for each compound, respectively. The mice were fasted for 12 h before administration, but were free to drink water. Test compounds were dissolved into a mixture of 5% DMSO、10% solutol and 85% physiological saline (v/v/v) for intravenous administration, and dissolved into a mixture of 5% DMSO、95% (0.5% Methylcellulose)

for oral administration. The test compounds were intravenously or orally injected into BALB/c mice at a dose of 1 mg/kg (5 mL/kg) and 5 mg/kg (10 mL/kg), respectively. Then plasma samples were collected into heparinized centrifuge tubes at time point : 0.083 h, 0.25 h, 0.5 h, 1 h, 2 h, 4 h, 8 h and 24 h after intravenous or oral administration (n = 3/group). The plasma samples were prepared by centrifugation at 6800 G for 6 min at 2 - 8 °C, then the resulting plasma was transferred to appropriately labeled tubes within 2 hour of blood collection/centrifugation and stored frozen at approximately -80°C. Method development and biological samples analysis for the test articles (Sodium heparin anticoagulant) were performed by testing facility by means of LC-MS/MS. The analytical results were confirmed using quality control samples for intra-assay variation. The pharmacokinetic parameters were calculated by a non-atrioventricular model using Phoenix WinNonlin 7.0 (Pharsight, USA).

Glosensor cAMP assay. HEK293 cells in 6-cm dishes were co-transfected with 2 μg EP4 and 2 μg pGloSensor™-22F cAMP plasmid for 24 h. Cells were harvested and seeded into 384 well plates (2×10^4 cells/well) (Costar, USA) in CO_2-independent medium (Gibco,

USA) with 4% GloSensor™ cAMP Reagent (Promega, USA). After incubation at rt for 1.5 h, cells were treated with different concentrations of compounds for an additional 15 min and then simulated with PGE_2. The luminescence was continuously measured using a Cytation 5 imaging reader (BioTek, USA) with an interval of 2 min for 30 min.

CRE-luciferase reporter assay. HEK293 cells were transfected with a CRE-luciferase plasmid and plated at a density of 2.5×10^4 cells per well in 48-well plates and allowed to adhere overnight. Cells were serum-starved for 2 h and treated with 10 nM PGE_2 in the absence or presence of indicated inhibitors for an additional 24 h (0.1% DMSO; negative vehicle). Subsequently, luciferase activity was determined by Dual Luciferase Assay kits (Promega, USA) with Cytation 5 imaging reader (BioTek, USA).

Western blotting. Cells were lysed with lysis buffer containing 10 μM EGTA, 5 mM EDTA, 1 mM Na_3VO_4, 1 mM NaF, 0.1% SDS, 20 mM Tris, 0.5% Triton X-100, 1% deoxycholate, 0.1% proteinase inhibitor cocktail and phosphorylated proteinase inhibitor cocktail (Calbiochem, Germany) and 1 mM PMSF. Extracted proteins were quantified using BCA assay (Thermo Fisher Scientific, USA). 50 μg protein sample was separated by 10%

SDS-polyacrylamide gels and then transferred to Nitrocellulose membranes (Millipore, USA) at 100V for 2 h. After blocking with 5% bovine serum albumin (BSA) for 1 h at rt, the membranes were incubated with primary antibodies, anti-phospho-ERK antibody (Thr202/Tyr204) (1 : 2000, Cell Signaling Technology, USA), anti-ERK antibody (1 : 1000, Cell Signaling Technology, USA), and anti-GAPDH antibody (1 : 10000, Sigma Aldrich, USA) at 4 °C overnight. The membranes were washed 3 times for 5 min with TBST buffer including 0.05% Tween-20, then were incubated with secondary antibody for 1 h in the dark. After washing 3 times for 4 min each in TBST, the membranes were scanned using the Odyssey Imager (Li-COR Biosciences, USA).

Q-PCR. Raw 264.7 cells were seeded into a 24-well plate and cultured in presence of recombinant mouse GM-CSF (10 ng/mL, Peprotech, USA) and recombinant mouse IL-4 (5 ng/mL, Peprotech, USA). Meanwhile, compound **4** (10 μM) or compound **59** (0.1 μM, 1 μM, 10 μM) was added in the presence of PGE_2 treatment (10 nM) for 24 h at 37 °C. RNA was collected by TRIzol reagent (Invitrogen, USA) and transcribed to cDNA by using HiScript Reverse Transcriptase (Vazyme, China) according to the manufacturer's

protocol. Q-PCR was carried out using the following protocol: 5 min at 95 °C, and 40 cycles of 30 s at 95 °C, 30 s at 58 °C and 30 s at 72 °C. β-Actin was used as vehicle for normalization. The primer sequences are listed in Table S2.

Animal Studies. BALB/c female mice (6-week-old) were purchased from National Rodent Laboratory Animal Resources (Shanghai, China). All animal studies were performed according to the Animal Care and Use Committee guidelines approved by East China Normal University. In order to establish the transplanted tumor model, 1×10^6 CT26 cells were subcutaneously injected into the backs of BALB/c mice. Once average tumor volume reached approximately 100 mm^3, mice were randomized into five groups: Vehicle (0.5% Carboxymethyl cellulose sodium, CMC-Na; p.o.; daily), compound **4** (150 $mg \cdot kg^{-1}$; p.o.; daily), and compound **59** (16, 50, and 150 $mg \cdot kg^{-1}$; p.o.; daily). Dosing volume for each mouse per day 100 μL. The tumor volume and body weight of mice were measured every two days. The tumor volume = length × $width^2$ × 0.5. At the end of the experiments, tumor tissues were extracted and weighed.

Flow cytometry. Fresh tumor tissues were cut into small sections on the ice and digested with 0.25 mg/mL collagenase I, 1 mg/mL collagenase IV (Gibco, USA), and 0.1 mg/mL DNAse (Roche, Switzerland) for 30 min at 37 °C, followed by red blood cells lysis and filtration through a 70-□m filter. Cells suspensions were incubated with indicated staining antibodies for 30 min in 4 °C in the presence of blocking antibodies anti-CD16/32 FcR. Anti-mouse CD16/32 antibody (93), anti-mouse CD45 (30-F11), anti-mouse/human CD11b (M1/70), and anti-mouse CD8 (53-6.7) antibody were purchased from BioLegend (USA). Flow cytometric analysis was conducted on FACS Calibur (BD Biosciences, USA) and all data were analyzed by FlowJo software (Tree Star, USA).

Immunofluorescence. The formalin-fixed tumor tissue sections were de-paraffinized and stained with anti-mouse CD8 antibody (1: 50, Abclonal, China) at 4 °C overnight. After washing with PBS three times, tissues were incubated with fluorochrome secondary antibodies and DAPI. Immunofluorescence images were acquired on a fluorescent inverted microscope (OLYMPUS, Japan), and co-localized by Image J (NIH, USA).

Statistical analysis. Data were calculated with GraphPad Prism (GraphPad, USA). Nonlinear regression analysis was performed to calculate EC_{50} (50% of the maximal effect concentration) and IC_{50} (50% of the maximal inhibitory concentration). All the data are presented as mean ± SEM of three independent experiments. Difference between groups were analyzed via Student's *t*-test or one-way ANOVA followed by Tukey-*post hoc* tests, and $P < 0.05$ was regarded as statistically significant (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

Antagonistic activity of compounds **59** and **4** in calcium flux assay; inhibitory activity of compounds **59** and **4** in β-arrestin recruitment assay; cytotoxic activity of selected compounds; the primer sequences of Q-PCR; crystal structure of compounds **11a**, **11g**

and **13b**; HPLC traces of compounds **15-65**; characterization of structural isomers by HMBC (PDF).

Molecular Formula Strings (CSV)

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ABBREVIATIONS USED

PGE_2, prostaglandin E_2; 15-PGDH, 15-hydroxyprostaglandin dehydrogenase; EP1-4, prostaglandin E_2 receptor 1-4; HATU, 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate; DIPEA, N,N-diisopropylethylamine; CRE, cAMP-response element; TGI, tumor growth inhibition.

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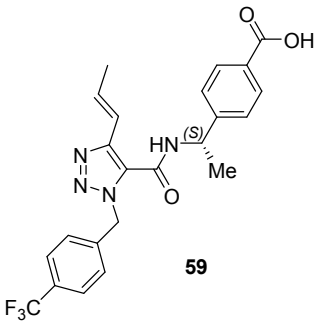

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Ca^{2+} flux IC_{50} = 6.1 nM
cAMP IC_{50} = 18.7 nM
β-Arrestin IC_{50} = 0.4 nM
IC_{50} for EP1 - EP3 > 10 μM
mPK: F = 48.0%, $t_{1/2,\ po}$ = 4.7 h
CYP Inhibition: low