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Synthesis and Structure–Activity Relationship Studies of Conformationally Flexible Tetrahydroisoquinolinyl Triazole Carboxamide and Triazole Substituted Benzamide Analogues as σ_2 Receptor Ligands

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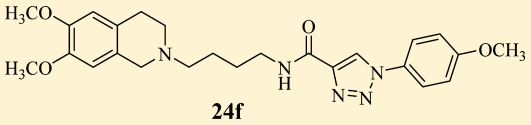
Supporting Information

ABSTRACT: Two novel classes of compounds targeting the sigma-2 (σ_2) receptor were synthesized, and their bioactivities to binding σ_1 and σ_2 receptors were measured. Four novel triazole carboxamide analogues, **24d**, **24e**, **24f**, and **39c**, demonstrated high affinity and selectivity for the σ_2 receptor. These data suggest ^{11}C-labeled versions of these compounds may be potential σ_2-selective radiotracers for imaging the proliferative status of solid tumors.



24f

$\sigma_1 = 39{,}157 \pm 2{,}771$ nM

$\sigma_2 = 5.5 + 1.9$ nM

ratio = 7719

$EC_{50} > 200$ μM

INTRODUCTION

Sigma receptors were first discovered in the 1970s and originally thought to be a type of opioid receptor.[1] Subsequent studies demonstrated that the sigma receptors are a distinct class of receptors that are expressed in the central nervous system (CNS) as well as in a variety of tissues and organs, including liver, kidneys, and endocrine glands.[2,3] Sigma receptors were pharmacologically identified as two subtypes, the sigma-1 (σ_1) and the sigma-2 (σ_2) receptors, using radioligand binding methods and biochemical analyses.[4,5] The σ_1 receptor has a molecular weight of ~25 kDa, and was purified, sequenced, and cloned from tissues of guinea pig, rat, mouse, and human, and shares 30% sequence homology with a sterol enzyme isomerase.[6−8] Investigations have shown that σ_1 receptor plays an important role in central nervous system function and is associated with various CNS disorders;[9,10] recent evidence suggests that it acts as a molecular chaperone in the endoplasmic reticulum and is involved in the regulation of oxidative stress and mitochondrial functions.[11,12] Ligands binding to σ_1 receptor regulate the release of neurotransmitters, and σ_1 receptor ligands have been developed as therapeutics for neuroprotection, anxiety, depression, psychosis, and learning and memory improvement.[13−18] The σ_1 receptor is considered a diagnostic and therapeutic target in CNS disorders associated with depression, anxiety, schizophrenia, drug abuse, and Alzheimer's disease.[19−21]

The σ_2 receptor has been described as a lipid raft protein that affects calcium signaling via sphingolipid products.[22] Although an endogenous ligand has not been identified, the biological function of the σ_2 receptor has long been linked to intracellular calcium release.[23−25] Most of what is known regarding the σ_2 receptor was obtained through the use of in vitro receptor binding studies aimed at the pharmacological characterization. The σ_2 receptor is found in many peripheral tissues including, kidneys, lung, and heart; it is highly expressed in the liver, proliferating normal tissues, and stem cells.[3,26,27] Little was known about the molecular properties of the σ_2 receptor until recent studies using a novel photoaffinity probe and confocal microscopy identified the progesterone receptor membrane component 1 (PGRMC1) protein complex as the σ_2 receptor binding site.[28]

While the role of this receptor in normal tissues is unclear, numerous studies have confirmed that the σ_2 receptor is an excellent biomarker of cell proliferation and a promising therapeutic target for inhibiting tumorigenesis.[29−35] It is known that σ_2 receptors are overexpressed in a wide variety of human and murine tumor cell lines and solid tumors such as breast adenocarcinoma, neuroblastoma, leukemia, glioblastoma, lung carcinomas, renal carcinoma, colon carcinoma, sarcoma, urinary bladder tumor, pancreas cancer, and prostate cancer.[29,36−48] Radiolabeled σ_2 receptor ligands can be used to assess the proliferative status of solid tumors with noninvasive imaging techniques, such as positron emission tomography (PET) and single photon emission computed tomography (SPECT), as tools for tumor diagnosis and tumor staging or to assist clinicians in selecting an appropriate therapy and to monitor response to treatment.[35,49−53]

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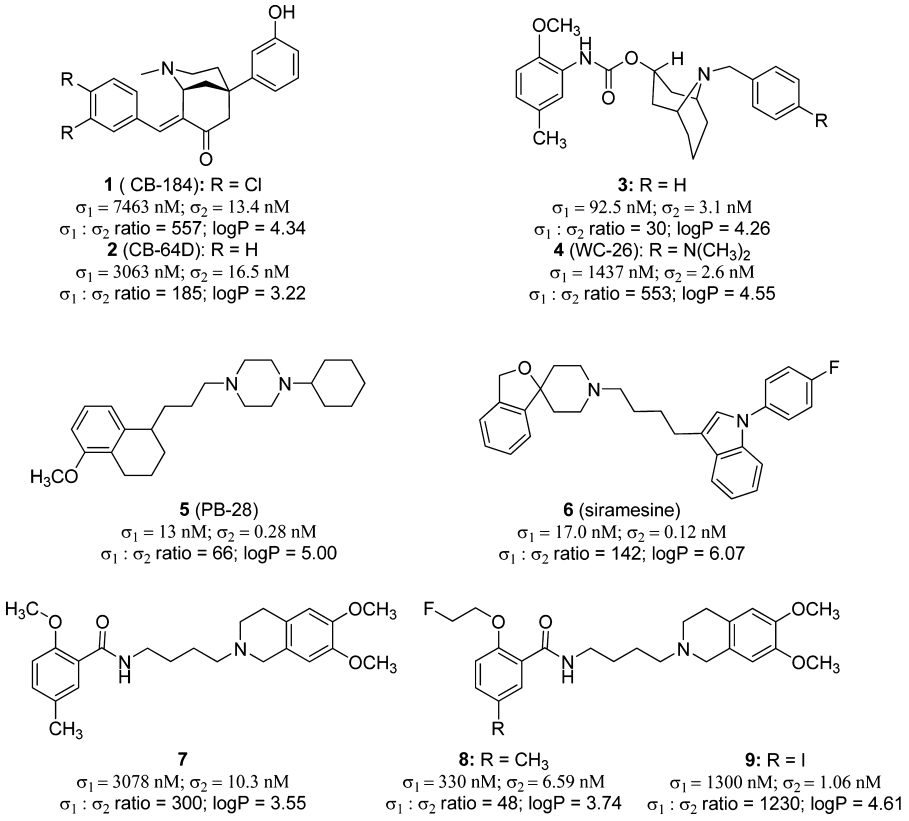

Figure 1. Representative compounds that are potent for σ_2 receptors.

The search for σ_2 receptor selective ligands has led to the identification of many compounds having modest to high affinity and selectivity for σ_2 vs σ_1 receptor. These compounds include morphanone analogues, CB-184 (**1**) and CB-64D (**2**),[54] piperazine analogues, PB28 (**5**),[42] siramesine (**6**),[55] as well as 9-azabicyclo[3.3.1]nonan-3α-yl phenylcarbamate analogues (**3** and **4**)[56,57] and the conformationally flexible tetrahydroisoquinolinyl benzamide analogues[52,53,58−61] (**7**−**9**) (Figure 1). Benzamide analogues **7**−**9** have high affinity and selectivity for σ_2 receptors in vitro and were radiolabeled. Biodistribution studies in EMT-6 tumor-bearing Balb/c mice of ^{11}C-, ^{18}F-, ^{76}Br-, and ^{125}I-labeled benzamide analogues, including **7**−**9**, demonstrated high tumor uptake and acceptable tumor/normal tissue ratios. The first human imaging studies of the σ_2 receptor ligand [^{18}F]**8** (^{18}F-ISO-1) were recently completed and demonstrate the potential utility of these compounds in imaging the proliferative status of solid tumors.[62]

The conformationally flexible tetrahydroisoquinolinyl benzamide analogues reported to date contain an electron-rich aromatic ring in the benzamide moiety.[67] There are no reports on the effect of placing a π-deficient heteroaromatic ring in this region of the benzamide analogues on σ_2 receptor affinity. As part of our continuing effort to develop improved σ_2 receptor ligands, we here report the structural modification of the benzamide moiety or carbon spacer group in conformationally flexible tetrahydroisoquinolinyl benzamide analogues. The three structural optimization strategies chosen involved (1) replacement of the benzamide ring with a 1*H*-[1,2,3]triazole or 1-substituted-[1,2,3]triazole 4-carboxamide ring; (2) shortening the spacer group of the triazole carboxamide analogues from four to two carbons should provide valuable structure−activity relationship data regarding the effects of conformational freedom of the alkyl chain linker between triazole carboxamide and tetrahydroisoquinoline moieties. (3) replacement of the 5-substituted group of the benzamide moiety of compounds **7**−**9** with a triazole group, or introduction of the triazole group in the aromatic ring in σ_2 receptor ligand *N*-(4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butyl)benzamide is expected to increase the binding affinity and selectivity for the σ_2 receptor. The results of this study revealed that introduction of a triazole ring in the benzamide moiety resulted in the identification of compounds having a high affinity and selectivity for σ_2 versus σ_1 receptors.

RESULTS

Chemistry. The synthesis of all σ_2 ligand compounds is shown in Schemes 1−3 and the synthesis of intermediates is shown in S1−S4 (Supporting Information [SI], Scheme S1−S4). The intermediate **12** was synthesized by reacting propargyl alcohol with compound **11** using sodium hydride as a base in THF, and **11** was obtained by mesylating diethylene glycol **10** with methanesulfonyl chloride. The intermediates of the primary amines, 2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethanamine **15a** and 2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butan-1-amine **15b**, were prepared by alkylation of the commercially available second amine **13** with 2-bromoacetonitrile or 4-bromobutanenitrile to give **14a** and **14b**, followed by the reduction of **14a** and **14b** with lithium aluminum hydride in THF. In the synthesis of compounds **19a**−**19c**, the amine groups of **16a**−**16c** were protected with Boc to give **17a**−**17c**, the phenol groups of **17a**−**17c** were alkylated with 1-bromo-2-fluoroethane in the presence of sodium hydride to afford **18a**−**18c**; this was followed by deprotection of the Boc group with ethyl acetate-HCl (3:1) to

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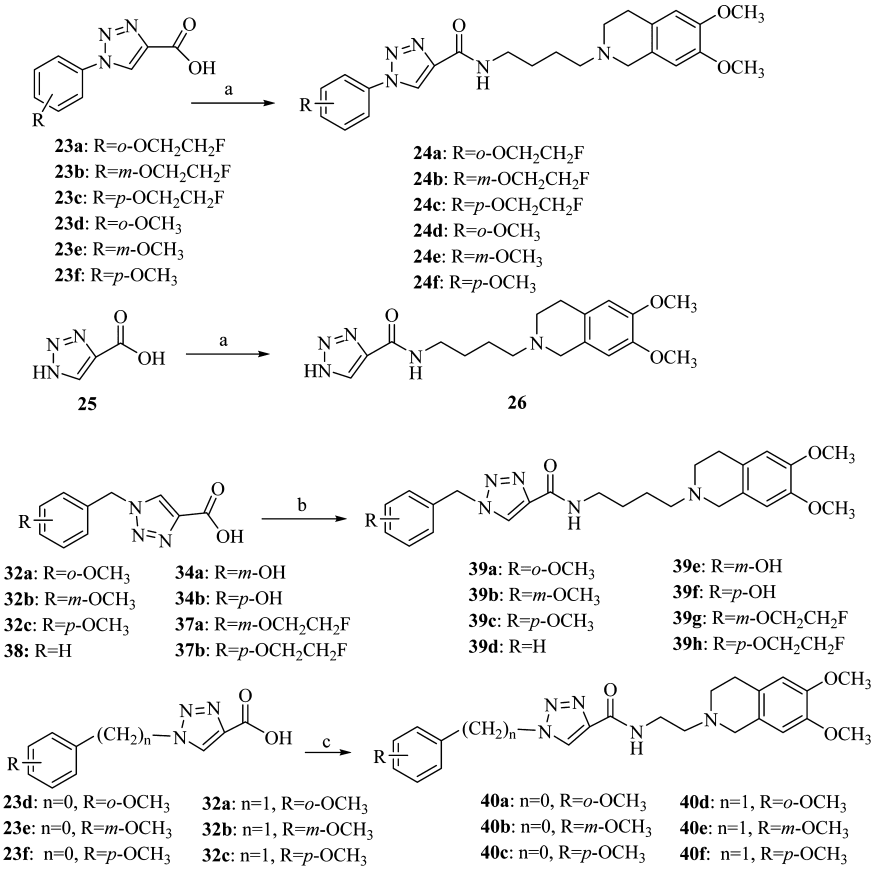
Scheme 1. Synthesis of Compounds 24a−24f, 26, 39a−39h, 44a−40f[a]



[a]Reagents and conditions: (a) **15b**, DCC, HOBt, CH_2Cl_2. (b) **15b**, CDI, CH_2Cl_2; (c) **15a**, DCC, HOBt, CH_2Cl_2.

give the required anilines **19a−19c,** respectively. The amino group of the 2-fluoroethoxy phenyl (**19a−19c**) and methoxy phenyl (**20a−20c**) were replaced with an azide group to give **21a−21f**. The Cu(I)-catalyzed (sodium ascorbate, copper sulfate, and BPDS) click reaction of ethyl propiolate with **21a−21f** afforded **22a−22f**. The substituted phenyl 1*H*-1,2,3-triazole-4-carboxylic acids **23a−23f** were prepared by basic hydrolysis of corresponding ethyl triazole carboxylates **22a−22f** (SI, Scheme S1).

Compound **29**, 3-(bromomethyl) phenyl acetate, was prepared from **27** at −78 °C in the presence of boron tribromide, followed by acetylation of **28** with acetic anhydride. The substituted benzyl azides **31a−31e**, prepared by azidation of the substituted benzyl halides **29**, **30a−30c** and **30e** with sodium azide, were treated with propiolic acid or methyl propiolate by click reaction to give the methoxy-substituted benzyl triazole 4-carboxylic acids **32a−32c** and acetoxy-substituted benzyl triazole 4-carboxylates **33a** and **33b,** respectively. The *meta*- and *para*-hydroxy benzyl triazole 4-carboxylic acids **34a** and **34b** were achieved by hydrolysis of **33a** and **33b** with sodium hydroxide. The selective hydrolysis of the phenol ester in **33a** and **33b** by sodium bicarbonate to give **35a** and **35b**, was followed by alkylation with 1-bromo-2-fluoroethane in the presence of sodium hydride to afford **36a** and **36b**, followed by hydrolyzing with sodium hydroxide to give *meta*- and *para*-(2-fluoroethoxy)benzyl triazole 4-carboxylic acids **37a** and **37b** (SI, Scheme S2).

The target compounds of 4-carbon spacer triazole carboxamides **24a−24f**, **26**, **39a−39h**, and 2-carbon spacer triazole carboxamides **40a−40f,** were synthesized by coupling amine **15a** or **15b** with the appropriate triazole 4-carboxylic acid (**23a−23f**, **25**, **32a−32c**, **34a**, **34b**, **37a**, **37b**, **38**) by activation with 1,1′-carbonyldiimidazole (CDI) or 1,3-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) as shown in Scheme 1.

The tosyloxyl groups of **41a−41c** were substituted with fluoride by nucleophilic substitution with tetrabutylammonium fluoride (TBAF) to give **42a−42c**, followed by azidation with sodium azide to give **43a−43c**. The triazole substituted benzoic acids **47b−47d** were synthesized by click reaction of **31b** and **31c** with 4-ethynylbenzoic acid or 3-ethynylbenzoic acid. Compound **49** was alkylated with 3-bromoprop-1-propyne to give **50**; and basic hydrolysis of **50** gave compound **51** (SI, Scheme S3).

Coupling **44** with **15b** to give **45**, followed by Cu(I)-catalyzed click reaction of **45** with **43a−43c** and **31a**, respectively, afforded the target triazole substituted benzamides **46a−46c**, and **48a**. Similarly, coupling **47b−47d** with **15b** gave the target triazole substituted benzamides **48b−48d**. Coupling **51** with **15b**, followed by click reaction of **52** with **43a** gave **53** (Scheme 2).

5-Iodosalicylic acid (**54**) was refluxed with dimethyl sulfate in the presence of potassium carbonate in acetone to afford methyl ester **56**, which was reacted with sodium azide in the presence of Cu(I) and L-proline to afford the azide compound **57**, which was treated with 3-dimethylamino-1-propyne, 5-ethynyl-1-methyl-1*H*-imidazole, and **12** in the presence of the Cu(I)-catalyst to afford triazole substituted benzoates **58a**, **58b**,

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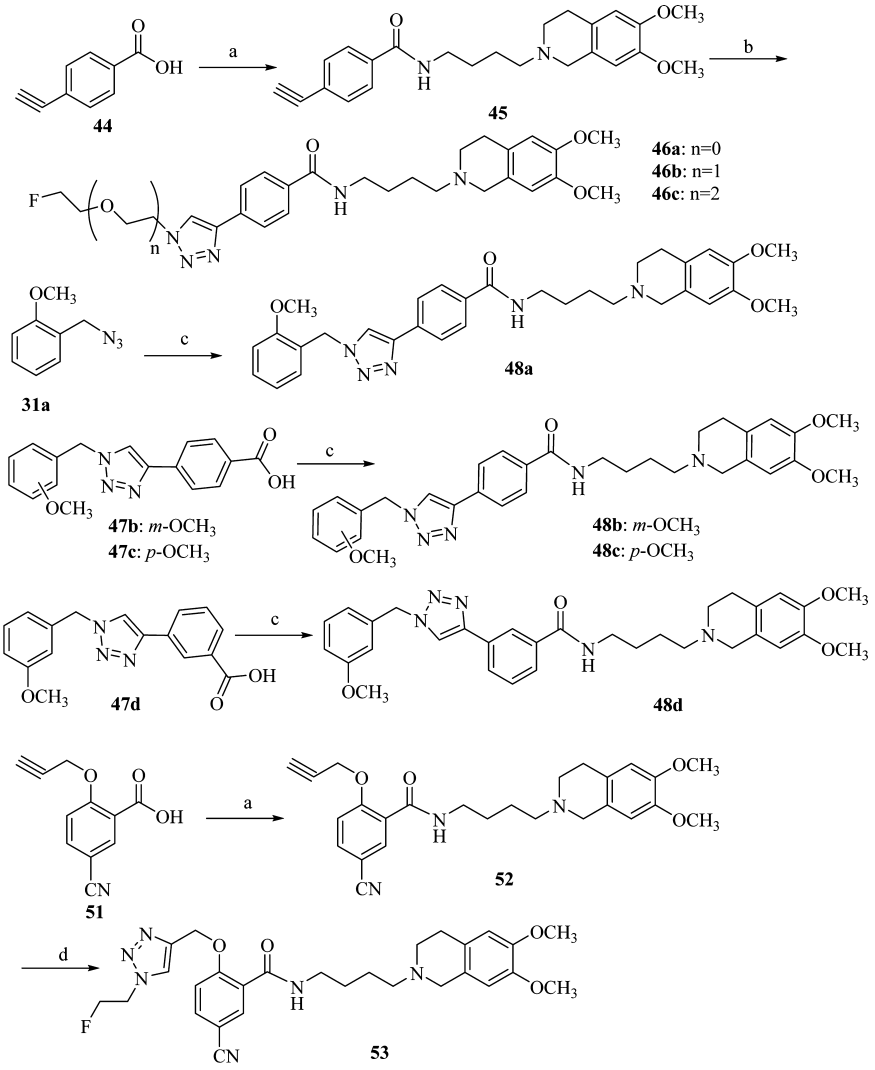
Scheme 2. Synthesis of Compounds 46a–46c, 48a–48d, 53[a]



[a]Reagents and conditions: (a) **15b**, DCC, HOBt, CH_2Cl_2; (b) **43a**, **43b**, **43c**, Cu(I), $(C_2H_5)_3N$, DMF; (c) **45**, Cu(I), $(C_2H_5)_3N$, DMF; (d) **43a**, CuSO4, sodium ascorbate, BPDS, DMF.

58d, respectively; compound **58d** was reacted with tetrabutylammonium fluoride to give **58c**. The triazole substituted benzoic acids **59a–59c** were obtained by basic hydrolysis of triazole substituted benzoates **58a–58c**. Methyl 5-iodosalicylate **55** was alkylated with 1-bromo-2-fluoroethane to give **61**, which was hydrolyzed to give **62**; coupling with **15b** afforded **63** (SI, Scheme S4).

Compounds **59a–59c** were coupled with **15b** to give **60a–6c**, respectively. Compound **65** was synthesized by substitution reaction of **63** with sodium azide in the presence of Cu(I), sodium ascorbate, and *N*,*N*′-dimethylethylenediamine to give **64**; click reaction of **64** with 3-dimethylamino-1-propyne afforded **65** (Scheme 3).

In Vitro Binding Studies. The σ_1 and σ_2 in vitro binding affinities of the new compounds were determined by the competitive inhibition method with tritiated σ ligands according to previously reported procedures.[63] The σ_1 binding sites were assayed using guinea pig brain membranes with the selective radioligand (+)-[^{3}H]pentazocine. The σ_2 binding sites were assayed in rat liver membranes, a rich source of these sites, with [^{3}H]DTG in the presence of (+)-pentazocine (100 nM) to mask σ_1 sites. The K_i values (nM) were measured from the competition curves. The results of the binding assays and the log *P* values for all novel compounds are shown in Table 1 and Table 2.

The basic conformationally flexible tetrahydroisoquinolinyl 1*H*-[1,2,3]triazole carboxamide analogue compound **26** has binding affinity for both σ_2 and σ_1 receptors. The 1-position *o*-, *m*-, *p*-methoxy phenyl substituted 1*H*-[1,2,3]triazole 4-carboxamide analogues **24d–24f** exhibited high affinity and selectivity for the σ_2 receptor, with K_i values of 1.5, 3.0, and 5.5 nM, and $K_i\sigma_1/K_i\sigma_2$ ratios of 8744-, 6731- and 7119-fold, respectively. The σ_2 receptor binding affinity trend is *o*- > *m*- > *p*-methoxy for the phenyl substituted analogues. Compared to the nonsubstituted 1*H*-[1,2,3]triazole carboxamide analogue **26**, the σ_2 binding affinity for compounds **24d–24f** were increased with 682, 341, and 186 fold, and the selectivity for σ_2 increased 817-, 629-, and 637-fold, respectively.

The 1-position *o*-, *m*-, *p*-methoxy benzyl substituted 1*H*-[1,2,3]triazole 4-carboxamide analogues **39a–39c** also showed high σ_2 affinity and selectivity with K_i values of 9.7, 10.7, and 5.0 nM and selectivity ratios of 2120-, 1183- and 674-fold, respectively. Removing the methoxy group in the benzyl moiety of **39a–39c** to give congener **39d**, slightly decreased the σ_2

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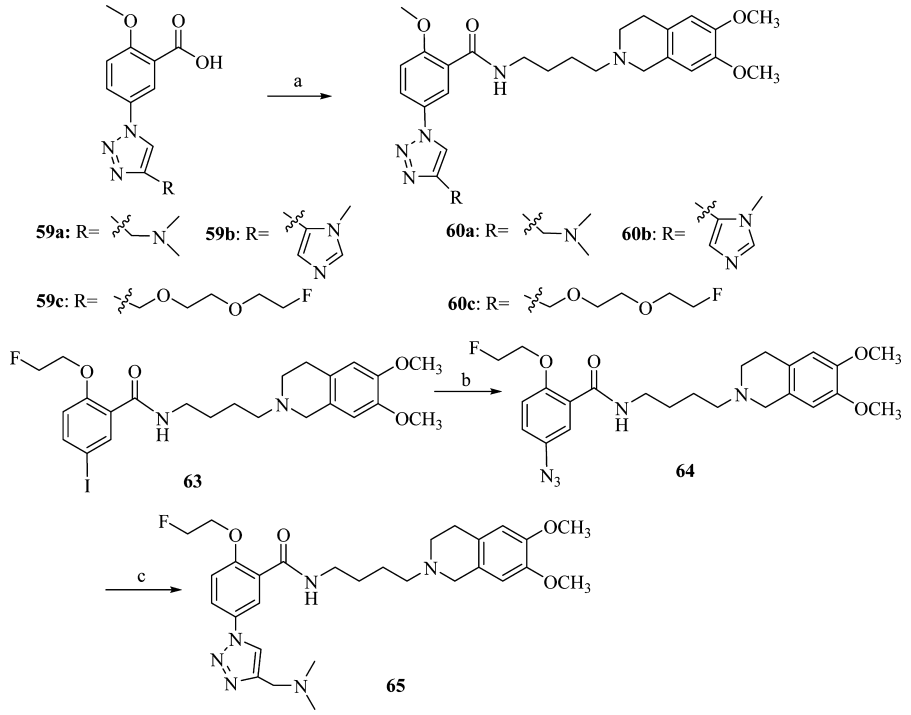
Scheme 3. Synthesis of Compounds 60a–60c, 65[a]



[a]Reagents and conditions: (a) **15b**, DCC, HOBt, CH_2Cl_2; (b) NaN_3, *N,N′*-dimethylethylenediamine, Cu(I), sodium ascorbate, DMF, 80 °C. (c) 3-Dimethylamino-1-propyne, $CuSO_4$, sodium ascorbate, BPDS, DMF.

Table 1. Binding Affinity of Triazole Carboxamide Analogues for σ_1 and σ_2 receptors

	K_i, nM[a]		selectivity	
compd	σ_1[b]	σ_2[c]	$K_i\sigma_1/K_i\sigma_2$	log *P*
24a	17116 ± 1247	24.0 ± 1.0	713	3.29
24b	17942 ± 2223	25.1 ± 1.1	715	3.29
24c	46324 ± 852	21.9 ± 0.5	2115	3.29
24d	13117 ± 227	1.5 ± 0.4	8744	3.10
24e	20195 ± 5194	3.0 ± 0.4	6731	3.10
24f	39157 ± 2771	5.5 ± 1.9	7119	3.10
26	10963 ± 176	1023 ± 23	10.7	1.32
39a	20573 ± 1953	9.7 ± 1.3	2120	3.17
39b	12665 ± 1023	10.7 ± 1.5	1183	3.17
39c	3345 ± 62	5.0 ± 1.2	669	3.17
39d	4476 ± 65	12.4 ± 1.62	361	3.57
39e	8082 ± 40	22.5 ± 0.7	359	2.9
39f	63545 ± 7443	11.6 ± 0.4	5478	2.9
39g	46254 ± 5117	62.1 ± 4.1	744	3.36
39h	21544 ± 6869	48.2 ± 3.2	447	3.36
40a	15472 ± 1573	150 ± 12	103	2.54
40b	76718 ± 29770	180 ± 9.6	426	2.54
40c	>100 000	410 ± 54	243	2.54
40d	18573 ± 275	156 ± 43	118	2.61
40e	80073 ± 21466	214 ± 11	373	2.61
40f	84510 ± 26542	93.2 ± 19	906	2.61
(+)-pentazocine	3.09 ± 0.21			
DTG		25.7 ± 1.4		4.31

[a]K_i values (mean ± SEM) were determined in at least three experiments. [b]K_i values for σ_1 receptors were measured on guinea pig brain membranes using [^{3}H](+)-pentazocine as the radioligand. [c]K_i values for σ_2 receptors were measured rat liver membranes using [^{3}H]-DTG as the radioligand in the presence of (+)-pentazocine.

binding affinity (K_i = 12.4 nM). In comparison to **26**, the σ_2 affinity for **39a–39d** increased 105-, 96-, 205-, and 82-fold, and selectivity increased 198-, 110-, 63- and 34-fold, respectively. The hydroxyl-substituted 1-benzyl [1,2,3]triazole 4-carboxamides analogues **39e** and **39f** showed 2-fold lower binding affinity for σ_2 receptor (K_i = 22.5 nM for **39e**; 11.6 nM for **39f**)

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Table 2. Binding Affinity of Triazole Substituted Benzamide Analogues for σ_1 and σ_2 receptors

compd	K_i, nM[a]		selectivity	log P
	σ_1[b]	σ_2[c]	$K_i\sigma_1/K_i\sigma_2$	
46a	71882 ± 3704	139 ± 12	517	3.43
46b	14703 ± 290	137 ± 3.0	107	3.27
46c	15968 ± 46	212 ± 3.0	75	3.11
48a	8580 ± 510	55.0 ± 0.1	156	4.84
48b	11479 ± 2186	66.2 ± 3.7	174	4.84
48c	12606 ± 1022	31.4 ± 2.9	406	4.84
48d	1752 ± 81	66.0 ± 2.2	26	4.84
52	3678 ± 554	12.2 ± 2.7	301	3.32
53	14100 ± 1053	31.9 ± 1.0	442	3.39
60a	22445 ± 2769	56.8 ± 2.3	395	3.04
60b	6359 ± 98	20.7 ± 1.5	307	2.36
60c	11479 ± 103	66.0 ± 2.8	174	2.92
65	21913 ± 314	94.0 ± 3.8	232	3.23
(+)-pentazocine	3.09 ± 0.21			
DTG		25.7 ± 1.4		4.31

[a] K_i values (mean ± SEM) were determined in at least three experiments. [b] K_i values for σ_1 receptors were measured on guinea pig brain membranes using [^{3}H](+)-pentazocine as the radioligand. [c] K_i values for σ_2 receptors were measured on rat liver membranes using [^{3}H]-DTG as the radioligand in the presence of (+)-pentazocine.

than corresponding methoxy-substituted congeners **39b** and **39c**.

Analogues **24a–24c**, **39g**, and **39h** were prepared by substituting the methoxy group of **24d–24f**, **39b**, and **39c** with a fluoroethoxy group. The compounds containing a fluoroethoxy group (**24a–24c**, **39g**, and **39h**) showed modest affinity for σ_2 receptors, with K_i values of 24.0, 25.1, 21.9, 62.1, and 48.2 nM respectively, which was lower than the compounds containing methoxy group (**24d–24f**, **39b**, and **39c**).

Analogues with a 2-carbon spacer linker, **40a–40f**, were synthesized to explore the effect of the alkyl chain length. These analogues had lower σ_2 receptor binding affinity than the 4-carbon spacer compounds. K_i values for **40a–40f** were 150, 180, 410, 156, 214, and 93.2 nM, respectively, which is 100-, 60-, 75-, 16-, 20- and 19-fold lower than the 4-carbon spacer congeners **24d–24f** and **39a–39c**.

Triazole-substituted conformationally flexible tetrahydroisoquinolinyl benzamide analogues were designed with the aim of determining the effect of the triazole group on the aromatic ring of the benzamide moiety. Although the σ_2 receptor binding affinity of **46a–46c** improved relative to the nonselective parent compound *N*-(4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butyl)benzamide (K_i = 5290 for σ_2; >10^7 for σ_1), **46a–46c** still had low binding affinities for σ_2 receptors with K_i values of 139, 137, 212 nM. The length of the fluoro-PEG group at 1-position of triazole ring in **46a–46c** only had a slightly positive effect on σ_2 receptor binding affinity. Compounds **48a–48c** were prepared by replacing the fluoro-PEG group of **46a–46c** with methoxy benzyl group, and **48a–48c** showed moderate binding affinity for σ_2 receptors with K_i values of 55.0, 66.2, and 31.4 nM, respectively. In addition, there were no remarkable differences in σ_2 receptor binding affinity between *o*-, *m*- and *p*-methoxy substituted congeners **48a–48c**. Compound **48d**, a congener of **48b**, in which the triazole group is located on the meta-position of the aromatic ring, also displayed moderate σ_2 receptor binding affinity with K_i value of 66.0 nM, the same as **48b**.

Compound **53** was prepared through replacement of the 5-methyl and 2-methoxy groups of lead compound **7** with a cyano group and a triazole group, respectively. This structural modification resulted in moderate σ_2 receptor binding affinity for **53** with K_i value of 31.9 nM, which was lower than those of its precursor **52** (K_i = 12.2 nM) and lead compound **7** (K_i =10.3 nM). The replacement of the 5-methyl of lead compound **7** with different triazole groups led to the synthesis of compounds **60a–60c**. These three new compounds exhibited moderate σ_2 receptor binding affinity with K_i values of 56.8, 20.7, and 66.0 nM, respectively, which apparently reduced σ_2 receptor binding affinity compared to that of lead compound **7**. Compound **65** was also synthesized by replacement of the 5-methyl or 5-iodo groups of lead compounds **8** and **9** with the substituted triazole group. The σ_2 receptor binding affinity of **65** remarkably decreased, with a K_i value of 94.0 nM, 14- and 89-fold lower than lead compound **8** (K_i = 6.59 nM) and **9** (K_i = 1.06 nM), respectively.

Functional Assay. In the novel conformationally flexible tetrahydroisoquinolinyl triazole carboxamide analogues, four compounds **24d–24f** and **39c** exhibited high σ_2 receptor binding affinity and excellent selectivity for σ_2 versus σ_1 receptor as well as the lower lipophilicity. These data showed that the corresponding ^{11}C-labeled analogues may be promising candidates for radiotracers imaging the σ_2 receptor status of solid tumors with PET.

Many σ_2 ligands with diverse structures have been shown to induce cell death in a variety of cancer cells by triggering caspase-dependent and independent cell death. Our group uses functional assays to define agonist/antagonist σ_2 receptor ligands based on the cytotoxicity of a novel ligand relative to that of siramesine.[64,65,68] The EC_{50} value of **24f** was determined for EMT-6 murine breast cancer cells using a cell viability assay.[68] As shown in Table 3, **24f** did not induce cytotoxicity, while siramesine exhibited potent cytotoxicity. Thus, **24f** is a σ_2 receptor antagonist.

Table 3. EC_{50} of Sigma Ligands in EMT-6 Cell Line Using MTS Assay

cmpd	EC_{50} ± SEM (24 h)	EC_{50} ± SEM (48 h)
24f	>200 μM	>200 μM
siramesine	14.9 ± 4.0 μM	5.3 ± 1.0 μM

DISCUSSION

The goal of the current study was to develop new σ_2 receptor ligands possessing a high affinity and selectivity for σ_2 receptor versus σ_1 receptor. 1*H*-[1,2,3]triazole, a π-deficient five-membered heterocyclic ring, is readily prepared from primary azides and terminal alkynes through the Huisgen 1,3-dipolar cycloaddition reaction, also known as a "click reaction". Triazole derivatives are known to possess remarkable pharmacological properties, such as antiallergic, antimicrobial, anticancer, anti-HIV, anti-inflammatory, and anticonvulsant activities, that have tremendous applications in drug discovery. The triazole moiety is stable to metabolic degradation and capable of hydrogen bonding, which could be favorable in binding biomolecular targets as well as in increasing solubility. It was reported that introduction of a triazole group in the amino acid structure of the tumor imaging agent improved the

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tumor uptake and tumor/background ratio in biodistribution and PET imaging studies. Therefore, a series of sigma receptor ligands containing a triazole group were prepared and their binding affinity for σ_2 and σ_1 receptor were measured.

The results of the binding affinity reveal that the 4-carbon spacer conformationally flexible tetrahydroisoquinolinyl triazole carboxamide analogues with substituted phenyl or benzyl group in the 1-position of the [1,2,3]triazole ring, such as **24d**−**24f**, **39a**−**39c**, and **39f**, displayed a higher or similar σ_2 receptor binding affinity and greater σ_1/σ_2 selectivity than lead compound **7**. On the other hand, 2-carbon spacer conformationally flexible tetrahydroisoquinolinyl triazole carboxamide analogues have lower σ_2 receptor binding affinity relative to corresponding 4-carbon spacer congeners, suggesting the alkyl chain linker in the tetrahydroisoquinolinyl triazole carboxamide analogues is sensitive for σ_2 receptor binding affinity.

The effects of triazole group on the benzene aromatic ring of the benzamide moiety analogues were studied. Although the analogues, which have a fluoro-PEG group or methoxy benzyl group at 1-position of triazole ring, improved the σ_2 receptor binding affinity compare with its nonsubstituted benzene parent compound, they still had lower binding affinity for σ_2 receptors than **7**. Similarly, replacement of the 5-methyl group of **7** with a different substituted triazole group led to reduction of the σ_2 receptor binding affinity.

Compound **24f** (σ_1 = 39157 nM; σ_2 = 5.5 nM) has high affinity and selectivity for that σ_2 receptor. Its EC_{50} value in EMT6 tumor cells (>200 μM in 24 h and >200 μM in 48 h, respectively) is much high than the EC_{50} value of siramesine (14.9 μM in 24 h and 5.3 μM in 48 h, respectively). These results show that **24f** is an antagonist at the σ_2 receptor. These data also suggest that ^{11}C-radiolabeled **24f** may be a useful PET radiotracer for imaging the σ_2 status of solid tumors. The high σ_2 receptor affinity and selectivity versus σ_1 receptors indicate that ^{11}C-labeled versions of **24d**, **24e**, and **39c** may also be potential PET radiotracers for imaging the σ_2 receptor status of solid tumors. The calculated log P value of these compounds (3.10−3.17), is also within the range expected to give a high uptake in tumors;[59] however, this method for determining the lipophilicity of a compounds is only an approximation and the actual octanol−water partition coefficient of the radiolabeled compounds needs to be measured directly. The evaluation of these putative PET radiotracers is currently ongoing in our group.

In conclusion, in the present study, two novel classes of compounds targeting the σ_2 receptor were synthesized. Four novel triazole carboxamide analogues **24d**, **24e**, **24f**, and **39c** demonstrated high binding affinity and excellent selectivity for σ_2 receptors. Compound **24f** was found to be an antagonist at σ_2 receptors. These studies suggest that ^{11}C-labeled versions of **24d**, **24e**, **24f**, and **39c** are potential radioligands for PET imaging studies of the σ_2 receptor.

EXPERIMENTAL SECTION

General Methods and Materials. All reagents and chemicals were obtained from standard commercial sources and used without further purification. All reactions were carried out by standard air-free and moisture-free techniques under an inert nitrogen atmosphere with dry solvents unless otherwise stated. Flash column chromatography was conducted using Scientific Adsorbents, Inc. silica gel, 60A, "40 Micron Flash" (32−63 μm). Melting points were determined using MEL-TEMP 3.0 apparatus and are uncorrected. Routine ^{1}H NMR spectra were recorded at 300 MHz on a Varian Mercury-VX spectrometer. All chemical shifts were reported as a part per million (δ ppm) and tetramethylsilane (TMS) as internal standard. Peak multiplicities are singlet, s; doublet, d; triplet t; double doublet, dd; multiplet, m; broad, br. All coupling constants (J) are given in hertz (Hz). HR-MS was performed on a Waters ZQ 4000 single quadrupole mass spectrometer equipped with an electrospray ionization (ESI) LC−MS interface. Elemental analyses (C, H, N) were determined by Atlantic Microlab, Inc. Elemental analysis, HR-MS and HPLC were used to determine the purity of the target compounds that were used for binding assay. The purity of all the final compounds was >95% as determined by analytical HPLC (Table S2, SI).

***N*-(4-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)butyl)-1-(2-(2-fluoroethoxy)phenyl)-1*H*-1,2,3-triazole-4-carboxamide (24a).** 1,3-Dicyclohexylcarbodiimide (290 mg, 1.41 mmol) and 1-hydroxybenzotriazole (190 mg, 1.41 mmol) were added to a solution of **23a** (296 mg, 1.18 mmol) and 15b (315 mg, 1.18 mmol) in 15 mL dichloromethane at 0 °C in ice−water bath. The reaction mixture was stirred overnight. After the reaction was completed as determined by TLC with ethyl acetate/methanol (2/1, v/v) mobile phase, 20 mL dichloromethane was added, and the mixture was filtered. The filtrate was sequentially washed with saturated sodium carbonate and brine and then dried over anhydrous sodium sulfate. After removal of the solvent under reduced pressure, the crude product was purified by silica gel column chromatography eluting with ethyl acetate/methanol (5/1, v/v) to afford **24a** as a colorless oil (219 mg, 37.3% yield). For in vitro binding experiments, the free base was converted into the corresponding oxalate salt, mp (oxalate salt): 175.0−175.6 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.74 (m, 4H), 2.57 (t, J = 5.5 Hz, 2H), 2.73 (t, J = 5.5 Hz, 2H), 2.84 (t, J = 5.5 Hz, 2H), 3.56 (t, J = 5.5 Hz, 2H), 3.57 (s, 2H), 3.84 (s, 6H), 4.28 (t, J = 3.9 Hz, 1H), 4.38 (t, J = 3.9 Hz, 1H), 4.64 (t, J = 3.9 Hz, 1H), 4.80 (t, J = 3.9 Hz, 1H), 6.54 (s, 1H), 6.59 (s, 1H), 7.11−7.19 (m, 2H), 7.41−7.49 (m, 2H), 7.79 (dd, J = 8.0, 1.6 Hz, 1H), 8.62 (s, 1H). Anal. ($C_{26}H_{32}FN_5O_4{\cdot}H_2C_2O_4$) C, H, N.

***N*-(4-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)butyl)-1-(3-(2-fluoroethoxy)phenyl)-1*H*-1,2,3-triazole-4-carboxamide (24b)** was prepared from **23b** (296 mg, 1.18 mmol) and **15b** (315 mg, 1.18 mmol) as described above for **24a**. The crude product was purified by silica gel column chromatography eluting with ethyl acetate/methanol (3/1, v/v) to afford **24b** as a white solid (221 mg, 40.1% yield), mp: 128.6−129.4 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.73 (m, 4H), 2.55 (t, J = 5.5 Hz, 2H), 2.70 (t, J = 5.5 Hz, 2H), 2.82 (t, J = 5.5 Hz, 2H), 3.54 (br s, 4H), 3.82 (s, 6H), 4.21 (t, J = 3.9 Hz, 1H), 4.31 (t, J = 3.9 Hz, 1H), 4.68 (t, J = 3.9 Hz, 1H), 4.84 (t, J = 3.9 Hz, 1H), 6.52 (s, 1H), 6.58 (s, 1H), 6.99 (d, J = 7.5 Hz, 1H), 7.34−7.43 (m, 3H), 7.71 (br s, 1H), 8.68 (s, 1H). Anal. ($C_{26}H_{32}FN_5O_4$) C, H, N.

***N*-(4-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)butyl)-1-(4-(2-fluoroethoxy)phenyl)-1*H*-1,2,3-triazole-4-carboxamide (24c)** was prepared from **23c** (296 mg, 1.18 mmol) and **15b** (315 mg, 1.18 mmol) as described above for **24a**. The crude product was purified by silica gel column chromatography eluting with ethyl acetate/methanol (5/1, v/v) to afford **24c** as a white solid (142 mg, 24.2% yield), mp: 162.5−163.8 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.73 (m, 4H), 2.56 (t, J = 5.5 Hz, 2H), 2.72 (t, J = 5.5 Hz, 2H), 2.83 (t, J = 5.5 Hz, 2H), 3.53−3.56 (br s, 4H), 3.83 (s, 6H), 4.22 (t, J = 3.0 Hz, 1H), 4.31 (t, J = 3.0 Hz, 1H), 4.71 (t, J = 3.0 Hz, 1H), 4.86 (t, J = 3.0 Hz, 1H), 6.53 (s, 1H), 6.59 (s, 1H), 7.06 (d, J = 8.1 Hz, 2H), 7.56 (t, J = 2.6 Hz, 1H), 7.66 (d, J = 8.1 Hz, 2H), 8.49 (s, 1H). Anal. ($C_{26}H_{32}FN_5O_4$) C, H, N.

***N*-(4-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)butyl)-1-(2-methoxyphenyl)-1*H*-1,2,3-triazole-4-carboxamide (24d)** was prepared from **23d** (329 mg, 1.5 mmol) and **15b** (476 mg, 1.8 mmol) as described above for **24a**. The crude product was purified by silica gel column chromatography eluting with ethyl acetate/methanol (4/1, v/v) to afford **24d** as a colorless oil (302 mg, 43.3% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.73 (m, 4H), 2.54 (t, J = 5.4 Hz, 2H), 2.70 (t, J = 5.4 Hz, 2H), 2.82 (t, J = 5.4 Hz, 2H), 3.54 (br s, 4H), 3.81 (s, 6H), 3.85 (s, 3H), 6.53 (s, 1H), 6.58 (s, 1H), 7.06−7.11 (m, 2H), 7.66 (t, J = 7.8 Hz, 1H), 7.72−7.78 (m, 2H), 8.65 (s, 1H). Anal. ($C_{25}H_{31}N_5O_4$) C, H, N.

***N*-(4-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)butyl)-1-(3-methoxyphenyl)-1*H*-1,2,3-triazole-4-carboxamide (24e)** was pre-

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pared from **23e** (219 mg, 1 mmol) and **15b** (264 mg, 1 mmol) as described above for **24a**. The crude product was purified by silica gel column chromatography eluting with ethyl acetate/dichloromethane/methanol (6/1/1, v/v/v) to afford **24e** as a white solid (124 mg, 26.6% yield), mp: 129.6−130.6 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.73−1.75 (m, 4H), 2.57 (t, J = 6.3 Hz, 2H), 2.73 (t, J = 5.4 Hz, 2H), 2.82 (t, J = 5.4 Hz, 2H), 3.55 (t, J = 6.3 Hz, 2H), 3.57 (s, 2H), 3.84 (s, 6H), 3.89 (s, 3H), 6.53 (s, 1H), 6.59 (s, 1H), 7.01 (dd, J = 8.4, 2.4 Hz, 1H), 7.28 (dd, J = 8.4, 2.4 Hz, 1H), 7.33 (t, J = 2.4 Hz, 1H), 7.44 (t, J = 8.4 Hz, 1H), 7.46 (overlapped, 1H), 8.51 (s, 1H). Anal. ($C_{25}H_{31}N_5O_4$) C, H, N.

***N*-(4-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)butyl)-1-(4-methoxyphenyl)-1*H*-1,2,3-triazole-4-carboxamide (24f)** was prepared from **23f** (219 mg, 1 mmol) and **15b** (264 mg, 1 mmol) as described above for **24a**. The crude product was purified by silica gel column chromatography eluting with ethyl acetate/methanol (4/1, v/v) to afford **24f** as a white solid (113 mg, 24.3% yield), mp: 164.9−165.6 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.73 (m, 4H), 2.57 (t, J = 6.8 Hz, 2H), 2.73 (t, J = 5.6 Hz, 2H), 2.84 (t, J = 5.6 Hz, 2H), 3.53−3.57 (m, 4H), 3.83 (s, 6H), 3.87 (s, 3H), 6.53 (s, 1H), 6.59 (s, 1H), 7.03 (d, J = 9.3 Hz, 2H), 7.49 (t, J = 5.6 Hz, 1H), 7.65 (d, J = 9.3 Hz, 2H), 8.43 (s, 1H). Anal. ($C_{25}H_{31}N_5O_4$) C, H, N.

N-(4-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)butyl)-1*H*-1,2,3-triazole-4-carboxamide (**26**) was prepared from 1*H*-1,2,3-triazole-4-carboxylic acid **25** (113 mg, 1 mmol) and **15b** (260 mg, 1 mmol) as described above for **24a**. The crude product was purified by silica gel column chromatography eluting with dichloromethane/methanol (5/1, v/v) to afford **26** as a white solid (54 mg, 15% yield), mp: 172−174 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.30−1.45 (m, 4H), 2.62−2.70 (m, 2H), 2.86 (s, 2H), 3.02−3.12 (m, 2H), 3.42−3.58 (m, 4H), 3.81 (s, 6H), 6.50 (s, 1H), 6.56 (s, 1H), 7.48 (br s, 1H), 8.13 (s, 1H). HR-MS (ESI, $[M + H]^+$) calcd for $C_{18}H_{25}N_5O_3$ + H, 360.2030; found, m/z 360.2027.

***N*-(4-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)butyl)-1-(2-methoxybenzyl)-1*H*-1,2,3-triazole-4-carboxamide (39a).** 1,1′-Carbonyldiimidazole (140 mg, 0.86 mmol) was added to a solution of **32a** (200 mg, 0.86 mmol) in 10 mL dichloromethane and 0.5 mL DMF at 0 °C. Then **15b** (206 mg, 0.78 mmol) in 10 mL dichloromethane was added, and the mixture was stirred overnight. Twenty mL dichloromethane was added, and the mixture was sequentially washed with sodium carbonate and brine and then dried with anhydrous sodium sulfate. After concentration under reduced pressure, the residue was purified by silica gel column chromatography eluting with ethyl acetate/methanol (10/2, v/v) to afford **39a** as a colorless oil (280 mg, 75% yield). For in vitro binding experiments, the free base was converted into the corresponding oxalate salt, mp (oxalate salt): 169−170 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.68 (br s, 4H), 2.53 (t, J = 5.7 Hz, 2H), 2.71 (t, J = 6.0 Hz, 2H), 2.83 (t, J = 5.7 Hz, 2H), 3.40−3.52 (m, 2H), 3.55 (s, 2H), 3.83 (s, 6H), 3.86 (s, 3H), 5.55 (s, 2H), 6.52 (s, 1H), 6.58 (s, 1H), 6.90−7.00 (m, 2H), 7.27−7.40 (m, 3H), 7.99 (s, 1H). Anal. ($C_{26}H_{33}N_5O_4 \cdot H_2C_2O_4$) C, H, N.

***N*-(4-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)butyl)-1-(3-methoxybenzyl)-1*H*-1,2,3-triazole-4-carboxamide (39b)** was prepared from **32b** (117 mg, 0.5 mmol) and **15b** (132 mg, 0.5 mmol) as described above for **39a**. The crude product was purified by silica gel column chromatography eluting with ethyl acetate/methanol (10/3, v/v) to afford **39b** as a white solid (120 mg, 50% yield), mp (oxalate salt): 167−169 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.65−1.75 (m, 4H), 2.56 (t, J = 3.3 Hz, 2H), 2.73 (t, J = 5.4 Hz, 2H), 2.84 (t, J = 5.7 Hz, 2H), 3.42−3.53 (m, 2H), 3.57 (s, 2H), 3.79 (s, 3H), 3.83 (s, 3H), 3.84 (s, 3H), 5.51 (s, 2H), 6.52 (s, 1H), 6.59 (s, 1H), 6.79 (t, J = 5.7 Hz, 1H), 6.83−6.93 (m, 2H), 7.27−7.36 (m, 2H), 7.95 (s, 1H). HR-MS (ESI, $[M + H]^+$) calcd for $C_{26}H_{33}N_5O_4$ + H, 480.2611; found, m/z 480.2623.

***N*-(4-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)butyl)-1-(4-methoxybenzyl)-1*H*-1,2,3-triazole-4-carboxamide (39c)** was prepared from **32c** (117 mg, 0.5 mmol) and **15b** (132 mg, 0.5 mmol) as described above for **39a**. The crude product was purified by silica gel column chromatography eluting with ethyl acetate/methanol (10/3, v/v) to afford **39c** as a white solid (112 mg, 47% yield), mp (oxalate salt): 171−173 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.60−1.75 (m, 4H), 2.45−2.60 (m, 2H), 2.70 (t, J = 5.4 Hz, 2H), 2.82 (t, J = 5.7 Hz, 2H), 3.42−3.50 (m, 2H), 3.54 (s, 2H), 3.80 (s, 3H), 3.83 (s, 6H), 5.47 (s, 2H), 6.51 (s, 1H), 6.58 (s, 1H), 6.88 (d, J = 6.0 Hz, 2H), 7.20−7.36 (m, 3H), 7.89 (s, 1H). HR-MS (ESI, $[M + H]^+$) calcd for $C_{26}H_{33}N_5O_4$ + H, 480.2611; found, m/z 480.2612.

1-Benzyl-*N*-(4-(6,7-dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-butyl)-1*H*-1,2,3-triazole-4-carboxamide (39d) was prepared from **38** (152 mg, 0.74 mmol) and **15b** (176 mg, 0.67 mmol) as described above for **39a**. The crude product was purified by silica gel column chromatography eluting with ethyl acetate/methanol (10/1, v/v) to afford **39d** as a white solid (122 mg, 40.5% yield), mp (oxalate salt): 176−178 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.56−1.80 (m, 4H), 2.55 (t, J = 5.0 Hz, 2H), 2.72 (t, J = 5.8 Hz, 2H), 2.84 (t, J = 5.8 Hz, 2H), 3.48 (d, J = 5.0 Hz, 2H), 3.56 (s, 2H), 3.83 (s, 3H), 3.84 (s, 6H), 5.54 (s, 2H), 6.52 (s, 1H), 6.59 (s, 1H), 7.25−7.36 (m, 3H), 7.36−7.44 (m, 3H), 7.93 (s, 1H). HR-MS (ESI, $[M + H]^+$) calcd for $C_{25}H_{31}N_5O_3$ + H, 450.2505; found, m/z 450.2519.

***N*-(4-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)butyl)-1-(3-hydroxybenzyl)-1*H*-1,2,3-triazole-4-carboxamide (39e)** was prepared from **34a** (370 mg, 1.69 mmol) and **15b** (458 mg, 1.73 mmol) as described above for **39a**. The crude product was purified by silica gel column chromatography eluting with ethyl acetate/methanol (10/1, v/v) to afford **39e** as a white solid (430 mg, 55% yield), mp (free base): 151−153 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.65 (br s, 4H), 2.56 (br s, 2H), 2.77 (t, J = 4.8 Hz, 2H), 2.83 (t, J = 4.8 Hz, 2H), 3.45 (t, J = 4.8 Hz, 2H), 3.59 (s, 2H), 3.83 (s, 6H), 5.40 (s, 2H), 6.53 (s, 1H), 6.57 (s, 1H), 6.61 (s, 1H), 6.72 (d, J = 7.8 Hz, 2H), 7.13 (t, J = 7.8 Hz, 1H), 7.80 (t, J = 6.0 Hz, 1H), 8.02 (s, 1H). HR-MS (ESI, $[M + H]^+$) calcd for $C_{25}H_{31}N_5O_4$ + H, 466.2454; found, m/z 466.2446.

***N*-(4-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)butyl)-1-(4-hydroxybenzyl)-1*H*-1,2,3-triazole-4-carboxamide (39f)** was prepared from **34b** (82 mg, 0.37 mmol) and **15b** (99 mg, 0.37 mmol) as described above for **39a**. The crude product was purified by silica gel column chromatography eluting with ethyl acetate/methanol (10/1, v/v) to afford **39f** as a white solid (46 mg, 27% yield), mp (free base): 119−120 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.70 (br s, 4H), 2.58 (br s, 2H), 2.78 (t, J = 5.1 Hz, 2H), 2.84 (t, J = 4.8 Hz, 2H), 3.48 (t, J = 5.7 Hz, 2H), 3.61 (s, 2H), 3.83 (s, 6H), 5.40 (s, 2H), 6.51 (s, 1H), 6.58 (s, 1H), 6.74 (d, J = 8.4 Hz, 2H), 7.06 (d, J = 8.4 Hz, 2H), 7.46 (t, J = 5.7 Hz, 1H), 7.91 (s, 1H). HR-MS (ESI, $[M + H]^+$) calcd for $C_{25}H_{31}N_5O_4$ + H, 466.2454; found, m/z 466.2443.

***N*-(4-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)butyl)-1-(3-(2-fluoroethoxy)benzyl)-1*H*-1,2,3-triazole-4-carboxamide (39g)** was prepared from **37a** (165 mg, 0.62 mmol) and **15b** (164 mg, 0.62 mmol) as described above for **39a**. The crude product was purified by silica gel column chromatography eluting with ethyl acetate/methanol (10/1, v/v) to afford **39g** as a white solid (230 mg, 72.5% yield), mp (oxalate salt): 165−167 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.70 (br s, 4H), 2.55 (br s, 2H), 2.72 (t, J = 5.7 Hz, 2H), 2.83 (t, J = 5.1 Hz, 2H), 3.48 (t, J = 6.0 Hz, 2H), 3.56 (s, 2H), 3.83 (s, 6H), 4.14 (t, J = 3.9 Hz, 1H), 4.23 (t, J = 3.9 Hz, 1H), 4.66 (t, J = 3.9 Hz, 1H), 4.82 (t, J = 3.9 Hz, 1H), 5.51 (s, 2H), 6.52 (s, 1H), 6.58 (s, 1H), 6.82 (s, 1H), 6.89 (d, J = 8.2 Hz, 1H), 6.92 (d, J = 8.2 Hz, 1H), 7.25−7.36 (m, 2H), 7.95 (s, 1H). Anal. ($C_{27}H_{34}FN_5O_4 \cdot H_2C_2O_4$) C, H, N.

***N*-(4-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)butyl)-1-(4-(2-fluoroethoxy)benzyl)-1*H*-1,2,3-triazole-4-carboxamide (39h)** was prepared from **37b** (156 mg, 0.59 mmol) and **15b** (155 mg, 0.59 mmol) as described above for **39a**. The crude product was purified by silica gel column chromatography eluting with ethyl acetate/methanol (10/1, v/v) to afford **39h** as a white solid (197 mg, 65.3% yield), mp (oxalate salt): 188−190 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.69 (br s, 4H), 2.54 (br s, 2H), 2.72 (t, J = 4.8 Hz, 2H), 2.8d (t, J = 4.8 Hz, 2H), 3.48 (t, J = 5.7 Hz, 2H), 3.56 (s, 2H), 3.84 (s, 6H), 4.17 (t, J = 3.9 Hz, 1H), 4.26 (t, J = 3.9 Hz, 1H), 4.68 (t, J = 3.9 Hz, 1H), 4.84 (t, J = 3.9 Hz, 1H), 5.48 (s, 2H), 6.52 (s, 1H), 6.59 (s, 1H), 6.93 (d, J = 8.4 Hz, 2H), 7.18−7.38 (m, 3H), 7.95 (s, 1H). Anal. ($C_{27}H_{34}FN_5O_4 \cdot H_2C_2O_4 \cdot 0.5H_2O$) C, H, N.

N-(2-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)ethyl)-1-(2-methoxyphenyl)-1*H*-1,2,3-triazole-4-carboxamide (**40a**) was prepared from **23d** (263 mg, 1.2 mmol) and **15a** (236 mg, 1 mmol) as described above for **24a**. The crude product was purified by silica gel column chromatography eluting with ethyl acetate/methanol (30/1, v/v) to afford **40a** as colorless gel (106 mg, 24.2% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.79−2.86 (m, 6H), 3.65 (s, 1H), 3.67−3.72 (m, 3H), 3.83 (s, 3H), 3.84 (s, 3H), 3.88 (s, 3H), 6.54 (s, 1H), 6.61 (s, 1H), 7.07−7.12 (m, 2H), 7.43 (t, J = 8.0 Hz, 1H), 7.76−7.78 (m, 2H), 8.64 (s, 1H). Anal. ($C_{23}H_{27}N_5O_4$) C, H, N.

N-(2-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)ethyl)-1-(3-methoxyphenyl)-1*H*-1,2,3-triazole-4-carboxamide (**40b**) was prepared from **23e** (236 mg, 1.2 mmol) and **15a** (236 mg, 1 mmol) as described above for **24a**. The crude product was purified by silica gel column chromatography eluting with ethyl acetate/methanol (20/1, v/v) to afford **40b** as white solid (224 mg, 51.2% yield), mp: 172.7−174.2 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.78−2.86 (m, 6H), 3.66 (s, 1H), 3.67−3.73 (m, 3H), 3.84 (s, 3H), 3.85 (s, 3H), 3.88 (s, 3H), 6.53 (s, 1H), 6.61 (s, 1H), 7.00 (dd, J = 8.1, 2.4 Hz, 1H), 7.25−7.28 (m, 1H), 7.33 (t, J = 2.4 Hz, 1H), 7.43 (t, J = 8.1 Hz, 1H), 7.70 (t, J = 5.2 Hz, 1H), 8.49 (s, 1H). Anal. ($C_{23}H_{27}N_5O_4$) C, H, N.

N-(2-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)ethyl)-1-(4-methoxyphenyl)-1*H*-1,2,3-triazole-4-carboxamide (**40c**) was prepared from **23f** (263 mg, 1.2 mmol) and **15a** (236 mg, 1 mmol) as described above for **24a**. The crude product was purified by silica gel column chromatography eluting with dichloromethane/methanol (50/1, v/v) to afford **40c** as white solid (119 mg, 27.2% yield), mp: 215.3−217.8 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.70−2.78 (m, 6H), 3.58 (s, 1H), 3.61−3.63 (m, 3H), 3.77 (s, 3H), 3.78 (s, 3H), 3.80 (s, 3H), 6.46 (s, 1H), 6.54 (s, 1H), 6.96 (d, J = 9.2 Hz, 2H), 7.55 (d, J = 9.2 Hz, 2H), 8.34 (s, 1H). Anal. ($C_{23}H_{27}N_5O_4{\cdot}H_2O$) C, H, N.

N-(2-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)ethyl)-1-(2-methoxybenthyl)-1*H*-1,2,3-triazole-4-carboxamide (**40d**) was prepared from **32a** (410 mg, 1.76 mmol) and **15a** (457 mg, 1.93 mmol) as described above for **24a**. The crude product was purified by silica gel column chromatography eluting with ethyl ether/methanol (10/1, v/v) to afford **40d** as white solid (405 mg, 51.0% yield), mp: 159.2−161.3 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.72−2.82 (m, 6H), 3.62−3.65 (m, 4H), 3.82 (s, 3H), 3.84 (s, 6H), 5.53 (s, 2H), 6.51 (s, 1H), 6.59 (s, 1H), 6.89−6.96 (m, 2H), 7.23 (d, J = 7.6 Hz, 1H), 7.34 (t, J = 7.6 Hz, 1H), 7.63 (t, J = 4.8 Hz, 1H), 8.01 (s, 1H). Anal. ($C_{24}H_{29}N_5O_4$) C, H, N.

N-(2-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)ethyl)-1-(3-methoxybenthyl)-1*H*-1,2,3-triazole-4-carboxamide (**40e**) was prepared from **32b** (373 mg, 1.60 mmol) and **15a** (416 mg, 1.76 mmol) as described above for **24a**. The crude product was purified by silica gel column chromatography eluting with ethyl acetate/methanol (20/1, v/v) to afford **40e** as white solid (395 mg, 54.7% yield), mp: 138.6−139.3 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.76−2.83 (m, 6H), 3.63 (m, 4H), 3.78 (s, 3H), 3.84 (s, 3H), 3.85 (s, 3H), 5.49 (s, 2H), 6.52 (s, 1H), 6.60 (s, 1H), 6.78 (s, 1H), 6.83−6.90 (m, 2H), 7.28 (m, 1H), 7.59 (br s, 1H), 7.96 (s, 1H). Anal. ($C_{24}H_{29}N_5O_4$) C, H, N.

N-(2-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)ethyl)-1-(4-methoxybenthyl)-1*H*-1,2,3-triazole-4-carboxamide (**40f**) was prepared from **32c** (410 mg, 1.76 mmol) and **15a** (457 mg, 1.93 mmol) as described above for **24a**. The crude product was purified by silica gel column chromatography eluting with ethyl acetate/methanol (25/1, v/v) to afford **40f** as a white solid (540 mg, 68.0% yield), mp: 142.8−143.6 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.73−2.83 (m, 6H), 3.60−3.66 (m, 4H), 3.81 (s, 3H), 3.83 (s, 3H), 3.85 (s, 3H), 5.46 (s, 2H), 6.52 (s, 1H), 6.60 (s, 1H), 6.89 (d, J = 8.5 Hz, 2H), 7.22 (d, J = 8.5 Hz, 2H), 7.57 (br s, 1H), 7.91 (s, 1H). Anal. ($C_{24}H_{29}N_5O_4$) C, H, N.

N-(4-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butyl)-4-ethynylbenzamide (**45**) was prepared from **44** (750 mg, 5.14 mmol) as described above for **24a**. The crude product was purified by silica gel column chromatography eluting with ethyl acetate/methanol (10/1, v/v) to afford **45** as a white solid (977 mg, 48.5% yield), mp: 171.3−171.6 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.77 (br s, 4H), 2.52−2.61 (m, 2H), 2.68−2.84 (m, 4H), 3.43−3.55 (m, 5H), 3.84 (s, 3H), 3.86 (s, 3H), 6.45 (s, 1H), 6.57 (s, 1H), 7.27 (d, J = 8.2 Hz, 2H), 7.56 (d, J = 8.2 Hz, 2H), 7.78 (br s, 1H).

N-(4-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butyl)-4-(1-(2-fluoroethyl)-1*H*-1,2,3-triazol-4-yl)benzamide (46a). 2-Fluoroethyl 4-methylbenzenesulfonate **42a** (310 mg, 1.4 mmol) and sodium azide (185 mg, 2 mmol) were mixed in 4 mL DMF with stirring overnight to form 1-azido-2-fluoroethane **43a**. This solution was filtered into 5 mL DMF solution of **45** (196 mg, 0.5 mmol), followed by the addition of copper(I) iodide (19 mg, 0.1 mmol) and 0.2 mL triethylamine with stirring for 12 h. The mixture was quenched with 70 mL ethyl acetate and 70 mL ethyl ether. After washing with saturated sodium carbonate, water, and brine, the organic phase was dried over anhydrous sodium sulfate. The crude product was purified by silica gel column chromatography eluting with methanol/dichloromethane/triethylamine (15/100/1, v/v/v) to afford **46a** as a white powder (113 mg, 47% yield), mp (oxalate salt): 211−213 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.80 (br s, 4H), 2.60 (t, J = 5.4 Hz, 2H), 2.74 (t, J = 5.1 Hz, 2H), 2.80 (d, J = 5.1 Hz, 2H), 3.50 (d, J = 5.4 Hz, 2H), 3.54 (s, 2H), 3.76 (s, 3H), 3.81 (s, 3H), 4.70 (t, J = 4.5 Hz, 1H), 4.80 (t, J = 4.5 Hz, 2H), 4.95 (t, J = 4.5 Hz, 1H), 6.44 (s, 1H), 6.57 (s, 1H), 7.59 (d, J = 8.4 Hz, 2H), 7.67 (d, J = 8.4 Hz, 2H), 7.82 (br s, 1H), 7.89 (s, 1H). Anal. ($C_{26}H_{32}FN_5O_3{\cdot}H_2C_2O_4{\cdot}0.25H_2O$) C, H, N.

N-(4-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butyl)-4-(1-(2-(2-fluoroethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)benzamide (**46b**) was prepared from **45** (196 mg, 0.5 mmol) and 1-azido-2-(2-fluoroethoxy)ethane **43b** as described above for **46a**. The crude product was purified by silica gel column chromatography eluting with methanol/dichloromethane/triethylamine (15/100/1, v/v/v) to afford **46b** as a white solid (90 mg, 34% yield), mp (oxalate salt): 190−192 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.78 (br s, 4H), 2.58 (t, J = 6.0 Hz, 2H), 2.73 (t, J = 5.4 Hz, 2H), 2.79 (d, J = 5.4 Hz, 2H), 3.50 (d, J = 6.0 Hz, 2H), 3.54 (s, 2H), 3.68 (t, J = 5.1 Hz, 1H), 3.76−3.80 (m, 1H), 3.78 (s, 3H), 3.81 (s, 3H), 3.96 (t, J = 5.1 Hz, 2H), 4.48 (t, J = 4.5 Hz, 1H), 4.59−4.69 (m, 3H), 6.46 (s, 1H), 6.57 (s, 1H), 7.54 (br s, 1H), 7.63−7.73 (m, 4H), 7.95 (s, 1H). Anal. ($C_{28}H_{36}FN_5O_4{\cdot}H_2C_2O_4{\cdot}0.5H_2O$) C, H, N.

N-(4-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butyl)-4-(1-(2-(2-(2-fluoroethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)-benzamide (**46c**) was prepared from **45** (196 mg, 0.5 mmol) and 1-azido-2-(2-(2-fluoroethoxy)ethoxy)ethane **43c** as described above for **46a**. The crude product was purified by silica gel column chromatography eluting with methanol/dichloromethane/triethylamine (15/100/1, v/v/v) to afford **46c** as a white solid (108 mg, 38% yield), mp (oxalate salt): 183−185 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.77 (br s, 4H), 2.57 (t, J = 6.3 Hz, 2H), 2.72 (t, J = 5.1 Hz, 2H), 2.80 (t, J = 4.8 Hz, 2H), 3.40−3.56 (m, 4H), 3.62−3.69 (m, 5H), 3.75 (t, J = 4.5 Hz, 1H), 3.78 (s, 3H), 3.82 (s, 3H), 3.94 (t, J = 5.1 Hz, 2H), 4.44 (t, J = 4.5 Hz, 1H), 4.58−4.65 (m, 3H), 6.47 (s, 1H), 6.57 (s, 1H), 7.43 (br s, 1H), 7.70 (br s, 4H), 8.00 (s, 1H). Anal. ($C_{30}H_{40}FN_5O_5{\cdot}H_2C_2O_4{\cdot}0.5H_2O$) C, H, N.

N-(4-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butyl)-4-(1-(2-methoxybenzyl)-1*H*-1,2,3-triazol-4-yl)benzamide (**48a**) was prepared from **45** (196 mg, 0.5 mmol) and **31a** (98 mg, 0.6 mmol) as described above for **24a**. The crude product was purified by silica gel column chromatography eluting with ethyl acetate/methanol (3/1, v/v) to afford **48a** as a white solid (243 mg, 87% yield), mp (oxalate salt): 154−156 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.76 (br s, 4H), 2.56 (t, J = 6.3 Hz, 2H), 2.71 (t, J = 5.4 Hz, 2H), 2.79 (t, J = 5.4 Hz, 2H), 3.44−3.56 (m, 4H), 3.74 (s, 3H), 3.78 (s, 3H), 3.91 (s, 3H), 5.61 (s, 2H), 6.41 (s, 1H), 6.55 (s, 1H), 6.97 (t, J = 8.4 Hz, 2H), 7.26 (br s, 1H), 7.36 (t, J = 8.4, 1H), 7.45 (br s, 1H), 7.64 (br s, 4H), 7.74 (s, 1H). Anal. ($C_{32}H_{37}N_5O_4{\cdot}H_2C_2O_4{\cdot}0.75H_2O$) C, H, N.

N-(4-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butyl)-4-(1-(3-methoxybenzyl)-1*H*-1,2,3-triazol-4-yl)benzamide (**48b**) was prepared from **47b** (107 mg, 0.35 mmol) and **15b** (92 mg, 0.35 mmol) as described above for **24a**. The crude product was purified by silica gel column chromatography eluting with ethyl acetate/methanol (5/1, v/v) to afford **48b** as a white solid (62 mg, 32% yield), mp (oxalate salt): 169−171 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.64 (br

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s, 4H), 2.56 (t, J = 6.3 Hz, 2H), 2.70 (t, J = 5.4 Hz, 2H), 2.78 (t, J = 5.4 Hz, 2H), 3.42−3.64 (m, 4H), 3.72 (s, 3H), 3.77 (s, 3H), 3.79 (s, 3H), 5.55 (s, 2H), 6.43 (s, 1H), 6.54 (s, 1H), 6.83−6.95 (m, 3H), 7.31 (t, J = 8.4 Hz, 1H), 7.54−7.67 (m, 5H), 7.69 (s, 1H). HR-MS (ESI, $[M + H]^+$) calcd for $C_{32}H_{37}N_5O_4$ + H, 556.2918; found, m/z 556.2917.

***N*-(4-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butyl)-4-(1-(4-methoxybenzyl)-1*H*-1,2,3-triazol-4-yl)benzamide (48c)** was prepared from **47c** (150 mg, 0.64 mmol) and **15b** (169 mg, 0.64 mmol) as described above for **24a**. The crude product was purified by silica gel column chromatography eluting with ethyl acetate/methanol (5/1, v/v) to afford **48c** as a white solid (266 mg, 75% yield), mp (oxalate salt): 175−176 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.64−1.76 (m, 4H), 2.57 (t, J = 6.3 Hz, 2H), 2.75 (t, J = 5.4 Hz, 2H), 2.84 (t, J = 5.4 Hz, 2H), 3.47 (d, J = 3.3 Hz, 2H), 3.59 (s, 2H), 3.81 (s, 3H), 3.83 (s, 3H), 3.84 (s, 3H), 5.47 (s, 2H), 6.52 (s, 1H), 6.59 (s, 1H), 6.90 (d, J = 8.7 Hz, 2H), 7.20−7.28 (m, 2H), 7.32 (br s, 1H), 7.65 (br s, 4H), 7.91 (s, 1H). HR-MS (ESI, $[M + H]^+$) calcd for $C_{32}H_{37}N_5O_4$ + H, 556.2918; found, m/z 556.2917.

***N*-(4-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butyl)-3-(1-(3-methoxybenzyl)-1*H*-1,2,3-triazol-4-yl)benzamide (48d)** was prepared from **47d** (168 mg, 0.54 mmol) and **15b** (144 mg, 0.54 mmol) as described above for **24a**. The crude product was purified by silica gel column chromatography eluting with ethyl acetate/methanol (5/2. v/v) to afford **48d** as a yellow solid (99 mg, 33% yield), mp (oxalate salt): 133−135 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.65 (br s, 4H), 2.61 (br s, 2H), 2.78 (t, J = 5.4 Hz, 4H), 3.46−3.68 (m, 4H), 3.79 (s, 3H), 3.80 (s, 3H), 3.83 (s, 3H), 5.54 (s, 2H), 6.43 (s, 1H), 6.52 (s, 1H), 6.84 (t, J = 1.2 Hz, 1H), 6.89 (t, J = 1.8 Hz, 1H), 6.91 (d, J = 8.4 Hz, 1H), 7.24 (t, J = 8.4 Hz, 1H), 7.31 (t, J = 8.4 Hz, 1H), 7.45 (br s, 1H), 7.55 (d, J = 8.4 Hz, 1H), 7.74 (s, 1H), 7.91 (d, J = 8.4, 1H), 8.13 (s, 1H). HR-MS (ESI, $[M + H]^+$) calcd for $C_{32}H_{37}N_5O_4$ + H, 556.2926; found, m/z 556.2917.

5-Cyano-*N*-(4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-butyl)-2-(prop-2-ynyloxy)benzamide (52) was prepared from **51** (180 mg, 0.896 mmol) and **15b** (236 mg, 0.896 mmol) as described above for **24a**. The crude product was purified by silica gel column chromatography eluting with ethyl acetate/methanol (4/1, v/v) to afford **52** as a pale yellow oil (114 mg, 28.5% yield). For the in vitro binding experiments, the free base was converted into the corresponding oxalate salt, mp (oxalate salt): 166.6−168.6 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.72 (br s, 4H), 2.55 (t, J = 6.6 Hz, 2H), 2.69 (m, 2H), 2.70 (s, 1H), 2.79 (t, J = 5.4 Hz, 2H), 3.51 (t, J = 5.4 Hz, 2H), 3.53 (s, 2H), 3.82 (s, 3H), 3.83 (s, 3H), 5.08 (s, 2H), 6.48 (s, 1H), 6.56 (s, 1H), 7.08 (d, J = 8.7 Hz, 1H), 7.68 (dd, J = 8.7, 2.1 Hz, 1H), 7.85 (t, J = 5.4 Hz, 1H), 8.78 (d, J = 2.1 Hz, 1H). Anal. ($C_{26}H_{29}N_3O_4 \cdot 1.5H_2C_2O_4$) C, H, N.

5-Cyano-*N*-(4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-butyl)-2-((1-(2-fluoroethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-benzamide (53) was prepared from **52** (80 mg, 0.179 mmol) and **43a** (39.8 mg, 0.447 mmol) as described above for **22a**, as a white solid (72 mg, 75% yield), mp: 147.3−148.1 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.60 (br s, 4H), 2.49 (t, J = 5.4 Hz, 2H), 2.66 (t, J = 5.4 Hz, 2H), 2.76 (t, J = 5.4 Hz, 2H), 3.43 (t, J = 5.4 Hz, 2H), 3.50 (s, 2H), 3.83 (s, 3H), 3.84 (s, 3H), 4.60 (t, J = 4.5 Hz, 1H), 4.67 (t, J = 4.5 Hz, 1H), 4.68 (t, J = 5.4 Hz, 1H), 4.83 (t, J = 5.4 Hz, 1H), 5.33 (s, 2H), 6.49 (s, 1H), 6.56 (s, 1H), 7.18 (d, J = 8.4 Hz, 1H), 7.67 (dd, J = 8.4, 2.3 Hz, 1H), 7.83 (s, 1H), 8.04 (t, J = 5.4 Hz, 1H), 8.38 (d, J = 2.3 Hz, 1H). Anal. ($C_{28}H_{33}FN_6O_4$) C, H, N.

***N*-(4-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butyl)-5-(4-((dimethylamino)methyl)-1*H*-1,2,3-triazol-1-yl)-2-methoxybenzamide (60a)** was prepared from **59a** (140 mg, 0.51 mmol) and **15b** (134 mg, 0.51 mmol) as described above for **24a**. The crude product was purified by silica gel column chromatography eluting with dichloromethane/methanol (10/1, v/v) to afford **60a** as a colorless grease (75 mg, 28% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.70 (br s, 4H), 2.32 (s, 6H), 2.55 (t, J = 5.4 Hz, 2H), 2.70 (t, J = 5.4 Hz, 2H), 2.80 (t, J = 5.4 Hz, 2H), 3.44−3.56 (m, 4H), 3.69 (s, 2H), 3.82 (s, 3H), 3.83 (s, 3H), 3.99 (s, 3H), 6.49 (s, 1H), 6.57 (s, 1H), 7.09 (d, J = 9.0 Hz, 1H), 7.92−8.04 (m, 3H), 8.38 (d, J = 3.0 Hz, 1H). Mp (oxalate salt): >250 °C. HR-MS (ESI, $[M + H]^+$) calcd for $C_{28}H_{38}N_6O_4$ + H, 523.3027; found, m/z 523.3023.

***N*-(4-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butyl)-2-methoxy-5-(4-(1-methyl-1*H*-imidazol-5-yl)-1*H*-1,2,3-triazol-1-yl)-benzamide (60b)** was prepared from **59b** (40 mg, 0.14 mmol) and **15b** (36 mg, 0.14 mmol) as described above for **24a**. The crude product was purified by silica gel column chromatography eluting with dichloromethane/methanol (10/1, v/v) to afford **60b** as a colorless grease (21 mg, 27% yield), mp (oxalate salt): 176−178 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.73 (br s, 4H), 2.52−2.62 (m, 2H), 2.70−2.79 (m, 2H), 2.79−2.88 (m, 2H), 3.52−3.60 (m, 4H), 3.83 (s, 3H), 3.84 (s, 3H), 3.98 (s, 3H), 4.02 (s, 3H), 6.49 (s, 1H), 6.58 (s, 1H), 7.15 (d, J = 9.0 Hz, 1H), 7.33 (s, 1H), 7.55 (s, 1H), 7.97−8.09 (m, 2H), 8.12 (s, 1H), 8.44 (d, J = 3.0 Hz, 1H). HR-MS (ESI, $[M + H]^+$) calcd for $C_{29}H_{35}N_7O_4$ + H, 546.2823; found, m/z 546.2820.

***N*-(4-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butyl)-5-(4-((2-(2-fluoroethoxy)ethoxy)methyl)-1*H*-1,2,3-triazol-1-yl)-2-methoxybenzamide (60c)** was prepared from **59c** (206 mg, 0.61 mmol) and **15b** (160 mg, 0.61 mmol) as described above for **24a**. The crude product was purified by silica gel column chromatography eluting with dichloromethane/methanol (10/1, v/v) to afford **60c** as a yellow grease (135 mg, 38% yield), mp (oxalate salt): 107−109 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.72 (br s, 4H), 2.52−2.62 (m, 2H), 2.68−2.78 (m, 2H), 2.79−2.87 (m, 2H), 3.46−3.60 (m, 4H), 3.66−3.80 (m, 6H), 3.83 (s, 3H), 3.84 (s, 3H), 4.00 (s, 3H), 4.51 (t, J = 4.0 Hz, 1H), 4.67 (t, J = 4.0 Hz, 1H), 4.78 (s, 2H), 6.49 (s, 1H), 6.58 (s, 1H), 7.10 (d, J = 9.0 Hz, 1H), 7.95−8.02 (m, 2H), 8.06 (s, 1H), 8.38 (d, J = 2.7 Hz, 1H). HR-MS (ESI, $[M + H]^+$) calcd for $C_{30}H_{40}FN_5O_6$ + H, 586.3035; found, m/z 586.3043.

5-Azido-*N*-(4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butyl)-2-(2-fluoroethoxy)benzamide (64). **63** (1.11 g, 2 mmol), sodium azide (260 mg, 4 mmol), *N*,*N*′-dimethylethylenediamine (25 mg, 0.3 mmol), copper(I) iodide (380 mg, 2 mmol) and sodium ascorbate (20 mg, 0.1 mmol) were added to 10 mL DMF with stirring and heated at 80 °C overnight. After cooling, 60 mL water was added. The mixture was extracted with ethyl acetate (60 mL × 3). The organic layer was washed with brine (180 mL), dried with anhydrous sodium sulfate, and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography eluting with dichloromethane/methanol (10/1, v/v) to afford **64** as a brown solid (474 mg, 50.2% yield), mp 45.2−46.3 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.60−1.80 (m, 4H), 2.84−2.96 (m, 2H), 3.40−3.62 (m, 8H), 3.90 (s, 6H), 4.26 (t, J = 4.2 Hz, 1H), 4.36 (t, J = 4.2 Hz, 1H), 4.71 (t, J = 4.2 Hz, 1H), 4.87 (t, J = 4.2 Hz, 1H), 6.62 (s, 1H), 6.69 (d, J = 8.6 Hz, 1H), 7.56 (s, 1H), 6.78 (dd, J = 8.6, 2.4 Hz, 1H), 7.83 (br s, 1H), 8.45 (d, J = 2.4 Hz, 1H).

***N*-(4-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butyl)-5-(4-((dimethylamino)methyl)-1*H*-1,2,3-triazol-1-yl)-2-(2-fluoroethoxy)benzamide (65)** was prepared from **64** (90 mg, 0.19 mmol) and 3-dimethylamino-1-propyne (16 mg, 0.19 mmol) as described above for **22a**, as a yellow grease (35 mg, 33% yield), mp (oxalate salt): >250 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.60−1.80 (m, 4H), 2.42 (s, 6H), 2.60−2.72 (m, 2H), 2.90−3.14 (m, 2H), 3.26−3.38 (m, 2H), 3.50−3.59 (m, 2H), 3.70−3.78 (m, 2H), 3.83 (s, 3H), 3.84 (s. 3H), 4.15 (s, 2H), 4.36 (t, J = 4.2 Hz, 1H), 4.46 (t, J = 4.2 Hz, 1H), 4.79 (t, J = 4.2 Hz, 1H), 4.95 (t, J = 4.2 Hz, 1H), 6.51 (s, 1H), 6.59 (s, 1H), 6.61 (s, 1H), 7.09 (d, J = 8.7 Hz, 1H), 8.00 (dd, J = 8.7, 2.7 Hz, 1H), 8.16 (s, 1H), 8.42 (d, J = 2.7 Hz, 1H). HR-MS (ESI, $[M + H]^+$) calcd for $C_{29}H_{39}N_6O_4$ + H, 555.3090; found, m/z 555.3096.

Sigma Receptor Binding Assays. The compounds were dissolved in DMSO or ethanol and then diluted in 50 mM Tris-HCl buffer containing 150 mM NaCl and 100 mM EDTA at pH 7.4 prior to performing the σ_1 and σ_2 receptor binding assays. The procedures for isolating the membrane homogenates and performing the σ_1 and σ_2 receptor binding assays have been previously described in detail.[63]

Briefly, the σ_1 receptor binding assays were conducted in 96-well plates using guinea pig brain membrane homogenates (~300 μg of protein) and ~5 nM (+)-[^{3}H]pentazocine (34.9 Ci/mmol, PerkinElmer, Boston, MA). The total incubation time was 90 min. Nonspecific binding was determined from samples that contained 10

μM cold haloperidol. After 90 min, the reaction was quenched by adding 150 μL ice-cold wash buffer (10 mM Tris-HCl, 150 mM NaCl, pH 7.4) using a 96-channel pipetter. The samples were harvested and filtered rapidly through a 96-well fiberglass filter plate (Millipore, Billerica, MA) that had been presoaked with 100 μL of 50 mM Tris-HCl buffer at pH 8.0 for 1 h. Each filter was washed three times with 200 μL of ice-cold wash buffer and the filter counted in a Wallac 1450 MicroBeta liquid scintillation counter (PerkinElmer, Boston, MA).

The σ_2 receptor binding assays were conducted using rat liver membrane homogenates (~300 μg of protein) and ~1 nM [^{3}H]DTG (58.1 Ci/mmol, PerkinElmer, Boston, MA) in the presence of 1 μM (+)-pentazocine to block σ_1 sites. The incubation time was 2 h at room temperature. Nonspecific binding was determined from samples that contained 10 μM cold haloperidol. All other procedures were identical to those described for the σ_1 receptor binding assay above.

Data from the competitive inhibition experiments were modeled using nonlinear regression analysis to determine the concentration that inhibits 50% of the specific binding of the radioligand (IC_{50}). Competitive curves were best fit to a one-site fit and gave pseudo-Hill coefficients of 0.6−1.0. K_i values were calculated using the method of Cheng and Prusoff[66] and are presented as the mean (±1 SEM). For these calculations, we used a K_d of 7.89 nM for (+)-[^{3}H]pentazocine and guinea pig brain and a K_d of 30.73 nM for [^{3}H]DTG and rat liver.

Functional Assay. EMT-6 mouse breast cancer cells were grown in DMEM containing 10% fetal bovine serum, 100 units/ml penicillin and 100 μg/mL streptomycin. EMT-6 cell line was maintained at 37 °C in a humidified incubator with a 5% CO_2 /95% air atmosphere. The cytotoxicity of the compounds on EMT-6 cell was measured using the CellTiter96 Aqueous One Solution (Promega, Madison, WI), which contains a tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt; MTS], according to the manufacturer's protocol. Briefly, cells were plated at 5×10^3 cells/well in 96-well plates 24 h prior to treatment with the compounds. Each compound was dissolved in DMSO and serially diluted in culture medium to acquire the desired concentrations. The final concentration of DMSO in the medium was no more than 1.0%. After a 24 or 48 h treatment with the compound, 20 μL CellTiter 96 Aqueous One Solution Reagent was added to each well, and the plate was incubated for 1−2 h at 37 °C. The plate was then read at 490 nm in a Victor^3 plate reader (PerkinElmer Life and Analytical Sciences, Shelton, CT). Cell viability (%) and cytotoxicity (%) at each concentration of the compound were calculated by formulas 1 and 2, respectively,

$$\text{Cell viability}(\%) = \text{OD}_{490,\sigma 2}/\text{OD}_{490,\text{control}} \times 100 \quad (1)$$

$$\text{Cytotoxicity}(\%) = 100 - \text{cell viability}(\%) \quad (2)$$

Where $\text{OD}_{490,\,\sigma 2}$ is the absorbance at 490 nm for σ_2 ligand-treated cells, and $\text{OD}_{490,\,\text{control}}$ is the absorbance at 490 nm for untreated cells. The EC_{50}, defined as the concentration of the σ_2 ligand required to inhibit cell proliferation by 50% relative to untreated cells, was determined from the dose−response curves generated using GraFit software, version 5 (Erithacus Software Limited, UK). All compounds were assayed in triplicate, and the EC_{50} values presented as the mean ± SEM of three independent experiments.

■ ASSOCIATED CONTENT

Supporting Information

The experimental detail corresponding to the synthesis of intermediates (**11**, **12**, **14a−14b**, **15a−15b**, **17a−17c**, **18a−18c**, **19a−19c**, **21a−21f**, **22a−22f**, **23a−23f**, **28**, **29**, **31a−31e**, **32a−32c**, **33a−33b**, **34a−34b**, **35a−35b**, **36a−36b**, **37a−37b**, **42a−42c**, **47b−47d**, **50**, **51**, **56**, **57**, **58a−58d**, **59a−59c**, **61**, **62**, **63**). Elemental analysis data of **24a−24f**, **39a**, **39g**, **39h**, **40a−40f**, **46a−46c**, **48a**, **52**, **53**. HPLC methods and spectra for **26**, **39b−39f**, **39h**, **46a−46c**, **48a−48d**, **52**, **60a−60c**, **65**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ ABBREVIATIONS USED

Anal, analysis; CNS, central nervous system; SAR, structure−activity relationship; σ_1, sigma-1; σ_2, sigma-2; DCC, *N*,*N*′-dicyclohexylcarbodiimide; HOBt, 1-hydroxybenzotriazole; CDI, 1,1′-carbonyldiimidazole; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; DTG, 1,3-ditolylguanidine; PET, positron emission tomography; THF, tetrahydrofuran; BPDS, bathophenanthrolinedisulfonic acid disodium salt hydrate; TBAF, tetrabutylammonium fluoride

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