International Journal of
Molecular Sciences

MDPI

Article

Design and Synthesis of Conformationally Flexible Scaffold as Bitopic Ligands for Potent D_3-Selective Antagonists

Ho Young Kim [1], Ji Youn Lee [1], Chia-Ju Hsieh [1], Michelle Taylor [2], Robert R. Luedtke [2] and Robert H. Mach [1,*]

[1] Vagelos Laboratories, Department of Radiology, University of Pennsylvania, 1012, 231 S. 34th Street, Philadelphia, PA 19104, USA
[2] Department of Pharmacology and Neuroscience, University of North Texas Health Science Center, Fort Worth, TX 76107, USA
* Correspondence: rmach@pennmedicine.upenn.edu

Abstract: Previous studies have confirmed that the binding of D_3 receptor antagonists is competitively inhibited by endogenous dopamine despite excellent binding affinity for D_3 receptors. This result urges the development of an alternative scaffold that is capable of competing with dopamine for binding to the D_3 receptor. Herein, an SAR study was conducted on metoclopramide that incorporated a flexible scaffold for interaction with the secondary binding site of the D_3 receptor. The alteration of benzamide substituents and secondary binding fragments with aryl carboxamides resulted in excellent D_3 receptor affinities (*Ki* = 0.8–13.2 nM) with subtype selectivity to the D_2 receptor ranging from 22- to 180-fold. The β-arrestin recruitment assay revealed that **21c** with 4-(pyridine-4-yl)benzamide can compete well against dopamine with the highest potency (IC_{50} = 1.3 nM). Computational studies demonstrated that the high potency of **21c** and its analogs was the result of interactions with the secondary binding site of the D_3 receptor. These compounds also displayed minimal effects for other GPCRs except moderate affinity for 5-HT_3 receptors and TSPO. The results of this study revealed that a new class of selective D_3 receptor antagonists should be useful in behavioral pharmacology studies and as lead compounds for PET radiotracer development.

Keywords: D_3 receptor antagonists; metoclopramide; bitopic ligand; β-arrestin recruitment assay; computational chemistry

Citation: Kim, H.Y.; Lee, J.Y.; Hsieh, C.-J.; Taylor, M.; Luedtke, R.R.; Mach, R.H. Design and Synthesis of Conformationally Flexible Scaffold as Bitopic Ligands for Potent D_3-Selective Antagonists. *Int. J. Mol. Sci.* **2023**, *24*, 432. https://doi.org/10.3390/ijms24010432

Academic Editors: Dorota Łażewska and Anna Stasiak

Received: 2 December 2022
Revised: 22 December 2022
Accepted: 24 December 2022
Published: 27 December 2022

1. Introduction

Targeting D_2 and D_3 receptors has been studied for the treatment of neuropsychiatric disorders such as schizophrenia, and substance use disorders and addiction [1–4]. However, preferential localization of D_3 receptors in limbic regions of the human brain suggested that D_3 receptors may be a suitable target for developing therapeutics for treating neuropsychiatric disorders [5,6]. Other studies have demonstrated that this receptor plays a role in mediating the motivational actions of psychostimulants such as cocaine and amphetamine, and D_3 antagonists have shown great promise in blocking cocaine self-administration in rodents and nonhuman primates [7,8]. The recent observation in the treatment of opioid use disorder has accelerated the need for the clinical evaluation of drugs targeting D_3 receptors [9–11].

The development of dopamine D_3-selective ligands continues to be a challenging area of medicinal chemistry research due to the high sequence homology of D_2 and D_3 receptor within the transmembrane (TM) domains (~79%) [12]. For developing receptor subtype selectivity, a "bitopic ligand" design has proven to be effective in the development of D_3-selective compounds [13,14]. In this approach, a protonated basic amine in different scaffolds forms a salt bridge with $Asp110^{3.32}$ of the D_3 receptor in the orthosteric binding site (OBS), which is important for high binding affinity and the potency [15]. A secondary pharmacophore

having an aromatic ring and appropriate linker group can result in high selectivity for the D_3 receptor by the interaction with the secondary binding site (SBS) [16–18].

The first D_3-selective scaffold contained an *N*-aryl piperazine moiety as the orthosteric binding fragment and an aryl carboxamide moiety with an alkyl linker as a secondary binding fragment [16,19–25]. This scaffold exhibited a sub nM binding affinity and good subtype selectivity for D_3 receptors versus D_2 receptors. However, these ligands also exhibited high binding affinity for other GPCRs (e.g., 5-HT or adrenergic receptors), which may lead the unwanted side effects [26–28]. Moreover, the in vivo properties of radiolabeled versions of this scaffold were not useful as PET radiotracers since they could not compete with endogenous dopamine for binding to the D_3 receptor in vivo [21,29]. Since the replacement of substituents on benzamide or secondary binding fragments did not result in a significant change in properties of the *N*-aryl piperazine congeners, many groups have pursued other scaffolds, including azabicyclo [3.1.0]hexane [30–32], azaspiro alkane [33], diazaspiro alkane [34], tranylcypromine [35], or phenylcyclopropylmethylamine (PCPMA) [36]). However, these can be limited for clinical use under certain circumstances due to the poor bioavailability or toxicity [37–39] or are still under investigation. Recently, D_2/D_3 receptor agonist- and antagonist-modified bitopic ligands were developed based on (+)-PD128,907 or PF-592379 for selective agonist [40] and eticlopride for D_2/D_3 receptor ligands [41]. These compounds had comparatively low selectivity for D_3 versus D_2 receptors.

In the current study, we designed a new class of D_3 receptor antagonist having the conformationally flexible scaffold of metoclopramide and the eticlopride-based benzamides (e.g., [^{18}F]fallypride, *K*i D_2 = 0.02 nM and D_3 = 0.19 nM [42,43], IC_{50} = 1.7 nM [29]; [^{11}C]FLB457, *K*i = 0.02 nM for D_2/D_3 receptors [44]) as lead compounds. Metoclopramide is largely used as an antiemetic; however, this compound also exhibited the low affinity for mixed D_2/D_3 receptors with the orthosteric binding fragment [45]. A combination of this flexible scaffold with well-established primary pharmocophore of the eticlopride-based benzamides was expected to achieve the high binding affinity and the potency for D_3 receptors. Since the basic amine in this scaffold is structurally flexible without ring strain, the secondary binding fragment can be extended to strongly interact with the SBS while the orhosteric binding fragment remains bound to the OBS. Comprehensive screening was investigated for off-target interactions with other GPCRs; computational studies were also performed to provide the rational for the excellent potency of developed D_3 receptor antagonists.

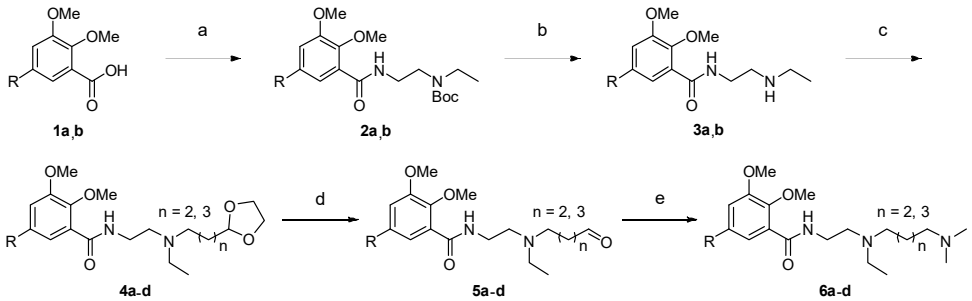
2. Results

2.1. Chemistry

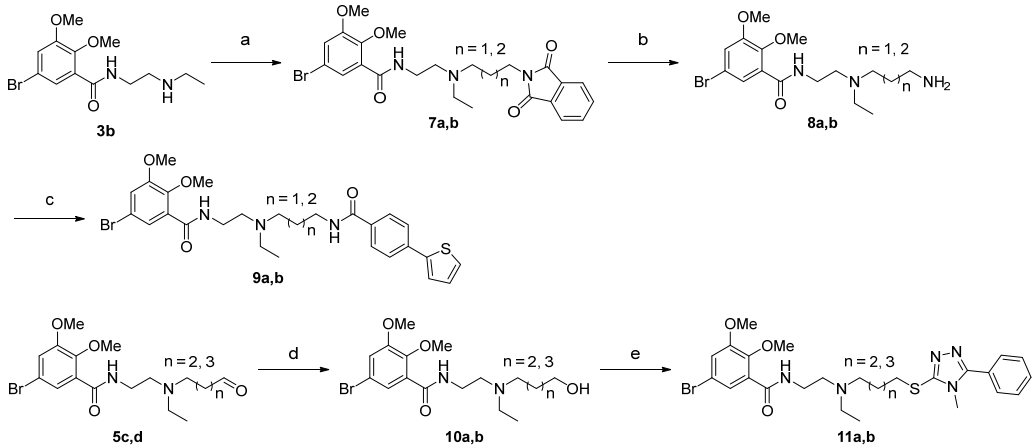
Synthesis of 3-fluoropropyl or bromo analogs which have a dimethyl *tert*-amine with different length of carbon linker is shown in Scheme 1. 5-(3-Fluoropropyl)-2,3-dimethoxybenzoic acid (**1a**) or 5-bromo-2,3-dimethoxybenzoic acid (**1b**) was conjugated with secondary amine Boc-protected *tert*-butyl (2-aminoethyl)(propyl)carbamate by amide coupling in a quantitative yield. After the removal of the Boc protecting group, free amine **3a** or **3b** was *N*-alkylated with 2-(3-bromopropyl) or 2-(4-bromobutyl)-1,3-dioxolane. Dioxolanes **4a** to **4d** were hydrolyzed using aqueous 4 N HCl at RT to give the aldehyde **5a** to **5d** which were conjugated with dimethylamine via reductive amination. 5-(3-Fluoropropyl) or 5-bromo-2,3-dimethoxybenzamide analogs having the dimethylamine moiety (**6a**–**d**) were obtained in 37–47% yield over the two-step synthesis.

The next series focused on preparing analogs having a spacer group with an aromatic ring system for interacting with the SBS. For the aromatic ring moiety, we tested 4-(thiophen-2-yl)benzamide or 4-methyl-5-phenyl-4*H*-1,2,4-triazole-3-thiol. The 4-(Thiophen-2-yl)benzamide fragment was chosen from our previous results. This aromatic ring system was observed in LS-3-134 and other structural congeners having a high D_3 affinity and excellent selectivity versus the D_2 receptor [46–48]. **3b** was *N*-alkylated with *N*-(3-bromopropyl) or *N*-(4-bromobutyl)phthalimide and then the protecting phthalimide **7a** or **7b** was hydrolyzed using hydrazine hydrate by heating for 3 h to give primary amine **8a** or **8b** (Scheme 2). 4-(Thiophen-2-yl)benzoic acid was converted to the corresponding acyl chlo-

ride using thionyl chloride at RT followed by treatment with **8a** or **8b** to give **9a** or **9b** in 50% or 20% yield, respectively. The triazole-thiol ether analogs were prepared by reduction of **5c** and **5d** to give alcohols **10a** and **10b**, which were converted to **11a** and **11b** using Mitsunobu reaction. The desired products **11a** or **11b** were obtained in 16% and 28% yield, respectively (Scheme 2).



Scheme 1. Synthesis of 3-fluoropropyl or bromo analogs **6a–d**. Reagents and conditions: (a) *tert*-butyl (2-aminoethyl) ethylcarbamate, HBTU, DIPEA, DMF, RT, 24 h; (b) TFA, CH_2Cl_2, 0 °C to RT, 1 h; (c) 2-(2-bromoethyl)-1,3-dioxolane or 2-(3-bromopropyl)-1,3-dioxolane, Na_2CO_3, MeCN, 65 °C, 72 h; (d) aq 4 N HCl, THF, RT, 3 h; (e) dimethylamine, sodium triacetoxy borohydride, dichloroethane, RT, 16 h.

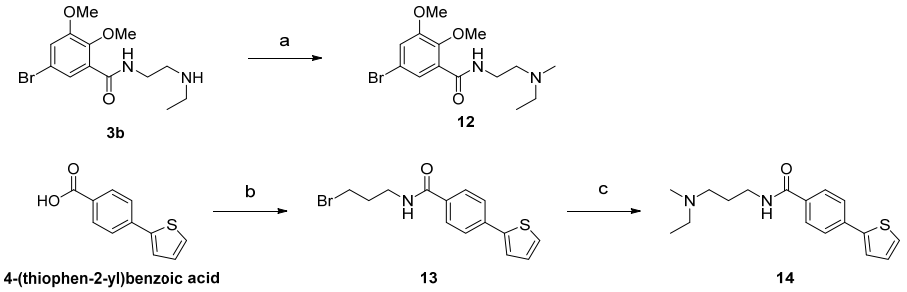


Scheme 2. Synthesis of **9a**,**b** or **11a**,**b** for the SBS interactions. Reagents and conditions: (a) *N*-(3-bromopropyl)phthalimide or *N*-(4-bromobutyl)phthalimide, K_2CO_3, DMF, 65 °C, 16 h; (b) hydrazine hydrate, EtOH, 75 °C, 3 h; (c) 4-(thiophen-2-yl)benzoic acid, $SOCl_2$, 3 h, then, **8a** or **8b**, CH_2Cl_2, RT, 16 h; (d) $NaB(OAc)_3$, RT, 16 h; (e) 4-methyl-5-phenyl-4*H*-1,2,4-triazole-3-thiol, DIAD, PPh_3, THF, RT, 24 h.

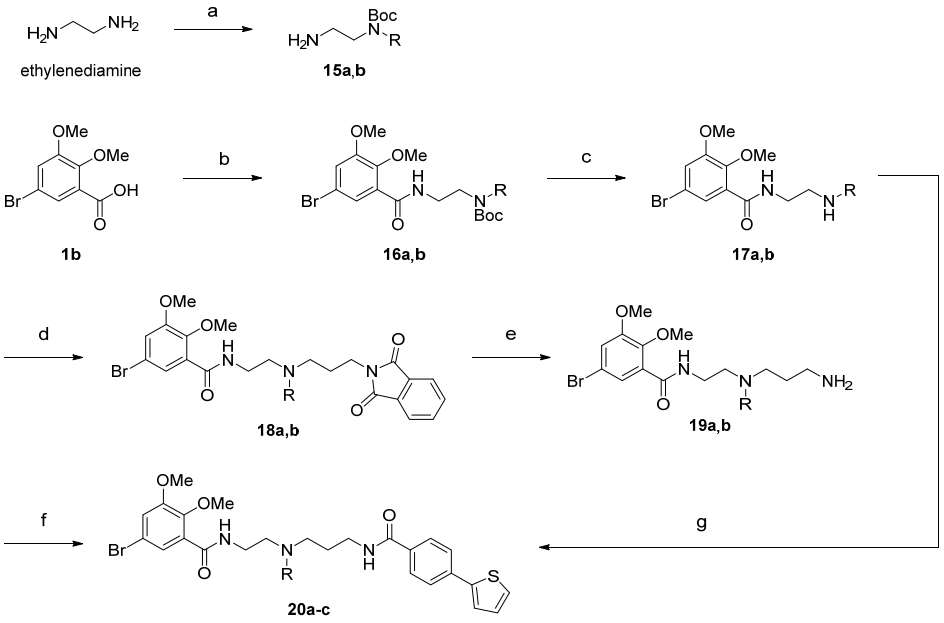
Inspection of the structure of **9a** reveals that two different benzamide fragments which share the *tert*-amine are capable of interacting with the OBS of the D_2 and D_3 receptors. Therefore, fragments **12** and **14** were synthesized for evaluation in in vitro binding studies (Scheme 3). **12** was synthesized by *N*-methylation from the secondary amine **3b** in 19% yield. For **14**, 4-(thiophen-2-yl)benzoic acid was conjugated with 3-bromopropylamine through acyl chlorination followed by *N*-alkylated with *N*-methylethanamine.

The next series probed the size of substituents on the *tert*-amine group. The pendent synthons for allyl (**15a**) and 4-fluorobenzyl (**15b**) were prepared from ethylenediamine (Scheme 4). For the synthesis of **15a**, one of the primary amines was protected with a trifluoroacetyl group and the other primary amine alkylated with allyl bromide. The secondary amine was protected as a *N*-Boc and the trifluoroacetyl group was removed. **15b** was synthesized in a similar method with **15a** except a reductive amination with 4-fluorobenzaldehyde was used. The prepared synthon **15a** or **15b** was conjugated with **1b**, and the *N*-Boc was removed to give intermediates **17a**,**b**. These intermediates were treated with *N*-propylphthalimide to give **18a**,**b**. Removal of the phthalimide group with hydrazine hydrate gave corresponding *N*-propyl intermediate **19a** (via reduction of the

N-allyl group) and the 4-fluorobenzyl analog **19b**. The intermediates **19a** and **19b** were conjugated with 4-(thiophen-2-yl)benzoic acid to give the desired products **20a** and **20c** in 71% or 32% yield, respectively. For the *N*-allyl analog, **17a** was directly *N*-alkylated with **13** to give **20b** in 21% yield.

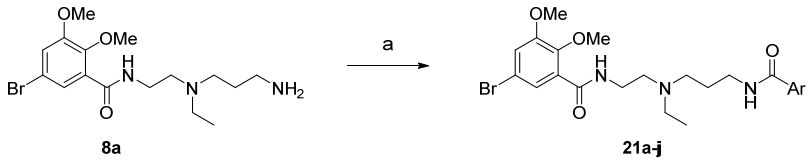


Scheme 3. Synthesis of **12** and **14** to determine the capability of the OBS binding. Reagents and conditions: (a) CH_3I, K_2CO_3, acetone, reflux, 16 h; (b) $SOCl_2$, CH_2Cl_2, RT, 3 h, then, 3-bromopropylamine hydrobromide, RT, 16 h (c) *N*-methylethanamine, K_2CO_3, DMF, 65 °C, 30 min.



Scheme 4. Synthesis of **20a**–**c** for different substituents on *tert*-amine. Reagents and conditions: (a) (1) ethyl trifluoroacetate, CH_2Cl_2, 0 °C to RT, 1 h; (2) [for **15a**] allyl bromide, Et_3N, MeOH, RT, 16 h, then, $(Boc)_2O$, 4 h; [for **15b**] 4-fluorobenzaldehyde, $NaB(OAc)_3$, then, $(Boc)_2O$, 4 h; (3) K_2CO_3, $MeOH/H_2O$ (9:1), reflux, 2 h (b) **15a** or **15b**, HBTU, DIPEA, DMF, RT, 24 h; (c) TFA, CH_2Cl_2, 0 °C to RT, 1 h; (d) *N*-(3-bromopropyl)phthalimide, K_2CO_3, DMF, 65 °C, 16 h; (e) hydrazine hydrate, EtOH, 75 °C, 2 h; (f) [for **20a** or **20c**] 4-(thiophen-2-yl)benzoic acid, $SOCl_2$, 3 h, then, **19a** or **19b**, CH_2Cl_2, RT, 16 h; (g) [for **20b**] **13**, K_2CO_3, DMF, 65 °C, 16 h.

To investigate the nature of the aromatic moiety for binding to the SBS, aryl carboxamides **21b**–**j** were synthesized using the same method described for the synthesis of **9a** but using different aryl carboxylic acids and the naphthamide **21a** was synthesized using 2-naphthoyl chloride using in the basic condition (Scheme 5). The desired benzamide analogs were obtained in yields ranging from 20 to 86%, respectively. The purity of all investigated compounds was confirmed prior to analysis and was greater than 95% on a 2695 Alliance LC-MS (Supplemental Table S1).



8a **21a-j**

Scheme 5. Synthesis of different aryl carboxamides **21a–j**. Reagents and conditions: (a) [for **21a**] 2-naphthoyl chloride, Et_3N, CH_2Cl_2, RT, 1 h; [for **21b** to **21j**] aryl carboxylic acids, $SOCl_2$, 3 h, then, **8a**, CH_2Cl_2, RT, 16 h.

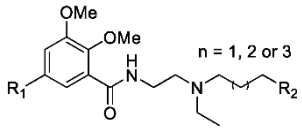
2.2. SAR Study

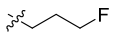
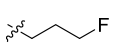
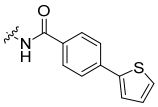
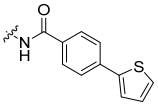
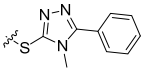
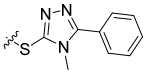
Two different assays were used to evaluate the properties of the analogs described above. The receptor binding affinity was measured by radioligand binding assays using [^{125}I]IABN with D_2 or D_3 receptors highly expressed HEK293 cells [49]. The functional activity of the analogs was determined using a β-arrestin recruitment assay. The assay was initially conducted in agonist binding mode to confirm that they function as antagonists at the D_3 receptor. Once this efficacy was confirmed, the assay was conducted in antagonist mode to determine the ability of the antagonist to compete with dopamine at the D_3 receptor. The results of the antagonist mode assay are reported as IC_{50} values [50–52]. Imax values were individually calculated from the assay and reliable with over 50% inhibition.

The first series of compounds evaluated were those synthesized in Schemes 1 and 2 (Table 1). The dimethyl amino analogs **6a–d** displayed a relatively low binding affinity for both D_2 and D_3 receptors. These data suggest that a basic amine moiety in the spacer group reduces affinity at both receptors. The observation that **6d** had a 10-fold higher affinity than its structural congener **6b** indicates that the Br-substituent is more preferred in the OBS than the corresponding fluoropropyl substituent. Compounds **9a**,**b** and **11a**,**b**, which have aromatic groups in the SBS, displayed a higher affinity at both D_2 and D_3 receptors. The 4-(thiophen-2-yl)benzamide analogs were more potent at the D_3 receptor than the corresponding 4-methyl-5-phenyl-4*H*-1,2,4-triazole-3-thiol analogs. These data suggest that benzamides are preferred in the SBS of the D_3 receptor for this scaffold. It is of interest to note that **9a** had ~170-fold higher affinity at the D_3 versus the D_2 receptor.

It is important to note that **9a** has two different modes in which it can bind to the D_3 receptor. The first mode has the bromobenzamide moiety binding to the OBS and the 4-(thiophen-2-yl)benzamide binding to the SBS. The second mode has the 4-(thiophen-2-yl)benzamide binding to the OBS and the bromobenzamide moiety binding to the SBS. In vitro binding studies revealed that fragment **12** showed non-selectively high *K*i values at both of dopamine receptor subtypes (*K*i D_2 = 89.2 ± 5.6 nM, D_3 = 21.8 ± 5.1 nM), whereas **14** did not show any binding affinity at D_2 and D_3 receptors (*K*i D_2 > 1000 nM and D_3 > 1000 nM). Moreover, the β-arrestin recruitment assay indicated that compound **18** is very potent for the D_3 receptor (IC_{50} = 4.6 ± 1.2 nM). These data are consistent with the first mode that the bromobenzamide moiety binds to the OBS and the 4-(thiophen-2-yl)benzamide binds to the SBS.

Table 1. Binding affinities and potency of R_1 and R_2 modified analogs with different length of the alkyl linker [a].



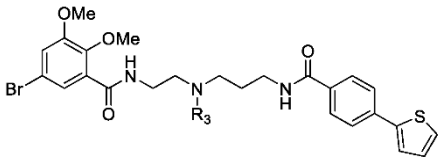
Cmpd	R_1	R_2	n	*Ki* ± SEM (nM) [b]		D_2/D_3	IC_{50} (nM) [c]	Imax (% Control) [d]
				D_3	D_2			
6a			2	1763 ± 942	4564 ± 2445	2.6	>1000	92.7 ± 5.4
6b			3	1236 ± 438	2810 ± 571	2.3	430 ± 467	98.4 ± 2.9
6c			2	1627 ± 165	5416 ± 1043	3.3	>1000	98.0 ± 5.6
6d			3	141 ± 26	449 ± 90	3.2	152 ± 185	94.0 ± 6.4
9a			1	1.0 ± 0.01	169 ± 4	169	14.0 ± 7.4	67.3 ± 18.9
9b			2	8.0 ± 0.8	103 ± 14	12.9	104 ± 136	70.8 ± 7.3
11a			2	125 ± 2	342 ± 23	2.7	NT [e]	NT
11b			3	22.2 ± 1.8	89.7 ± 3.6	4.6	NT	NT

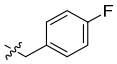
[a] All compounds were converted to HCl salts prior to tests. [b] mean ± SEM; mean *Ki* ± SEM values were measured using [^{125}I]IABN in D_2 or D_3 receptors highly expressed HEK cells. The radioligand binding assay was performed by three individual experiments. [c] The potency for D_3 receptors was expressed as mean ± SD by three individual experiments. [d] Imax was obtained from a percentage of the maximum inhibition of a dopamine at EC_{80} concentration in the same assay. [e] NT; not tested.

Table 2 shows the effect of the size of the *N*-alkyl group in the *tert*-amine on the D_2 and D_3 receptor binding. Our results indicate that the *N*-ethyl substituent **9a** showed the highest binding affinity and subtype selectivity at the D_3 receptor versus the D_2 receptor. There was a slight decrease in affinity in going from propyl to allyl groups, whereas the 4-fluorobenzyl group resulted in a large loss in affinity at both D_2 and D_3 receptors (Table 2). When the size of substituents was increased, the binding affinity and subtype selectivity was decreased. This reduction in affinity also translated to the β-arrestin recruitment assay. That is, there was a trend of decreased potency in the order of **9a** (IC_{50} = 14.0 ± 7.4 nM) > **20a** (IC_{50} = 26.5 ± 12.9 nM) > **20b** (IC_{50} = 51.6 ± 40.8 nM). Based on this SAR study, the *N*-ethyl group is the preferred alkyl group with respect to binding to the OBS.

A number of compounds were prepared to explore the nature of the interaction between the aromatic ring and the SBS. Previous studies with the *N*-aryl piperazine analogs revealed that a wide range of aromatic rings are tolerated in the SBS with respect to D_3 affinity, but the overall D_3 versus D_2 selectivity can be influenced by the nature of this interaction. The results of this study are shown in Table 3. All compounds had good affinity at D_3 receptors, with *Ki* values ranging between 0.8 and 13.2 nM. The D_2 affinities ranged between 107 and 525 nM, resulting in a D_3 selectivity ratio (i.e., D_2/D_3 ratio) ranging from 22.1- to 180-fold. The effect of the nature of the aromatic ring in the SBS on the ability of the antagonist to compete with dopamine in the β-arrestin assay was somewhat unexpected. For example, both **21a** and **21c** have ~1 nM affinity for the D_3 receptor in the radioligand binding assay, but the potency of **21c** in the β-arrestin recruitment assay was 10-fold higher than that of **21a** (IC_{50} = 1.3 vs. 16.4 nM).

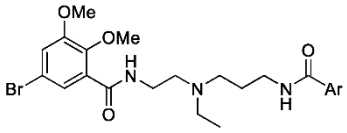
Table 2. Binding affinities of different sized substituents on the *tert*-amine [a].



Cmpd	R_3	*Ki* ± SEM (nM) [b]		D_2/D_3	IC_{50} (nM) [c]	Imax (% Control) [d]
		D_3	D_2			
9a		1.0 ± 0.01	169 ± 4	169	14.0 ± 7.4	67.3 ± 18.9
20a		2.7 ± 0.4	259 ± 23	110	26.5 ± 12.9	88.1 ± 20.4
20b		5.8 ± 1.0	243 ± 15	46.7	51.6 ± 40.8	100.6 ± 7.9
20c		299 ± 101	574 ± 170	1.9	NT [e]	NT

[a] All compounds were converted to HCl salts prior to tests. [b] mean ± SEM; mean *Ki* ± SEM values were measured using [^{125}I]IABN in D_2 or D_3 receptors highly expressed HEK cells. The radioligand binding assay was performed by three individual experiments. [c] The potency for D_3 receptors was expressed as mean ± SD by three individual experiments. [d] Imax was obtained from a percentage of the maximum inhibition of a dopamine at EC_{80} concentration in the same assay. [e] NT; not tested.

Table 3. R_3 optimization based on carboxamide moieties [a].



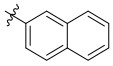
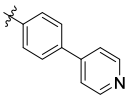
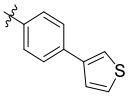
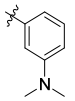
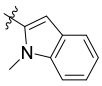
Cmpd	Ar	*Ki* ± SEM (nM) [b]		D_2/D_3	IC_{50} (nM) [c]	Imax (% Control) [d]	cLogP [e]
		D_3	D_2				
21a		1.2 ± 0.2	169 ± 12	141	16.4 ± 7.7	66.1 ± 17.6	4.12
21b		13.2 ± 0.5	525 ± 72	39.8	19.6 ± 23.7	75.2 ± 4.8	3.21
21c		1.1 ± 0.1	107 ± 5	97.3	1.3 ± 1.0	78.2 ± 18.0	3.47
21d		0.8 ± 0.2	148 ± 9	180.5	9.3 ± 12.0	57.7 ± 26.9	2.72
21e		2.8 ± 0.5	142 ± 16	50.7	4.3 ± 2.5	85.4 ± 6.0	2.41
21f		6.1 ± 0.6	327 ± 32	53.7	2.7 ± 0.6	85.7 ± 13.1	1.79
21g		2.5 ± 0.3	312 ± 18	125	36.8 ± 35.7	50.1 ± 20.9	4.78
21h		11.2 ± 1.4	248 ± 19	22.1	4.1 ± 2.1	73.8 ± 14.9	3.41

Table 3. *Cont.*

Cmpd	Ar	*Ki* ± SEM (nM) [b]		D_2/D_3	IC_{50} (nM) [c]	Imax (% Control) [d]	cLogP [e]
		D_3	D_2				
21i		7.0 ± 0.5	304 ± 7	43.4	2.7 ± 0.2	99.6 ± 20.7	3.05
21j		3.1 ± 0.5	192 ± 22	61.9	4.8 ± 0.8	81.5 ± 10.1	2.96

[a] All compounds were converted to HCl salts prior to tests. [b] mean ± SEM; mean *Ki* ± SEM values were measured using [^{125}I]IABN in D_2 or D_3 receptors highly expressed HEK cells. The radioligand binding assay was performed by three individual experiments. [c] The potency for the D_3 receptor was expressed as mean ± SD by three individual experiments. [d] Imax was obtained from a percentage of the maximum inhibition of a dopamine at EC_{80} concentration in the same assay. [e] NT; not tested.

2.3. Molecular Docking and Molecular Dynamics Simulations (MDS)

To understand the favorable binding profiles of the metoclopramide analogs, molecular docking and MDS studies were performed using different *N*-alkyl compounds (**9a**, **20a**, **20b**, **20c** and **21c**) with the D_3 receptor (PDB: 3PBL) (Table 4). These compounds were chosen because they are close structural analogs and have a wide range in D_3 receptor affinity (1–300 nM). As reported in previous studies [13,29,53], the binding pose that formed a bridge hydrogen bond between the carboxylate of ASP110$^{3.32}$ and the protonated nitrogen was considered to be critical for high binding affinity for the D_3 receptor. The distance between the protonated nitrogen ranged between 2.6 and 2.9 Å, and **9a** was found to have the closest interaction (2.6 Å). The estimated binding energies were not significantly different for each compound (−9.74 to −10.22 kcal/mol). Therefore, the difference in D_3 affinity of the five compounds cannot be explained by the distance between ASP110$^{3.32}$ and the protonated nitrogen atom, and the calculated binding energies from docking studies.

Table 4. Molecular docking and MDS results of selected analogs.

Cmpd	Docking		MDS
	Distance to ASP110 (Å)	Binding Energy (kcal/mol)	Ligand RMSD (Å)
fallypride [a]	2.7	−7.71	2.08 ± 0.33
9a	2.6	−9.74	3.18 ± 0.54
20a	2.9	−10.11	2.18 ± 0.74
20b	2.8	−10.00	2.29 ± 0.60
20c	2.9	−10.22	3.00 ± 0.82
21c	2.7	−10.10	2.45 ± 0.49

[a] Values were obtained from the previous study [29].

In MDS studies, the root mean square distance (RMSD) was calculated over 50–200 ns in five copies of the MDS production (Table 4). The first time frame (0 ns) of the production run was used as the reference position to determine the stability of each compound in the binding site. **21c** presented the lowest standard deviation of RMSD (2.45 ± 0.49 Å) indicating the least amount of movement in the binding site. A relatively higher amount of motion (3.00 ± 0.82 Å) with **20c** is consistent with the lower binding affinity for D_3 receptors. These results indicate the MDS studies correlate better with D_3 affinity than the results of docking studies.

The representative binding pose of the MDS production run is displayed in Figure 1. Within the OBS of the D_3 receptor, all the selected compounds were engaged in multiple interactions. The hydrogen bond with ASP110$^{3.32}$ and π staking interactions with PHE345$^{6.52}$ were observed with all five compounds. However, a halogen bond between VAL189$^{5.39}$ and the bromine of the 5-bromo-2,3-dimethoxybenzene moiety was observed for **9a**, **21c**, and **20c** (Figure 1a,b,e, respectively). It is of interest to note the **21c**, the most potent compound in the β-arrestin recruitment assay, which predicts the ability to compete with endogenous

dopamine, had a cation–π interaction between the protonated nitrogen and PHE106$^{3.28}$ residue (Figure 1b).

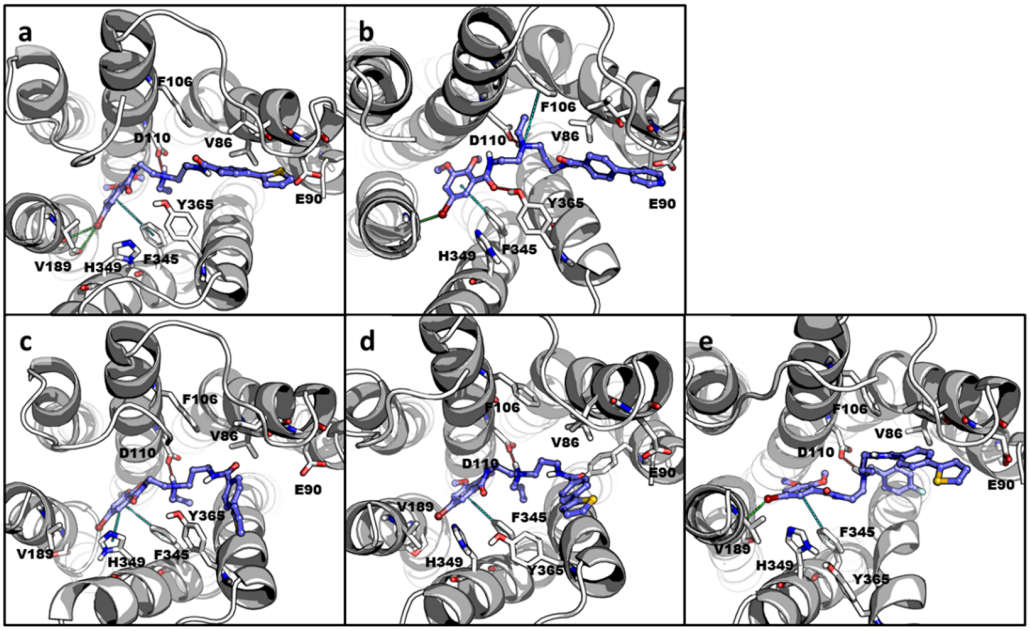


Figure 1. Representative poses of MDS for (**a**) **9a**, (**b**) **21c**, (**c**) **20a**, (**d**) **20b**, and (**e**) **20c**. The predicted interactions of each compound with residues in the OBS and the SBS of the D_3 receptor were distinguished by the color. Red: hydrogen; cyan: π-interactions; green: halogen bond.

The summary of overall frequency of contacts from the MDS studies, including hydrophobic interactions, hydrogen bonds, the salt bridge, halogen bonds, and π-interactions, is shown in Figure 2. All five compounds formed stable interactions (frequency of contact > 0.6) with most of residues in the OBS (i.e., ASP110$^{3.32}$, VAL111$^{3.33}$, CYS114$^{3.36}$, SER196$^{5.46}$, PHE345$^{6.51}$, and THR369$^{7.39}$). The frequency of all interactions in the OBS of **20c**, which exhibited the lowest binding affinity for D_3 receptors, was lower than the higher-affinity compounds. As mentioned above, **21c** showed a high frequency of contacts with PHE106$^{3.28}$ including approximately 95% of hydrophobic interactions and 10% of cation–π interactions over the MDS production runs.

Consistent with previous modeling studies, the formation of key interactions between ASP110$^{3.32}$ and the protonated nitrogen of the ligand stabilized the binding pose of **9a**, **20a**, **20b**, and **20c** (frequency of contact > 0.998) by 97.8% to 99.4% of the hydrogen bond formation. However, the frequency of contacts between ASP110$^{3.32}$ and **20c** was relatively lower (frequency of contact = 0.990) and formed only 68.6% of hydrogen bonds over the MDS production runs.

In the SBS, **9a**, **20a** and **21c** that exhibited high subtype selectivity, presented a moderate to high probability (frequency of contacts = 0.4–0.9) of interaction with VAL86$^{2.61}$, LEU89$^{2.64}$, GLY93^{EL1}, and GLY94^{EL1}. In addition, the pyridine of **21c** formed a hydrophobic interaction with GLU90$^{2.65}$ (frequency of interaction = 0.563). In contrast to our expectations, 90% of the hydrophobic interactions that formed with VAL86$^{2.61}$ were from the 4-fluorobenzyl group whereas 10% of the interactions were from the 4-(thiophen-2-yl)benzamide moiety.

The average frequency of the overall interactions in the binding sites (i.e., OBS and SBS) was correlated with D_3 receptor binding affinity ($r = -0.8756$, and $p = 0.0517$). In addition, the average frequency of interaction in the OBS was significantly correlated with the IC_{50} values from the β-arrestin recruitment assay ($r = -0.9934$, and $p = 0.0066$).

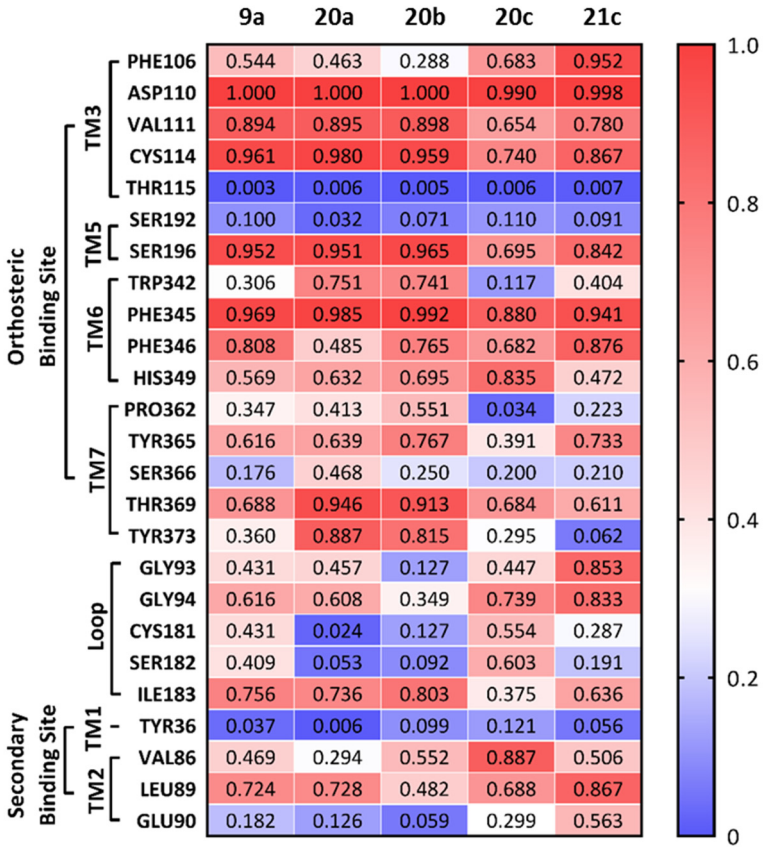


Figure 2. Summary of frequency of all contacts between selected ligands and residues in the D_3 receptor binding sites (the OBS and SBS).

2.4. Comprehensive Screening for Other GPCRs

Based on the results in the dopamine receptor radioligand binding assays, nine flexible-based compounds were selected for further evaluation for off-target binding with other GPCRs through the Psychoactive Drug Screening Program (PDSP) (Supplemental Table S2) [54]. Previous studies with the *N*-aryl piperazine analogs showed high binding affinity for serotonin 5-HT_{1A} and 5-HT_{2B} receptors. For example, many of the *N*-aryl piperazine-based analogs that our group developed in the past for either the D_2 or D_3 receptor had high affinity for the 5-HT_{1A} receptor [55–58]. It is of interest to note that none of the panel submitted for evaluation had a high affinity for the 5-HT_{1A} receptor or any of the other GPCRs in the screening assay (Supplemental Table S2). Compounds **21a**, **21c**, and **21i** had modest affinity for the 5-HT_3 receptor (*K*i values 29–58 nM). Furthermore, a relatively high affinity of compounds **20a**, **21a**, **21e**, **21g**, and **21i** for the peripheral benzodiazepine receptor (PBR) was observed. This mitochondrial-based protein is typically used as a target for imaging neuroinflammation. The results of the PDSP-binding assays also confirmed the data obtained in our lab for the binding of this panel of nine compounds to D_2 and D_3 receptors (Supplemental Table S2).

3. Discussion

The goal of the current study was to identify a new scaffold for D_3-selective antagonists that must display a high affinity and selectivity for D_3 versus D_2 receptors in the radioligand binding assays, but also a high potency in a β-arrestin recruitment assay, which measures the ability of a compound to compete with dopamine in binding to the D_3 receptor [21,29]. Previous studies have shown that a PET radiotracer developed in our lab having a high affinity for the D_3 receptor (Kd~50 pM) and excellent selectivity versus the D_2 receptor (>150-fold) was not able to image D_3 receptors in vivo without pretreatment with drugs that reduce synaptic levels of dopamine [59].

For the current study, we chose metoclopramide as the lead compound for our SAR studies. Metoclopramide was chosen as the lead compound for this study because it has a modest affinity for both D_2 and D_3 receptors and it should be possible to make analogs of this compound having an improved D_3 binding affinity while minimizing D_2 receptor affinity by interacting with the SBS. The results of SAR indicated that 5-bromo-2,3-dimethoxybenzamide, the moiety from FLB457, was more favorable for binding to the OBS, which is important for determining affinity for both D_3 and D_2 receptors. The size of fragments in **9a**,**b** or **11a**,**b** that interact with the SBP residues of the D_3 receptor are important for high selectivity for D_3 versus D_2 selectivity [60]. It is of interest to note that the appropriate length of linker between the basic amine and the secondary binding fragment was one carbon shorter than other known D_3 receptor antagonists such as *N*-arylpiperazine congeners. The D_3 receptor binding affinity was also affected by steric hindrance of the substituent on the basic amine.

A number of the compounds reported here exhibited excellent D_3 binding affinity (ranging from 0.8 to 13.2 nM) and excellent selectivity (22.1- to 180-fold) for D_3 vs. D_2 receptors. Although analogs such as **9a**, **21a**, **21d**, and **21g** exhibit high binding affinity and subtype selectivity for the D_3 receptor, **21c** was identified as the best-in-series candidate because of its high D_3 affinity and selectivity, and excellent potency in the β-arrestin recruitment assay (IC_{50} = 1.3 nM). This IC_{50} value was comparable with fallypride that is widely used as a non-selective PET probe for D_2/D_3 receptors and can bind to D_3 receptors in the presence of endogenous dopamine (fallypride, IC_{50} = 1.7 nM) [29]. Moreover, the computational modeling studies demonstrated that the high potency of **21c** may result from the short distance of the bridge-bond with $ASP110^{3.32}$ and the high-frequency contacts between **21c** and residues in the OBS and SBS in the D_3 receptor.

Since metoclopramide was previously used in drug development and led to the identification of compounds having a diverse range of pharmacologic activity including mixed 5-HT_3 antagonists/5-HT_4 agonists (e.g., zacopride, BRL 24682) and D_2 antagonists (e.g., clebopride, BRL 25594) [45], there was a concern that the conformational flexibility of our compounds could result in significant off-target bindings to other G-proteins. By the comprehensive screening from PDSP, these compounds possess minimal affinity for other GPCRs except a moderate affinity for 5-HT_3 receptors (29–58 nM). Interestingly, **21d**, which has an indole carboxamide as a secondary binding fragment, exhibited nM binding affinity for the histamine H_1 receptor (0.95 nM). Other compounds acquired affinity for the translocator protein (TSPO); however, it is not clear if this off-target binding would be problematic for using these compounds in D_3 receptor binding assays or behavioral studies. Further studies are ongoing in our lab to prepare radiolabeled versions of **21c** for imaging D_3 receptors in the brain, and SAR studies are being conducted that aim to improve the properties of this new scaffold as a means of identifying potential D_3 receptor selective PET radiotracers.

4. Materials and Methods

4.1. General

5-(3-Fluoropropyl)-2,3-dimethoxybenzoic acid (**1a**) was prepared from methyl 5-allyl-3-methoxy salicylate via methylation for phenol, oxidation of the allyl group, fluorination, and hydrolysis of methyl ester [61]. 5-Bromo-2,3-dimethoxybenzoic acid (**1b**) was prepared from 5-bromo-2-hydroxy-3-methoxy benzoic acid via methylation for phenol and oxidation of aldehyde to carboxylic acid using silver catalyst [62]. For the OBS binding, ***tert***-butyl (2-aminoethyl) ethylcarbamate was prepared from ***N***-ethylethylenediamine via the primary amine protection, the secondary amine protection and the primary amine de-protection according to the literature [63]. 2-(2-Bromoethyl)-1,3-dioxolane and 2-(3-bromopropyl)-1,3-dioxolane were prepared via reduction followed by cyclization [64]. The other reagents and solvents were purchased from Sigma-Aldrich, TCI, Matrix Scientific, Advanced chemtech, Fisher chemical, Ambeed, Chembridge corporation, Acros organics, and Decon laboratories and used as received (Supplemental Table S3). Reactions were monitored by thin layer chromatography (TLC) using TLC silica gel 60W F254S plates and the spots were detected

under UV light (254 nm) or developed using ninhydrin. Flash column chromatography was carried out on a Biotage Isolera One with a dual wavelength UV-vis detector. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker NEO-400 spectrometer (Bruker, Billerica, MA, USA). Chemical shifts (δ) were recorded in parts per million (ppm) relative to the deuterated solvent as an internal reference. Mass spectra (m/z) were recorded on a 2695 Alliance LC-MS (Waters Corporation, Milford, MA, USA) using positive electrospray ionization (ESI^+). High resolution mass spectra (HRMS, *m/z*) were acquired on a waters LCT premier mass spectrometer (Waters Corporation, Milford, MA, USA). PathHunter^{TM} β-arrestin recruitment assay kit and the Chinese hamster ovary CHO-K1 cell line were purchased from DiscoverX (Fremont, CA, USA).

4.2. Chemistry

tert-Butyl ethyl(2-(5-(3-fluoropropyl)-2,3-dimethoxybenzamido)ethyl)carbamate (**2a**) In a mixture of **1a** (1 g, 4.13 mmol), ***tert***-butyl (2-aminoethyl) ethylcarbamate, (1.55 g, 8.26 mmol) and HBTU (1.55 g, 6.2 mmol) in DMF (20 mL), DIPEA (1.08 mL, 6.2 mmol) was added. The mixture was stirred for 24 h at RT. After completion of the reaction, the mixture was diluted with EtOAc and washed with water and brine. The volatiles were removed under reduced pressure and the crude product was purified by flash chromatography on silica gel (EtOAc/hexane = 1:2) to afford **2a** (1.15 g, 68% yield) as a yellow oil. (^{1}H NMR, 400 MHz, $CDCl_3$): δ = 8.12 (br, 1H), 7.45 (s, 1H), 6.81 (s, 1H), 4.43 (t, *J* = 5.9 Hz, 1H), 4.31 (t, *J* = 5.9 Hz, 1H), 3.81 (s, 3H), 3.80 (s, 3H), 3.53 (q, *J* = 6.1 Hz, 2H), 3.37 (t, *J* = 6.3 Hz, 2H), 3.22 (d, *J* = 6.2 Hz, 2H), 2.66 (t, *J* = 7.4 Hz, 2H), 2.00–1.87 (m, 2H), 1.37 (s, 9H), 1.05 (t, *J* = 7.0 Hz, 3H); (^{13}C NMR, 100 MHz, $CDCl_3$): δ = 165.4, 152.3, 145.8, 137.2, 83.6, 82.0, 79.4, 61.1, 56.0, 45.7, 31.8, 31.6, 31.0$_3$, 30.9$_8$, 28.2; ESI-MS *m/z* calculated for $C_{21}H_{34}FN_2O_5^+$ $[M+H]^+$ 413.5; found 413.6.

tert-Butyl (2-(5-bromo-2,3-dimethoxybenzamido)ethyl)(ethyl)carbamate (**2b**) **2b** was synthesized using **1b** (5 g, 19.15 mmol) in the same procedure as **2a** and purified by flash chromatography on silica gel (EtOAc/hexane = 1:2) to afford **2b** (8 g, 97% yield) as a yellow oil. (^{1}H NMR, 400 MHz, CD_3CN): δ = 7.96 (br, 1H), 7.57 (d, *J* = 2.2 Hz, 1H), 7.24 (d, *J* = 2.3 Hz, 1H), 3.85 (s, 3H), 3.83 (s, 3H), 3.48 (q, *J* = 6.0 Hz, 2H), 3.37 (t, *J* = 6.0 Hz, 2H), 3.23 (q, *J* = 7.0 Hz, 2H), 1.38 (s, 9H), 1.07 (t, *J* = 7.0 Hz, 3H) (^{13}C NMR, 100 MHz, CD_3CN): δ = 164.8, 154.9, 147.9, 125.1, 119.2, 117.1, 79.8, 62.0, 57.2, 39.4, 28.6; ESI-MS *m/z* calculated for $C_{18}H_{27}BrN_2O_5^+$ $[M]^+$ 431.3; found 431.4.

N-(2-(Ethylamino)ethyl)-5-(3-fluoropropyl)-2,3-dimethoxybenzamide (**3a**) In a solution of **2a** (1.15 g, 2.79 mmol) in 15 mL of CH_2Cl_2, TFA (15 mL, 196 mmol) was slowly added at 0 °C. The reaction mixture was warmed to RT and stirred for 1 h. The volatiles were removed followed by the residue was dissolved in CH_2Cl_2 and the organic layer washed by aq saturated $NaHCO_3$ solution. The inorganic layer was extracted by CH_2Cl_2 and the combined layer was washed by brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo to afford **3a** (850 mg, 98% yield) as a yellow oil. The crude product was used for the next step without further purification. (^{1}H NMR, 400 MHz, CD_3CN): δ = 8.26 (br, 1H), 7.36 (d, *J* = 2.2 Hz, 1H), 7.02 (d, *J* = 2.1 Hz, 1H), 4.52 (t, *J* = 6.0 Hz, 1H), 4.40 (t, *J* = 6.0 Hz, 1H), 3.86 (s, 3H), 3.84 (s, 3H), 3.43 (q, *J* = 5.8 Hz, 2H), 2.78 (t, *J* = 6.1 Hz, 2H), 2.71 (t, *J* = 7.6 Hz, 2H), 2.65 (q, *J* = 7.1 Hz, 2H), 2.00–1.96 (m, 2H), 1.37 (s, 9H), 1.08 (t, *J* = 7.1 Hz, 3H); (^{13}C NMR, 100 MHz, CD_3CN): δ = 165.9, 153.9, 146.8, 138.7, 128.1, 122.4, 116.7, 85.2, 61.8, 56.8, 49.4, 44.5, 40.2, 32.9, 31.8, 15.7; ESI-MS *m/z* calculated for $C_{16}H_{26}FN_2O_3^+$ $[M+H]^+$ 313.4; found 313.5.

Bromo-*N*-(2-(ethylamino)ethyl)-2,3-dimethoxybenzamide (**3b**) **3b** was synthesized using **2b** (8 g, 18.55 mmol) in the same procedure as **3a** and purified by flash chromatography on silica gel (CH_2Cl_2/7 N NH_3 in MeOH = 20:1) to afford 3b (6 g, 98% yield) as a yellow oil. (^{1}H NMR, 400 MHz, CD_3CN): δ = 8.24 (br, 1H), 7.60 (d, *J* = 2.4 Hz, 1H), 7.26 (d, *J* = 2.4 Hz, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 3.43 (q, *J* = 5.7 Hz, 2H), 2.80 (t, *J* = 6.0 Hz, 2H), 2.66 (q, *J* = 7.1 Hz, 2H), 1.08 (t, *J* = 7.1 Hz, 3H) (^{13}C NMR, 100 MHz, CD_3CN): δ = 164.5, 155.0,

148.0, 129.8, 125.2, 119.3, 117.2, 62.0, 57.2, 49.1, 44.4, 40.1, 15.4; ESI-MS *m/z* calculated for $C_{13}H_{21}BrN_2O_3^+$ $[M]^+$ 331.2; found 331.4.

N-(2-((3-(1,3-Dioxolan-2-yl)propyl)(ethyl)amino)ethyl)-5-(3-fluoropropyl)-2,3-dimethoxybenzamide (**4a**) In a solution of **3a** (300 mg, 0.96 mmol) in 9.6 mL of MeCN, 2-(3-bromopropyl)-1,3-dioxolane (281 mg, 1.44 mmol) and Na_2CO_3 (254 mg, 2.4 mmol) were added. The reaction mixture was stirred for 24 h at 65 °C and another 1 eq of 2-(3-bromopropyl)-1,3-dioxolane (187 mg, 0.96 mmol) was added. The reaction mixture was stirred for 48 h at 65 °C. After the completion of the reaction which was checked by TLC, aq saturated $NaHCO_3$ solution was added and the crude product was extracted with EtOAc. The organic layer was washed by brine, dried over $MgSO_4$, filtered and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH = 10:1) to afford 10a (240 mg, 59% yield) as a yellow oil. (^{1}H NMR, 400 MHz, CD_3CN): δ = 8.19 (br, 1H), 7.30 (d, *J* = 2.1 Hz, 1H), 6.94 (d, *J* = 2.0 Hz, 1H), 4.70 (t, *J* = 4.4 Hz, 1H), 4.44 (t, *J* = 6.0 Hz, 1H), 4.32 (t, *J* = 6.0 Hz, 1H), 3.79–3.75 (m, 8H), 3.69–3.65 (m, 2H), 3.35 (q, *J* = 5.8 Hz, 2H), 2.63 (t, *J* = 7.6 Hz, 2H), 2.55 (t, *J* = 6.1 Hz, 2H), 2.51 (q, *J* = 7.1 Hz, 2H), 2.44 (t, *J* = 7.2 Hz, 2H), 1.89–1.86 (m, 2H), 1.56–1.42 (m, 4H), 0.94 (t, *J* = 7.1 Hz, 3H) (^{13}C NMR, 100 MHz, CD_3CN): δ = 165.2, 153.4, 146.4, 138.2, 127.4, 122.0, 116.2, 104.7, 84.7, 83.1, 65.1, 61.4, 56.3, 53.3, 52.6, 47.6, 37.8, 32.4, 32.2, 32.0, 31.4, 31.3, 22.1, 15.6; ESI-MS *m/z* calculated for $C_{22}H_{36}FN_2O_5^+$ $[M+H]^+$ 427.5; found 427.6.

N-(2-((4-(1,3-Dioxolan-2-yl)butyl)(ethyl)amino)ethyl)-5-(3-fluoropropyl)-2,3-dimethoxybenzamide (**4b**) **4b** was synthesized using **3a** (346 mg, 1.10 mmol) and 2-(4-bromobutyl)-1,3-dioxolane (577 mg, 2.76 mmol) in the same procedures as 4a and obtained 282 mg (58% yield) as a yellow oil. (^{1}H NMR, 400 MHz, CD_3CN): δ = 8.34 (br, 1H), 7.41 (d, *J* = 2.0 Hz, 1H), 7.06 (d, *J* = 2.0 Hz, 1H), 4.75 (t, *J* = 4.4 Hz, 1H), 4.56 (t, *J* = 6.0 Hz, 1H), 4.44 (t, *J* = 6.0 Hz, 1H), 3.90–3.88 (m, 8H), 3.79–3.76 (m, 2H), 3.49 (q, *J* = 5.7 Hz, 2H), 2.77–2.66 (m, 6H), 2.57 (t, *J* = 6.5 Hz, 2H), 2.10–2.00 (m, 2H), 1.64–1.52 (m, 4H), 1.46–1.38 (m, 2H), 1.09 (t, *J* = 7.1 Hz, 3H) (^{13}C NMR, 100 MHz, CD_3CN): δ = 165.3, 153.4, 146.4, 138.2, 127.4, 121.9, 116.2, 104.7, 84.7, 83.1, 65.0, 61.4, 56.3, 53.4, 52.6, 47.8, 37.6, 34.0, 32.4, 32.2, 31.4, 31.3, 22.2; ESI-MS *m/z* calculated for $C_{23}H_{38}FN_2O_5^+$ $[M+H]^+$ 441.6; found 441.7.

N-(2-((3-(1,3-Dioxolan-2-yl)propyl)(ethyl)amino)ethyl)-5-bromo-2,3-dimethoxybenzamide (**4c**) **4c** was synthesized using **3b** (320 mg, 0.97 mmol) in the same procedures as 4a and obtained 269 mg (62% yield) as a yellow oil. (^{1}H NMR, 400 MHz, CD_3CN): δ = 8.23 (br, 1H), 7.62 (d, *J* = 2.4 Hz, 1H), 7.25 (d, *J* = 2.4 Hz, 1H), 4.77 (t, *J* = 4.8 Hz, 1H), 3.85–3.82 (m, 8H), 3.75–3.71 (m, 2H), 3.40 (q, *J* = 5.7 Hz, 2H), 2.59 (t, *J* = 6.1 Hz, 2H), 2.55 (q, *J* = 7.1 Hz, 2H), 2.48 (t, *J* = 7.2 Hz, 2H), 1.59–1.49 (m, 4H), 0.99 (t, *J* = 7.1 Hz, 3H) (^{13}C NMR, 100 MHz, CD_3CN): δ = 164.0, 154.9, 148.0, 129.6, 125.3, 119.3, 117.2, 105.2, 65.5, 62.0, 57.2, 53.7, 52.9, 48.0, 38.4, 32.5, 22.7, 12.2; ESI-MS *m/z* calculated for $C_{19}H_{29}BrN_2O_5^+$ $[M]^+$ 445.4; found 445.5.

N-(2-((4-(1,3-Dioxolan-2-yl)butyl)(ethyl)amino)ethyl)-5-bromo-2,3-dimethoxybenzamide (**4d**) **4d** was synthesized using **3b** (309 mg, 0.93 mmol) in the same procedures as 4a and obtained 412 mg (96% yield) as a yellow oil. (^{1}H NMR, 400 MHz, CD_3CN): δ = 8.25 (br, 1H), 7.62 (d, *J* = 2.4 Hz, 1H), 7.25 (d, *J* = 2.4 Hz, 1H), 4.70 (t, *J* = 4.8 Hz, 1H), 3.85–3.82 (m, 8H), 3.74–3.70 (m, 2H), 3.41 (q, *J* = 5.8 Hz, 2H), 2.61 (t, *J* = 6.1 Hz, 2H), 2.57 (q, *J* = 7.1 Hz, 2H), 2.47 (t, *J* = 7.1 Hz, 2H), 1.58–1.53 (m, 2H), 1.51–1.44 (m, 2H), 1.40–1.32 (m, 2H), 1.00 (t, *J* = 7.1 Hz, 3H) (^{13}C NMR, 100 MHz, CD_3CN): δ = 164.1, 154.9, 148.0, 129.6, 125.3, 119.3, 117.1, 105.2, 65.5, 62.0, 57.2, 53.8, 52.9, 48.1, 38.3, 34.6, 27.7, 22.8, 12.0; ESI-MS *m/z* calculated for $C_{20}H_{31}BrN_2O_5^+$ $[M]^+$ 459.4; found 459.5.

N-(2-(Ethyl(4-oxobutyl)amino)ethyl)-5-(3-fluoropropyl)-2,3-dimethoxybenzamide (**5a**) In a solution of **4a** (86 mg 0.2 mmol), in 2 mL of THF, 2 mL of aq 4 N HCl was slowly added. The reaction mixture was stirred for 3 h at RT and then, neutralized by 4 mL of aq 2 N NaOH solution. The crude product was extracted with EtOAc and the organic layer was washed by aq saturated $NaHCO_3$ solution, water and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo to afford 11a (74 mg, 96% yield) as a colorless oil. 11a was used without further purification for the next step. ESI-MS *m/z* calculated for $C_{20}H_{32}FN_2O_4^+$ $[M+H]^+$ 383.5; found 383.5.

N-(2-(Ethyl(5-oxopentyl)amino)ethyl)-5-(3-fluoropropyl)-2,3-dimethoxybenzamide (**5b**) **5b** was synthesized using **4b** (42 mg, 0.1 mmol) in the same procedures as **5a** and obtained 33 mg (88% yield) as a colorless oil. ESI-MS *m/z* calculated for $C_{21}H_{34}FN_2O_4^+$ $[M+H]^+$ 397.5; found 397.7.

5-Bromo-*N*-(2-(ethyl(4-oxobutyl)amino)ethyl)-2,3-dimethoxybenzamide (**5c**) **5c** was synthesized using **4c** (74 mg, 0.17 mmol) in the same procedures as **5a** and obtained 64 mg (96% yield) as a colorless oil. ESI-MS *m/z* calculated for $C_{17}H_{25}BrN_2O_4^+$ $[M]^+$ 401.3; found 401.4.

5-Bromo-*N*-(2-(ethyl(5-oxopentyl)amino)ethyl)-2,3-dimethoxybenzamide (**5d**) **5d** was synthesized using **4d** (90 mg, 0.2 mmol) in the same procedures as **5a** and obtained 63 mg (78% yield) as a colorless oil. ESI-MS *m/z* calculated for $C_{18}H_{27}BrN_2O_4^+$ $[M]^+$ 415.3; found 415.4.

N-(2-((4-(Dimethylamino)butyl)(ethyl)amino)ethyl)-5-(3-fluoropropyl)-2,3-dimethoxybenzamide (**6a**) In a mixture of **5a** (74 mg, 0.19 mmol) and 2 M solution of dimethylamine in THF (0.97 mL, 1.93 mmol) in 2 mL of dichloroethane, sodium triacetoxyborohydride (205 mg, 0.97 mmol) was added. The mixture was stirred at RT for 16 h. After completion of the reaction, the mixture was diluted with EtOAc and washed by aq saturated $NaHCO_3$ solution and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel (CH_2Cl_2/7 N NH_3 in MeOH = 20:1) to afford 6a (33 mg, 42% yield) as a colorless oil. (^{1}H NMR, 400 MHz, MeOD): δ = 7.30 (d, *J* = 1.9 Hz, 1H), 7.06 (d, *J* = 1.9 Hz, 1H), 4.51 (t, *J* = 5.9 Hz, 1H), 4.39 (t, *J* = 5.9 Hz, 1H), 3.90 (s, 3H), 3.88 (s, 3H), 3.51 (d, *J* = 6.6 Hz, 2H), 2.76–2.69 (m, 4H), 2.65 (q, *J* = 7.1 Hz, 2H), 2.56 (t, *J* = 6.9 Hz, 2H), 2.33 (t, *J* = 7.3 Hz, 2H), 2.22 (s, 6H), 2.07–1.94 (m, 2H), 1.54–1.50 (m, 4H), 1.10 (t, *J* = 7.1 Hz, 3H) (^{13}C NMR, 100 MHz, MeOD): δ = 168.1, 154.3, 147.3, 139.2, 128.1, 122.3, 117.1, 84.9, 83.2, 62.0, 60.7, 56.7, 54.5, 53.2, 45.5, 38.6, 33.5, 33.3, 32.3_2, 32.2_7, 26.3, 26.1, 12.0; ESI-MS *m/z* calculated for $C_{22}H_{39}FN_3O_3^+$ $[M+H]^+$ 412.6; found 412.6 HRMS (ESI) for $C_{22}H_{39}FN_3O_3^+$ $[M+H]^+$ requires 412.2975; found 412.2972.

N-(2-((5-(Dimethylamino)pentyl)(ethyl)amino)ethyl)-5-(3-fluoropropyl)-2,3 -dimethoxybenzamide (**6b**) **6b** was synthesized using **5b** (33 mg, 0.08 mmol) in the same procedures as 6a and obtained 14 mg (42% yield) as a colorless oil. (^{1}H NMR, 400 MHz, MeOD): δ = 7.31 (d, *J* = 1.9 Hz, 1H), 7.07 (d, *J* = 1.8 Hz, 1H), 4.51 (t, *J* = 5.9 Hz, 1H), 4.40 (t, *J* = 5.9 Hz, 1H), 3.91 (s, 3H), 3.88 (s, 3H), 3.51 (d, *J* = 6.6 Hz, 2H), 2.77–2.69 (m, 4H), 2.65 (q, *J* = 7.2 Hz, 2H), 2.54 (t, *J* = 7.4 Hz, 2H), 2.31 (t, *J* = 7.7 Hz, 2H), 2.23 (s, 6H), 2.08–1.95 (m, 2H), 1.58–1.48 (m, 4H), 1.38–1.31 (m, 2H), 1.10 (t, *J* = 7.1 Hz, 3H) (^{13}C NMR, 100 MHz, MeOD): δ = 168.1, 154.3, 147.3, 139.2, 128.1, 122.4, 117.2, 84.9, 83.2, 62.0, 60.7, 56.7, 54.5, 53.2, 45.4, 38.6, 33.5, 33.3, 32.3_3, 32.2_7, 28.2, 28.0, 26.5, 22.2, 12.0; ESI-MS *m/z* calculated for $C_{23}H_{41}FN_3O_3^+$ $[M+H]^+$ 426.6; found 426.6 HRMS (ESI) for $C_{23}H_{41}FN_3O_3^+$ $[M+H]^+$ requires 426.3132; found 426.3136.

5-Bromo-*N*-(2-((4-(dimethylamino)butyl)(ethyl)amino)ethyl)-2,3-dimethoxybenzamide (**6c**) **6c** was synthesized using **5c** (64 mg, 0.16 mmol) in the same procedures as **6a** and obtained 34 mg (49% yield) as a colorless oil. (^{1}H NMR, 400 MHz, MeOD): δ = 7.53 (d, *J* = 2.3 Hz, 1H), 7.31 (d, *J* = 2.3 Hz, 1H), 3.89 (s, 3H), 3.88 (s, 3H), 3.48 (d, *J* = 6.5 Hz, 2H), 2.68 (d, *J* = 6.5 Hz, 2H), 2.62 (q, *J* = 7.1 Hz, 2H), 2.53 (t, *J* = 6.7 Hz, 2H), 2.32 (t, *J* = 7.2 Hz, 2H), 2.22 (s, 6H), 1.50 (t, *J* = 3.5 Hz, 4H), 1.07 (t, *J* = 7.1 Hz, 3H) (^{13}C NMR, 100 MHz, MeOD): δ = 166.5, 155.4, 148.4, 130.0, 125.3, 119.7, 117.7, 62.1, 60.7, 57.1, 54.5, 53.1, 45.4, 38.7, 26.3, 26.1, 12.0; ESI-MS *m/z* calculated for $C_{19}H_{32}BrN_3O_3^+$ $[M]^+$ 430.4; found 430.5 HRMS (ESI) for $C_{19}H_{32}BrN_3O_3^+$ $[M]^+$ requires 430.1705; found 430.1703.

5-Bromo-*N*-(2-((5-(dimethylamino)pentyl)(ethyl)amino)ethyl)-2,3-dimethoxybenzamide (**6d**) **6d** was synthesized using **5d** (63 mg, 0.15 mmol) in the same procedures as 6a and obtained 31 mg (46% yield) as a colorless oil. (^{1}H NMR, 400 MHz, MeOD): δ = 7.54 (d, *J* = 2.4 Hz, 1H), 7.31 (d, *J* = 2.3 Hz, 1H), 3.89 (s, 3H), 3.88 (s, 3H), 3.48 (d, *J* = 6.5 Hz, 2H), 2.67 (d, *J* = 6.5 Hz, 2H), 2.62 (q, *J* = 7.2 Hz, 2H), 2.51 (t, *J* = 7.4 Hz, 2H), 2.27 (t, *J* = 7.7 Hz, 2H), 2.21 (s, 6H), 1.55–1.45 (m, 4H), 1.35–1.28 (m, 2H), 1.07 (t, *J* = 7.1 Hz, 3H) (^{13}C NMR, 100 MHz, MeOD): δ = 166.5, 155.3, 148.4, 130.0, 125.3, 119.7, 117.7, 62.1, 60.8, 57.1, 54.5, 53.0,

45.5, 38.7, 28.3, 28.1, 26.6, 12.0; ESI-MS *m/z* calculated for $C_{20}H_{34}BrN_3O_3^+$ $[M]^+$ 444.4; found 444.5 HRMS (ESI) for $C_{20}H_{34}BrN_3O_3^+$ $[M]^+$ requires 444.1862; found 444.1872.

5-Bromo-*N*-(2-((3-(1,3-dioxoisoindolin-2-yl)propyl)(ethyl)amino)ethyl)-2,3-dimethoxybenzamide (**7a**) In a solution of **3b** (2 g, 6.04 mmol) in 20 mL of DMF, *N*-(3-bromopropyl)phthalimide (3.4 g, 12.08 mmol) and K_2CO_3 (2.1 g, 15.1 mmol) were added. The mixture was heated to 65 °C and stirred for 16 h. The reaction mixture was cooled to RT and diluted with EtOAc. The mixture was washed by aq saturated $NaHCO_3$ solution, water and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel (CH_2Cl_2/7 N NH_3 in MeOH = 40:1) to afford **7a** (1.82 g, 57% yield) as a white solid. (^{1}H NMR, 400 MHz, acetone-d_6): δ = 8.32 (br, 1H), 7.82 (s, 4H), 7.66 (d, *J* = 2.4 Hz, 1H), 7.28 (d, *J* = 2.5 Hz, 1H), 3.92 (s, 3H), 3.91 (s, 3H), 3.73 (t, *J* = 7.1 Hz, 2H), 3.47 (q, *J* = 6.0 Hz, 2H), 2.66 (t, *J* = 6.2 Hz, 2H), 2.62–2.58 (m, 4H), 1.91–1.84 (m, 2H), 1.02 (t, *J* = 7.2 Hz, 3H) (^{13}C NMR, 100 MHz, acetone-d_6): δ = 168.9, 163.7, 154.9, 148.1, 135.0, 133.3, 129.7, 125.5, 123.7, 119.0, 116.9, 61.8, 57.0, 53.2, 51.6, 47.9, 38.4, 36.9, 27.1, 12.0; ESI-MS *m/z* calculated for $C_{24}H_{28}BrN_3O_5^+$ $[M]^+$ 518.4; found 518.5.

5-Bromo-*N*-(2-((4-(1,3-dioxoisoindolin-2-yl)butyl)(ethyl)amino)ethyl)-2,3-dimethoxybenzamide (**7b**) **7b** was synthesized using **3b** (180 mg, 0.54 mmol) and *N*-(4-bromobutyl)phthalimide (305 mg, 1.08 mmol) in the same procedures as 7a and obtained 220 mg (77% yield) as a colorless oil. (^{1}H NMR, 400 MHz, acetone-d_6): δ = 8.32 (br, 1H), 7.83 (s, 4H), 7.67 (d, *J* = 2.4 Hz, 1H), 7.26 (d, *J* = 2.5 Hz, 1H), 3.90 (s, 3H), 3.87 (s, 3H), 3.73 (t, *J* = 7.1 Hz, 2H), 3.46 (q, *J* = 5.8 Hz, 2H), 2.65 (t, *J* = 6.1 Hz, 2H), 2.62–2.54 (m, 4H), 1.76–1.69 (m, 2H), 1.58–1.51 (m, 2H), 1.04 (t, *J* = 7.1 Hz, 3H) (^{13}C NMR, 100 MHz, acetone-d_6): δ = 168.9, 163.7, 154.9, 148.1, 135.0, 133.2, 129.6, 125.5, 123.7, 119.1, 116.9, 61.8, 57.0, 53.5, 53.1, 48.1, 38.4, 27.2, 25.3, 12.2, 0.1; ESI-MS *m/z* calculated for $C_{25}H_{30}BrN_3O_5^+$ $[M]^+$ 532.4; found 532.5.

N-(2-((3-Aminopropyl)(ethyl)amino)ethyl)-5-bromo-2,3-dimethoxybenzamide (**8a**) In a solution of **7a** (1.82 g, 3.42 mmol) in 34 mL of EtOH, hydrazine hydrate (519 μL, 10.25 mmol) was added. The mixture was heated at 75 °C for 3 h and cooled to RT. The mixture was diluted with EtOAc and washed by aq saturated $NaHCO_3$ solution and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel (CH_2Cl_2/7 N NH_3 in MeOH = 10:1) to afford **8a** (1.03 g, 78% yield) as a yellow oil. (^{1}H NMR, 400 MHz, MeOD): δ = 7.52 (d, *J* = 2.3 Hz, 1H), 7.31 (d, *J* = 2.4 Hz, 1H), 3.89 (d, *J* = 2.7 Hz, 6H), 3.49 (t, *J* = 6.6 Hz, 2H), 2.68 (t, *J* = 7.0 Hz, 4H), 2.63 (q, *J* = 7.2 Hz, 2H), 2.57 (t, *J* = 7.2 Hz, 2H), 1.70–1.63 (m, 2H), 1.08 (t, *J* = 7.1 Hz, 3H) (^{13}C NMR, 100 MHz, MeOD): δ = 166.6, 155.4, 148.3, 130.1, 125.2, 119.8, 117.7, 62.1, 57.1, 53.2, 52.4, 48.6, 41.1, 38.7, 30.9, 12.0; ESI-MS *m/z* calculated for $C_{16}H_{26}BrN_3O_3^+$ $[M]^+$ 388.3; found 388.4.

N-(2-((4-Aminobutyl)(ethyl)amino)ethyl)-5-bromo-2,3-dimethoxybenzamide (**8b**) **8b** was synthesized using **7b** (220 mg, 0.41 mmol) in the same procedures as **8a** and obtained 165 mg (77% yield) as a yellow oil. (^{1}H NMR, 400 MHz, MeOD): δ = 7.53 (d, *J* = 2.2 Hz, 1H), 7.30 (d, *J* = 2.2 Hz, 1H), 3.89 (s, 3H), 3.88 (s, 3H), 3.47 (t, *J* = 6.6 Hz, 2H), 2.69–2.59 (m, 6H), 2.52 (t, *J* = 6.6 Hz, 2H), 1.56–1.44 (m, 4H), 1.07 (t, *J* = 7.1 Hz, 3H) (^{13}C NMR, 100 MHz, MeOD): δ = 166.5, 155.3, 148.3, 130.0, 125.3, 119.7, 117.7, 62.1, 57.1, 54.5, 53.1, 48.7, 42.6, 38.7, 31.8, 25.5, 12.1; ESI-MS *m/z* calculated for $C_{17}H_{28}BrN_3O_3^+$ $[M]^+$ 402.3; found 402.4.

5-Bromo-*N*-(2-(ethyl(3-(4-(thiophen-2-yl)benzamido)propyl)amino)ethyl)-2,3-dimethoxybenzamide (**9a**) Thionyl chloride (1.38 mL, 18.9 mmol) was added to 4-(thiophen-2-yl)benzoic acid (129 mg, 0.63 mmol) in a vial. The mixture was stirred at RT for 3 h and the volatiles were removed under the reduced pressure. **8a** (163 mg, 0.42 mmol) in 4.2 mL of CH_2Cl_2 and Et_3N (0.15 mL, 1.05 mmol) were added and the mixture was stirred for 16 h. After completion of the reaction, the volatiles were removed under the reduced pressure and the crude product was purified by flash chromatography on silica gel (CH_2Cl_2/7 N NH_3 in MeOH = 40:1) to afford **9a** (120 mg, 50% yield) as a yellow oil. (^{1}H NMR, 400 MHz, MeOD): δ = 7.78 (d, *J* = 8.6 Hz, 2H), 7.67 (d, *J* = 8.5 Hz, 2H), 7.50 (d, *J* = 2.4 Hz, 1H), 7.47 (dd, J_1 = 3.6 Hz, J_2 = 1.0 Hz, 1H), 7.43 (dd, J_1 = 5.1 Hz, J_2 = 1.0 Hz, 1H), 7.24 (d, *J* = 2.4

Hz, 1H), 7.11 (dd, J_1 = 5.0 Hz, J_2 = 3.7 Hz, 1H), 3.85 (s, 3H), 3.83 (s, 3H), 3.50 (t, *J* = 6.4 Hz, 2H), 3.43 (t, *J* = 6.8 Hz, 2H), 2.70 (t, *J* = 6.4 Hz, 2H), 2.67–2.62 (m, 4H), 1.86–1.79 (m, 2H), 1.07 (t, *J* = 7.1 Hz, 3H) (^{13}C NMR, 100 MHz, MeOD): δ = 169.6, 166.7, 155.3, 148.3, 144.3, 138.9, 134.4, 129.9, 129.5, 129.1, 127.2, 126.6, 125.6, 125.3, 119.8, 117.7, 62.1, 57.0, 53.4, 52.3, 39.7, 38.8, 27.8, 11.9; ESI-MS *m/z* calculated for $C_{27}H_{33}BrN_3O_4S^+$ $[M+H]^+$ 575.5; found 575.5 HRMS (ESI) for $C_{27}H_{33}BrN_3O_4S^+$ $[M+H]^+$ 574.1375; found 574.1381.

5-Bromo-*N*-(2-(ethyl(4-(4-(thiophen-2-yl)benzamido)butyl)amino)ethyl)-2,3-dimethoxybenzamide (**9b**) **9b** was synthesized using **8b** (120 mg, 0.3 mmol) in the same procedures as **9a** and obtained 35 mg (20% yield) as a colorless oil. (^{1}H NMR, 400 MHz, MeOD): δ = 7.80 (d, *J* = 8.5 Hz, 2H), 7.68 (d, *J* = 8.6 Hz, 2H), 7.52 (d, *J* = 2.4 Hz, 1H), 7.49 (dd, J_1 = 3.6 Hz, J_2 = 1.1 Hz, 1H), 7.45 (dd, J_1 = 5.1 Hz, J_2 = 1.1 Hz, 1H), 7.24 (d, *J* = 2.4 Hz, 1H), 7.12 (dd, J_1 = 5.1 Hz, J_2 = 3.6 Hz, 1H), 3.83 (s, 3H), 3.82 (s, 3H), 3.49 (t, *J* = 6.6 Hz, 2H), 3.41 (t, *J* = 6.6 Hz, 2H), 2.70 (t, *J* = 6.5 Hz, 2H), 2.64 (q, *J* = 7.2 Hz, 2H), 2.59 (t, *J* = 7.4 Hz, 2H), 1.68–1.56 (m, 4H), 1.08 (t, *J* = 7.1 Hz, 3H) (^{13}C NMR, 100 MHz, MeOD): δ = 169.7, 166.6, 155.3, 148.3, 144.3, 138.9, 134.5, 130.0, 129.5, 129.2, 127.2, 126.6, 125.6, 125.3, 119.8, 117.7, 62.1, 57.0, 54.2, 53.1, 48.8, 40.8, 38.6, 28.5, 25.5, 11.9; ESI-MS *m/z* calculated for $C_{28}H_{35}BrN_3O_4S^+$ $[M+H]^+$ 589.6; found 589.4 HRMS (ESI) for $C_{28}H_{35}BrN_3O_4S^+$ $[M+H]^+$ 588.1532; found 588.1555.

5-Bromo-*N*-(2-(ethyl(4-hydroxybutyl)amino)ethyl)-2,3-dimethoxybenzamide (**10a**) In a solution of **5c** (586 mg, 1.46 mmol) in 15 mL of CH_2Cl_2, sodium triacetoxyborohydride (774 mg, 3.65 mmol) was added. The mixture was stirred at RT for 16 h. After completion of the reaction, the mixture was diluted with EtOAc and washed by aq saturated $NaHCO_3$ solution and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel (CH_2Cl_2/7 N NH_3 in MeOH = 20:1) to afford 10a (300 mg, 51% yield) as a colorless oil. (^{1}H NMR, 400 MHz, MeOD): δ = 7.52 (d, *J* = 2.4 Hz, 1H), 7.30 (d, *J* = 2.4 Hz, 1H), 3.89 (s, 3H), 3.88 (s, 3H), 3.55 (t, *J* = 6.0 Hz, 2H), 3.49 (t, *J* = 6.7 Hz, 2H), 2.70 (t, *J* = 6.7 Hz, 2H), 2.64 (q, *J* = 7.2 Hz, 2H), 2.55 (t, *J* = 7.0 Hz, 2H), 1.61–1.54 (m, 4H), 1.08 (t, *J* = 7.2 Hz, 3H) (^{13}C NMR, 100 MHz, MeOD): δ = 166.7, 155.4, 148.3, 130.1, 125.2, 119.8, 117.7, 63.0, 62.1, 57.1, 54.7, 53.1, 48.7, 38.6, 31.9, 24.8, 11.9; ESI-MS *m/z* calculated for $C_{17}H_{28}BrN_2O_4^+$ $[M+H]^+$ 404.3; found 404.3.

5-Bromo-N-(2-(ethyl(5-hydroxypentyl)amino)ethyl)-2,3-dimethoxybenzamide (**10b**) **10b** was synthesized using **5d** (51 mg, 0.12 mmol) in the same procedures as **10a** and obtained 25 mg (29% yield) as a colorless oil. (^{1}H NMR, 400 MHz, MeOD): δ = 7.52 (d, *J* = 2.3 Hz, 1H), 7.30 (d, *J* = 2.4 Hz, 1H), 3.89 (s, 3H), 3.88 (s, 3H), 3.53 (t, *J* = 6.6 Hz, 2H), 3.48 (t, *J* = 6.6 Hz, 2H), 2.69 (t, *J* = 6.6 Hz, 2H), 2.63 (q, *J* = 7.2 Hz, 2H), 2.54 (t, *J* = 7.5 Hz, 2H), 1.58–1.49 (m, 4H), 1.40–1.34 (m, 2H), 1.08 (t, *J* = 7.1 Hz, 3H) (^{13}C NMR, 100 MHz, MeOD): δ = 166.7, 155.4, 148.4, 130.1, 125.2, 119.8, 117.7, 63.0, 62.1, 57.1, 54.7, 53.1, 48.8, 38.7, 33.7, 27.9, 25.0, 12.0; ESI-MS *m/z* calculated for $C_{18}H_{29}BrN_2O_4^+$ $[M]^+$ 417.3; found 417.4.

5-Bromo-*N*-(2-(ethyl(4-((4-methyl-5-phenyl-4*H*-1,2,4-triazol-3-yl)thio)butyl)amino)ethyl)-2,3-dimethoxybenzamide (**11a**) In a mixture of **10a** (100 mg, 0.25 mmol), 4-methyl-5-phenyl-4*H*-1,2,4-triazole-3-thiol (57 mg, 0.3 mmol), and PPh_3 (98 mg, 0.37 mmol) in 2.5 mL of THF, DIAD (73 μL, 0.37 mmol) was slowly added. The mixture was stirred at RT for 24 h. The mixture was diluted with EtOAc and washed by aq saturated $NaHCO_3$ solution and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH = 15:1) to afford 11a (23 mg, 16% yield) as a colorless oil. (^{1}H NMR, 400 MHz, MeOD): δ = 7.67–7.65 (m, 2H), 7.58–7.54 (m, 3H), 7.51 (d, *J* = 2.4 Hz, 1H), 7.27 (d, *J* = 2.4 Hz, 1H), 4.28 (t, *J* = 6.9 Hz, 2H), 3.85 (s, 6H), 3.61 (s, 3H), 3.50 (t, *J* = 6.4 Hz, 2H), 2.73 (t, *J* = 6.4 Hz, 2H), 2.72–2.63 (m, 4H), 1.98–1.90 (m, 2H), 1.63–1.56 (m, 2H), 1.09 (t, *J* = 7.1 Hz, 3H) (^{13}C NMR, 100 MHz, MeOD): δ = 168.5, 166.7, 155.3, 152.4, 148.4, 132.2, 130.3, 129.9, 129.8, 127.3, 125.3, 119.8, 117.7, 62.2, 57.1, 54.0, 53.2, 38.6, 33.7, 27.1, 24.8, 11.8; ESI-MS *m/z* calculated for $C_{26}H_{35}BrN_5O_3S^+$ $[M+H]^+$ 577.6; found 577.3 HRMS (ESI) for $C_{26}H_{35}BrN_5O_3S^+$ $[M+H]^+$ 576.1644; found 576.1639.

5-Bromo-*N*-(2-(ethyl(5-((4-methyl-5-phenyl-4*H*-1,2,4-triazol-3-yl)thio)pentyl)amino)ethyl)-2,3-dimethoxybenzamide (**11b**) **11b** was synthesized using **10b** (25 mg, 0.06 mmol) in the same procedures as **11a** and purified by flash chromatography on silica gel (CH_2Cl_2/MeOH/7 N NH_3 in MeOH = 20:1:0.1). 11b was obtained 10 mg (28% yield) as a colorless oil. (^{1}H NMR, 400 MHz, MeOD): δ = 7.69–7.67 (m, 2H), 7.58–7.53 (m, 3H), 7.52 (d, *J* = 2.4 Hz, 1H), 7.29 (d, *J* = 2.4 Hz, 1H), 4.23 (t, *J* = 7.0 Hz, 2H), 3.88 (s, 3H), 3.87 (s, 3H), 3.62 (s, 3H), 3.49 (t, *J* = 6.5 Hz, 2H), 2.72 (t, *J* = 6.4 Hz, 2H), 2.66 (q, *J* = 7.1 Hz, 2H), 2.57 (t, *J* = 7.4 Hz, 2H), 1.94–1.87 (m, 2H), 1.63–1.56 (m, 2H), 1.44–1.36 (m, 2H), 1.08 (t, *J* = 7.1 Hz, 3H) (^{13}C NMR, 100 MHz, MeOD): δ = 168.4, 166.7, 155.4, 152.4, 148.4, 132.2, 130.3, 130.0, 129.9, 127.4, 125.3, 119.9, 117.7, 62.2, 57.1, 54.4, 53.2, 48.9, 38.6, 33.7, 29.0, 27.3, 25.3, 11.9; ESI-MS *m/z* calculated for $C_{27}H_{36}BrN_5O_3S^+$ $[M]^+$ 590.6; found 590.6 HRMS (ESI) for $C_{27}H_{37}BrN_5O_3S^+$ [M]+ 590.1800; found 590.1787.

5-Bromo-*N*-(2-(ethyl(methyl)amino)ethyl)-2,3-dimethoxybenzamide (**12**) In a solution of **3b** (40 mg, 0.12 mmol) in 5 mL of acetone, CH_3I (7.5 μL, 0.12 mmol) and K_2CO_3 (36 mg, 0.26 mmol) were added. The mixture was refluxed for 16 h and cooled to RT. The volatiles were removed under the reduced pressure and the crude product was purified by flash chromatography on silica gel (CH_2Cl_2/7 N NH_3 in MeOH = 40:1) to afford 12 (7.8 mg, 19% yield) as a colorless oil. (^{1}H NMR, 400 MHz, MeOD): δ = 7.51 (d, *J* = 2.4 Hz, 1H), 7.31 (d, *J* = 2.4 Hz, 1H), 3.89 (s, 3H), 3.88 (s, 3H), 3.52 (t, *J* = 6.7 Hz, 2H), 2.63 (t, *J* = 6.7 Hz, 2H), 2.54 (q, *J* = 7.2 Hz, 2H), 2.31 (s, 3H), 1.11 (t, *J* = 7.2 Hz, 3H) (^{13}C NMR, 100 MHz, MeOD): δ = 166.8, 155.4, 148.3, 130.3, 125.2, 119.8, 117.7, 62.1, 57.0, 56.6, 52.6, 41.8, 38.4, 12.4; ESI-MS *m/z* calculated for $C_{14}H_{21}BrN_2O_3^+$ $[M]^+$ 345.2; found 345.3 HRMS (ESI) for $C_{14}H_{22}BrN_2O_3^+$ $[M+H]^+$ 345.0814; found 345.0813.

N-(3-Bromopropyl)-4-(thiophen-2-yl)benzamide (**13**) **13** was synthesized using 4-(thiophen-2-yl)benzoic acid (150 mg, 0.73 mmol) and 3-bromopropylamine hydrobromide (161 mg, 0.73 mmol) in the same procedures as **9a** and obtained 35 mg (15% yield) as a white solid. (^{1}H NMR, 400 MHz, DMSO-d_6): δ = 8.57 (t, *J* = 5.5 Hz, 1H), 7.89 (d, *J* = 8.5 Hz, 2H), 7.75 (d, *J* = 8.5 Hz, 2H), 7.64–7.61 (m, 2H), 7.17 (dd, J_1 = 5.1 Hz, J_2 = 3.7 Hz, 1H), 3.59 (t, *J* = 6.6 Hz, 2H), 3.39 (t, *J* = 6.6 Hz, 2H), 2.12–2.05 (m, 2H) (^{13}C NMR, 100 MHz, DMSO-d_6): δ = 166.2, 142.8, 140.2, 136.7, 129.6, 129.2, 128.6, 127.2, 126.0, 125.5, 38.4, 33.0, 18.9; ESI-MS *m/z* calculated for $C_{14}H_{14}BrNOS^+$ $[M]^+$ 324.2; found 324.2.

N-(3-(Ethyl(methyl)amino)propyl)-4-(thiophen-2-yl)benzamide (**14**) **14** was synthesized using **13** (35 mg, 0.11 mmol) and *N*-methylethanamine (12.8 mg, 0.22 mmol) in the same procedures as **13a** and obtained 18 mg (55% yield) as a white solid. (^{1}H NMR, 400 MHz, MeOD): δ = 7.89 (d, *J* = 8.6 Hz, 2H), 7.75 (d, *J* = 8.5 Hz, 2H), 7.52 (dd, J_1 = 3.6 Hz, J_2 = 1.0 Hz, 1H), 7.37 (dd, J_1 = 5.1 Hz, J_2 = 1.0 Hz, 1H), 7.13 (dd, J_1 = 5.1 Hz, J_2 = 3.7 Hz, 1H), 3.58–3.45 (m, 2H), 3.36–3.24 (m, 2H), 3.22–3.11 (m, 2H), 2.88 (s, 3H), 2.10–2.01 (m, 2H), 1.37 (t, *J* = 7.3 Hz, 3H) (^{13}C NMR, 100 MHz, MeOD): δ = 170.4, 144.1, 139.4, 133.7, 129.6, 129.3, 127.4, 126.7, 125.8, 54.6, 52.8, 39.8, 37.7, 26.1, 9.7; ESI-MS *m/z* calculated for $C_{17}H_{24}N_2OS^+$ $[M+2H]^+$ 304.5; found 304.2 HRMS (ESI) for $C_{17}H_{23}N_2OS^+$ $[M+H]^+$ 303.1531; found 303.1516.

tert-Butyl allyl(2-aminoethyl)carbamate (**15a**) In a solution of ethylenediamine (2.8 mL, 41.6 mmol) in 100 mL of CH_2Cl_2, ethyl trifluoroacetate (4.9 mL, 41.6 mmol) in 100 mL of CH_2Cl_2 was added dropwise at 0 °C. The mixture was warmed to RT and stirred for 1 h. The solvent was removed under the reduced pressure and the residue was dissolved with 210 mL of MeOH. Allyl bromide (3.6 mL, 41.6 mmol) and Et_3N (6.4 mL, 46 mmol) were added slowly into the mixture and the mixture was stirred for 16 h. Then, $(Boc)_2O$ (9.6 mL, 41.6 mmol) was added and the mixture was stirred for another 4 h. The volatiles were removed under the reduced pressure and the residue was dissolved EtOAc. The organic layer was washed by aq 0.5 N HCl solution and brine, dried over $MgSO_4$, filtered and concentrated in vacuo. Deprotection of trifluoroacetyl group was performed according to the reported method [63] and 2.8 g of **15a** (34% yield) was obtained as a yellow oil. The crude product was used for the next step without further purification.

tert-Butyl (2-aminoethyl)(4-fluorobenzyl)carbamate (**15b**) In a solution of *N*-(2-aminoethyl)-2,2,2-trifluoroacetamide (6.5 g, 41.6 mmol) in 200 mL of CH_2Cl_2, 4-fluorobenzaldehyde (4.46 mL, 41.6 mmol) and sodium triacetoxyborohydride (17.6 g, 83 mmol) were added. The mixture was stirred for 16 h at RT and washed by aq saturated $NaHCO_3$ solution and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel (CH_2Cl_2/7 N NH_3 in MeOH = 20:1) to afford an intermediate (1.7 g, 6.5 mmol) as a colorless oil. Protection of Boc group and deprotection of trifluoroacetyl group were performed according to the reported method [63] and 1.6 g of 15b (15% yield) was obtained as a colorless oil. The crude product was used for the next step without further purification.

tert-Butyl allyl(2-(5-bromo-2,3-dimethoxybenzamido)ethyl)carbamate (**16a**) **16a** was synthesized using **1b** (783 mg, 3 mmol) and **15a** (1.2 g, 6 mmol) in the same procedures as **2a** and obtained 783 mg (60% yield) as a colorless oil. (^{1}H NMR, 400 MHz, CD_3CN): δ = 8.04 (br, 1H), 7.69 (d, *J* = 2.3 Hz, 1H), 7.37 (d, *J* = 2.3 Hz, 1H), 5.96–5.87 (m, 1H), 5.26–5.19 (m, 2H), 3.97 (s, 3H), 3.95 (s, 3H), 3.94 (br, 2H), 3.59 (q, *J* = 6.0 Hz, 2H), 3.49 (t, *J* = 5.9 Hz, 2H), 1.48 (s, 9H) (^{13}C NMR, 100 MHz, CD_3CN): δ = 164.8, 154.9, 147.9, 125.1, 119.3, 117.1, 116.7, 80.2, 62.0, 57.3, 39.1, 28.6; ESI-MS *m/z* calculated for $C_{19}H_{27}BrN_2O_5^+$ $[M]^+$ 443.3; found 443.3.

tert-Butyl (2-(5-bromo-2,3-dimethoxybenzamido)ethyl)(4-fluorobenzyl)carbamate (**16b**) **16b** was synthesized using **1b** (500 mg, 1.92 mmol) and **15b** (771 mg, 2.87 mmol) in the same procedures as **2a** and obtained 670 mg (68% yield) as a colorless oil. (^{1}H NMR, 400 MHz, CD_3CN): δ = 7.90 (br, 1H), 7.57 (d, *J* = 2.4 Hz, 1H), 7.29–7.25 (m, 3H), 7.05 (t, *J* = 8.8 Hz, 2H), 4.42 (s, 2H), 3.86 (s, 3H), 3.83 (s, 3H), 3.48 (q, *J* = 6.0 Hz, 2H), 3.39 (br, 2H), 1.38 (s, 9H) (^{13}C NMR, 100 MHz, CD_3CN): δ = 164.9, 164.1, 161.7, 156.8, 155.0, 148.0, 136.1, 130.3, 125.2, 119.4, 117.1, 116.2, 116.0, 80.6, 62.0, 57.3, 46.7, 39.0, 28.6; ESI-MS *m/z* calculated for $C_{23}H_{28}BrFN_2O_5^+$ $[M]^+$ 511.4; found 511.3.

N-(2-(Allylamino)ethyl)-5-bromo-2,3-dimethoxybenzamide (**17a**) **17a** was synthesized using **16a** (350 mg, 0.79 mmol) in the same procedures as **3a** and obtained 250 mg (92% yield) as a colorless oil. (^{1}H NMR, 400 MHz, MeOD): δ = 7.45 (d, *J* = 2.4 Hz, 1H), 7.30 (d, *J* = 2.3 Hz, 1H), 5.96–5.86 (m, 1H), 5.26–5.12 (m, 2H), 3.89 (s, 3H), 3.87 (s, 3H), 3.51 (t, *J* = 6.4 Hz, 2H), 3.28 (t, *J* = 1.2 Hz, 1H), 3.27 (t, *J* = 1.3 Hz, 1H), 2.81 (t, *J* = 6.4 Hz, 2H) (^{13}C NMR, 100 MHz, CD_3CN): δ = 167.4, 155.4, 148.1, 137.2, 131.0, 124.9, 119.6, 117.4, 117.1, 62.1, 57.1, 52.9, 40.4; ESI-MS *m/z* calculated for $C_{14}H_{19}BrN_2O_3^+$ $[M]^+$ 343.2; found 343.5.

5-Bromo-*N*-(2-((4-fluorobenzyl)amino)ethyl)-2,3-dimethoxybenzamide (**17b**) **17b** was synthesized using **16b** (656 mg, 1.28 mmol) in the same procedures as **3a** and obtained 480 mg (91% yield) as a colorless oil. (^{1}H NMR, 400 MHz, MeOD): δ = 7.45 (d, *J* = 2.4 Hz, 1H), 7.39–7.35 (m, 2H), 7.29 (d, *J* = 2.4 Hz, 1H), 7.04 (t, *J* = 8.8 Hz, 2H), 3.89 (s, 3H), 3.83 (s, 3H), 3.79 (s, 2H), 3.53 (t, *J* = 6.2 Hz, 2H), 2.83 (t, *J* = 6.2 Hz, 2H) (^{13}C NMR, 100 MHz, MeOD): δ = 167.4, 164.9, 162.5, 155.3, 148.1, 136.8, 131.6, 131.5, 130.8, 125.0, 119.6, 117.7, 116.3, 116.1, 62.1, 57.1, 53.5, 40.3; ESI-MS *m/z* calculated for $C_{18}H_{20}BrFN_2O_3^+$ $[M]^+$ 411.3; found 411.3.

N-(2-(Allyl(3-(1,3-dioxoisoindolin-2-yl)propyl)amino)ethyl)-5-bromo-2,3-dimethoxybenzamide (**18a**) **18a** was synthesized using **17a** (240 mg, 0.7 mmol) in the same procedures as **7a** and obtained 100 mg (27% yield) as a colorless oil. (^{1}H NMR, 400 MHz, acetone-d_6): δ = 8.29 (br, 1H), 7.83 (s, 4H), 7.65 (d, *J* = 2.4 Hz, 1H), 7.28 (d, *J* = 2.4 Hz, 1H), 5.92–5.85 (m, 1H), 5.21–5.16 (m, 1H), 5.09–5.06 (m, 1H), 3.92 (s, 3H), 3.91 (s, 3H), 3.72 (t, *J* = 7.1 Hz, 2H), 3.48 (q, *J* = 6.0 Hz, 2H), 3.20 (d, *J* = 1.6 Hz, 2H), 2.68 (t, *J* = 6.2 Hz, 2H), 2.62 (t, *J* = 6.9 Hz, 2H), 1.92–1.85 (m, 2H) (^{13}C NMR, 100 MHz, acetone-d_6): δ = 168.9, 163.8, 154.9, 148.1, 136.7, 135.0, 133.3, 129.8, 125.5, 123.7, 119.0, 117.8, 116.9, 61.8, 57.4, 57.0, 53.4, 51.7, 38.2, 36.8, 26.9; ESI-MS *m/z* calculated for $C_{25}H_{29}BrN_3O_5^+$ $[M+H]^+$ 531.4; found 531.4.

5-Bromo-*N*-(2-((3-(1,3-dioxoisoindolin-2-yl)propyl)(4-fluorobenzyl)amino)ethyl)-2,3-dimethoxybenzamide (**18b**) **18b** was synthesized using **17b** (270 mg, 0.66 mmol) in the same procedures as **7a** and obtained 333 mg (84% yield) as a colorless oil. (^{1}H NMR, 400 MHz, MeOD): δ = 7.80–7.75 (m, 4H), 7.44 (d, *J* = 2.4 Hz, 1H), 7.29 (dd, J_1 = 8.5 Hz,

J_2 = 5.5 Hz, 2H), 7.26 (d, J = 2.4 Hz, 1H), 6.85 (t, J = 8.8 Hz, 2H), 3.87 (s, 3H), 3.84 (s, 3H), 3.68 (t, J = 7.0 Hz, 2H), 3.59 (s, 2H), 3.47 (t, J = 6.0 Hz, 2H), 2.66 (t, J = 6.1 Hz, 2H), 2.53 (t, J = 6.8 Hz, 2H), 1.91–1.84 (m, 2H) (^{13}C NMR, 100 MHz, MeOD): δ = 170.0, 166.6, 164.6, 162.2, 155.3, 148.3, 135.4, 133.4, 132.0$_3$, 130.9$_5$, 130.1, 125.3, 124.2, 119.7, 117.7, 116.0, 115.8, 62.2, 58.6, 57.1, 53.9, 52.0, 38.8, 37.1, 27.0; ESI-MS *m/z* calculated for $C_{29}H_{30}BrFN_3O_5^+$ $[M+H]^+$ 599.5; found 599.3.

N-(2-((3-Aminopropyl)(propyl)amino)ethyl)-5-bromo-2,3-dimethoxybenzamide (**19a**) **19a** was synthesized using **18a** (100 mg, 0.19 mmol) in the same procedures as **8a** and obtained 25 mg (33% yield) as a colorless oil. (^{1}H NMR, 400 MHz, MeOD): δ = 7.52 (d, J = 2.3 Hz, 1H), 7.31 (d, J = 2.3 Hz, 1H), 3.89 (s, 3H), 3.88 (s, 3H), 3.48 (t, J = 6.5 Hz, 2H), 2.70–2.66 (m, 4H), 2.56 (t, J = 7.1 Hz, 2H) 2.50–2.46 (m, 2H), 1.70–1.62 (m, 2H), 1.57–1.47 (m, 2H), 0.91 (t, J = 7.4 Hz, 3H) (^{13}C NMR, 100 MHz, MeOD): δ = 166.6, 155.3, 148.3, 130.1, 125.2, 119.8, 117.7, 62.1, 57.4, 57.1, 53.9, 53.1, 41.0, 38.8, 30.9, 21.2, 12.3; ESI-MS *m/z* calculated for $C_{17}H_{28}BrN_3O_3^+$ $[M]^+$ 402.3; found 402.4.

N-(2-((3-Aminopropyl)(4-fluorobenzyl)amino)ethyl)-5-bromo-2,3-dimethoxybenzamide (**19b**) **19b** was synthesized using **18b** (156 mg, 0.26 mmol) in the same procedures as 8a and obtained 61 mg (50% yield) as a colorless oil. (^{1}H NMR, 400 MHz, MeOD): δ = 7.50 (d, J = 2.4 Hz, 1H), 7.34 (dd, J_1 = 8.5 Hz, J_2 = 5.6 Hz, 2H), 7.31 (d, J = 2.4 Hz, 1H), 6.97 (7, J = 8.8 Hz, 2H), 3.90 (s, 3H), 3.85 (s, 3H), 3.60 (s, 2H), 3.49 (t, J = 6.2 Hz, 2H), 2.67–2.61 (m, 4H), 2.53 (t, J = 7.0 Hz, 2H), 1.70–1.63 (m, 2H) (^{13}C NMR, 100 MHz, MeOD): δ = 166.5, 164.7, 162.3, 155.3, 148.3, 136.6, 132.0, 131.9, 130.0, 125.3, 119.7, 117.7, 116.1, 115.9, 62.2, 58.9, 57.8, 52.5, 40.9, 31.1; ESI-MS *m/z* calculated for $C_{21}H_{27}BrFN_3O_3^+$ $[M]^+$ 468.4; found 468.5.

5-Bromo-2,3-dimethoxy-*N*-(2-(propyl(3-(4-(thiophen-2-yl)benzamido)propyl)amino)ethyl) benzamide (**20a**) **20a** was synthesized using **19a** (25 mg, 0.06 mmol) in the same procedures as **9a** and obtained 25 mg (71% yield) as a colorless oil. (^{1}H NMR, 400 MHz, MeOD): δ = 7.79 (d, J = 8.4 Hz, 2H), 7.69 (d, J = 8.4 Hz, 2H), 7.50 (d, J = 2.4 Hz, 1H), 7.49 (d, J = 3.7 Hz, 1H), 7.44 (d, J = 5.0 Hz, 1H), 7.25 (d, J = 2.4 Hz, 1H), 7.12 (dd, J_1 = 5.0 Hz, J_2 = 3.7 Hz, 1H), 3.86 (s, 3H), 3.84 (s, 3H), 3.50 (t, J = 6.3 Hz, 2H), 3.43 (t, J = 6.8 Hz, 2H), 2.71 (t, J = 6.3 Hz, 2H), 2.64 (t, J = 6.8 Hz, 2H), 2.53–2.49 (m, 2H), 1.86–1.79 (m, 2H), 1.57–1.48 (m, 2H), 0.91 (t, J = 7.3 Hz, 3H) (^{13}C NMR, 100 MHz, MeOD): δ = 169.7, 166.7, 155.3, 148.3, 144.3, 138.9, 134.4, 130.0, 129.5, 129.1, 127.2, 126.6, 125.6, 125.3, 119.8, 117.7, 62.1, 57.4, 57.1, 54.1, 52.9, 39.6, 38.9, 27.9, 21.2, 12.3; ESI-MS *m/z* calculated for $C_{28}H_{35}BrN_3O_4S^+$ $[M+H]^+$ 589.6; found 589.4 HRMS (ESI) for $C_{28}H_{35}BrN_3O_4S^+$ $[M+H]^+$ 588.1532; found 588.1527.

N-(2-(Allyl(3-(4-(thiophen-2-yl)benzamido)propyl)amino)ethyl)-5-bromo-2,3-dimethoxybenzamide (**20b**) **20b** was synthesized using **13** (36 mg, 0.11 mmol) and **17a** (38 mg, 0.11 mmol) in the same procedures as **7a** and obtained 7 mg (21% yield) as a colorless oil. 20 mg of **17a** (20 mg, 0.06 mmol) was recovered. (^{1}H NMR, 400 MHz, MeOD): δ = 7.79 (d, J = 8.6 Hz, 2H), 7.69 (d, J = 8.5 Hz, 2H), 7.50–7.49 (m, 2H), 7.45 (dd, J_1 = 5.1 Hz, J_2 = 1.0 Hz, 1H), 7.26 (d, J = 2.4 Hz, 1H), 7.13 (dd, J_1 = 5.1 Hz, J_2 = 3.7 Hz, 1H), 5.97–5.86 (m, 1H), 5.26–5.21 (m, 1H), 5.18–5.14 (m, 1H), 3.87 (s, 3H), 3.85 (s, 3H), 3.51 (t, J = 6.3 Hz, 2H), 3.43 (t, J = 6.8 Hz, 2H), 3.22 (d, J = 6.6 Hz, 2H), 2.72 (t, J = 6.3 Hz, 2H), 2.65 (t, J = 6.9 Hz, 2H), 1.87–1.80 (m, 2H) (^{13}C NMR, 100 MHz, MeOD): δ = 169.7, 166.7, 155.3, 148.3, 144.3, 138.9, 136.4, 134.4, 130.0, 129.5, 129.1, 127.2, 126.6, 125.6, 125.3, 119.8, 118.8, 117.7, 62.2, 58.2, 57.1, 53.7, 52.5, 39.6, 38.8, 27.9; ESI-MS *m/z* calculated for $C_{28}H_{33}BrN_3O_4S^+$ $[M+H]^+$ 587.6; found 587.3 HRMS (ESI) for $C_{28}H_{33}BrN_3O_4S^+$ $[M+H]^+$ 586.1375; found 586.1377.

5-Bromo-*N*-(2-((4-fluorobenzyl)(3-(4-(thiophen-2-yl)benzamido)propyl)amino)ethyl)-2,3-dimethoxybenzamide (**20c**) **20c** was synthesized using **19b** (60 mg, 0.13 mmol) in the same procedures as **9a** and obtained 40 mg (32% yield) as a colorless oil. (^{1}H NMR, 400 MHz, MeOD): δ = 7.75 (d, J = 8.6 Hz, 2H), 7.68 (d, J = 8.6 Hz, 2H), 7.49 (dd, J_1 = 3.6 Hz, J_2 = 1.1 Hz, 1H), 7.47 (d, J = 2.4 Hz, 1H), 7.44 (dd, J_1 = 5.1 Hz, J_2 = 1.0 Hz, 1H), 7.33 (dd, J_1 = 8.6 Hz, J_2 = 5.5 Hz, 2H), 7.27 (d, J = 2.4 Hz, 1H), 7.12 (dd, J_1 = 5.1 Hz, J_2 = 3.6 Hz, 1H), 6.92 (t, J = 8.8 Hz, 2H), 3.86 (s, 3H), 3.83 (s, 3H), 3.62 (s, 2H), 3.50 (t, J = 6.0 Hz, 2H), 3.41 (t, J = 6.8 Hz, 2H), 2.68 (t, J = 6.1 Hz, 2H), 2.60 (t, J = 6.8 Hz, 2H), 1.88–1.81 (m, 2H) (^{13}C NMR, 100 MHz, MeOD): δ = 169.7, 166.7, 164.7, 162.3, 155.3, 148.3, 144.3, 138.9, 136.5$_4$, 136.5$_0$, 134.4,

132.1, 132.0, 130.2, 129.6, 129.5, 129.2, 127.2, 126.6, 125.6, 125.2, 119.7, 117.7, 116.1, 115.9, 62.2, 58.8, 57.1, 54.0, 52.3, 39.3, 38.9, 27.8; ESI-MS *m/z* calculated for $C_{32}H_{33}BrFN_3O_4S^+$ $[M]^+$ 654.6; found 654.6 HRMS (ESI) for $C_{32}H_{34}BrFN_3O_4S^+$ $[M+H]^+$ 654.1437; found 654.1447.

N-(3-((2-(5-Bromo-2,3-dimethoxybenzamido)ethyl)(ethyl)amino)propyl)-2-naphthamide (**21a**) In a solution of **8a** (30 mg, 0.08 mmol) in 2 mL of CH_2Cl_2, 2-naphthoyl chloride (22 mg, 0.12 mmol) and Et_3N (12.9 µL, 0.09 mmol) were added. The reaction mixture was stirred for 1 h at RT followed by 2 mL of MeOH was added. After the mixture was stirred for 10 min, the crude product was purified by flash chromatography on silica gel (CH_2Cl_2/7 N NH_3 in MeOH = 80:1) to afford 21a (37 mg, 86% yield) as a colorless oil. (^{1}H NMR, 400 MHz, MeOD): δ = 8.31 (s, 1H), 7.95–89 (m, 3H), 7.83 (dd, J_1 = 8.6 Hz, J_2 = 1.7 Hz, 1H), 7.60–7.53 (m, 2H), 7.48 (d, *J* = 2.4 Hz, 1H), 7.22 (d, *J* = 2.4 Hz, 1H), 3.85 (s, 3H), 3.83 (s, 3H), 3.53–3.48 (m, 4H), 2.72 (t, *J* = 6.4 Hz, 2H), 2.70–2.64 (m, 4H), 1.90–1.83 (m, 2H), 1.09 (t, *J* = 7.1 Hz, 3H) (^{13}C NMR, 100 MHz, MeOD): δ = 170.3, 166.7, 155.3, 148.3, 136.4, 134.2, 133.1, 130.1, 130.0, 129.4, 128.9, 128.8, 128.0, 125.2, 124.9, 119.7, 117.7, 62.1, 57.0, 53.4, 52.3, 39.8, 38.8, 27.9, 11.9; ESI-MS *m/z* calculated for $C_{27}H_{32}BrN_3O_4^+$ $[M]^+$ 542.5; found 542.5 HRMS (ESI) for $C_{27}H_{33}BrN_3O_4^+$ $[M+H]^+$ 542.1654; found 542.1649.

N-(3-((2-(5-Bromo-2,3-dimethoxybenzamido)ethyl)(ethyl)amino)propyl)quinoline -4-carboxamide (**21b**) **21b** was synthesized using **8a** (30 mg, 0.08 mmol) and 4-quinolinecarboxylic acid (21 mg, 0.12 mmol) in the same procedures as **9a** and obtained 27 mg (65% yield) as a colorless oil. (^{1}H NMR, 400 MHz, MeOD): δ = 8.90 (d, *J* = 4.4 Hz, 1H), 8.16 (d, *J* = 8.4 Hz, 1H), 8.08 (d, *J* = 8.4 Hz, 1H), 7.84–7.80 (m, 1H), 7.68–7.64 (m, 1H), 7.54 (d, *J* = 4.4 Hz, 1H), 7.50 (d, *J* = 2.4 Hz, 1H), 7.28 (d, *J* = 2.4 Hz, 1H), 3.88 (s, 3H), 3.86 (s, 3H), 3.56–3.49 (m, 4H), 2.75–2.66 (m, 6H), 1.93–1.85 (m, 2H), 1.10 (t, *J* = 7.1 Hz, 3H) (^{13}C NMR, 100 MHz, MeOD): δ = 169.8, 166.7, 155.3, 151.2, 149.3, 148.3, 144.6, 131.7, 130.1, 129.9, 129.1, 126.7, 126.1, 125.3, 120.3, 119.8, 117.7, 62.2, 57.1, 53.3, 52.2, 39.5, 38.8, 28.0, 12.0; ESI-MS *m/z* calculated for $C_{26}H_{31}BrN_4O_4^+$ $[M]^+$ 543.5; found 543.4 HRMS (ESI) for $C_{26}H_{32}BrN_4O_4^+$ $[M+H]^+$ 543.1607; found 543.1622.

5-Bromo-*N*-(2-(ethyl(3-(4-(pyridin-4-yl)benzamido)propyl)amino)ethyl)-2,3-dimethoxybenzamide (**21c**) **21c** was synthesized using **8a** (30 mg, 0.08 mmol) and 4-(4-pyridyl)benzoic acid (24 mg, 0.12 mmol) in the same procedures as **9a** and obtained 33 mg (75% yield) as a colorless oil. (^{1}H NMR, 400 MHz, MeOD): δ = 8.61 (dd, J_1 = 4.6 Hz, J_2 = 1.6 Hz, 2H), 7.92 (d, *J* = 8.4 Hz, 2H), 7.82 (d, *J* = 8.5 Hz, 2H), 7.75 (dd, J_1 = 4.6 Hz, J_2 = 1.6 Hz, 2H), 7.49 (d, *J* = 2.3 Hz, 1H), 7.25 (d, *J* = 2.4 Hz, 1H), 3.87 (s, 3H), 3.84 (s, 3H), 3.51 (t, *J* = 6.4 Hz, 2H), 3.46 (t, *J* = 6.8 Hz, 2H), 2.71 (t, *J* = 6.4 Hz, 2H), 2.70–2.63 (m, 4H), 1.88–1.81 (m, 2H), 1.08 (t, *J* = 7.1 Hz, 3H) (^{13}C NMR, 100 MHz, MeOD): δ = 169.5, 166.7, 155.3, 150.9, 149.7, 148.3, 141.8, 136.6, 130.0, 129.3, 128.4, 125.3, 123.4, 119.8, 117.7, 62.1, 57.1, 53.4, 52.3, 40.6, 39.8, 38.8, 27.8, 11.9; ESI-MS *m/z* calculated for $C_{28}H_{33}BrN_4O_4^+$ $[M]^+$ 569.5; found 569.5 HRMS (ESI) for $C_{28}H_{34}BrN_4O_4^+$ $[M+H]^+$ 569.1763; found 569.1775.

N-(3-((2-(5-Bromo-2,3-dimethoxybenzamido)ethyl)(ethyl)amino)propyl)-1*H*-indole-2-carboxamide (**21d**) **21d** was synthesized using **8a** (30 mg, 0.08 mmol) and indole-2-carboxylic acid (19 mg, 0.12 mmol) in the same procedures as **9a** and obtained 17 mg (42% yield) as a yellow oil. (^{1}H NMR, 400 MHz, MeOD): δ = 7.57 (d, *J* = 8.0 Hz, 1H), 7.50 (d, *J* = 2.4 Hz, 1H), 7.42 (dd, J_1 = 8.3 Hz, J_2 = 0.6 Hz, 1H), 7.23 (d, *J* = 2.4 Hz, 1H), 7.22–7.18 (m, 1H), 7.06–7.02 (m, 1H), 7.00 (d, *J* = 0.6 Hz, 1H), 3.86 (s, 3H), 3.83 (s, 3H), 3.51 (t, *J* = 6.4 Hz, 2H), 3.44 (t, *J* = 6.9 Hz, 2H), 2.71 (t, *J* = 6.4 Hz, 2H), 2.68–2.63 (m, 4H), 1.87–1.80 (m, 2H), 1.08 (t, *J* = 7.1 Hz, 3H) (^{13}C NMR, 100 MHz, MeOD): δ = 166.8, 164.3, 155.3, 148.3, 138.3, 132.4, 130.0, 129.1, 125.2, 125.1, 122.8, 121.2, 119.7, 117.7, 113.1, 104.2, 62.1, 57.0, 53.4, 52.3, 39.1, 38.8, 28.1, 11.9; ESI-MS *m/z* calculated for $C_{25}H_{31}BrN_4O_4^+$ $[M]^+$ 531.5; found 531.4 HRMS (ESI) for $C_{25}H_{32}BrN_4O_4^+$ $[M+H]^+$ 531.1607; found 531.1596.

N-(3-((2-(5-Bromo-2,3-dimethoxybenzamido)ethyl)(ethyl)amino)propyl)imidazopyridine-2-carboxamide (**21e**) **21e** was synthesized using **8a** (30 mg, 0.08 mmol) and imidazo[1,2-a]pyridine-2-carobxylic acid (20 mg, 0.12 mmol) in the same procedures as 9a and obtained 14 mg (34% yield) as a colorless oil. (^{1}H NMR, 400 MHz, MeOD): δ = 8.40 (d, *J* = 6.8 Hz, 1H), 8.13 (s, 1H), 7.45 (d, *J* = 9.2 Hz, 1H), 7.34–7.30 (m, 1H), 7.10 (d, *J* = 2.4 Hz, 1H), 7.04

(d, *J* = 2.3 Hz, 1H), 6.93 (td, J_1 = 6.7 Hz, J_2 = 0.6 Hz, 1H), 3.78 (s, 3H), 3.77 (s, 3H), 3.58 (t, *J* = 5.8 Hz, 2H), 3.50 (t, *J* = 6.3 Hz, 2H), 2.73 (t, *J* = 5.6 Hz, 2H), 2.70–2.66 (m, 4H), 1.86–1.79 (m, 2H), 1.10 (t, *J* = 7.1 Hz, 3H) (^{13}C NMR, 100 MHz, MeOD): δ = 167.6, 164.8, 155.0, 147.7, 146.2, 140.4, 131.3, 128.7, 128.1, 124.3, 119.0, 118.5, 117.1, 116.0, 114.9, 62.0, 56.9, 53.9, 53.8, 48.6, 40.4, 38.8, 26.8, 11.8; ESI-MS *m/z* calculated for $C_{24}H_{30}BrN_5O_4^+$ $[M]^+$ 532.4; found 532.4 HRMS (ESI) for $C_{24}H_{31}BrN_5O_4^+$ $[M+H]^+$ 532.1559; found 532.1559.

N-(3-((2-(5-Bromo-2,3-dimethoxybenzamido)ethyl)(ethyl)amino)propyl)isonicotinamide (**21f**) **21f** was synthesized using **8a** (30 mg, 0.08 mmol) and isonicotinic acid (15 mg, 0.12 mmol) in the same procedures as **9a** and obtained 15 mg (40% yield) as a colorless oil. (^{1}H NMR, 400 MHz, MeOD): δ = 8.66 (dd, J_1 = 4.5 Hz, J_2 = 1.7 Hz, 2H), 7.73 (dd, J_1 = 4.5 Hz, J_2 = 1.7 Hz, 2H), 7.49 (d, *J* = 2.4 Hz, 1H), 7.28 (d, *J* = 2.4 Hz, 1H), 3.87 (d, *J* = 2.4 Hz, 6H), 3.50 (t, *J* = 6.5 Hz, 2H), 3.45 (t, *J* = 6.9 Hz, 2H), 2.70 (t, *J* = 6.4 Hz, 2H), 2.67–2.61 (m, 4H), 1.86–1.79 (m, 2H), 1.08 (t, *J* = 7.1 Hz, 3H) (^{13}C NMR, 100 MHz, MeOD): δ = 167.7, 166.7, 155.3, 151.1, 148.3, 144.1, 130.1, 125.2, 123.0, 119.8, 117.7, 62.1, 57.1, 53.4, 52.2, 39.7, 38.8, 28.0, 12.0; ESI-MS *m/z* calculated for $C_{22}H_{29}BrN_4O_4^+$ $[M]^+$ 493.4; found 493.4 HRMS (ESI) for $C_{22}H_{30}BrN_4O_4^+$ $[M+H]^+$ 493.1450; found 493.1451.

5-Bromo-*N*-(2-(ethyl(3-(4-(thiophen-3-yl)benzamido)propyl)amino)ethyl)-2,3-dimethoxybenzamide (**21g**) **21g** was synthesized using **8a** (30 mg, 0.08 mmol) and 4-(thiophen-3-yl)benzoic acid (25 mg, 0.12 mmol) in the same procedures as 9a and obtained 6 mg (15% yield) as a colorless oil. (^{1}H NMR, 400 MHz, MeOD): δ = 7.81 (d, *J* = 8.5 Hz, 2H), 7.74 (d, *J* = 2.1 Hz, 1H), 7.72 (d, *J* = 8.5 Hz, 2H), 7.51 (d, *J* = 2.2 Hz, 2H), 7.50 (d, *J* = 2.4 Hz, 1H), 7.25 (d, *J* = 2.4 Hz, 1H), 3.86 (s, 3H), 3.84 (s, 3H), 3.51 (t, *J* = 6.5 Hz, 2H), 3.45 (t, *J* = 6.8 Hz, 2H), 2.72 (t, *J* = 6.4 Hz, 2H), 2.69–2.63 (m, 4H), 1.87–1.80 (m, 2H), 1.09 (t, *J* = 7.1 Hz, 3H) (^{13}C NMR, 100 MHz, MeOD): δ = 169.9, 166.8, 155.3, 148.3, 142.5, 140.3, 134.1, 130.0, 129.0, 127.9, 127.3, 127.2, 125.2, 122.9, 119.8, 117.7, 62.1, 57.1, 54.0, 52.4, 39.7, 38.8, 27.9, 11.9; ESI-MS *m/z* calculated for $C_{27}H_{33}BrN_3O_4S^+$ $[M+H]^+$ 575.5; found 575.2 HRMS (ESI) for $C_{27}H_{33}BrN_3O_4S^+$ $[M+H]^+$ 574.1375; found 574.1381.

5-Bromo-*N*-(2-((3-(3-(dimethylamino)benzamido)propyl)(ethyl)amino)ethyl)-2,3-dimethoxybenzamide (**21h**) **21h** was synthesized using **8a** (30 mg, 0.08 mmol) and 3-dimethylaminobenzoic acid (20 mg, 0.12 mmol) in the same procedures as **9a** and obtained 3 mg (7% yield) as a colorless oil. (^{1}H NMR, 400 MHz, MeOD): δ = 8.23 (t, *J* = 1.8 Hz, 1H), 7.99 (d, *J* = 7.9 Hz, 1H), 7.85 (dd, J_1 = 7.9 Hz, J_2 = 2.3 Hz, 1H), 7.70 (t, *J* = 8.0 Hz, 1H), 7.52 (d, *J* = 2.4 Hz, 1H), 7.32 (d, *J* = 2.4 Hz, 1H), 3.91 (s, 3H), 3.89 (s, 3H), 3.83 (t, *J* = 5.9 Hz, 2H), 3.55 (t, *J* = 6.4 Hz, 2H), 3.47–3.43 (m, 2H), 3.43–3.37 (m, 4H), 3.34 (s, 6H), 2.17–2.10 (m, 2H), 1.39 (t, *J* = 7.2 Hz, 3H) (^{13}C NMR, 100 MHz, MeOD): δ = 168.8, 168.3, 155.3, 148.5, 144.7, 137.7, 132.1, 129.8, 129.5, 125.3, 124.8, 121.0, 120.2, 117.6, 62.2, 57.2, 55.0, 53.4, 52.1, 47.1, 38.0, 36.5, 25.6, 9.2; ESI-MS *m/z* calculated for $C_{25}H_{35}BrN_4O_4^+$ $[M]^+$ 535.5; found 535.4 HRMS (ESI) for $C_{25}H_{36}BrN_4O_4^+$ $[M+H]^+$ 535.1920; found 535.1934.

N-(3-((2-(5-Bromo-2,3-dimethoxybenzamido)ethyl)(ethyl)amino)propyl)thiophen- e-3-carboxamide (**21i**) **21i** was synthesized using **8a** (30 mg, 0.08 mmol) and 3-thiophenecaroboxylic acid (15 mg, 0.12 mmol) in the same procedures as **9a** and obtained 24 mg (63% yield) as a colorless oil. (^{1}H NMR, 400 MHz, MeOD): δ = 7.99 (dd, J_1 = 2.7 Hz, J_2 = 1.6 Hz, 1H), 7.50 (d, *J* = 2.4 Hz, 1H), 7.47–7.43 (m, 2H), 7.28 (d, *J* = 2.4 Hz, 1H), 3.87 (s, 6H), 3.50 (t, *J* = 6.5 Hz, 2H), 3.39 (t, *J* = 7.0 Hz, 2H), 2.71 (t, *J* = 6.4 Hz, 2H), 2.68–2.61 (m, 4H), 1.84–1.77 (m, 2H), 1.07 (t, *J* = 7.1 Hz, 3H) (^{13}C NMR, 100 MHz, MeOD): δ = 166.7, 165.7, 155.3, 148.3, 138.7, 130.1, 129.7, 127.6, 127.5, 125.2, 119.7, 117.7, 62.1, 57.1, 53.3, 52.2, 48.7, 39.2, 38.7, 27.9, 11.9; ESI-MS *m/z* calculated for $C_{21}H_{29}BrN_3O_4S^+$ $[M+H]^+$ 499.4; found 499.2 HRMS (ESI) for $C_{21}H_{29}BrN_3O_4S^+$ $[M+H]^+$ 498.1062; found 498.1060.

N-(3-((2-(5-Bromo-2,3-dimethoxybenzamido)ethyl)(ethyl)amino)propyl)-1-methyl-1*H*-indole-2-carboxamide (**21j**) **21j** was synthesized using **8a** (30 mg, 0.08 mmol) and 1-methylindole-2-carboxylic acid (21 mg, 0.12 mmol) in the same procedures as **9a** and obtained 8 mg (20% yield) as a yellow oil. (^{1}H NMR, 400 MHz, MeOD): δ = 7.58 (d, *J* = 8.0 Hz, 1H), 7.50 (d, *J* = 2.3 Hz, 1H), 7.43 (d, *J* = 8.4 Hz, 1H), 7.29 (t, *J* = 7.3 Hz, 1H), 7.25 (d, *J* = 2.4 Hz, 1H), 7.10 (t, *J* = 7.6 Hz, 1H), 6.95 (s, 1H), 4.00 (s, 3H), 3.87 (s, 3H), 3.85 (s, 3H),

3.54 (t, *J* = 6.4 Hz, 2H), 3.44 (t, *J* = 6.8 Hz, 2H), 2.76 (t, *J* = 6.3 Hz, 2H), 2.74–2.67 (m, 4H), 1.89–1.82 (m, 2H), 1.12 (t, *J* = 7.1 Hz, 3H) (^{13}C NMR, 100 MHz, MeOD): δ = 166.8, 165.2, 155.3, 148.3, 140.6, 133.5, 130.0, 127.8, 125.2, 125.1, 122.9, 121.5, 119.7, 117.7, 111.2, 105.8, 62.1, 57.0, 54.9, 53.4, 52.3, 39.1, 38.7, 31.9, 27.9, 11.8; ESI-MS *m/z* calculated for $C_{26}H_{34}BrN_4O_4^+$ $[M+H]^+$ 546.5; found 546.3 HRMS (ESI) for $C_{26}H_{34}BrN_4O_4^+$ $[M+H]^+$ 545.1763; found 545.1745.

4.3. Statistical Analysis

4.3.1. Radioligand Binding Assays

Ki values for D_2 and D_3 receptors were measured using [^{125}I]IABN in human D_2 and D_3 receptors expressed in HEK cells, respectively. A filtration binding assay was used to characterize membrane-associated receptor binding properties [49]. The details for the procedures were described in the literature [26].

4.3.2. β-Arrestin Recruitment Assay

CHO-K1 cells which were overexpressed human D_3 receptors were cultured in assaycomplete^{TM} cell culture kit 107. Cells were seeded at a density of 25,000 cells per well of 96-well plate, and incubated at 5% CO_2, 37 °C. Two days later, test compounds were dissolved in DMSO, and diluted with 11-point series in phosphate-buffered saline (PBS). Prepared compounds were added to the cells, and it was incubated for 30 min at 5% CO_2, 37 °C. Then, cells were treated with 30 nM (EC_{80}) of dopamine, and the plate was incubated another 90 min. PathHunter^{TM} detection reagent was added to each well, and then plate was incubated for 80 min at RT in the dark. The chemiluminescent signal was measured by PerkinElmer Enspire plate reader (PerkinElmer, Boston, MA). Data were analyzed by Prism followed by non-linear regression.

4.3.3. Molecular Docking and Molecular Dynamics Simulations (MDS)

The 4 compounds with different ***N***-alkyl groups (**9a**, **20a**, **20b**, and **20c**) and the best candidate **21c** were selected and performed for molecular docking and MDS studies on the D_3 receptor. The protonated status at physiological pH of each compound was predicted by using Open Babel v3.1.0 [65]. Then, the molecular docking studies and MDS were performed by following the previous protocols [29]. In brief, molecular docking studies were performed via the AutoDock 4.2 [66] plugin on PyMOL (pymol.org). The X-ray structure of the D_3 receptor (PDB: 3PBL, resolution: 2.89 Å) was obtained from the RCSB Protein Data Bank (www.rcsb.org (accessed on 19 May 2022)). Heteroatoms were removed from the crystal structure and polar hydrogens were added. Non-polar hydrogens were removed from selected compounds. A grid box with a dimension of 30 × 30 × 28.2 Å^3 was applied for covering OBS and SBS bindings. The Lamarckian Genetic Algorithm with a maximum of 2,500,000 energy evaluations was used to calculated 100 protein–ligand binding poses for each compound. The D_3 receptor−ligand complex that reproduced the crystallographic ligand binding pose with good docking score was subjected for the evaluation. The CHARMM-GUI web-sever [67] was used for MDS preparation. The topology and parameter files of protonated compounds were generated by the Ligand Reader and Modeler module [68,69]. The Bilayer Membrane Builder module [70,71] was used for building the MDS system with FF19SB force field. The protein–ligand complexes generated from docking studies were aligned to the D_3 receptor structure obtained from the Orientations of Protein in Membranes (OPM) database [72], and the POPC membrane were placed by using the OPM D_3 receptor model. The protein, ligand, and membrane complexes were solvated in a TIP3P water box, and then Monte-Carlo sampling was used to add 0.15 M NaCl for charge neutralization. The MDS studies were performed via Amber18 [73] on the high-performance computing (HPC) cluster at Center for Biomedical Image Computing and Analytics at the University of Pennsylvania. The input files of system minimization, 6 steps equilibration including 2 steps NVT ensemble and 4 steps NPT ensemble, and 5 copies of 200 ns production run for MDS were generated from the last step of Membrane Builder [70,71] on the CHARMM-GUI web-sever [67].

The 50 to 200 ns of production simulation with a total of 7500 frames (1500 frames of 5 production simulation copies) for each compound were used for further MDS analysis. The interactions between a ligand and protein in the production simulations were calculated by using the software BINANA v2.1 [74].

5. Conclusions

A new scaffold was designed based on metoclopramide and identified having high affinity and subtype selectivity for the D_3 receptor versus the D_2 receptor. Initially, **9a** having 4-(thiophen-2-yl)benzamide was recognized as a lead compound showing high binding affinity and subtype selectivity for the D_3 receptor (*K*i D_2 = 169 nM and D_3 = 1 nM). Although different aryl carboxamides exhibited excellent binding affinities preferring D_3 receptors, **21c** was the most potent (IC_{50} = 1.3 nM) for competing with dopamine in the β-arrestin recruitment assay. Furthermore, the comprehensive screening of **21c** revealed the minimal off-target binding for other CNS targets. Molecular docking or MDS demonstrated that interactions between **21c** and the D_3 receptor were comparable with fallypride that was known for potent D_2/D_3 antagonists. These results suggested that **21c** may have a greater potential for competing with synaptic dopamine for binding to the D_3 receptor. Overall, this novel scaffold can be developed as high-affinity D_3 receptor antagonists that bind with low affinity at D_2 receptors and other CNS receptors.

Supplementary Materials: The supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijms24010432/s1.

Author Contributions: Conceptualization, H.Y.K. and R.H.M.; Methodology, H.Y.K., J.Y.L., C.-J.H. and M.T.; Validation, R.R.L. and R.H.M.; Formal analysis, H.Y.K., J.Y.L., C.-J.H. and R.H.M.; Investigation, H.Y.K., J.Y.L., C.-J.H., M.T. and R.H.M.; Resources, R.R.L. and R.H.M.; Data curation, R.R.L. and R.H.M.; Writing—original draft, H.Y.K., J.Y.L., C.-J.H. and R.H.M.; Writing—review and editing, H.Y.K., J.Y.L., R.R.L. and R.H.M. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by a grant from National Institute on Drug Abuse, grant number DA029840.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Off-target binding profiles for GPCRs were generously provided by the National Institute of Mental Health's Psychoactive Drug Screening Program, Contact# HHSN-271-2018-00023-C (NIMH PDSP). The NIMH PDSP is Directed by Bryan L. Roth at the University of North Carolina at Chapel Hill and Project Officer Jamie Driscoll at NIMH, Bethesda MD, USA. The molecular dynamic simulation studies were conducted on the high-performance computing cluster (https://www.med.upenn.edu/cbica/cubic.html) at the University of Pennsylvania Center for Biomedical Image Computing and Analytics and supported by the National Institutes of Health, Grant Number: 1S10OD023495-01.

Conflicts of Interest: The authors declare no competing financial interest.

Abbreviations

aq: aqueous; Boc, *tert*-butoxycarbonyl; br, broad; DIAD, diisopropyl azodicarboxylate; DMF, *N*, *N*-dimethylformamide; GPCR, G-protein coupled receptor; HBTU, hexafluorophosphate benzotriazole tetramethyl uronium; IC_{50}, half-maximum inhibitory concentration; *K*i, inhibition constant; MeCN, acetonitrile; RT, room temperature; SAR, structure–activity relationship; TFA, trifluoroacetic acid; THF, tetrahydrofuran; PPh_3, triphenylphosphine.

References

1. Luedtke, R.R.; Rangel-Barajas, C.; Malik, M.; Reichert, D.E.; Mach, R.H. Bitropic D3 Dopamine Receptor Selective Compounds as Potential Antipsychotics. *Curr. Pharm. Des.* **2015**, *21*, 3700–3724. [CrossRef]
2. Strange, P.G. Antipsychotic drug action: Antagonism, inverse agonism or partial agonism. *Trends Pharmacol. Sci.* **2008**, *29*, 314–321. [CrossRef] [PubMed]
3. Volkow, N.D.; Fowler, J.S.; Wang, G.J.; Swanson, J.M. Dopamine in drug abuse and addiction: Results from imaging studies and treatment implications. *Mol. Psychiatry* **2004**, *9*, 557–569. [CrossRef] [PubMed]
4. Gilbert, J.G.; Newman, A.H.; Gardner, E.L.; Ashby, C.R., Jr.; Heidbreder, C.A.; Pak, A.C.; Peng, X.Q.; Xi, Z.X. Acute administration of SB-277011A, NGB 2904, or BP 897 inhibits cocaine cue-induced reinstatement of drug-seeking behavior in rats: Role of dopamine D3 receptors. *Synapse* **2005**, *57*, 17–28. [CrossRef] [PubMed]
5. Sokoloff, P.; Le Foll, B. The dopamine D3 receptor, a quarter century later. *Eur. J. Neurosci.* **2017**, *45*, 2–19. [CrossRef]
6. Sokoloff, P.; Giros, B.; Martres, M.P.; Bouthenet, M.L.; Schwartz, J.C. Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. *Nature* **1990**, *347*, 146–151. [CrossRef] [PubMed]
7. Newman, A.H.; Blaylock, B.L.; Nader, M.A.; Bergman, J.; Sibley, D.R.; Skolnick, P. Medication discovery for addiction: Translating the dopamine D3 receptor hypothesis. *Biochem. Pharmacol.* **2012**, *84*, 882–890. [CrossRef] [PubMed]
8. Newman, A.H.; Grundt, P.; Nader, M.A. Dopamine D3 receptor partial agonists and antagonists as potential drug abuse therapeutic agents. *J. Med. Chem.* **2005**, *48*, 3663–3679. [CrossRef]
9. You, Z.B.; Gao, J.T.; Bi, G.H.; He, Y.; Boateng, C.; Cao, J.; Gardner, E.L.; Newman, A.H.; Xi, Z.X. The novel dopamine D3 receptor antagonists/partial agonists CAB2-015 and BAK4-54 inhibit oxycodone-taking and oxycodone-seeking behavior in rats. *Neuropharmacology* **2017**, *126*, 190–199. [CrossRef]
10. Newman, A.H.; Xi, Z.X.; Heidbreder, C. *Current Perspectives on Selective Dopamine D(3) Receptor Antagonists/Partial Agonists as Pharmacotherapeutics for Opioid and Psychostimulant Use Disorders*; Springer: Berlin/Heidelberg, Germany, 2022.
11. You, Z.B.; Bi, G.H.; Galaj, E.; Kumar, V.; Cao, J.; Gadiano, A.; Rais, R.; Slusher, B.S.; Gardner, E.L.; Xi, Z.X.; et al. Dopamine D(3)R antagonist VK4-116 attenuates oxycodone self-administration and reinstatement without compromising its antinociceptive effects. *Neuropsychopharmacology* **2019**, *44*, 1415–1424. [CrossRef]
12. Boeckler, F.; Gmeiner, P. Dopamine D3 receptor ligands: Recent advances in the control of subtype selectivity and intrinsic activity. *Biochim. Biophys. Acta* **2007**, *1768*, 871–887. [CrossRef] [PubMed]
13. Wang, Q.; Mach, R.H.; Luedtke, R.R.; Reichert, D.E. Subtype selectivity of dopamine receptor ligands: Insights from structure and ligand-based methods. *J. Chem. Inf. Model.* **2010**, *50*, 1970–1985. [CrossRef] [PubMed]
14. Newman, A.H.; Beuming, T.; Banala, A.K.; Donthamsetti, P.; Pongetti, K.; LaBounty, A.; Levy, B.; Cao, J.; Michino, M.; Luedtke, R.R.; et al. Molecular determinants of selectivity and efficacy at the dopamine D3 receptor. *J. Med. Chem.* **2012**, *55*, 6689–6699. [CrossRef] [PubMed]
15. Chien, E.Y.; Liu, W.; Zhao, Q.; Katritch, V.; Han, G.W.; Hanson, M.A.; Shi, L.; Newman, A.H.; Javitch, J.A.; Cherezov, V.; et al. Structure of the human dopamine D3 receptor in complex with a D2/D3 selective antagonist. *Science* **2010**, *330*, 1091–1095. [CrossRef]
16. Yuan, J.; Chen, X.; Brodbeck, R.; Primus, R.; Braun, J.; Wasley, J.W.; Thurkauf, A. NGB 2904 and NGB 2849: Two highly selective dopamine D3 receptor antagonists. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2715–2718. [CrossRef]
17. Reavill, C.; Taylor, S.G.; Wood, M.D.; Ashmeade, T.; Austin, N.E.; Avenell, K.Y.; Boyfield, I.; Branch, C.L.; Cilia, J.; Coldwell, M.C.; et al. Pharmacological actions of a novel, high-affinity, and selective human dopamine D(3) receptor antagonist, SB-277011-A. *J. Pharmacol. Exp. Ther.* **2000**, *294*, 1154–1165.
18. Mach, R.H.; Huang, Y.; Freeman, R.A.; Wu, L.; Vangveravong, S.; Luedtke, R.R. Conformationally-flexible benzamide analogues as dopamine D3 and sigma 2 receptor ligands. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 195–202. [CrossRef]
19. Pilla, M.; Perachon, S.; Sautel, F.; Garrido, F.; Mann, A.; Wermuth, C.G.; Schwartz, J.C.; Everitt, B.J.; Sokoloff, P. Selective inhibition of cocaine-seeking behaviour by a partial dopamine D3 receptor agonist. *Nature* **1999**, *400*, 371–375. [CrossRef]
20. Stemp, G.; Ashmeade, T.; Branch, C.L.; Hadley, M.S.; Hunter, A.J.; Johnson, C.N.; Nash, D.J.; Thewlis, K.M.; Vong, A.K.; Austin, N.E.; et al. Design and synthesis of trans-N-[4-[2-(6-cyano-1,2,3, 4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-4-quinolinecarboxamide (SB-277011): A potent and selective dopamine D(3) receptor antagonist with high oral bioavailability and CNS penetration in the rat. *J. Med. Chem.* **2000**, *43*, 1878–1885. [CrossRef]
21. Mach, R.H.; Tu, Z.; Xu, J.; Li, S.; Jones, L.A.; Taylor, M.; Luedtke, R.R.; Derdeyn, C.P.; Perlmutter, J.S.; Mintun, M.A. Endogenous dopamine (DA) competes with the binding of a radiolabeled D(3) receptor partial agonist in vivo: A positron emission tomography study. *Synapse* **2011**, *65*, 724–732. [CrossRef]
22. Maramai, S.; Gemma, S.; Brogi, S.; Campiani, G.; Butini, S.; Stark, H.; Brindisi, M. Dopamine D3 Receptor Antagonists as Potential Therapeutics for the Treatment of Neurological Diseases. *Front. Neurosci.* **2016**, *10*, 451. [CrossRef] [PubMed]
23. Shaik, A.B.; Kumar, V.; Bonifazi, A.; Guerrero, A.M.; Cemaj, S.L.; Gadiano, A.; Lam, J.; Xi, Z.X.; Rais, R.; Slusher, B.S.; et al. Investigation of Novel Primary and Secondary Pharmacophores and 3-Substitution in the Linking Chain of a Series of Highly Selective and Bitopic Dopamine D3 Receptor Antagonists and Partial Agonists. *J. Med. Chem.* **2019**, *62*, 9061–9077. [CrossRef] [PubMed]
24. Lee, B.; Taylor, M.; Griffin, S.A.; McInnis, T.; Sumien, N.; Mach, R.H.; Luedtke, R.R. Evaluation of Substituted N-Phenylpiperazine Analogs as D3 vs. D2 Dopamine Receptor Subtype Selective Ligands. *Molecules* **2021**, *26*, 3182. [CrossRef] [PubMed]

25. Bonifazi, A.; Newman, A.H.; Keck, T.M.; Gervasoni, S.; Vistoli, G.; Del Bello, F.; Giorgioni, G.; Pavletic, P.; Quaglia, W.; Piergentili, A. Scaffold Hybridization Strategy Leads to the Discovery of Dopamine D(3) Receptor-Selective or Multitarget Bitopic Ligands Potentially Useful for Central Nervous System Disorders. *ACS Chem. Neurosci.* **2021**, *12*, 3638–3649. [CrossRef]
26. Reilly, S.W.; Riad, A.A.; Hsieh, C.J.; Sahlholm, K.; Jacome, D.A.; Griffin, S.; Taylor, M.; Weng, C.C.; Xu, K.; Kirschner, N.; et al. Leveraging a Low-Affinity Diazaspiro Orthosteric Fragment to Reduce Dopamine D3 Receptor (D3R) Ligand Promiscuity across Highly Conserved Aminergic G-Protein-Coupled Receptors (GPCRs). *J. Med. Chem.* **2019**, *62*, 5132–5147. [CrossRef]
27. Wassouf, Z.; Schulze-Hentrich, J.M. Alpha-synuclein at the nexus of genes and environment: The impact of environmental enrichment and stress on brain health and disease. *J. Neurochem.* **2019**, *150*, 591–604. [CrossRef]
28. Zingales, V.; Torrisi, S.A.; Leggio, G.M.; Bucolo, C.; Drago, F.; Salomone, S. Pharmacological and Genetic Evidence of Dopamine Receptor 3-Mediated Vasoconstriction in Isolated Mouse Aorta. *Biomolecules* **2021**, *11*, 418. [CrossRef]
29. Hsieh, C.J.; Riad, A.; Lee, J.Y.; Sahlholm, K.; Xu, K.; Luedtke, R.R.; Mach, R.H. Interaction of Ligands for PET with the Dopamine D3 Receptor: In Silico and In Vitro Methods. *Biomolecules* **2021**, *11*, 529. [CrossRef]
30. Micheli, F.; Arista, L.; Bonanomi, G.; Blaney, F.E.; Braggio, S.; Capelli, A.M.; Checchia, A.; Damiani, F.; Di-Fabio, R.; Fontana, S.; et al. 1,2,4-Triazolyl azabicyclo [3.1.0]hexanes: A new series of potent and selective dopamine D(3) receptor antagonists. *J. Med. Chem.* **2010**, *53*, 374–391. [CrossRef]
31. Mugnaini, M.; Iavarone, L.; Cavallini, P.; Griffante, C.; Oliosi, B.; Savoia, C.; Beaver, J.; Rabiner, E.A.; Micheli, F.; Heidbreder, C.; et al. Occupancy of brain dopamine D3 receptors and drug craving: A translational approach. *Neuropsychopharmacology* **2013**, *38*, 302–312. [CrossRef]
32. Murphy, A.; Nestor, L.J.; McGonigle, J.; Paterson, L.; Boyapati, V.; Ersche, K.D.; Flechais, R.; Kuchibatla, S.; Metastasio, A.; Orban, C.; et al. Acute D3 Antagonist GSK598809 Selectively Enhances Neural Response During Monetary Reward Anticipation in Drug and Alcohol Dependence. *Neuropsychopharmacology* **2017**, *42*, 1925–1926. [CrossRef] [PubMed]
33. Micheli, F.; Bacchi, A.; Braggio, S.; Castelletti, L.; Cavallini, P.; Cavanni, P.; Cremonesi, S.; Cin, M.D.; Feriani, A.; Gehanne, S.; et al. 1,2,4-Triazolyl 5-Azaspiro[2.4]heptanes: Lead Identification and Early Lead Optimization of a New Series of Potent and Selective Dopamine D3 Receptor Antagonists. *J. Med. Chem.* **2016**, *59*, 8549–8576. [CrossRef] [PubMed]
34. Reilly, S.W.; Griffin, S.; Taylor, M.; Sahlholm, K.; Weng, C.C.; Xu, K.; Jacome, D.A.; Luedtke, R.R.; Mach, R.H. Highly Selective Dopamine D3 Receptor Antagonists with Arylated Diazaspiro Alkane Cores. *J. Med. Chem.* **2017**, *60*, 9905–9910. [CrossRef] [PubMed]
35. Chen, J.; Levant, B.; Jiang, C.; Keck, T.M.; Newman, A.H.; Wang, S. Tranylcypromine substituted cis-hydroxycyclobutylnaphthamides as potent and selective dopamine D(3) receptor antagonists. *J. Med. Chem.* **2014**, *57*, 4962–4968. [CrossRef]
36. Tan, L.; Zhou, Q.; Yan, W.; Sun, J.; Kozikowski, A.P.; Zhao, S.; Huang, X.P.; Cheng, J. Design and Synthesis of Bitopic 2-Phenylcyclopropylmethylamine (PCPMA) Derivatives as Selective Dopamine D3 Receptor Ligands. *J. Med. Chem.* **2020**, *63*, 4579–4602. [CrossRef]
37. Bennacef, I.; Salinas, C.A.; Bonasera, T.A.; Gunn, R.N.; Audrain, H.; Jakobsen, S.; Nabulsi, N.; Weinzimmer, D.; Carson, R.E.; Huang, Y.; et al. Dopamine D3 receptor antagonists: The quest for a potentially selective PET ligand. Part 3: Radiosynthesis and in vivo studies. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5056–5059. [CrossRef]
38. Keck, T.M.; John, W.S.; Czoty, P.W.; Nader, M.A.; Newman, A.H. Identifying Medication Targets for Psychostimulant Addiction: Unraveling the Dopamine D3 Receptor Hypothesis. *J. Med. Chem.* **2015**, *58*, 5361–5380. [CrossRef]
39. Appel, N.M.; Li, S.H.; Holmes, T.H.; Acri, J.B. Dopamine D3 Receptor Antagonist (GSK598809) Potentiates the Hypertensive Effects of Cocaine in Conscious, Freely-Moving Dogs. *J. Pharmacol. Exp. Ther.* **2015**, *354*, 484–492. [CrossRef]
40. Battiti, F.O.; Cemaj, S.L.; Guerrero, A.M.; Shaik, A.B.; Lam, J.; Rais, R.; Slusher, B.S.; Deschamps, J.R.; Imler, G.H.; Newman, A.H.; et al. The Significance of Chirality in Drug Design and Synthesis of Bitopic Ligands as D3 Receptor (D3R) Selective Agonists. *J. Med. Chem.* **2019**, *62*, 6287–6314. [CrossRef]
41. Shaik, A.B.; Boateng, C.A.; Battiti, F.O.; Bonifazi, A.; Cao, J.; Chen, L.; Chitsazi, R.; Ravi, S.; Lee, K.H.; Shi, L.; et al. Structure Activity Relationships for a Series of Eticlopride-Based Dopamine D2/D3 Receptor Bitopic Ligands. *J. Med. Chem.* **2021**, *64*, 15313–15333. [CrossRef]
42. Mukherjee, J.; Yang, Z.Y.; Das, M.K.; Brown, T. Fluorinated benzamide neuroleptics–III. Development of (S)-N-[(1-allyl-2-pyrrolidinyl)methyl]-5-(3-[18F]fluoropropyl)-2, 3-dimethoxybenzamide as an improved dopamine D-2 receptor tracer. *Nucl. Med. Biol.* **1995**, *22*, 283–296. [CrossRef] [PubMed]
43. Mukherjee, J.; Constantinescu, C.C.; Hoang, A.T.; Jerjian, T.; Majji, D.; Pan, M.L. Dopamine D3 receptor binding of (18)F-fallypride: Evaluation using in vitro and in vivo PET imaging studies. *Synapse* **2015**, *69*, 577–591. [CrossRef] [PubMed]
44. Halldin, C.; Farde, L.; Hogberg, T.; Mohell, N.; Hall, H.; Suhara, T.; Karlsson, P.; Nakashima, Y.; Swahn, C.G. Carbon-11-FLB 457: A radioligand for extrastriatal D2 dopamine receptors. *J. Nucl. Med.* **1995**, *36*, 1275–1281. [PubMed]
45. Yang, D.; Kefi, S.; Audinot, V.; Millan, M.J.; Langlois, M. Benzamides derived from 1,2-diaminocyclopropane as novel ligands for human D2 and D3 dopamine receptors. *Bioorg. Med. Chem.* **2000**, *8*, 321–327. [CrossRef]
46. Chen, P.J.; Taylor, M.; Griffin, S.A.; Amani, A.; Hayatshahi, H.; Korzekwa, K.; Ye, M.; Mach, R.H.; Liu, J.; Luedtke, R.R.; et al. Design, synthesis, and evaluation of N-(4-(4-phenyl piperazin-1-yl)butyl)-4-(thiophen-3-yl)benzamides as selective dopamine D3 receptor ligands. *Bioorg. Med. Chem. Lett.* **2019**, *29*, 2690–2694. [CrossRef]
47. Tu, Z.; Li, S.; Cui, J.; Xu, J.; Taylor, M.; Ho, D.; Luedtke, R.R.; Mach, R.H. Synthesis and pharmacological evaluation of fluorine-containing D(3) dopamine receptor ligands. *J. Med. Chem.* **2011**, *54*, 1555–1564. [CrossRef]

48. Rangel-Barajas, C.; Malik, M.; Taylor, M.; Neve, K.A.; Mach, R.H.; Luedtke, R.R. Characterization of [(3) H]LS-3-134, a novel arylamide phenylpiperazine D3 dopamine receptor selective radioligand. *J. Neurochem.* **2014**, *131*, 418–431. [CrossRef]
49. Luedtke, R.R.; Freeman, R.A.; Boundy, V.A.; Martin, M.W.; Huang, Y.; Mach, R.H. Characterization of (125)I-IABN, a novel azabicyclononane benzamide selective for D2-like dopamine receptors. *Synapse* **2000**, *38*, 438–449. [CrossRef]
50. Wang, T.; Li, Z.; Cvijic, M.E.; Krause, C.; Zhang, L.; Sum, C.S. Measurement of beta-Arrestin Recruitment for GPCR Targets. In *Assay Guidance Manual*; Markossian, S., Grossman, A., Brimacombe, K., Arkin, M., Auld, D., Austin, C., Baell, J., Chung, T.D.Y., Coussens, N.P., Dahlin, J.L., et al., Eds.; Bethesda: Rockville, MD, USA, 2004.
51. Lefkowitz, R.J.; Shenoy, S.K. Transduction of receptor signals by beta-arrestins. *Science* **2005**, *308*, 512–517. [CrossRef]
52. Zhao, X.; Jones, A.; Olson, K.R.; Peng, K.; Wehrman, T.; Park, A.; Mallari, R.; Nebalasca, D.; Young, S.W.; Xiao, S.H. A homogeneous enzyme fragment complementation-based beta-arrestin translocation assay for high-throughput screening of G-protein-coupled receptors. *J. Biomol. Screen* **2008**, *13*, 737–747. [CrossRef]
53. Hayatshahi, H.S.; Xu, K.; Griffin, S.A.; Taylor, M.; Mach, R.H.; Liu, J.; Luedtke, R.R. Analogues of Arylamide Phenylpiperazine Ligands To Investigate the Factors Influencing D3 Dopamine Receptor Bitropic Binding and Receptor Subtype Selectivity. *ACS Chem. Neurosci.* **2018**, *9*, 2972–2983. [CrossRef] [PubMed]
54. Besnard, J.; Ruda, G.F.; Setola, V.; Abecassis, K.; Rodriguiz, R.M.; Huang, X.P.; Norval, S.; Sassano, M.F.; Shin, A.I.; Webster, L.A.; et al. Automated design of ligands to polypharmacological profiles. *Nature* **2012**, *492*, 215–220. [CrossRef] [PubMed]
55. Chu, W.; Tu, Z.; McElveen, E.; Xu, J.; Taylor, M.; Luedtke, R.R.; Mach, R.H. Synthesis and in vitro binding of N-phenyl piperazine analogs as potential dopamine D3 receptor ligands. *Bioorg. Med. Chem.* **2005**, *13*, 77–87. [CrossRef] [PubMed]
56. Vangveravong, S.; Zhang, Z.; Taylor, M.; Bearden, M.; Xu, J.; Cui, J.; Wang, W.; Luedtke, R.R.; Mach, R.H. Synthesis and characterization of selective dopamine D(2) receptor ligands using aripiprazole as the lead compound. *Bioorg. Med. Chem.* **2011**, *19*, 3502–3511. [CrossRef] [PubMed]
57. Xu, J.; Vangveravong, S.; Li, S.; Fan, J.; Jones, L.A.; Cui, J.; Wang, R.; Tu, Z.; Chu, W.; Perlmutter, J.S.; et al. Positron emission tomography imaging of dopamine D2 receptors using a highly selective radiolabeled D2 receptor partial agonist. *Neuroimage* **2013**, *71*, 168–174. [CrossRef]
58. Peng, X.; Wang, Q.; Mishra, Y.; Xu, J.; Reichert, D.E.; Malik, M.; Taylor, M.; Luedtke, R.R.; Mach, R.H. Synthesis, pharmacological evaluation and molecular modeling studies of triazole containing dopamine D3 receptor ligands. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 519–523. [CrossRef]
59. Vilkman, H.; Kajander, J.; Aalto, S.; Vahlberg, T.; Nagren, K.; Allonen, T.; Syvalahti, E.; Hietala, J. The effects of lorazepam on extrastriatal dopamine D(2/3)-receptors-A double-blind randomized placebo-controlled PET study. *Psychiatry Res.* **2009**, *174*, 130–137. [CrossRef]
60. Lober, S.; Hubner, H.; Tschammer, N.; Gmeiner, P. Recent advances in the search for D3- and D4-selective drugs: Probes, models and candidates. *Trends Pharmacol. Sci.* **2011**, *32*, 148–157. [CrossRef]
61. Gao, M.; Wang, M.; Mock, B.H.; Glick-Wilson, B.E.; Yoder, K.K.; Hutchins, G.D.; Zheng, Q.H. An improved synthesis of dopamine D2/D3 receptor radioligands [11C]fallypride and [18F]fallypride. *Appl. Radiat. Isot.* **2010**, *68*, 1079–1086. [CrossRef]
62. Pettit, G.R.; Piatak, D.M. Hydrogen Bromide-Acetic Acid Demethylation of 2,3-Dimethoxy-6-bromobenzoic Acid. An Example of Concomitant Bromine Migration1,2. *J. Org. Chem.* **1960**, *25*, 721–725. [CrossRef]
63. Komiya, C.; Aihara, K.; Morishita, K.; Ding, H.; Inokuma, T.; Shigenaga, A.; Otaka, A. Development of an Intein-Inspired Amide Cleavage Chemical Device. *J. Org. Chem.* **2016**, *81*, 699–707. [CrossRef] [PubMed]
64. Varseev, G.N.; Maier, M.E. A novel palladium-catalyzed arylation-dehydroaromatization reaction: Synthesis of 7-aryltetralones. *Org. Lett.* **2005**, *7*, 3881–3884. [CrossRef]
65. O'Boyle, N.M.; Banck, M.; James, C.A.; Morley, C.; Vandermeersch, T.; Hutchison, G.R. Open Babel: An open chemical toolbox. *J. Cheminform.* **2011**, *3*, 33. [CrossRef] [PubMed]
66. Morris, G.M.; Huey, R.; Lindstrom, W.; Sanner, M.F.; Belew, R.K.; Goodsell, D.S.; Olson, A.J. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J. Comput. Chem.* **2009**, *30*, 2785–2791. [CrossRef] [PubMed]
67. Lee, J.; Cheng, X.; Swails, J.M.; Yeom, M.S.; Eastman, P.K.; Lemkul, J.A.; Wei, S.; Buckner, J.; Jeong, J.C.; Qi, Y.; et al. CHARMM-GUI Input Generator for NAMD, GROMACS, AMBER, OpenMM, and CHARMM/OpenMM Simulations Using the CHARMM36 Additive Force Field. *J. Chem. Theory Comput.* **2016**, *12*, 405–413. [CrossRef] [PubMed]
68. Jo, S.; Kim, T.; Iyer, V.G.; Im, W. CHARMM-GUI: A web-based graphical user interface for CHARMM. *J. Comput. Chem.* **2008**, *29*, 1859–1865. [CrossRef] [PubMed]
69. Kim, S.; Lee, J.; Jo, S.; Brooks, C.L., 3rd; Lee, H.S.; Im, W. CHARMM-GUI ligand reader and modeler for CHARMM force field generation of small molecules. *J. Comput. Chem.* **2017**, *38*, 1879–1886. [CrossRef]
70. Klauda, J.B.; Venable, R.M.; Freites, J.A.; O'Connor, J.W.; Tobias, D.J.; Mondragon-Ramirez, C.; Vorobyov, I.; MacKerell, A.D., Jr.; Pastor, R.W. Update of the CHARMM all-atom additive force field for lipids: Validation on six lipid types. *J. Phys. Chem. B* **2010**, *114*, 7830–7843. [CrossRef]
71. Venable, R.M.; Sodt, A.J.; Rogaski, B.; Rui, H.; Hatcher, E.; MacKerell, A.D., Jr.; Pastor, R.W.; Klauda, J.B. CHARMM all-atom additive force field for sphingomyelin: Elucidation of hydrogen bonding and of positive curvature. *Biophys. J.* **2014**, *107*, 134–145. [CrossRef]
72. Lomize, M.A.; Pogozheva, I.D.; Joo, H.; Mosberg, H.I.; Lomize, A.L. OPM database and PPM web server: Resources for positioning of proteins in membranes. *Nucleic Acids Res.* **2012**, *40*, D370–D376. [CrossRef]

73. Case, D.B.-S.I.; Brozell, S.; Cerutti, D.; Cheatham III, T.; Cruzeiro, V.; Darden, T.; Duke, R.; Ghoreishi, D.; Gilson, M.; Gohlke, H.; et al. *AMBER 18*; University of California: San Francisco, CA, USA, 2018.
74. Durrant, J.D.; McCammon, J.A. BINANA: A novel algorithm for ligand-binding characterization. *J. Mol. Graph. Model.* **2011**, *29*, 888–893. [CrossRef] [PubMed]