European Journal of Medicinal Chemistry 198 (2020) 112368

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Synthesis, in vitro evaluation and molecular docking of a new class of indolylpropyl benzamidopiperazines as dual AChE and SERT ligands for Alzheimer's disease

Julio Rodríguez-Lavado [a], Carlos Gallardo-Garrido [a], Michael Mallea [a], Victor Bustos [b], Rodrigo Osorio [a], Martín Hödar-Salazar [c], Hery Chung [d], Ramiro Araya-Maturana [e], Marcos Lorca [f, g], C. David Pessoa-Mahana [d], Jaime Mella-Raipán [g, h], Claudio Saitz [a], Pablo Jaque [a], Miguel Reyes-Parada [i, j], Patricio Iturriaga-Vásquez [c, k, **], Hernán Pessoa-Mahana [a, *]

[a] Departamento de Química Orgánica y Fisicoquímica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Olivos, 1007, Santiago, Chile
[b] Laboratory of Cellular and Molecular Neuroscience, The Rockefeller University, New York, USA
[c] Departamento de Ciencias Químicas y Recursos Naturales, Facultad de Ingeniería Ciencias, Universidad de la Frontera, Temuco, Chile
[d] Departamento de Farmacia, Facultad de Química, Pontificia Universidad Católica de Chile, Santiago, Chile
[e] Instituto de Química de Recursos Naturales, Universidad de Talca, Talca, Chile
[f] Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares, Universidade de Santiago de Compostela, Santiago de Compostela, Spain
[g] Instituto de Química y Bioquímica, Facultad de Ciencias, Universidad de Valparaíso, Valparaíso, Chile
[h] Centro de Investigación Farmacopea Chilena (CIFAR), Universidad de Valparaíso, Santa Marta, Valparaíso, Chile
[i] Centro de Investigación Biomédica y Aplicada (CIBAP), Escuela de Medicina, Facultad de Ciencias Médicas, Universidad de Santiago de Chile, Chile
[j] Facultad de Ciencias de la Salud, Universidad Autónoma de Chile, Santiago, Chile
[k] Center of Excellence in Biotechnology Research Applied to the Environment, Universidad de La Frontera, Temuco, Chile

ARTICLE INFO

Article history:
Received 13 January 2020
Received in revised form
11 April 2020
Accepted 20 April 2020
Available online 29 April 2020

Keywords:
Alzheimer's disease
Multitarget
Dual-active compounds
AChE
SERT
Indole derivatives

ABSTRACT

During the last decade, the one drug-one target strategy has resulted to be inefficient in facing diseases with complex ethiology like Alzheimer's disease and many others. In this context, the multitarget paradigm has emerged as a promising strategy. Based on this consideration, we aim to develop novel molecules as promiscuous ligands acting in two or more targets at the same time. For such purpose, a new series of indolylpropyl-piperazinyl oxoethyl-benzamido piperazines were synthesized and evaluated as multitarget-directed drugs for the serotonin transporter (SERT) and acetylcholinesterase (AChE). The ability to decrease β-amyloid levels as well as cell toxicity of all compounds were also measured. In vitro results showed that at least four compounds displayed promising activity against SERT and AChE. Compounds **18** and **19** (IC_{50} = 3.4 and 3.6 μM respectively) exhibited AChE inhibition profile in the same order of magnitude as donepezil (DPZ, IC_{50} = 2.17 μM), also displaying nanomolar affinity in SERT. Moreover, compounds **17** and **24** displayed high SERT affinities (IC_{50} = 9.2 and 1.9 nM respectively) similar to the antidepressant citalopram, and significant micromolar AChE activity at the same time. All the bioactive compounds showed a low toxicity profile in the range of concentrations studied. Molecular docking allowed us to rationalize the binding mode of the synthesized compounds in both targets. In addition, we also show that compounds **11** and **25** exhibit significant β-amyloid lowering activity in a cell-based assay, **11** (50% inhibition, 10 μM) and **25** (35% inhibition, 10 μM).

These results suggest that indolylpropyl benzamidopiperazines based compounds constitute promising leads for a multitargeted approach for Alzheimer's disease.

* Corresponding author. Departamento de Química Orgánica y Fisicoquímica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile.
** Corresponding author. Departamento de Ciencias Químicas y Recursos Naturales, Facultad de Ingeniería Ciencias, Universidad de la Frontera, Temuco, Chile.
E-mail addresses: patricio.iturriaga@ufrontera.cl (P. Iturriaga-Vásquez), hpessoa@ciq.uchile.cl (H. Pessoa-Mahana).

https://doi.org/10.1016/j.ejmech.2020.112368
0223-5234/

Abbreviation

AD	Alzheimer's Disease
Aβ	β-Amyloid
AChE	Acetylcholinesterase
SERT	Serotonin transporter
APP	Amyloid precursor protein
MTDL	Multi-Target-Direct-Ligand
NMDA	*N*-Methyl-D-Aspartate
DPZ	Donepezil
BOC	*N-tert*butoxycarbonyl
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
PAS	Peripherial Anionic Site
PDB	Protein Data Bank

1. Introduction

Alzheimer's disease (AD) is a chronic, progressive and fatal neurodegenerative disorder affecting cognition, behavior and function, being one of the most common causes of mental deterioration in elderly people. Indeed, around 50–60% of the overall dementias correspond to AD. World Health Organization estimates that about 46.8 million of people worldwide currently suffer from AD, thus becoming a major public health concern as the world's population ages [1]. The number of AD patients is expected to double every 20 years, and thus the population with AD is estimaded to reach 74.7 million in 2030 [1]. AD often starts with mild symptoms but ends with severe brain damage and people with dementia lose their abilities at different rates [1–4]. In spite of multiple efforts, the detailed pathogenesis of AD remains so far unclear, and despite huge social and economic incentives, big pharmaceutical companies are pulling out AD research programs given the continuous failed results in the clinical phases [5–7]. At present, cholinesterase inhibitors donepezil, rivastigmine, galantamine, and the NMDA receptor antagonist memantine, are the only approved treatments used in AD [8]. However, these treatments only improve the symptoms associated with this disease without modifying the underlying pathology [9,10].

The multifactorial nature of the disease has been a problem for developing novel drugs with therapeutic potential [11–14]. At present, different hypotheses concerning AD are being considered [15]. These include, abnormal deposits of amyloid β (Aβ) proteins [16,17], intracellular tau protein accumulation involving twisted fibers [18,19], inflammation and oxidative stress [20,21], cholinergic neuron damage (cholinergic hypothesis) [22,23], and more recently the serotonin hypothesis [24,25].

Currently, several different targets have been identified as possible sites of action for anti AD drugs. These include, among others, β and γ-secretase [26–29], acethylcholinesterase (AChE) and butyrylcholinesterase (BuChE) [22,23], γ-aminobutyric acid (GABA) [30], dopamine D_2 receptor [31,32], serotonin 5-HT_6 and 5-HT_4 receptors [33–35], serotonin transporter (SERT) [36], SFRP1 [37], ApoE [27] and a large list of other different targets [13,38–41].

In this context, the multi-target strategy has powerfully drawn attention, especially in the last 15 years, as a potential solution for the treatment of diseases with complex etiology like AD [14,42–45] The current paradigm 'one drug-one target' has now been shifted by the mentioned multitarget approach. Thus, complex disorders like AD are more likely to be treated or alleviated through simultaneous modulation of two or more protein targets [46–48].

Indole and benzamide derivatives have drawn attention for their affinity on the serotonin systems [49,50]. In addition, other studies have reported an AChE inhibitory activity in compounds containing similar structural frameworks [51–53]. Interestingly, new data suggest that the decrease in cholinergic activity and the accumulation of β-amyloid plaques are related [54].

In consequence, based on the multi-target directed ligands (MTDL's) strategy, we decided to explore the development of novel compounds acting as promiscuous ligands [35,55–60]. In such sense, a series of indolylpropyl benzamidopiperazines have been synthesized and biologically evaluated at the following targets/functions: AChE, SERT, amyloid-beta levels, and cell viability.

2. Results and discussion

2.1. Chemistry

Substituted *N*-benzamidepiperazines **1a-k** were synthesized according to previously reported procedures [61–65], and were reacted with 4-chloroacetyl-piperazine-1-carboxylic acid *tert*-butyl ester and K_2CO_3 to give *N*-Boc protected bis piperazinyl derivatives **2a-k** in good to excelent yields (75–90%, Scheme 1). After purification by column chromatography, Boc removal was achieved by dissolving **2a-k** in a 1:1 TFA-CH_2Cl_2 mixture at room temperature for 2 h. The resulting unprotected free amines were immediately reacted with tosylates **A, B** and **C** [59] using K_2CO_3 in dry MeCN in a two-step one-pot procedure. After purification, compounds **3**–**35** were obtained in moderate to excelent yields (61–89%, Scheme 2). ^1H and ^{13}C NMR assignement and conclusions drawn from it, confirmed the structure of intermediates and final molecules. All the compounds displayed in ^1H NMR spectra characteristic singlets at downfield corresponding to the indolic NH along with two sets of differentiated aromatic signals, between 7.80 and 6.80 ppm corresponding to the indole and benzoyl rings, while piperazine methylenes signals appeared as broad singlets at highfield. Methylene and propylene connecting chains provided the expected highfield signals confirming the final structure. ESI-HRMS characterization provided the molecular ions for each single intermediate **2a**-**k** and the final compounds **3**–**35**.

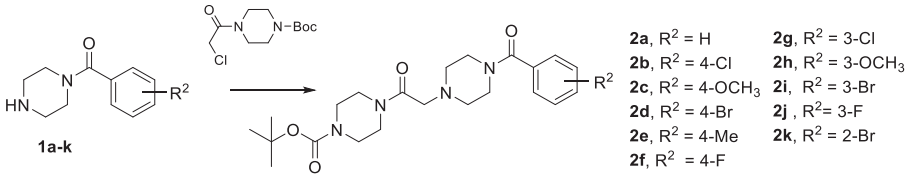
2.2. Acetylcholinesterase inhibition

The in vitro anticholinesterase activity of compounds **3**–**35** was determined using AChE from human blood plasma. In this study, donepezil (DPZ) was used as an AChE inhibitor control while citalopram was used for comparative purposes. The IC_{50} values (μM) are summarized in Table 1. All compounds were soluble at pH = 7.4 in the studied concentration range (See Experimental Section).

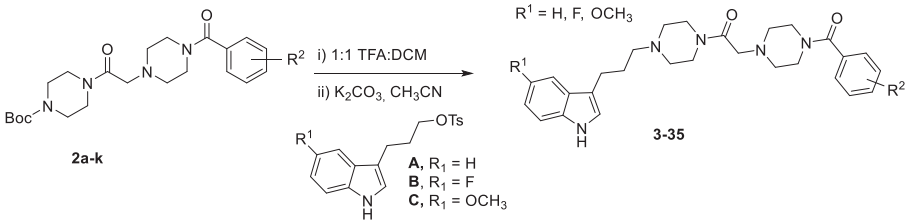
Our results revealed that nearly half of the synthesized compounds exhibited a significant activity against AChE. The unsubstituted compound **3** ($R^1 = R^2 = H$) displayed no inhibitory AChE properties in the whole range of concentrations studied ($IC_{50} > 50$ μM).

Compounds **25**–**35** which bear the strong electron donating methoxyl group at the 5-indolic position, resulted to be inactive, excepting compound **34**, which showed a moderate inhibitory activity ($IC_{50} = 37.3$ μM). Thus, the presence of a methoxyl group at this position led to low activity or inactive compounds.

On the other hand, the presence of a hydrogen or fluorine atom at the 5-indolic position, led to moderate to very active compounds, depending on the benzamide substituent. Thus, compounds **11** ($R^1 = H$, $R^2 = 3$-Br, $IC_{50} = 23.0$ μM) and **13** ($R^1 = H$, $R^2 = 2$-Br, $IC_{50} = 20.2$ μM) displayed moderate activity. Interestigly, switching R^1 from H to F in these analogues, led to less active compounds **22** ($R^1 = F$, $R^2 = 3$-Br, $IC_{50} = 37.3$ μM) and **24** ($R^1 = F$, $R^2 = 2$-Br,



Scheme 1. Reagents and conditions: K_2CO_3, 4-chloroacetyl-piperazine-1-carboxylic acid tert-butyl ester, dry CH_3CN, reflux (80 °C), 16 h. Yields (75–90%).



Scheme 2. Reagents and conditions: i) 1:1 TFA-CH_2Cl_2, rt, 2 h ii) Tosylates **A, B** or **C**, K_2CO_3, dry CH_3CN, reflux (80 °C), 16 h. 61–89% (two step overall yield).

Table 1
Values for AChE inhibition, SERT affinity and toxicity profiles for all measured compounds.

Compound	R^1	R^2	cLogP[a]	IC_{50} *h*-AChE[b] (μM)	IC_{50} *h*-SERT[c] (nM)	Cell Viability IC_{50} HEK-293 (μM)	Cell Viability IC_{50} SH-SY5Y (μM)
Donepezil	–	–	3.76	2.17 ± 0.15	>1000	>200	>200
Citalopram	–	–	4.21	73.3	3.0 ± 0.2	>200	>200
3	H	H	2.49	>50	>1000	>200	>200
4	H	4-Cl	3.01	35.2 ± 5.8	>1000	98.2 ± 7.6	139.0 ± 14.4
5	H	4-OMe	2.24	25.1 ± 3.3	>1000	155.6 ± 29.1	147.9 ± 19.5
6	H	4-Br	3.26	35.7 ± 9.4	>1000	71.9 ± 6.4	59.3 ± 6.0
7	H	4-Me	2.96	29.4 ± 6.9	>1000	158.5 ± 10.3	>200
8	H	4-F	2.63	39.4 ± 5.1	>1000	140.9 ± 18.9	188.4 ± 52.3
9	H	3-Cl	3.01	32.4 ± 7.3	>1000	99.1 ± 7.4	120.2 ± 18.8
10	H	3-OMe	2.24	>50	>1000	142.9 ± 6.1	>200
11	H	3-Br	3.28	23.0 ± 2.7	>1000	80.7 ± 5.3	80.5 ± 7.8
12	H	3-F	2.63	>50.0	>1000	181.6 ± 30.4	165.2 ± 7.7
13	H	2-Br	3.28	20.2 ± 4.0	>1000	133.4 ± 8.6	82.6 ± 12.6
14	F	H	2.63	>50	320 ± 87	147.6 ± 13.6	145.5 ± 21.3
15	F	4-Cl	3.15	22.3 ± 3.4	546 ± 213	44.5 ± 5.2	125.0 ± 18.6
16	F	4-OMe	2.38	14.4 ± 1.9	133 ± 35	140.9 ± 18.9	160.7 ± 21.7
17	F	4-Br	3.42	39.4 ± 5.0	9.2 ± 1.4	60.5 ± 4.3	47.4 ± 11.1
18	F	4-Me	3.10	3.6 ± 0.4	122 ± 32	140.9 ± 18.9	118.9 ± 13.0
19	F	4-F	2.77	3.4 ± 0.4	212 ± 61	101.9 ± 6.7	121.1 ± 8.0
20	F	3-Cl	3.15	18.4 ± 2.2	149 ± 54	66.2 ± 5.2	59.8 ± 5.2
21	F	3-OMe	2.38	19.4 ± 0.1	83 ± 4.0	87.1 ± 7.4	79.4 ± 11.4
22	F	3-Br	3.42	37.3 ± 7.1	181 ± 64	40.6 ± 2.7	71.6 ± 9.5
23	F	3-F	2.77	>50	69 ± 33	83.0 ± 6.5	168.7 ± 23.4
24	F	2-Br	3.42	33.3 ± 4.9	1.9 ± 0.5	77.1 ± 4.7	79.1 ± 14.3
25	OMe	H	2.24	>50	>1000	>200	>200
26	OMe	4-Cl	2.75	>50	>1000	117.2 ± 15.4	158.5 ± 15.0
27	OMe	4-OMe	1.98	>50	>1000	190.1 ± 27.3	189.7 ± 11.4
28	OMe	4-Br	3.03	>50	>1000	80.5 ± 3.8	150.3 ± 12.2
29	OMe	4-Me	2.70	>50	>1000	146.9 ± 15.9	>200
30	OMe	4-F	2.38	>50	>1000	>200	>200
31	OMe	3-Cl	2.75	>50	>1000	142.6 ± 7.2	>200
32	OMe	3-OMe	1.98	>50	>1000	>200	>200
33	OMe	3-Br	3.03	>50	>1000	57.0 ± 5.7	105.0 ± 9.4
34	OMe	3-F	2.36	37.3 ± 8.2	>1000	>200	>200
35	OMe	2-Br	3.03	>50	>1000	162.9 ± 9.3	111.2 ± 11.7

[a] Calculated with ChemAxon/chemicalize add-in from MarvinSketch 20.4.
[b] from blood plasma.
[c] HEK-293 cells (PerkinElmer).

IC_{50} = 33.3 μM). Furthermore, compounds **18** (R^1 = F, R^2 = 4-CH_3, IC_{50} = 3.6 μM) and **19** (R^1 = F, R^2 = 4-F, IC_{50} = 3.4 μM) exhibited the best AChE inhibitory properties (Fig. 1), with IC_{50} values very similar to that of DPZ (Fig. 3, IC_{50} = 2.17 μM).

Remarkably, the replacement of the substituents at the C-4 of the benzamide moiety (4-Me in **18** or 4-F in **19**) by a hydrogen atom or methoxyl group lead to inactive compounds (**14** and **16**).

For the series **3**–**24** (R^1 = H, F) the highest activities were observed when the substitution occurs in the 4-position of the benzamide ring. Nevertheless, good activities were also present with substituents at the 3-position (compounds **11**, **20**, **21**, **22**, and **34**) or even in the 2-position (compounds **13** and **24**) indicating that indolylpropyl benzamidopiperazines with an appropriate substitution pattern can result in relevant AChE inhibitory activity,

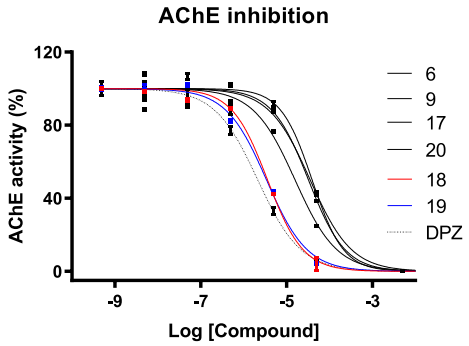


Fig. 1. Inhibition curve of compounds **18** (IC_{50} = 3.6 μM) and **19** (IC_{50} = 3.4 μM) and other selected compounds against AChE from human blood plasma. Each determination was made in triplicate and the data were expressed as the mean ± SD.

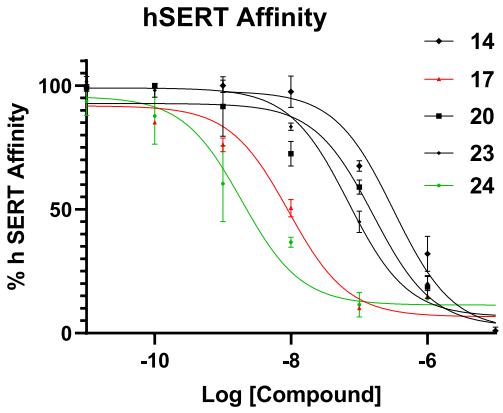


Fig. 2. hSERT affinity curve of compound **14**, **17**, **20**, **23** and **24**, showing IC_{50} in the nanomolar range. Each determination was made in triplicate and the data were expressed as the mean ± SD.

offering a remarkable broad chemical space for further optimization.

To complement these results, cell viability assays were carried out by a standard MTT test in HEK-293 and SH-SY5Y cell lines. Most of compounds displayed a relatively low toxicity (in most cases with IC_{50} values in the medium to high micromolar range), showing in the case of most active compounds (**18** and **19**) a potency difference of more than 30-fold when comparing their AChE inhibitory properties and their ability to produce cell toxicity. Remarkably both compounds are compatible with blood brain barrier (BBB) crossing properties according to calculated LogP values. (Table 1).

2.3. Serotonin transporter affinity

Among the 33 tested molecules, only those carrying a fluorine atom in the 5-position of the indole ring were able to displace [H^3]-paroxetine from hSERT in binding experiments. This finding was previously described by Heinrich et al. [49] and Evrard et al. [66] and corroborated by us in similar systems [57,59], where this small and electron-withdrawing atom in the indolic moiety would be the main responsible for high hSERT affinity. In our case, this condition was mandatory in order to achieve high binding affinities. As shown in Table 1, all 5-fluorinated compounds displayed nanomolar affinity towards this protein. The parent compound **14** carrying no substitution at the benzamide moiety, displayed the second lowest affinity (IC_{50} = 320 nM), while compounds **18** (R^2 = 4-CH_3, IC_{50} = 122 nM), **19** (R^2 = 4-F, IC_{50} = 212 nM), **20** (R^2 = 3-Cl, IC_{50} = 149 nM), **23** (R^2 = 3-F, IC_{50} = 69 nM) and **24** (R^2 = 2-Br) showed affinities in the nanomolar range. Moreover, compounds **17** (R^2 = 4-Br, IC_{50} = 9.2 nM) and **24** (R^2 = 2-Br, IC_{50} = 1.9 nM) showed the highest potency of the series (Fig. 2), with IC_{50} values similar to the widely used antidepressant citalopram (Fig. 3), employed as positive control. In this series, the best affinity was exhibited by compounds containing the bulky bromine substituent on the 4 or 2 position, while the corresponding 3-brominated derivative showed a lower affinity. Interestingly, compounds **17**, **18**, **19** and **24** also displayed a good inhibition profile in AChE. As can be deducted from Table 1, none hSERT active ligand was toxic in the IC_{50} activity range.

2.4. Docking simulations

In order to rationalize the pharmacological results of the designed compounds in hAChE and hSERT, docking studies were carried out and the ligand-protein molecular interactions were analized in the light of their experimental affinities. Before to carry out the docking studies, we investigated which are the protonated

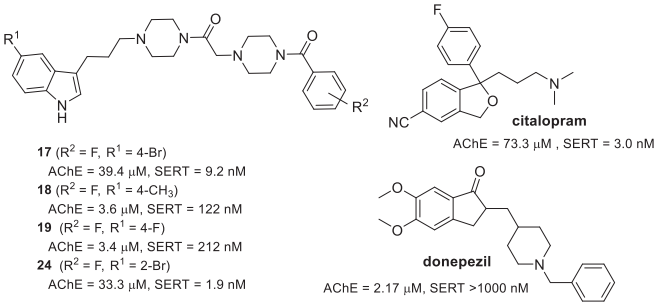


Fig. 3. AChE inhibition and SERT for more active compounds. Citalopram and Donepezil were used as validation.

states of the designed compounds at pH = 7.4 (Supplementary Material). To answer to this question, a theoretical approach, based on quantum chemical calculations, for aqueous pKa calculations was applied, by coupling accurate electronic structure methods for gas-phase energy changes with solvation methods as is explained in detail in the Supplementary Material. The results for the two first deprontation ($pKa_{1(a)}$ = 7.48 vs. $pKa_{1(b)}$ = 8.02) show that the compounds can be found as an equilibrium mixture of di- and mono-protonated species, being the diprotonated state in major ratio (see Fig. S8). Therefore, since at physiological pH the diprotonated species is predicted to be predominant, all docking simulations were performed considering this state.

2.4.1. hAChE

Docking simulations were carried out for the two most active derivatives **18** (IC_{50} = 3.6 μM) and **19** (IC_{50} = 3.4 μM) and other selected compounds (Supplementary Material) in the human AChE structure (PDB 4EY7). Docking poses for compounds **18** and **19** span the length of the enzyme cavity without reaching the deeper catalytic site similar to the described binding mode of DPZ [67,68] (Fig. 4). In both cases, the indole moiety binds at the bottom of the cavity where it can participate in aromatic π-stacking interactions with Trp86 and Tyr337. The protonated propyl piperazine nitrogen is located midway down the gorge near the described enzyme bottleneck formed by Tyr124 and Tyr341 [69] where it can form a hydrogen bond with Tyr124 and a possible π-cation interaction with the aromatic residue Tyr341 [70,71]. On the other side, the second piperazine ring interacts with peripherial anionic site (PAS) residues at the entrance of the active-site gorge [72]. Here the charged benzamide piperazine nitrogen can form a π-cation

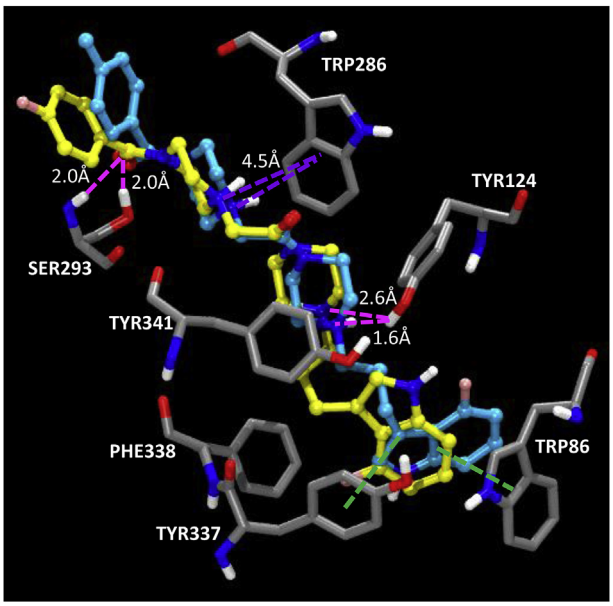


Fig. 4. Overlapped docking poses obtained for the two most active compounds in AchE (PDB 4EY7). Compounds **18** (cyan) and **19** (yellow). Main interactiong residues are shown in gray sticks. Magenta dashed lines represent hydrogen bonds, green dashed lines reoresent aromatic interactions and π-cation interactions are denoted by purple dashed lines. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

interaction with Trp286 while the benzamide moiety extends towards the surface of the enzyme and can form interactions with surface loops residues. Particularly, the benzamide carboyl group can form hydrogen bonds with Ser293 at the entrance of the binding cavity. These stabilizing interactions observed in compounds **18** and **19** were not observed in less active derivatives (Supplementary Material).

2.4.2. hSERT

Docking simulations were carried out for the two most active derivatives **17** (IC_{50} = 9.2 nM) and **24** (IC_{50} = 1.9 nM) and other selected compounds (Supplementary Material) in the human SERT structure (PDB 5I73). The binding modes show that both compounds interact with the binding site adopting a U shape conformation. In the case of compound **24**, an additional H-bond is observed between the carbonyl group of the amide, and the Tyr95 residue, which justifies its higher potency. Compound **17** shows a π-stacking interaction between the benzamide fragment and Trp103 and a halogen bond with Arg104. In the case of compound **24**, the π-stacking occurs between the benzamide fragment and the Tyr176 residue, and a halogen bond with the carbonyl backbone of Tyr95. Both compounds exhibit a π-stacking interaction between the indole ring and Phe335, which allows the stabilization of the bonding of the molecules to the hSERT pocket and facilitates the formation of the H-bond between the indole hydrogen and Glu494. A π-stacking interaction between cationic hydrogen of piperazine and Phe335 is observed for both analogues. A new π-stacking interaction between the piperazine cationic nitrogen of compound **17** and Tyr176 is observed. The most potent compounds (**17** and **24**) contain a bromine atom at positions 4 or 2, respectively, and adopted a common docking pose which favors π-stacking interactions with Trp103 and Tyr176 respectively (Fig. 5). In addition, a hydrogen bond interaction was observed between Arg104 and the bromine atom (Fig. 5). These drug-target interactions stabilize the complex as compared to the less active molecules **3** and **25** (Supplementary Material), and are in agreement with those described in the crystal structure of the hSERT in complex with paroxetine [73]. Noteworthy, compounds **17** and **24** showed the best binding energy values with a salt brigde, an hydrogen bond and a π -stacking interaction stabilizing their binding modes (see ESI, Table S2). On the other hand, compounds **3** and **25** showed poor binding energies and low affinities. Thus, theoretical data are in agreement with the obtained IC_{50} values in the binding experiments.

2.5. Effect of compounds on Abeta levels in cells in culture

N2A mouse neuroblastome cells stably transfected with APP-695 were incubated for 16 h with 10 μM of selected compounds. As shown in Fig. 6 compounds **11** and **25** were able to lower Aβ40 levels by 50% (**11**, ***p < 0.001) and 37% (**25**, **p < 0.01). For **11**, Western blot analysis indicated that the C99 C-terminal fragment of APP was decreased, without change in total APP levels (Supplementary Material) In the conditions of these experiments, toxicity was not observed. Further experiments are needed to determine if the decrease in C99 is the result of decreased beta-cleavage or increased degradation. These results show that in addition to modulating neurotransmitter signaling, these compounds also modulate APP metabolism, given that the inhibition mechanism of **11** and **25** towards Aβ40 levels is still unknown, no correlation can be made between activity of such compound in AChE/SERT and Aβ40.

3. Conclusions

This study demonstrates the possibility to generate a series of hSERT and AChE ligands with promising capabilities as dual-target AD modulators. Among the 33 studied compounds, nine of them display affinities for both targets. The 5-Fluorine substitution at the indole moiety always resulted in hSERT nanomolar affinity and remarkably, these indolic-fluorinated compounds are also between the best structures for AChE inhibition. Compounds **17**, **18**, **19** and **24** exhibited IC_{50} values in the micromolar and nanomolar range for AChE and SERT, respectivel. In this study, none of the best performing molecules on the mentioned targets demonstrated to be active as β-amyloid deposition inhibitors, while two compounds of the whole series presented promissing anti Aβ properties, especially compound **11**. These results suggest that indolylpropyl benzamidopiperazines-based compounds could contribute to the field of multitarget directed ligands for Alzheimer's Disease, although much more efforts are needed to develop novel molecules with multitarget action that may reduce the progression of AD.

4. Experimental part

4.1. General methods

All commercially available reagents were purchased from Merck AG or Aldrich and used without further purification. TLC was carried out on aluminum sheets coated with *Sílica Gel 60 F_{254} Merck* (0.25 mm). Column chromatography was performed on Chromagel (SDS silice 60 AC.C 35–70 μm or 70–200 μm). Melting points were recorded by MP90 digital melting point apparatus (Mettler Toledo, Ohio, USA) and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a *Bruker AVANCE 300*. The chemical shifts (δ) and coupling constants (J) are expressed in parts per million and Hertz, respectively. High Resolution Mass Spectra (HRMS) were obtained on an orthogonal time-of-flight and electrospray ionization source (ESI-ToF) mass spectrometer (QTof Micro, Micromass UK) or on a Thermo Q exactive focus.

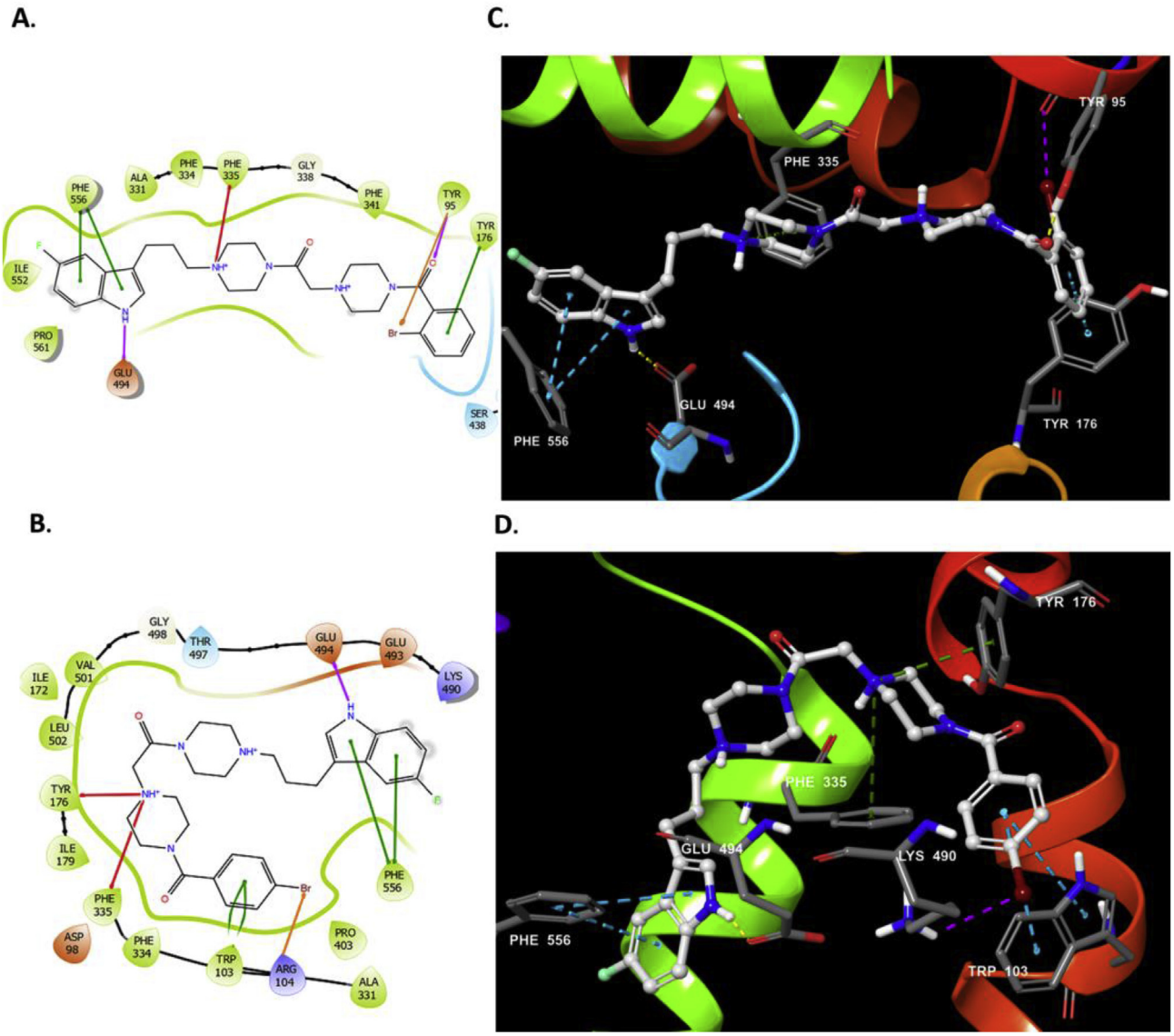


Fig. 5. A, B) Two-dimensional diagrams of the main interactions between the ligand and the residues involved in the recognition of the ligand to the h-SERT binding site (PDB: 5I73) (in A compound **24**, in B compound **17**). Magenta lines represent hydrogen bonds, blue-red lines to salt bridges and green line π-stacking. Amino acids in green indicate hydrophobic residues, in red, negatively charged, in sky-blue polar and in purple positively charged. C) Best pose obtained of compound **24**, and D) Best pose for **17**. The ligand is represented in gray. Segmented lines correspond to each interaction between the ligand and the receptor residues (in yellow hydrogen bonds, in purple salt bridges, in magenta bond with halogen, and in sky-blue π-stacking). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

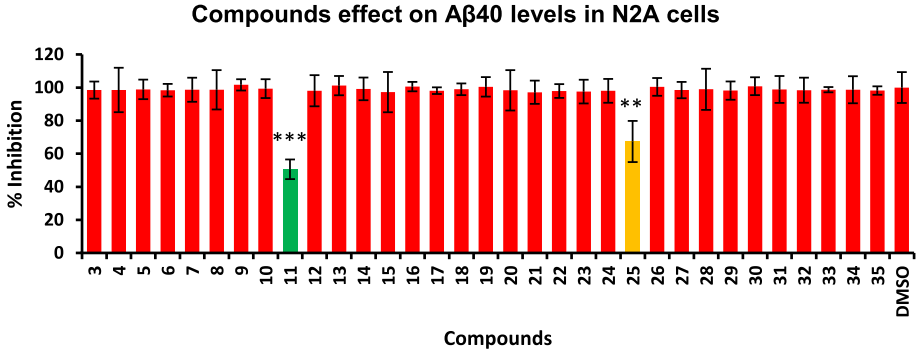


Fig. 6. % of Aβ40 inhibition on N2A cells at 10 μM of all compounds. Results shown as mean ± SD. **p < 0.01, ***p < 0.001, Statistical test: one-way ANOVA and post-hoc Dunnett's multiple comparison test vs untreated group.

4.1.1. General procedures for synthesis of derivatives ***2a-k***

To a solution of the corresponding *N*-piperazinyl benzamide **1a-k** in dry CH_3CN (10 mL/mmol of **1a-k**) was added 4-chloroacetyl-piperazine-1-carboxylic acid tert-butyl ester (1 eq) and K_2CO_3 (3 eq) and the resulting suspension was stirred at reflux temperature (80 °C) under N_2 for 16 h. Solvent was evaporated under reduced pressure and AcOEt (20 mL) and water (20 mL) were added. The organic layer was collected, dried ($MgSO_4$) and concentrated. The residue was purified by column chromatography CH_2Cl_2:MeOH 30:1 → 9:1 yielding the pure product with good to excellent yields (69–91%).

4.1.1.1. Tert-butyl 4-(2-(4-benzoylpiperazin-1-yl)acetyl)piperazine-1-carboxylate ***(2a)***. From 0.55 g (2.9 mmol) of **1a** were obtained 0.85 g (71%) of **2a** as a white solid. Mp.: 128–130 °C; ^{1}H-RMN (300 MHz, $CDCl_3$, 298 K): δ = 7.23 (m, 5 H, Ar), 3.63 (bs, 2 H, CH_2Pip), 3.40 (bs, 4H, CH_2Pip), 3.26 (m, 6 H, CH_2Pip), 3.26 (s, 2 H, COCH_2N), 2.38 (bs, 4 H, CH_2Pip), 1.30 (s, 9 H, C(CH_3)$_3$). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 170.3, 167.2 (*C*ONH), 154.5 ($CO_{carbamate}$), 135.6 (COC_q), 129.7, 128.5 (2C, CHAr), 127.0 (2C, CHAr), 80.3 (*C*(CH$_3$)$_3$), 60.9 (COCH_2N), 53.1 (2C, *C*H$_2$Pip), 45.5 (2C, *C*H$_2$Pip), 42.0 (2C, *C*H$_2$Pip), 41.6 (2C, *C*H$_2$Pip), 28.4 (3C, C(CH_3)$_3$. HRMS: (ESI-TOF) *m*/*z* calcd. for $C_{22}H_{32}N_4O_4$: 416.2424. Found: 416.2434.

4.1.1.2. Tert-butyl 4-(2-(4-(4-chlorobenzoyl)piperazin-1-yl)acetyl) piperazine-1-carboxylate ***(2b)***. From 0.395 g (1.76 mmol) of **1a** were obtained 0.69 g (87%) of **2b** as a white solid. Mp.: 112–113 °C; ^{1}H-RMN (300 MHz, $CDCl_3$, 298 K): δ = 7.37 (m, 4 H, Ar), 3.78 (bs, 2 H, CH_2Pip), 3.67–3.31 (m, 10 H, CH_2Pip), 3.25 (s, 2 H, COCH_2N), 2.54 (bs, 4 H, CH_2Pip), 1.48 (s, 9 H, C(CH_3)$_3$). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 169.2, 167.6 (*C*ONH), 154.6 ($CO_{carbamate}$), 135.9 (C_qCl), 133.9 (COC_q), 128.8 (2C, CHAr), 128.6 (2C, *C*HAr), 80.5 (*C*(CH$_3$)$_3$), 60.7 (COCH_2N), 53.0 (2C, *C*H$_2$Pip), 45.5 (2C, *C*H$_2$Pip), 42.0 (2C, *C*H$_2$Pip), 41.6 (2C, *C*H$_2$Pip), 28.4 (3C, C(CH_3)$_3$. HRMS: (ESI-TOF) *m*/*z* calcd. for $C_{22}H_{31}ClN_4O_4$: 450.2034. Found: 450.2052.

4.1.1.3. Tert-butyl 4-(2-(4-(4-methoxybenzoyl)piperazin-1-yl)acetyl) piperazine-1-carboxylate ***(2c)***. From 0.452 g (2.00 mmol) of **1c** were obtained 0.65 g (71%) of **2c** as a pale brown solid. Mp.: 119–121 °C; ^{1}H-RMN (300 MHz, $CDCl_3$): δ = 7.37 (d, 2 H, $J_{H,H}$ = 7.9 Hz, Ar), 6.91 (d, 2 H, $J_{H,H}$ = 8.5 Hz, Ar), 3.83 (s, 3 H, CH_3), 3.74–3.34 (m, 12 H, CH_2Pip), 3.24 (s, 2 H, COCH_2N), 2.53 (bs, 4 H, CH_2Pip), 1.47 (s, 9 H, C(CH_3)$_3$). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 170.3, 167.6 (*C*ONH), 160.8 (C_qOCH_3), 154.6 ($CO_{carbamate}$), 129.1 (4C, *C*HAr), 127.6 (COC_q), 113.23 (COC_q), 80.4 (*C*(CH$_3$)$_3$), 61.0 (COCH_2N), 55.4 (OCH_3), 53.1 (2C, *C*H$_2$Pip), 45.5 (2C, *C*H$_2$Pip), 43.5 (2C, *C*H$_2$Pip), 41.6 (2C, *C*H$_2$Pip), 28.4 (3C, C(CH_3)$_3$). HRMS: (ESI-TOF) *m*/*z* calcd. for $C_{23}H_{34}N_4O_5$: 446.2529. Found: 446.2518.

4.1.1.4. Tert-butyl 4-(2-(4-(4-bromobenzoyl)piperazin-1-yl)acetyl) piperazine-1-carboxylate ***(2d)***. From 1.75 g (6.50 mmol) of **1d** were obtained 2.42 g (75%) of **2d** as a white solid. Mp.: 148–150 °C; ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 7.54 (d, 2 H, $J_{H,H}$ = 8.5 Hz, Ar), 7.28 (d, 2 H, $J_{H,H}$ = 8.5 Hz, Ar), 3.78 (bs, 4 H, CH_2Pip), 3.56 (m, 4 H, CH_2Pip), 3.46 (m, 4 H, CH_2Pip), 3.24 (m, 2 H, COCH_2N), 2.54 (bs, 4 H, CH_2Pip), 1.47 (s, 9 H, C(CH_3)$_3$). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 169.3, 167.6 (*C*ONH), 154.5 ($CO_{carbamate}$), 134.4 (COC_q), 131.7 (2C, *C*HAr), 128.8 (2C, *C*HAr), 124.1 (C_qBr), 80.5 (*C*(CH$_3$)$_3$), 60.8 (COCH_2N), 53.1 (2C, *C*H$_2$Pip), 47.5 (2C, *C*H$_2$Pip), 45.4 (2C, *C*H$_2$Pip), 41.6 (2C, *C*H$_2$Pip), 28.3 (3C, C(CH_3)$_3$). HRMS: (ESI-TOF) *m*/*z* calcd. for $C_{22}H_{31}BrN_4O_4$: 494.1529. Found: 494.1538.

4.1.1.5. Tert-butyl 4-(2-(4-(4-methylbenzoyl)piperazin-1-yl)acetyl) piperazine-1-carboxylate ***(2e)***. From 0.48 g (2.35 mmol) of **1e** were obtained 0.79 g (78%) of **2e** as a white solid. Mp.: 102–104 °C; ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 7.30 (d, 2 H, $J_{H,H}$ = 8.3 Hz, Ar), 7.22 (d, 2 H, $J_{H,H}$ = 8.3 Hz, Ar), 3.78 (bs, 4 H, CH_2Pip), 3.46 (m, 4 H, CH_2Pip), 3.52 (m, 4 H, CH_2Pip), 3.24 (m, 2 H, COCH_2N), 2.53 (bs, 4 H, CH_2Pip), 2.38 (s, 3 H, CH_3), 1.47 (s, 9 H, C(CH_3)$_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.5, 167.8 (*C*ONH), 154.6 ($CO_{carbamate}$), 139.9 (CH_3C_q), 132.2 (COC_q), 129.1 (2C, *C*HAr), 127.2 (2C, *C*HAr), 80.3 (*C*(CH$_3$)$_3$), 61.0 (COCH_2N), 53.2 (2C, *C*H$_2$Pip), 45.5 (2C, *C*H$_2$Pip), 43.9 (2C, *C*H$_2$Pip), 41.7 (2C, *C*H$_2$Pip), 28.3 (3C, C(CH_3)$_3$) 21.4 (CH_3). HRMS: (ESI-TOF) *m*/*z* calcd. for $C_{23}H_{34}N_4O_4$: 430.2528. Found: 430.2507.

4.1.1.6. Tert-butyl 4-(2-(4-(4-fluorobenzoyl)piperazin-1-yl)acetyl) piperazine-1-carboxylate ***(2f)***. From 1.00 g (4.80 mmol) of compound **1f** were obtained 1.81 g (87%) of compound **2f** as a white solid. Mp.: 128–130 °C; ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.38 (m, 2 H, Ar), 7.07 (t, 2 H, $J_{H,H}$ = 8.7 Hz, Ar), 3.74 (bs, 4 H, CH_2Pip), 3.54 (m, 4 H, CH_2Pip), 3.40 (m, 4 H, CH_2Pip), 3.22 (m, 2 H, COCH_2N), 2.53 (bd, 4 H, CH_2Pip), 1.45 (s, 9 H, C(CH_3)$_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.4, 167.5 (*C*ONH), 165.1 (C_qF), 154.5 ($CO_{carbamate}$), 131.6, 131.5, 129.3, 115.7, 115.4, (*C*HAr), 80.4 (*C*(CH$_3$)$_3$), 60.8 (COCH_2N), 55.4 (2C, *C*H$_2$Pip), 45.4 (2C, *C*H$_2$Pip), 43.7 (2C, *C*H$_2$Pip), 41.6 (2C, *C*H$_2$Pip), 28.4 (3C, C(CH_3)$_3$). HRMS: (ESI-TOF) *m*/*z* calcd. for $C_{22}H_{31}FN_4O_4$: 434.2329. Found: 434.2337.

4.1.1.7. Tert-butyl 4-(2-(4-(3-chlorobenzoyl)piperazin-1-yl)acetyl) piperazine-1-carboxylate ***(2g)***. From 2.08 g (9.28 mmol) of compound **1g** were obtained 3.21 g (77%) of compound **2g** as a pale brown solid. Mp.: 84–86 °C; ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 7.40 (m, 2 H, CHAr), 7.35 (t, 1 H, $J_{H,H}$ = 7.7 Hz, CHAr), 7.72 (bs, 1 H, CHAr), 4.06–3.66 (bs, 4 H, CH_2Pip), 3.84 (s, 2 H, COCH_2N), 3.54 (m, 2 H, CH_2Pip), 3.48–3.31 (m, 6 H, CH_2Pip), 3.25 (Bs, 4 H, CH_2Pip), 1.46 (s, 9 H, C(CH_3)$_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 168.8, 163.4 (*C*ONH), 154.4 ($CO_{carbamate}$), 136.1 (COC_q), 134.9 (CqCl), 130.6, 130.2, 127.3, 125.1 (*C*HAr), 80.6 (*C*(CH$_3$)$_3$), 56.6 (COCH_2N), 51.9 (2C, *C*H$_2$Pip), 45.0 (2C, *C*H$_2$Pip), 43.5 (2C, *C*H$_2$Pip), 41.7 (2C, *C*H$_2$Pip), 28.3 (3C, C(CH_3)$_3$). HRMS: (ESI-TOF) *m*/*z* calcd. for $C_{22}H_{31}ClN_4O_4$: 450.2034. Found: 450.2041.

4.1.1.8. Tert-butyl 4-(2-(4-(3-methoxybenzoyl)piperazin-1-yl)acetyl) piperazine-1-carboxylate ***(2h)***. From 0.70 g (3.17 mmol) of compound **1h** were obtained 1.21 g (85%) of compound **2h** as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 7.25 (t, 1 H, $J_{H,H}$ = 7.27 Hz, Ar), 7.89 (m, 3 H, Ar), 3.08 (s, 3 H, OCH_3), 3.77 (bs, 4 H, CH_2Pip), 3.55 (m, 4 H, CH_2Pip), 3.41 (m, 4 H, CH_2Pip), 3.22 (s, 2 H, COCH_2N), 2.55 (bd, 4 H, CH_2Pip), 1.47 (s, 9 H, C(CH_3)$_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.0, 167.7 (*C*ONH), 159.7 (C_qOCH_3), 154.5 ($CO_{carbamate}$), 136.9 (COC_q), 129.5, 119.5, 115.6, 112.3 (*C*HAr), 80.3 (*C*(CH$_3$)$_3$), 60.9 (COCH_2N), 55.4 (CH_3), 53.1 (2C, *C*H$_2$Pip), 45.4 (2C, *C*H$_2$Pip), 43.6 (2C, *C*H$_2$Pip), 41.6 (2C, *C*H$_2$Pip), 28.4 (3C, C(CH_3)$_3$). HRMS: (ESI-TOF) *m*/*z* calcd. for $C_{23}H_{34}N_4O_5$: 446.2529. Found: 446.2535.

4.1.1.9. Tert-butyl 4-(2-(4-(3-bromobenzoyl)piperazin-1-yl)acetyl) piperazine-1-carboxylate ***(2i)***. From 0.52 g (1.93 mmol) of compound **1i** were obtained 0.87 g (91%) of compound **2i** as a pale yellow solid. Mp.: 128–129 °C; ^{1}H-RMN (300 MHz, $CDCl_3$, 298 K): δ = 7.46 (m, 2 H, CHAr), 7.26–7.16 (m, 2 H, CHAr), 3.71 (bs, 2 H, CH_2Pip), 3.47 (m, 4 H, CH_2Pip), 3.35 (m, 4 H, CH_2Pip), 3.16 (s, 4 H, COCH_2N), 2.47 (bs, 4 H, CH_2Pip), 1.39 (s, 9 H, C(CH_3)$_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 168.5, 167.5 (*C*ONH), 154.5 ($CO_{carbamate}$), 137.5 (COCq), 132.8, 130.1, 130.0, 125.5, (*C*HAr), 122.6 (C_qBr), 80.4 (*C*(CH$_3$)$_3$), 60.8 (COCH_2N), 53.6 (2C, *C*H$_2$Pip), 45.4 (2C, *C*H$_2$Pip), 43.7 (2C, *C*H$_2$Pip), 41.6 (2C, *C*H$_2$Pip), 28.4 (3C, C(CH_3)$_3$). HRMS: (ESI-TOF) *m*/*z* calcd. for $C_{22}H_{31}BrN_4O_4$: 494.1529. Found: 494.1535.

*4.1.1.10. Tert-butyl 4-(2-(4-(3-fluorobenzoyl)piperazin-1-yl)acetyl) piperazine-1-carboxylate **(2j)**.* From 0.19 g (0.91 mmol) of compound **1j** were obtained 0.34 g (83%) of compound **2j**. as a white solid. Mp.: 116–118 °C ^{1}H-RMN (300 MHz, $CDCl_3$, 298 K): δ = 7.36 (m, 1 H, CHAr), 7.15 (dt, 1 H, $J_{H,H}$ = 1.09 Hz, $J_{H,H}$ = 7.6 Hz, CHAr), 7.12–7.06 (m, 2 H, CHAr), 3.76 (bs, 2 H, CH_2Pip), 3.54 (m, 4 H, CH_2Pip), 3.42 (m, 6 H, CH_2Pip), 3.22 (s, 2 H, COCH_2N), 2.55 (m, 4 H, CH_2Pip), 1.45 (s, 9 H, C(CH_3)$_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 168.8 (d, $^4J_{C,F}$ = 2.1 Hz, *C*ON), 167.5 (*C*ON), 162.6 (d, $^1J_{C,F}$ = 249 Hz, CqF), 154.5 ($CO_{carbamate}$), 137.6 (d, $^3J_{C,F}$ = 6.5 Hz, COCq), 130.3 (d, $^3J_{C,F}$ = 7.5 Hz, *C*HAr), 122.7 (d, $^4J_{C,F}$ = 2.1 Hz, *C*HAr), 116.8 (d, $^2J_{C,F}$ = 21.3 Hz, *C*HAr), 114.3 (d, $^2J_{C,F}$ = 22.4 Hz, *C*HAr), 80.4 (*C*(CH$_3$)$_3$), 60.8 (COCH_2N), 53.0 (2C, *C*H_2Pip), 45.4 (2C, *C*H_2Pip), 43.3 (2C, *C*H_2Pip), 41.6 (2C, *C*H_2Pip), 28.4 (3C, C(CH_3)$_3$). HRMS: (ESI-TOF) *m*/*z* calcd. for $C_{22}H_{31}FN_4O_4$: 434.2329. Found: 434.2333.

*4.1.1.11. Tert-butyl 4-(2-(4-(2-bromobenzoyl)piperazin-1-yl)acetyl) piperazine-1-carboxylate **(2k)**.* From 1.02 g (3.79 mmol) of compound **1k** were obtained 1.41 g (75%) of compound **2k** as a white solid. Mp.: 115–116 °C. ^{1}H-RMN (300 MHz, $CDCl_3$, 298 K): δ = 7.58 (d, 1 H, $J_{H,H}$ = 8.2 Hz, CHAr), 7.33 (t, 1 H, $J_{H,H}$ = 7.5 Hz, CHAr), 7.23 (t, 2 H, $J_{H,H}$ = 7.5 Hz, CHAr), 3.82 (m, 2 H, CH_2Pip), 3.55 (m, 4 H, CH_2Pip), 3.40 (m, 6 H, CH_2Pip), 3.21 (s, 2 H, COCH_2N), 2.59 (m, 4 H, CH_2Pip), 1.45 (s, 9 H, C(CH_3)$_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 167.6 (*C*ONH), 154.5 ($CO_{carbamate}$), 137.5 (COCq), 132.8, 130.3, 127.7 (*C*HAr), 119.1 (C_qBr), 80.4 (*C*(CH$_3$)$_3$), 60.9 (COCH_2N), 53.0, 52.7, 46.6, 45.5, 43.5, 43.3, 41.6, 41.4 (*C*H_2Pip), 28.4 (C(CH_3)$_3$). HRMS: (ESI-TOF) *m*/*z* calcd. for $C_{22}H_{31}BrN_4O_4$: 494.1529. Found: 494.1537.

*4.1.2. General procedures for synthesis of derivatives **3**–**35***

To a solution of **2a-k** in dichlorometane (5 mL/mmol of **2a-k**) was added trifluoroacetic acid in 1:1 ratio. The solution was stirred at rt for 2 h. After reaction completion upon TLC, solvents were evaporated by an air flow for 1 h, until neutral pH of the steam. After that, dry MeCN (20 mL/mmol of starting material **2a-k**) and K_2CO_3 (6 Eq) were added, followed by addition of the appropriate tosylate **A, B** or **C**. The suspension was refluxed (80 °C) under N_2 and stirred for 16 h. Solvent was evaporated, EtOAc (20 mL) and water (2 × 20 mL) were added, and the organic layer was dried ($MgSO_4$) and concentrated. The residue was purified by column chromatography CH_2Cl_2:MeOH 30:1 → 9:1 yielding the pure product with good to moderate to good yields (58–79%).

*4.1.2.1. 1-(4-(3-(1H-indol-3-yl)propyl)piperazin-1-yl)-2-(4-benzoylpiperazin-1-yl)ethan-1-one **(3)**.* From 0.16 g (0.38 mmol) of compound **2a** and tosylate **A** were obtained 0.15 g (83%) of compound **3** as a white solid. Mp.: 69–71 °C.; ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.04 (s, 1 H, NH$_{Ind}$), 7.59 (d, 1 H, $J_{H,H}$ = 8.0 Hz, Ar$_{Ind}$), 7.39 (m, 5 H, C_6H_5), 7.33 (t, 1 H, $J_{H,H}$ = 7.3 Hz, Ar$_{Ind}$), 7.17 (t, 1 H, $J_{H,H}$ = 7.4 Hz, Ar$_{Ind}$) 7.09 (t, 1 H, $J_{H,H}$ = 7.4 Hz, Ar$_{Ind}$), 6.95 (bs, 1 H, Ar$_{Ind}$), 3.80 (bs, 2 H, CH_2Pip), 3.60 (dt, 4 H, $J_{H,H}$ = 4.0, $J_{H,H}$ = 16.8 Hz, CH_2Pip), 3.44 (bs, 2 H, CH_2Pip), 3.22 (s, 2 H, COCH_2N), 2.79 (t, 2 H, $J_{H,H}$ = 7.3 Hz, ArCH$_2$CH$_2$CH_2N), 2.53 (m, 8 H, CH_2Pip), 2.42 (m, 2 H, ArCH_2CH$_2$CH$_2$N), 1.91 (q, 2 H, $J_{H,H}$ = 7.7 Hz, CH$_2$CH_2CH$_2$). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.4 (N*C*OAr), 167.4 (N*C*OCH$_2$), 136.4 (C_qNH), 135.7 ($C_q$benz), 129.7 (2C, *C*Hbenz), 128.5 (2C, *C*Hbenz), 127.0 (*C*Hbenz), 127.4 (C_{qInd}) 121.8, 121.2, 119.0, 118.0, 116.0 (CHAr$_{Ind}$), 111.1 (C_{qInd} CH$_2$CH$_2$CH$_2$), 60.7 (COCH$_2$N), 58.1 (CH$_2$CH$_2$*C*H$_2$N), 53.6 (2C, *C*H$_2$Pip), 53.0 (2C, *C*H$_2$Pip), 45.5 (2C, *C*H$_2$Pip), 41.8 (2C, *C*H$_2$Pip), 27.2 (ArCH$_2$*C*H$_2$CH$_2$N), 22.8 (Ar*C*H$_2$CH$_2$CH$_2$N). HRMS: (ESI-TOF) *m*/*z* calcd. for $C_{28}H_{35}N_5O_2$: 473.2791. Found: 473.2799.

*4.1.2.2. 1-(4-(3-(1H-indol-3-yl)propyl)piperazin-1-yl)-2-(4-(4-chlorobenzoyl)piperazin-1-yl)ethan-1-one **(4)**.* From 0.20 g (0.44 mmol) of compound **2b** and tosylate **A** were obtained 0.162 g (72%) of compound **4** as a white solid. Mp.: 118–122 °C.; ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.10 (s, 1 H, NH$_{Ind}$), 7.59 (d, 1 H, $J_{H,H}$ = 7.9 Hz, Ar$_{Ind}$), 7.42–7.31 (m, 5 H, C_6H_4, Ar$_{Ind}$), 7.18 (td, 1 H, $J_{H,H}$ = 1.13 Hz, $J_{H,H}$ = 7.3 Hz, Ar$_{Ind}$), 7.10 (td, 1 H, $J_{H,H}$ = 1.13 Hz, $J_{H,H}$ = 7.3 Hz, Ar$_{Ind}$), 6.97 (d, 1 H, $J_{H,H}$ = 1.5 Hz, Ar$_{Ind}$), 3.79 (bs, 2 H, CH_2Pip), 3.60 (m, 4 H, CH_2Pip), 3.44 (bs, 2 H, CH_2Pip), 3.22 (s, 2 H, COCH_2N), 2.79 (t, 2 H, $J_{H,H}$ = 7.3 Hz, ArCH$_2$CH$_2$CH_2N), 2.54 (m, 8 H, CH_2Pip), 2.44 (m, 2 H, ArCH_2CH$_2$CH$_2$N), 1.92 (q, 2 H, $J_{H,H}$ = 7.5 Hz, CH$_2$CH_2CH$_2$). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 169.3 (N*C*OAr), 167.3 (N*C*OCH$_2$), 136.3 (C_qNH), 135.9 (C_qCl), 133.9 (COC$_q$), 128.8 (2C, *C*Hbenz), 128.6 (2C, *C*Hbenz), 127.0 (CH$_{qInd}$), 121.9, 121.2, 119.1, 118.8, 116.0 (CHAr$_{Ind}$), 111.1 (C_{qInd}CH$_2$CH$_2$CH$_2$), 60.6 (COCH$_2$N), 58.1 (CH$_2$CH$_2$*C*H$_2$N), 53.5 (2C, *C*H$_2$Pip), 53.0 (2C, *C*H$_2$Pip), 45.5 (2C, *C*H$_2$Pip), 41.8 (2C, *C*H$_2$Pip), 27.2 (ArCH$_2$*C*H$_2$CH$_2$N), 22.8 (ArCH$_2$CH$_2$CH$_2$N). HRMS: (ESI-TOF) *m*/*z* calcd. for $C_{28}H_{34}ClN_5O_2$: 507.2401. Found: 507.2404.

*4.1.2.3. 1-(4-(3-(1H-indol-3-yl)propyl)piperazin-1-yl)-2-(4-(4-methoxybenzoyl)piperazin-1-yl)ethan-1-one **(5)**.* From 0.14 g (0.31 mmol) of compound **2c** and tosylate **A** were obtained 0.137 g (88%) of compound **5** as a white solid. Mp.: 108–109 °C.; ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.22 (s, 1 H, NH$_{Ind}$), 7.60 (d, 1 H, $J_{H,H}$ = 8.0 Hz, Ar$_{Ind}$), 7.37 (d, 2 H, $J_{H,H}$ = 8.7 Hz, Ar$_{benz}$), 7.32 (s, 1 H, Ar$_{Ind}$), 7.17 (td, 1 H, $J_{H,H}$ = 1.0 Hz, $J_{H,H}$ = 7.4 Hz, Ar$_{Ind}$), 7.10 (td, 1 H, $J_{H,H}$ = 1.0 Hz, $J_{H,H}$ = 7.3 Hz, Ar$_{Ind}$), 6.97 (d, 1 H, $J_{H,H}$ = 2 Hz, Ar$_{Ind}$), 6.90 (d, 2 H, $J_{H,H}$ = 8.7 Hz, Ar$_{benz}$), 3.82 (s, 3 H, OCH$_3$), 3.62 (m, 6 H, CH_2Pip), 3.22 (s, 2 H, COCH_2N), 2.79 (t, 2 H, $J_{H,H}$ = 7.5 Hz, ArCH$_2$CH$_2$CH_2N), 2.53 (bs, 4 H, CH_2Pip), 2.43 (m, 6 H, CH_2Pip, ArCH_2CH$_2$CH$_2$N), 1.91 (q, 2 H, $J_{H,H}$ = 7.5 Hz, CH$_2$CH_2CH$_2$). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 170.4 (N*C*OAr), 167.4 (N*C*OCH$_2$), 160.8 (C_qOCH$_3$), 136.3 (C_qNH), 129.2 (2C, *C*HAr$_{benz}$), 127.6 (CH$_{qInd}$), 127.4 (COC$_q$), 121.9, 121.2, 119.0, 118.8, 116.0 (CHAr$_{Ind}$), 113.7 (2C, *C*HAr$_{benz}$), 113.7111.1 (C_{qInd}CH$_2$CH$_2$CH$_2$), 60.8 (COCH$_2$N), 58.2 (CH$_2$CH$_2$*C*H$_2$N), 55.4 (OCH$_3$), 53.6 (2C, *C*H$_2$Pip), 53.1 (2C, *C*H$_2$Pip), 45.5 (2C, *C*H$_2$Pip), 41.8 (2C, *C*H$_2$Pip), 27.2 (ArCH$_2$*C*H$_2$CH$_2$N), 22.9 (ArCH$_2$CH$_2$CH$_2$N). HRMS: (ESI-TOF) *m*/*z* calcd. for $C_{29}H_{37}N_5O_3$: 503.2896. Found: 503.2890.

*4.1.2.4. 1-(4-(3-(1H-indol-3-yl)propyl)piperazin-1-yl)-2-(4-(4-bromobenzoyl)piperazin-1-yl)ethan-1-one **(6)**.* From 0.20 g (0.40 mmol) of compound **2d** and tosylate **A** were obtained 0.19 g (86%) of compound **6** as a pale brown solid. Mp.: 185–187 °C.; ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 8.30 (s, 1 H, NH$_{Ind}$), 7.59 (d, 1 H, $J_{H,H}$ = 7.5 Hz, Ar$_{Ind}$), 7.53 (d, 2 H, $J_{H,H}$ = 8.4 Hz, Ar$_{benz}$), 7.33 (s, 1 H, Ar$_{Ind}$), 7.27 (d, 2 H, $J_{H,H}$ = 8.4 Hz, Ar$_{Ind}$), 7.17 (dt, 1 H, $J_{H,H}$ = 1.0 Hz, $J_{H,H}$ = 7.0 Hz, Ar$_{Ind}$), 7.09 (dt, 1 H, $J_{H,H}$ = 1.0 Hz, $J_{H,H}$ = 7.0 Hz, Ar$_{Ind}$), 6.95 (bs, 1 H, Ar$_{Ind}$), 3.71 (bs, 2 H, CH_2Pip), 3.63 (m, 2 H, CH_2Pip), 3.57 (m, 2 H, CH_2Pip), 3.43 (bs, 2 H, CH_2Pip), 3.22 (s, 2 H, COCH_2N), 2.79 (t, 2 H, $J_{H,H}$ = 7.4 Hz, ArCH$_2$CH$_2$CH_2N), 2.53 (bs, 4 H, CH_2Pip), 2.43 (m, 6 H, CH_2Pip, ArCH_2CH$_2$CH$_2$N), 1.91 (q, 2 H, $J_{H,H}$ = 7.4 Hz, CH$_2$CH_2CH$_2$). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 169.3 (N*C*OAr), 167.3 (N*C*OCH$_2$), 136.4 (C_qNH), 134.4 (COC$_q$), 131.8 (2C, *C*HAr$_{benz}$), 128.8 (2C, *C*HAr$_{benz}$), 127.4 (C_{qInd}), 124.1 (C_qBr), 121.8, 121.3, 119.1, 118.8, 115.9 (CHAr$_{Ind}$), 111.1 (C_{qInd}CH$_2$CH$_2$CH$_2$), 60.5 (COCH$_2$N), 57.3 (CH$_2$CH$_2$*C*H$_2$N), 53.6 (2C, *C*H$_2$Pip), 53.0 (2C, *C*H$_2$Pip), 45.5 (2C, *C*H$_2$Pip), 41.7 (2C, *C*H$_2$Pip), 29.7 (ArCH$_2$*C*H$_2$CH$_2$N), 22.8 (ArCH$_2$CH$_2$CH$_2$N). HRMS: (ESI-TOF) *m*/*z* calcd. for $C_{28}H_{34}BrN_5O_2$: 551.1896. Found: 551.1906.

*4.1.2.5. 1-(4-(3-(1H-indol-3-yl)propyl)piperazin-1-yl)-2-(4-(4-methylbenzoyl)piperazin-1-yl)ethan-1-one **(7)**.* From 0.13 g (0.30 mmol) of compound **2e** and tosylate **A** were obtained 0.116 g (77%) of compound **7** as a pale yellow solid. Mp.: 88–90 °C.; ^{1}H NMR (300 MHz, CD_3OD, 298 K): δ = 7.56 (m, 1 H, Ar$_{Ind}$), 7.39–7.01

(m, 8 H, Ar*Ind*, Ar*benz*, NH*Indol*), 6.98 (m, 1 H, $J_{H,H}$ = 8.7 Hz, Ar*Ind*), 3.74 (bs, 2 H, C*H*$_2$Pip), 3.59 (m, 2 H, C*H*$_2$Pip), 3.57 (m, 2 H, C*H*$_2$Pip), 3.41 (m, 4 H, C*H*$_2$Pip), 3.21 (bs, 2 H, COC*H*$_2$N), 2.78 (m, 2 H, ArCH$_2$CH$_2$C*H*$_2$N), 2.65−2.39 (m, 10 H, C*H*$_2$Pip, ArC*H*$_2$CH$_2$CH$_2$N, CH$_3$), 1.92 (m, 2 H, CH$_2$C*H*$_2$CH$_2$). ^{13}C NMR (75 MHz, CD$_3$OD, 298 K): δ = 171.0 (NCOAr), 167.7 (NCOCH$_2$), 140.2 (C$_q$CH$_3$), 134.4 (C$_q$NH), 132.1 (COC$_q$), 129.1 (2C, CHAr*benz*), 127.0 (2C, C*H*Ar*benz*), 127.4 (C$_{qInd}$), 121.5, 121.4, 118.7, 118.5, 115.1 (CHAr$_{Ind}$), 111.1 (C$_{qInd}$CH$_2$CH$_2$CH$_2$), 60.4 (COCH$_2$N), 58.1 (CH$_2$CH$_2$C*H*$_2$N), 53.4 (2C, C*H*$_2$Pip), 53.3 (2C, C*H*$_2$Pip), 45.5 (2C, C*H*$_2$Pip), 41.5 (2C, C*H*$_2$Pip), 26.8 (ArCH$_2$C*H*$_2$CH$_2$N), 22.1 (ArCH$_2$CH$_2$CH$_2$N), 21.2 (CH$_3$). HRMS: (ESI-TOF) *m*/*z* calcd. for C$_{29}$H$_{37}$N$_5$O$_2$: 487.2947. Found: 487.2950.

4.1.2.6. 1-(4-(3-(1H-indol-3-yl)propyl)piperazin-1-yl)-2-(4-(4-fluorobenzoyl)piperazin-1-yl)ethan-1-one ***(8)***. From 0.20 g (0.46 mmol) of compound **2f** and tosylate **A** were obtained 0.149 g (64%) of compound **8** as a pale yellow solid. Mp.: 82−83 °C.; ^{1}H NMR (300 MHz, CDCl$_3$, 298 K): δ = 8.11 (s, 1 H, NH*Ind*), 7.59 (d, 1 H, $J_{H,H}$ = 7.6 Hz, Ar*Ind*), 7.44−7.31 (m, 3 H, Ar*benz*, Ar*Ind*), 7.18 (dt, 1 H, $J_{H,H}$ = 1.0 Hz, $J_{H,H}$ = 7.1 Hz, Ar*Ind*), 7.13−7.04 (m, 3 H, Ar*benz*, Ar*Ind*), 6.97 (bs, 1 H, Ar*Ind*), 3.75 (bs, 2 H, C*H*$_2$Pip), 3.60 (m, 4 H, C*H*$_2$Pip), 3.47 (m, 2 H, C*H*$_2$Pip), 3.22 (s, 2 H, COC*H*$_2$N), 2.79 (t, 2 H, $J_{H,H}$ = 7.4 Hz, ArCH$_2$CH$_2$C*H*$_2$N), 2.54 (bs, 4 H, C*H*$_2$Pip), 2.43 (m, 6 H, C*H*$_2$Pip, ArC*H*$_2$CH$_2$CH$_2$N), 1.91 (q, 2 H, $J_{H,H}$ = 7.4 Hz, CH$_2$C*H*$_2$CH$_2$). ^{13}C NMR (75 MHz, CDCl$_3$, 298 K): δ = 169.5 (NCOAr), 167.3 (NCOCH$_2$), 165.1 (C$_q$F), 136.3 (C$_q$NH), 131.6 (COC$_q$), 129.4 (2C, C*H*Ar*benz*), 127.4 (C$_{qInd}$), 121.9, 121.2, 119.1, 118.8, 116.0 (CHAr$_{Ind}$), 115.6 (2C, CHAr*benz*), 111.1 (C$_{qInd}$CH$_2$CH$_2$CH$_2$), 60.5 (COCH$_2$N), 57.3 (CH$_2$CH$_2$C*H*$_2$N), 53.5 (2C, C*H*$_2$Pip), 53.0 (2C, C*H*$_2$Pip), 45.5 (2C, C*H*$_2$Pip), 41.8 (2C, C*H*$_2$Pip), 27.2 (ArCH$_2$C*H*$_2$CH$_2$N), 22.9 (ArC*H*$_2$CH$_2$CH$_2$N). HRMS: (ESI-TOF) *m*/*z* calcd. for C$_{28}$H$_{34}$FN$_5$O$_2$: 491.2697. Found: 491.2690.

4.1.2.7. 1-(4-(3-(1H-indol-3-yl)propyl)piperazin-1-yl)-2-(4-(3-chlorobenzoyl)piperazin-1-yl)ethan-1-one ***(9)***. From 0.20 g (0.44 mmol) of compound **2g** and tosylate **A** were obtained 0.164 g (73%) of compound **9** as a white solid. Mp.: 148−150 °C.; ^{1}H NMR (300 MHz, CDCl$_3$, 298 K): δ = 8.14 (s, 1 H, NH*Ind*), 7.59 (d, 1 H, $J_{H,H}$ = 7.6 Hz, Ar*Ind*), 7.42−7.29 (m, 4 H, Ar*benz*, Ar*Ind*), 7.09 (dt, 1 H, $J_{H,H}$ = 1.0 Hz, $J_{H,H}$ = 7.0 Hz, Ar*Ind*), 7.18 (dt, 1 H, $J_{H,H}$ = 1.0 Hz, $J_{H,H}$ = 7.1 Hz, Ar*Ind*), 7.10 (dt, 1 H, $J_{H,H}$ = 1.0 Hz, $J_{H,H}$ = 7.1 Hz, Ar*Ind*), 6.96 (bs, 1 H, Ar*Ind*), 3.76 (bs, 2 H, C*H*$_2$Pip), 3.61 (m, 4 H, C*H*$_2$Pip), 3.47 (m, 2 H, C*H*$_2$Pip), 3.22 (s, 2 H, COC*H*$_2$N), 2.79 (t, 2 H, $J_{H,H}$ = 7.6 Hz, ArCH$_2$CH$_2$C*H*$_2$N), 2.54 (bs, 4 H, C*H*$_2$Pip), 2.43 (m, 6 H, C*H*$_2$Pip, ArC*H*$_2$CH$_2$CH$_2$N), 1.91 (q, 2 H, $J_{H,H}$ = 7.6 Hz, CH$_2$C*H*$_2$CH$_2$). ^{13}C NMR (75 MHz, CDCl$_3$, 298 K): δ = 168.7 (NCOAr), 167.2 (NCOCH$_2$), 137.4 (COC$_q$), 136.4 (C$_q$NH), 134.6 (C$_q$Cl), 129.4 (2C, C*H*Ar*benz*), 127.5 (C$_{qInd}$), 127.2 (C*H*Ar*benz*), 125.1 (C*H*Ar*benz*), 121.9, 121.2, 119.1, 118.8, 116.0 (CHAr$_{Ind}$), 111.1 (C$_{qInd}$CH$_2$CH$_2$CH$_2$), 60.6 (COCH$_2$N), 58.1 (CH$_2$CH$_2$CH$_2$N), 53.5 (2C, CH$_2$Pip), 53.0 (2C, CH$_2$Pip), 45.5 (2C, CH$_2$Pip), 41.8 (2C, C*H*$_2$Pip), 27.2 (ArCH$_2$C*H*$_2$CH$_2$N), 22.8 (ArC*H*$_2$CH$_2$CH$_2$N). HRMS: (ESI-TOF) *m*/*z* calcd. for C$_{28}$H$_{34}$ClN$_5$O$_2$: 507.2401. Found: 507.2411.

4.1.2.8. 1-(4-(3-(1H-indol-3-yl)propyl)piperazin-1-yl)-2-(4-(3-methoxybenzoyl)piperazin-1-yl)ethan-1-one ***(10)***. From 0.18 g (0.40 mmol) of compound **2h** and tosylate **A** were obtained 0.149 g (75%) of compound **10** as a pale brown oil. ^{1}H NMR (300 MHz, CDCl$_3$, 298 K): δ = 8.14 (s, 1 H, NH*Ind*), 7.59 (d, 1 H, $J_{H,H}$ = 7.7 Hz, Ar*Ind*), 7.36−7.26 (m, 2 H, Ar*benz*), 7.17 (dt, 1 H, $J_{H,H}$ = 1.0 Hz, $J_{H,H}$ = 7.5 Hz, Ar*Ind*), 7.09 (dt, 1 H, $J_{H,H}$ = 1.0 Hz, $J_{H,H}$ = 7.5 Hz, Ar*Ind*), 7.00−6.89 (m, 4 H, Ar*benz*, Ar*Ind*), 3.80 (s, 3 H, OCH$_3$), 3.77 (bs, 2 H, C*H*$_2$Pip), 3.62 (m, 4 H, C*H*$_2$Pip), 3.44 (m, 2 H, C*H*$_2$Pip), 3.21 (s, 2 H, COC*H*$_2$N), 2.79 (t, 2 H, $J_{H,H}$ = 7.5 Hz, ArCH$_2$CH$_2$C*H*$_2$N), 2.56 (bs, 4 H, C*H*$_2$Pip), 2.47 (m, 6 H, C*H*$_2$Pip, ArC*H*$_2$CH$_2$CH$_2$N), 1.93 (q, 2 H, $J_{H,H}$ = 7.5 Hz, CH$_2$C*H*$_2$CH$_2$). ^{13}C NMR (75 MHz, CDCl$_3$, 298 K): δ = 170.1 (NCOAr), 167.4 (NCOCH$_2$), 159.7 (C$_q$OCH$_3$), 136.9 (COC$_q$), 136.4 (C$_q$NH), 129.6 (2C, C*H*Ar*benz*), 127.4 (C$_{qInd}$), 121.9, 121.2, 119.1, 119.0, 115.8 (CHAr$_{Ind}$), 115.6 (CHAr*benz*), 122.4 (CHAr$_{benz}$), 111.1 (C$_{qInd}$CH$_2$CH$_2$CH$_2$), 60.7 (COCH$_2$N), 58.0 (CH$_2$CH$_2$C*H*$_2$N), 55.4 (OCH$_3$), 53.4 (2C, CH$_2$Pip), 52.9 (2C, CH$_2$Pip), 45.3 (2C, CH$_2$Pip), 41.5 (2C, C*H*$_2$Pip), 26.9 (ArCH$_2$C*H*$_2$CH$_2$N), 22.7 (ArC*H*$_2$CH$_2$CH$_2$N). HRMS: (ESI-TOF) *m*/*z* calcd. for C$_{29}$H$_{37}$N$_5$O$_3$: 503.2896. Found: 503.2892.

4.1.2.9. 1-(4-(3-(1H-indol-3-yl)propyl)piperazin-1-yl)-2-(4-(3-bromobenzoyl)piperazin-1-yl)ethan-1-one ***(11)***. From 0.20 g (0.40 mmol) of compound **2i** and tosylate **A** were obtained 0.183 g (82%) of compound **11** as a pale brown oil. ^{1}H NMR (300 MHz, CDCl$_3$, 298 K): ^{1}H NMR (300 MHz, CDCl$_3$, 298 K): δ = 8.08 (s, 1 H, NH*Ind*), 7.62−7.50 (m, 3 H, Ar*benz*, Ar*Ind*), 7.38−7.24 (m, 3 H, Ar*benz*, Ar*Ind*), 7.18 (dt, 1 H, $J_{H,H}$ = 1.1 Hz, $J_{H,H}$ = 7.3 Hz, Ar*Ind*), 7.10 (dt, 1 H, $J_{H,H}$ = 1.3 Hz, $J_{H,H}$ = 7.9 Hz, Ar*benz*), 6.95 (d, 1 H, $J_{H,H}$ = 2.0 Hz, Ar*Ind*), 3.79 (bs, 2 H, C*H*$_2$Pip), 3.61 (m, 4 H, C*H*$_2$Pip), 3.43 (m, 2 H, C*H*$_2$Pip), 3.23 (s, 2 H, COC*H*$_2$N), 2.79 (t, 2 H, $J_{H,H}$ = 7.4 Hz, ArCH$_2$CH$_2$C*H*$_2$N), 2.58 (bs, 4 H, C*H*$_2$Pip), 2.43 (m, 6 H, C*H*$_2$Pip, ArC*H*$_2$CH$_2$CH$_2$N), 1.91 (q, 2 H, $J_{H,H}$ = 7.4 Hz, CH$_2$C*H*$_2$CH$_2$). ^{13}C NMR (75 MHz, CDCl$_3$, 298 K): δ = 168.6 (NCOAr), 167.4 (NCOCH$_2$), 137.6 (COC$_q$), 136.3 (C$_q$NH), 132.8 (C*H*Ar*benz*), 130.2, 130.0 (C*H*Ar*benz*), 127.4 (C$_{qInd}$), 125.6 (C$_q$Br), 122.8 (C*H*Ar*benz*), 121.9, 121.2, 119.1 (CHAr$_{Ind}$), 118.8 (Cq$_{Ind}$), 116.1 (CHAr$_{Ind}$), 111.1 (C$_{qInd}$CH$_2$CH$_2$CH$_2$), 60.6 (COCH$_2$N), 58.1 (CH$_2$CH$_2$C*H*$_2$N), 53.5, 53.0, 45.5, 41.8 (C*H*$_2$Pip), 27.1 (ArCH$_2$C*H*$_2$CH$_2$N), 22.8 (ArC*H*$_2$CH$_2$CH$_2$N). HRMS: (ESI-TOF) *m*/*z* calcd. for C$_{28}$H$_{34}$BrN$_5$O$_2$: 551.1896. Found: 551.1899.

4.1.2.10. 1-(4-(3-(1H-indol-3-yl)propyl)piperazin-1-yl)-2-(4-(3-fluorobenzoyl)piperazin-1-yl)ethan-1-one ***(12)***. From 0.15 g (0.33 mmol) of compound **2j** and tosylate **A** were obtained 0.138 g (84%) of compound **12** as a pale yellow oil. ^{1}H NMR (300 MHz, CDCl$_3$, 298 K): δ = 8.17 (s, 1 H, NH*Ind*), 7.59 (d, 1 H, $J_{H,H}$ = 7.5 Hz, Ar*Ind*), 7.40−7.29 (m, 2 H, Ar*benz*, Ar*Ind*), 7.21−7.00 (m, 5 H, Ar*benz*, Ar*Ind*), 6.96 (bs, 1 H, Ar*Ind*), 3.79 (bs, 2 H, C*H*$_2$Pip), 3.60 (m, 4 H, C*H*$_2$Pip), 3.42 (m, 2 H, C*H*$_2$Pip), 3.22 (s, 2 H, COC*H*$_2$N), 2.78 (t, 2 H, $J_{H,H}$ = 7.5 Hz, ArCH$_2$CH$_2$C*H*$_2$N), 2.59 (bs, 4 H, C*H*$_2$Pip), 2.43 (m, 6 H, C*H*$_2$Pip, ArC*H*$_2$CH$_2$CH$_2$N), 1.91 (q, 2 H, $J_{H,H}$ = 7.6 Hz, CH$_2$C*H*$_2$CH$_2$). ^{13}C NMR (75 MHz, CDCl$_3$, 298 K): δ = 168.8 (NCOAr), 167.3 (NCOCH$_2$), 162.5 (d, C$_q$F), 137.7 (d, COC$_q$), 136.4 (C$_q$NH), 130.3 (d, C*H*Ar*benz*), 127.4 (C$_{qInd}$), 122.7 (d, C*H*Ar*benz*), 121.9, 121.2, 119.1, 118.8, 116.0 (CHAr$_{Ind}$), 116.9 (d, CHAr$_{benz}$), 116.0 (CHAr*benz*), 114.4 (d, C*H*Ar*benz*), 111.1 (C$_{qInd}$CH$_2$CH$_2$CH$_2$), 60.6 (COCH$_2$N), 58.2 (CH$_2$CH$_2$C*H*$_2$N), 53.5 (2C, C*H*$_2$Pip), 53.0 (2C, C*H*$_2$Pip), 45.5 (2C, C*H*$_2$Pip), 41.8 (2C, C*H*$_2$Pip), 27.2 (ArCH$_2$C*H*$_2$CH$_2$N), 22.9 (ArC*H*$_2$CH$_2$CH$_2$N). HRMS: (ESI-TOF) *m*/*z* calcd. for C$_{28}$H$_{34}$FN$_5$O$_2$: 491.2697. Found: 491.2687.

4.1.2.11. 1-(4-(3-(1H-indol-3-yl)propyl)piperazin-1-yl)-2-(4-(2-bromobenzoyl)piperazin-1-yl)ethan-1-one ***(13)***. From 0.15 g (0.303 mmol) of compound **2k** and tosylate **A** were obtained 0.136 g (81%) of compound **13** as a pale yellow solid. Mp.: 75−77 °C.; ^{1}H NMR (300 MHz, CDCl$_3$, 298 K): ^{1}H NMR (300 MHz, CDCl$_3$, 298 K): δ = 8.05 (s, 1 H, NH*Ind*), 7.58 (m, 2 H, Ar*benz*), 7.39−7.30 (m, 2 H, Ar*benz*, Ar*Ind*), 7.28−7.20 (m, 2 H, Ar*benz*, Ar*Ind*), 7.18 (dt, 1 H, $J_{H,H}$ = 1.0 Hz, $J_{H,H}$ = 7.1 Hz, Ar*Ind*), 7.10 (dt, 1 H, $J_{H,H}$ = 1.0 Hz, $J_{H,H}$ = 7.1 Hz, Ar*Ind*), 6.97 (bs, 1 H, Ar*Ind*), 3.84 (t, 2 H, $J_{H,H}$ = 5.0 Hz, C*H*$_2$Pip), 3.62 (m, 4 H, C*H*$_2$Pip), 3.27 (m, 2 H, C*H*$_2$Pip), 3.22 (s, 2 H, COC*H*$_2$N), 2.79 (t, 2 H, $J_{H,H}$ = 7.5 Hz, ArCH$_2$CH$_2$C*H*$_2$N), 2.70−2.35 (m, 10 H, C*H*$_2$Pip, ArC*H*$_2$CH$_2$CH$_2$N), 1.91 (q, 2 H, $J_{H,H}$ = 7.5 Hz, CH$_2$C*H*$_2$CH$_2$). ^{13}C NMR (75 MHz, CDCl$_3$, 298 K): δ = 167.6 (NCOAr), 167.3 (NCOCH$_2$), 137.9 (COC$_q$), 136.4 (C$_q$NH), 132.8, 130.4 (C*H*Ar*benz*), 127.7 (2C, C*H*Ar*benz*), 127.5 (C$_{qInd}$), 121.9, 121.2 (CHAr$_{ind}$), 119.1 (2C, CHAr$_{ind}$), 116.1 (CHAr$_{Ind}$), 118.8 (C$_q$Br), 111.1 (C$_{qInd}$CH$_2$CH$_2$CH$_2$), 60.7 (COCH$_2$N), 58.1 (CH$_2$CH$_2$C*H*$_2$N), 53.5 (2C, C*H*$_2$Pip), 53.0 (2C, C*H*$_2$Pip), 45.5 (2C, C*H*$_2$Pip), 41.8 (2C, C*H*$_2$Pip), 27.2 (ArCH$_2$C*H*$_2$CH$_2$N), 22.8

(ArCH$_2$CH$_2$CH$_2$N). HRMS: (ESI-TOF) *m/z* calcd. for $C_{28}H_{34}BrN_5O_2$: 551.1896. Found: 551.1895.

*4.1.2.12. 2-(4-benzoylpiperazin-1-yl)-1-(4-(3-(5-fluoro-1H-indol-3-yl)propyl)piperazin-1-yl)ethan-1-one **(14)**.* From 0.16 g (0.38 mmol) of compound **2a** and tosylate **B** were obtained 0.11 g (61%) of compound **14** as a white solid. Mp.: 74–75 °C.; ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 8.11 (s, 1 H, NH$_{Ind}$), 7.23 (bs, 5 H, Ar$_{benz}$), 7.11–7.03 (m, 2 H, Ar$_{Ind}$), 6.83 (bs, 1 H, Ar$_{Ind}$), 6.74 (dt, 1 H, $J_{H,H}$ = 2.0 Hz, $J_{H,H}$ = 9 Hz, Ar$_{Ind}$), 3.64 (bs, 2 H, CH_2Pip), 3.44 (m, 4 H, CH_2Pip), 3.28 (m, 2 H, CH_2Pip), 3.05 (s, 2 H, COCH_2N), 2.55 (t, 2 H, $J_{H,H}$ = 7.7 Hz, ArCH$_2$CH$_2$CH_2N), 2.39 (bs, 2 H, CH_2Pip), 2.31 (bs, 2 H, CH_2Pip), 2.22 (m, 6 H, CH_2Pip, ArCH_2CH$_2$CH$_2$N), 1.69 (q, 2 H, $J_{H,H}$ = 7.7 Hz, CH$_2$CH_2CH$_2$). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 169.3 (NCOAr), 166.3 (NCOCH$_2$), 157.5 (d, $^1J_{C,F}$ = 235 Hz, CqF$_{Ind}$), 134.6 (COC$_q$), 131.8 (C$_q$Ind), 128.4 (2C, CHAr$_{benz}$), 127.6 (CHAr$_{Ind}$), 126.9 (2C, CHAr$_{benz}$), 123.0 (CHAr$_{benz}$), 122.1 (CHAr$_{Ind}$), 116.1 (d, $^4J_{C,F}$ = 4.5 Hz, CAr$_{Ind}$), 111.7 (d, $^3J_{C,F}$ = 4.5 Hz, CAr$_{Ind}$), 110.1 (d, $^2J_{C,F}$ = 26.5 Hz, C$_q$IndCH$_2$CH$_2$CH$_2$), 103.6 (d, $^2J_{C,F}$ = 23 Hz, CAr$_{Ind}$), 60.6 (COCH$_2$N), 57.9 (CH$_2$CH$_2$CH$_2$N), 53.5 (2C, CH$_2$Pip), 53.0 (2C, CH$_2$Pip), 45.4 (2C, CH$_2$Pip), 41.7 (2C, CH$_2$Pip), 27.1 (ArCH$_2$CH$_2$CH$_2$N), 22.6 (ArCH$_2$CH$_2$CH$_2$N). HRMS: (ESI-TOF) *m/z* calcd. for $C_{28}H_{34}FN_5O_2$: 491.2697. Found: 491.2705.

*4.1.2.13. 2-(4-(4-chlorobenzoyl)piperazin-1-yl)-1-(4-(3-(5-fluoro-1H-indol-3-yl)propyl)piperazin-1-yl)ethan-1-one **(15)**.* From 0.20 g (0.44 mmol) of compound **2b** and tosylate **B** were obtained 0.192 g (83%) of compound **15** as a white solid. Mp.: 78–80 °C.; ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 8.31 (s, 1 H, NH$_{Ind}$), 7.37 (d, 2 H, $J_{H,H}$ = 8.4 Hz, Ar$_{benz}$), 7.32 (d, 2 H, $J_{H,H}$ = 8.4 Hz, Ar$_{benz}$), 7.22 (m, 2 H, Ar$_{Ind}$), 6.99 (bs, 1 H, Ar$_{Ind}$), 6.74 (dt, 1 H, $J_{H,H}$ = 2.3 Hz, $J_{H,H}$ = 9.0 Hz, Ar$_{Ind}$), 3.76 (bs, 2 H, CH_2Pip), 3.59 (m, 4 H, CH_2Pip), 3.43 (bs, 2 H, CH_2Pip), 3.22 (s, 2 H, COCH_2N), 2.73 (t, 2 H, $J_{H,H}$ = 7.5 Hz, ArCH$_2$CH$_2$CH_2N), 2.53 (bs, 4 H, CH_2Pip), 2.41 (m, 6 H, CH_2Pip, ArCH_2CH$_2$CH$_2$N), 1.87 (q, 2 H, $J_{H,H}$ = 7.7 Hz, CH$_2$CH_2CH$_2$). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 169.3 (NCOAr), 166.7 (NCOCH$_2$), 157.6 (d, $^1J_{C,F}$ = 235 Hz, CqF$_{Ind}$), 135.6 (C$_q$Cl), 133.9 (COC$_q$), 132.8 (C$_q$NH$_{Ind}$), 128.8 (2C, CHAr$_{benz}$), 128.6 (2C, CHAr$_{benz}$), 127.8 (d, $^3J_{C,F}$ = 9.5 Hz, CHAr$_{Ind}$), 123.1 (CHAr$_{Ind}$), 116.1 (d, $^4J_{C,F}$ = 5.0 Hz, CAr$_{Ind}$), 111.7 (d, $^3J_{C,F}$ = 5.0 Hz, CHAr$_{Ind}$), 110.2 (d, $^2J_{C,F}$ = 26.2 Hz, C$_q$IndCH$_2$CH$_2$CH$_2$), 103.2 (d, $^2J_{C,F}$ = 23 Hz, CAr$_{Ind}$), 60.6 (COCH$_2$N), 57.9 (CH$_2$CH$_2$CH$_2$N), 53.5 (2C, CH$_2$Pip), 53.0 (2C, CH$_2$Pip), 45.4 (2C, CH$_2$Pip), 41.7 (2C, CH$_2$Pip), 27.0 (ArCH$_2$CH$_2$CH$_2$N), 22.7 (ArCH$_2$CH$_2$CH$_2$N). HRMS: (ESI-TOF) *m/z* calcd. for $C_{28}H_{33}ClFN_5O_2$: 525.2307. Found: 525.2311.

*4.1.2.14. 1-(4-(3-(5-fluoro-1H-indol-3-yl)propyl)piperazin-1-yl)-2-(4-(4-methoxybenzoyl) piperazin-1-yl)ethan-1-one **(16)**.* From 0.14 g (0.31 mmol) of compound **2c** and tosylate **B** were obtained 0.141 g (86%) of compound **16** as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 8.41 (s, 1 H, NH$_{Ind}$), 7.37 (d, 2 H, $J_{H,H}$ = 9.0 Hz, Ar$_{benz}$), 7.26–7.19 (m, 2 H, Ar$_{Ind}$), 7.00 (bs, 1 H, Ar$_{Ind}$), 6.93 (m, 1 H, Ar$_{ind}$), 6.89 (d, 2 H, $J_{H,H}$ = 9.0 Hz, Ar$_{benz}$), 3.82 (s, 3 H, OCH$_3$), 3.75–3.53 (m, 8 H, CH_2Pip), 3.22 (s, 2 H, COCH_2N), 2.73 (t, 2 H, $J_{H,H}$ = 7.5 Hz, ArCH$_2$CH$_2$CH_2N), 2.54 (bs, 4 H, CH_2Pip), 2.41 (m, 6 H, CH_2Pip, ArCH_2CH$_2$CH$_2$N), 1.87 (q, 2 H, $J_{H,H}$ = 7.5 Hz, CH$_2$CH_2CH$_2$). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 171.2 (NCOAr), 170.3 (NCOCH$_2$), 160.8 (C$_q$OCH$_3$), 157.5 (d, $^1J_{C,F}$ = 235 Hz, CqF$_{Ind}$), 132.8 (C$_q$NH$_{Ind}$), 129.1 (2C, CHAr$_{benz}$), 127.8 (d, $^3J_{C,F}$ = 9.5 Hz, CHAr$_{Ind}$), 127.6 (COC$_q$), 123.2 (CH$_{Ind}$), 116.1 (d, $^4J_{C,F}$ = 5.0 Hz, CAr$_{Ind}$), 113.74 (2C, CHAr$_{benz}$), 111.7 (d, $^3J_{C,F}$ = 9.5 Hz, C$_q$IndCH$_2$CH$_2$CH$_2$), 110.1 (d, $^2J_{C,F}$ = 25.0 Hz, CHAr$_{Ind}$) 103.6 (d, $^2J_{C,F}$ = 23.0 Hz, CAr$_{Ind}$), 60.7 (COCH$_2$N), 58.0 (CH$_2$CH$_2$CH$_2$N), 55.4 (OCH$_3$), 53.5 (2C, CH$_2$Pip), 53.0 (2C, CH$_2$Pip), 45.4 (2C, CH$_2$Pip), 41.8 (2C, CH$_2$Pip), 27.1 (ArCH$_2$CH$_2$CH$_2$N), 22.7 (ArCH$_2$CH$_2$CH$_2$N). HRMS: (ESI-TOF) *m/z* calcd. for $C_{29}H_{36}FN_5O_3$: 521.2802. Found: 521.2808.

*4.1.2.15. 2-(4-(4-bromobenzoyl)piperazin-1-yl)-1-(4-(3-(5-fluoro-1H-indol-3-yl)propyl)piperazin-1-yl)ethan-1-one **(17)**.* From 0.16 g (0.32 mmol) of compound **2d** and tosylate **B** were obtained 0.163 g (88%) of compound **17** as a pale brown solid. Mp.: 200–202 °C.; ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 8.45 (s, 1 H, NH$_{Ind}$), 7.53 (d, 2 H, $J_{H,H}$ = 7.5 Hz, Ar$_{benz}$), 7.26 (d, 2 H, $J_{H,H}$ = 7.5 Hz, Ar$_{benz}$), 7.26–7.18 (m, 2 H, Ar$_{Ind}$), 7.00 (bs, 1 H, Ar$_{Ind}$), 6.89 (dt, 1 H, $J_{H,H}$ = 9.0 Hz $J_{H,H}$, = 2.5 Hz, Ar$_{Ind}$), 3.77 (bs, 2 H, CH_2Pip), 3.68–3.56 (m, 4 H, CH$_2$Pip), 3.43 (bs, 2 H, CH$_2$Pip), 3.22 (s, 2 H, COCH_2N), 2.73 (t, 2 H, $J_{H,H}$ = 7.5 Hz, ArCH$_2$CH$_2$CH_2N), 2.54 (m, 4 H, CH_2Pip), 2.42 (m, 6 H, CH_2Pip, ArCH_2CH$_2$CH$_2$N), 1.87 (q, 2 H, $J_{H,H}$ = 7.5 Hz, CH$_2$CH_2CH$_2$). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 169.3 (NCOAr), 167.3 (NCOCH$_2$), 157.8 (d, $^1J_{C,F}$ = 235 Hz, CqF$_{Ind}$), 134.4 (COC$_q$), 132.8 (C$_q$NH$_{Ind}$), 131.8 (2C, CHAr$_{benz}$), 128.8 (2C, CHAr$_{benz}$), 127.8 (d, $^3J_{C,F}$ = 9.5 Hz, CHAr$_{Ind}$), 124.1 (C$_q$Br), 123.1 (CHAr$_{Ind}$), 116.0 (d, $^4J_{C,F}$ = 5.0 Hz, C$_q$IndCH$_2$CH$_2$CH$_2$), 111.8 (d, $^3J_{C,F}$ = 9.5 Hz, CAr$_{Ind}$), 110.1 (d, $^2J_{C,F}$ = 25.0 Hz, CHAr$_{Ind}$), 103.7 (d, $^2J_{C,F}$ = 25.0 Hz, CHAr$_{Ind}$), 60.5 (COCH$_2$N), 58.0 (CH$_2$CH$_2$CH$_2$N), 53.5 (2C, CH$_2$Pip), 52.9 (2C, CH$_2$Pip), 45.4 (2C, CH$_2$Pip), 41.8 (2C, CH$_2$Pip), 27.1 (ArCH$_2$CH$_2$CH$_2$N), 22.7 (ArCH$_2$CH$_2$CH$_2$N). HRMS: (ESI-TOF) *m/z* calcd. for $C_{28}H_{33}BrFN_5O_2$: 569.1802. Found: 569.1812.

*4.1.2.16. 1-(4-(3-(5-fluoro-1H-indol-3-yl)propyl)piperazin-1-yl)-2-(4-(4-methylbenzoyl)piperazin-1-yl)ethan-1-one **(18)**.* From 0.15 g (0.35 mmol) of compound **2e** and tosylate **B** were obtained 0.145 g (82%) of compound **18** as a white solid. Mp.: 94–96 °C.; ^{1}H NMR (300 MHz, CD_3OD, 298 K): 7.34–7.24 (m, 5 H, Ar$_{ind}$, Ar$_{benz}$), 7.20 (dd, 1 H, $J_{H,H}$ = 7.9 Hz, $J_{H,H}$ = 2.5 Hz, Ar$_{Ind}$), 7.1 (s, 1 H, Ar$_{Ind}$), 6.85 (dt, 1 H, $J_{H,H}$ = 9.2 Hz $J_{H,H}$, = 2.5 Hz, Ar$_{ind}$), 3.76 (bs, 2 H, CH_2Pip), 3.65 (m, 4 H, CH_2Pip), 3.48 (bs, 2 H, CH$_2$Pip), 3.28 (s, 2 H, COCH_2N), 2.76 (t, 2 H, $J_{H,H}$ = 7.5 Hz, ArCH$_2$CH$_2$CH_2N), 2.65–2.42 (m, 10 H, CH_2Pip, ArCH_2CH$_2$CH$_2$N), 2.39 (s, 3 H, CH$_3$), 1.93 (q, 2 H, $J_{H,H}$ = 7.5 Hz, CH$_2$CH_2CH$_2$). ^{13}C NMR (75 MHz, CD_3OD, 298 K): δ = 172.6 (NCOAr), 169.9 (NCOCH$_2$), 158.7 (d, $^1J_{C,F}$ = 235 Hz, CqF$_{Ind}$), 141.6 (C$_q$CH$_3$), 134.7 (COC$_q$), 133.6 (CH$_{Ind}$), 130.2 (2C, CHAr$_{benz}$), 128.1 (2C, CHAr$_{benz}$), 129.0 (d, $^3J_{C,F}$ = 9.5 Hz, Cq$_{Ind}$), 125.0 (CHAr$_{Ind}$), 115.9 (d, $^4J_{C,F}$ = 5.0 Hz, C$_q$Ind), 112.9 (d, $^3J_{C,F}$ = 9.5 Hz, CHAr$_{Ind}$), 110.8 (d, $^2J_{C,F}$ = 25.0 Hz, CHAr$_{Ind}$), 103.9 (d, $^2J_{C,F}$ = 25.0 Hz, CHAr$_{Ind}$), 60.9 (COCH$_2$N), 59.0 (CH$_2$CH$_2$CH$_2$N), 54.3 (2C, CH$_2$Pip), 53.8 (2C, CH$_2$Pip), 46.1 (2C, CH$_2$Pip), 42.4 (2C, CH$_2$Pip), 27.9 (ArCH$_2$CH$_2$CH$_2$N), 23.6 (ArCH$_2$CH$_2$CH$_2$N), 21.3 (CH$_3$). HRMS: (ESI-TOF) *m/z* calcd. for $C_{29}H_{36}FN_5O_2$: 505.2853. Found: 505.2860.

*4.1.2.17. 1-(4-(3-(5-fluoro-1H-indol-3-yl)propyl)piperazin-1-yl)-2-(4-(4-fluorobenzoyl)piperazin-1-yl)ethan-1-one **(19)**.* From 0.20 g (0.46 mmol) of compound **2f** and tosylate **B** were obtained 0.195 g (83%) of compound **19** as a pale yellow solid. Mp.: 107–108 °C.; ^{1}H NMR (300 MHz, CD_3OD, 298 K): 7.48 (m, 2 H, Ar$_{benz}$), 7.32–7.16 (m, 4 H, Ar$_{benz}$, Ar$_{Ind}$), 7.11 (s, 1 H, Ar$_{ind}$), 6.85 (dt, 1 H, $J_{H,H}$ = 9.2 Hz $J_{H,H}$, = 2.5 Hz, Ar$_{ind}$), 3.77 (bs, 2 H, CH_2Pip), 3.65 (m, 4 H, CH_2Pip), 3.48 (bs, 2 H, CH$_2$Pip), 3.29 (s, 2 H, COCH_2N), 2.77 (t, 2 H, $J_{H,H}$ = 7.5 Hz, ArCH$_2$CH$_2$CH_2N), 2.65–2.42 (m, 10 H, CH_2Pip, ArCH_2CH$_2$CH$_2$N), 1.94 (q, 2 H, $J_{H,H}$ = 7.5 Hz, CH$_2$CH_2CH$_2$). ^{13}C NMR (75 MHz, CD_3OD, 298 K): δ = 170.1 (NCOAr), 168.4 (NCOCH$_2$), 165.4 (CqF$_{benz}$), 160.5 (d, $^1J_{C,F}$ = 231 Hz, CqF$_{Ind}$), 133.3 (COC$_q$), 131.4 (d, $^4J_{C,F}$ = 3.3 Hz, CqNH$_{Ind}$), 129.3 (d, 2C, $^4J_{C,F}$ = 9.0 Hz, CHAr$_{benz}$), 127.5 (CHAr$_{Ind}$), 123.6 (CHAr$_{Ind}$), 115.2 (d, 2C, $^2J_{C,F}$ = 22.5 Hz, CHAr$_{benz}$), 114.3 (d, $^4J_{C,F}$ = 5.0 Hz, Cq$_{Ind}$), 111.5 (d, 2C, $^2J_{C,F}$ = 10.0 Hz, CHAr$_{Ind}$), 108.8 (d, $^2J_{C,F}$ = 25.0 Hz, C$_q$Ind), 102.5 (d, $^2J_{C,F}$ = 25.0 Hz, CHAr$_{Ind}$), 59.5 (COCH$_2$N), 57.7 (CH$_2$CH$_2$CH$_2$N), 52.9 (2C, CH$_2$Pip), 52.4 (2C, CH$_2$Pip), 44.7 (2C, CH$_2$Pip), 41.0 (2C, CH$_2$Pip), 26.6 (ArCH$_2$CH$_2$CH$_2$N), 22.3 (ArCH$_2$CH$_2$CH$_2$N). HRMS: (ESI-TOF) *m/z* calcd. for $C_{28}H_{33}F_2N_5O_2$: 509.2602. Found: 509.2610.

*4.1.2.18. 2-(4-(3-chlorobenzoyl)piperazin-1-yl)-1-(4-(3-(5-fluoro-1H-indol-3-yl)propyl)piperazin-1-yl)ethan-1-one **(20)**.* From 0.20 g (0.44 mmol) of compound **2g** and tosylate **B** were obtained 0.155 g (67%) of compound **20** as a white solid. Mp.: 166–167 °C.; ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 8.20 (s, 1 H, NH_{Ind}), 7.43–7.20 (m, 6 H, Ar_{Ind}, Ar_{benz}), 7.01 (bs, 1 H, Ar_{Ind}), 6.92 (dt, 1 H, $J_{H,H}$ = 9.0 Hz $J_{H,H}$, = 2.2 Hz, Ar_{ind}), 3.79 (bs, 2 H, CH_2Pip), 3.60 (m, 4 H, CH_2Pip), 3.43 (bs, 2 H, CH_2Pip), 3.23 (s, 2 H, COCH_2N), 2.74 (t, 2 H, $J_{H,H}$ = 7.5 Hz, ArCH$_2$CH$_2$CH_2N), 2.59 (bs, 2 H, CH_2Pip), 2.50 (bs, 2 H, CH_2Pip), 2.42 (m, 6 H, CH_2Pip, ArCH_2CH$_2$CH$_2$N), 1.88 (q, 2 H, $J_{H,H}$ = 7.5 Hz, CH$_2$CH_2CH$_2$). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 168.8 (NCOAr), 167.3 (NCOCH$_2$), 157.7 (d, $^1J_{C,F}$ = 234 Hz, CqF$_{Ind}$), 137.7 (Cq$_{Ind}$), 134.6 (C_qCl), 132.8 (COC$_q$), 129.9 (2C, CHAr$_{benz}$), 127.8 (d, $^3J_{C,F}$ = 9.5 Hz, C$_{qInd}$), 127.2 (CHAr$_{benz}$), 125.1 (CHAr$_{benz}$), 123.1 (CHAr$_{benz}$), 116.2 (d, $^4J_{C,F}$ = 3.5 Hz, C_{qInd}CH$_2$CH$_2$CH$_2$), 111.7 (d, $^3J_{C,F}$ = 10.0 Hz CHAr$_{Ind}$), 110.2 (d, $^2J_{C,F}$ = 23.0 Hz, CHAr$_{ind}$), 103.8 (d, $^2J_{C,F}$ = 25.0 Hz, CHAr$_{Ind}$), 60.6 (COCH$_2$N), 57.9 (CH$_2$CH$_2$CH_2N), 53.6 (2C, CH_2Pip), 53.0 (2C, CH_2Pip), 45.8 (2C, CH_2Pip), 41.8 (2C, CH_2Pip), 27.1 (ArCH$_2$CH_2CH$_2$N), 22.7 (ArCH$_2$CH$_2$CH$_2$N). HRMS: (ESI-TOF) *m/z* calcd. for $C_{28}H_{33}ClFN_5O_2$: 525.2307. Found: 525.2319.

*4.1.2.19. 1-(4-(3-(5-fluoro-1H-indol-3-yl)propyl)piperazin-1-yl)-2-(4-(3-methoxybenzoyl) piperazin-1-yl)ethan-1-one **(21)**.* From 0.18 g (0.40 mmol) of compound **2h** and tosylate **B** were obtained 0.157 g (75%) of compound **21** as a pale brown oil. ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 8.25 (s, 1 H, NH_{Ind}), 7.29 (t, 1 H, $J_{H,H}$ = 2.2 Hz, Ar_{benz}), 7.25–7.19 (m, 2 H, Ar_{Ind}, Ar_{benz}), 7.00 (bs, 1 H, Ar_{Ind}), 6.96–6.87 (m, 4 H, Ar_{benz}, Ar_{ind}), 3.80 (s, 3 H, OCH_3), 3.78 (bs, 2 H, CH_2Pip), 3.61 (m, 4 H, CH_2Pip), 3.44 (bs, 2 H, CH_2Pip), 3.22 (s, 2 H, COCH_2N), 2.73 (t, 2 H, $J_{H,H}$ = 7.5 Hz, ArCH$_2$CH$_2$CH_2N), 2.57 (bs, 2 H, CH_2Pip), 2.48 (bs, 2 H, CH_2Pip), 2.42 (m, 6 H, CH_2Pip, ArCH_2CH$_2$CH$_2$N), 1.88 (q, 2 H, $J_{H,H}$ = 7.5 Hz, CH$_2$CH_2CH$_2$). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 170.1 (NCOAr), 167.4 (NCOCH$_2$), 159.6 (C_qOCH$_3$), 157.7 (d, $^1J_{C,F}$ = 236 Hz, CqF$_{Ind}$), 136.9 (COC$_q$), 132.8 (C_qNH$_{Ind}$), 129.6 (CHAr$_{benz}$), 127.8 (d, $^3J_{C,F}$ = 9.5 Hz, C$_{qInd}$), 123.1 (CHAr$_{ind}$), 119.0 (CHAr$_{benz}$), 116.1 (C_{qInd}CH$_2$CH$_2$CH$_2$), 115.6 (CHAr$_{benz}$), 112.4 (CHAr$_{benz}$), 111.7 (d, $^3J_{C,F}$ = 9.5 Hz CHAr$_{Ind}$), 110.1 (d, $^2J_{C,F}$ = 28.0 Hz, CHAr$_{ind}$), 103.3 (d, $^2J_{C,F}$ = 23.0 Hz, CHAr$_{Ind}$), 60.7 (COCH$_2$N), 57.9 (CH$_2$CH$_2$CH_2N), 55.4 (OCH_3), 53.6 (2C, CH_2Pip), 53.0 (2C, CH_2Pip), 45.4 (2C, CH_2Pip), 41.7 (2C, CH_2Pip), 27.0 (ArCH$_2$CH$_2$CH$_2$N), 22.7 (ArCH$_2$CH$_2$CH$_2$N). HRMS: (ESI-TOF) *m/z* calcd. for $C_{29}H_{36}FN_5O_3$: 521.2802. Found: 521.2813.

*4.1.2.20. 2-(4-(3-bromobenzoyl)piperazin-1-yl)-1-(4-(3-(5-fluoro-1H-indol-3-yl)propyl)piperazin-1-yl)ethan-1-one **(22)**.* From 0.184 g (0.37 mmol) of compound **2i** and tosylate **B** were obtained 0.163 g (78%) of compound **22** as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 8.28 (s, 1 H, NH_{Ind}), 7.57–7.50 (m, 2 H, Ar_{benz}), 7.35–7.18 (m, 4 H, Ar_{Ind}, Ar_{benz}), 7.00 (d, 1 H, $J_{H,H}$ = 2.2 Hz, Ar_{Ind}), 6.92 (dt, 1 H, $J_{H,H}$ = 9.1 Hz $J_{H,H}$, = 2.3 Hz, Ar_{ind}), 3.79 (bs, 2 H, CH_2Pip), 3.60 (m, 4 H, CH_2Pip), 3.43 (bs, 2 H, CH_2Pip), 3.23 (s, 2 H, COCH_2N), 2.79 (t, 2 H, $J_{H,H}$ = 7.4 Hz, ArCH$_2$CH$_2$CH_2N), 2.59 (bs, 2 H, CH_2Pip), 2.50 (bs, 2 H, CH_2Pip), 2.42 (m, 6 H, CH_2Pip, ArCH_2CH$_2$CH$_2$N), 1.88 (q, 2 H, $J_{H,H}$ = 7.5 Hz, CH$_2$CH_2CH$_2$). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 168.3 (NCOAr), 167.3 (NCOCH$_2$), 157.6 (d, $^1J_{C,F}$ = 235 Hz, CqF$_{Ind}$), 137.6 (COC$_q$), 132.8 (CHAr$_{Benz}$), 130.2 (2C, CHAr$_{benz}$), 130.0 (2C, CHAr$_{benz}$), 127.8 (d, $^3J_{C,F}$ = 9.6 Hz, C$_{qInd}$), 125.5 (CHAr$_{benz}$), 123.1 (CHAr$_{ind}$), 122.7 (C_qBr), 116.2 (d, $^4J_{C,F}$ = 5.0 Hz, CHAr$_{Ind}$), 111.7 (d, $^3J_{C,F}$ = 9.7 Hz CHAr$_{ind}$), 110.1 (d, $^2J_{C,F}$ = 26.0 Hz, C_{qInd}CH$_2$CH$_2$CH$_2$), 103.7 (d, $^2J_{C,F}$ = 23.0 Hz, CHAr$_{Ind}$), 60.6 (COCH$_2$N), 57.9 (CH$_2$CH$_2$CH$_2$N), 53.5 (2C, CH_2Pip), 53.0 (2C, CH_2Pip), 45.5 (2C, CH_2Pip), 41.8 (2C, CH_2Pip), 27.1 (ArCH$_2$CH$_2$CH$_2$N), 22.7 (ArCH$_2$CH$_2$CH$_2$N). HRMS: (ESI-TOF) *m/z* calcd. for $C_{28}H_{33}FBrN_5O_2$: 569.1802. Found: 569.1809.

*4.1.2.21. 1-(4-(3-(5-fluoro-1H-indol-3-yl)propyl)piperazin-1-yl)-2-(4-(3-fluorobenzoyl)piperazin-1-yl)ethan-1-one **(23)**.* From 0.20 g (0.46 mmol) of compound **2j** and tosylate **B** were obtained 0.18 g (77%) of compound **23** as a pale brown solid. Mp.: 140–141 °C.; ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 8.36 (s, 1 H, NH_{Ind}), 7.37 (m, 1 H, Ar_{benz}), 7.28–7.19 (m, 2 H, Ar_{Ind}, Ar_{benz}), 7.19–7.06 (m, 3 H, Ar_{ind}, Ar_{benz}), 7.00 (bs, 1 H, Ar_{Ind}), 6.91 (dt, 1 H, $J_{H,H}$ = 9.0 Hz $J_{H,H}$, = 2.5 Hz, Ar_{ind}), 3.80 (bs, 2 H, CH_2Pip), 3.61 (m, 4 H, CH_2Pip), 3.42 (bs, 2 H, CH_2Pip), 3.23 (s, 2 H, COCH_2N), 2.73 (t, 2 H, $J_{H,H}$ = 7.5 Hz, ArCH$_2$CH$_2$CH_2N), 2.59 (bs, 2 H, CH_2Pip), 2.49 (bs, 2 H, CH_2Pip), 2.42 (m, 6 H, CH_2Pip, ArCH_2CH$_2$CH$_2$N), 1.88 (q, 2 H, $J_{H,H}$ = 7.5 Hz, CH$_2$CH_2CH$_2$). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 168.9 (d, $^4J_{C,F}$ = 2.2 Hz, NCOAr), 167.3 (NCOCH$_2$), 162.4 (d, $^1J_{C,F}$ = 249.3 Hz, C_qF), 157.7 (d, $^1J_{C,F}$ = 235 Hz, CqF$_{Ind}$), 137.6 (d, $^3J_{C,F}$ = 236 Hz, COC$_q$), 132.8 (C_qNH$_{Ind}$), 130.4 (d, $^3J_{C,F}$ = 8.3 Hz, CHAr$_{benz}$), 127.8 (d, $^3J_{C,F}$ = 9.5 Hz, C$_{qInd}$), 123.1 (CHAr$_{ind}$), 122.6 (d, $^4J_{C,F}$ = 3.4 Hz, CHAr$_{benz}$), 116.9 (d, $^3J_{C,F}$ = 25.0 Hz, CHAr$_{benz}$), 116.1 (d, $^3J_{C,F}$ = 5 Hz, CHAr$_{Ind}$), 114.3 (d, $^2J_{C,F}$ = 23 Hz, CHAr$_{ind}$), 111.7 (d, $^3J_{C,F}$ = 9.5 Hz CHAr$_{Ind}$), 110.1 (d, $^2J_{C,F}$ = 27.0 Hz, C_{qInd}CH$_2$CH$_2$CH$_2$), 103.3 (d, $^2J_{C,F}$ = 23.0 Hz, CHAr$_{Ind}$), 60.6 (COCH$_2$N), 58.0 (CH$_2$CH$_2$CH$_2$N), 53.5 (2C, CH_2Pip), 53.0 (2C, CH_2Pip), 45.4 (2C, CH_2Pip), 41.7 (2C, CH_2Pip), 27.1 (ArCH$_2$CH$_2$CH$_2$N), 22.7 (ArCH$_2$CH$_2$CH$_2$N). HRMS: (ESI-TOF) *m/z* calcd. for $C_{28}H_{33}F_2N_5O_2$: 509.2602. Found: 509.2609.

*4.1.2.22. 2-(4-(2-bromobenzoyl)piperazin-1-yl)-1-(4-(3-(5-fluoro-1H-indol-3-yl)propyl)piperazin-1-yl)ethan-1-one **(24)**.* From 0.136 g (0.27 mmol) of compound **2k** and tosylate **B** were obtained 0.12 g (77%) of compound **24** as a pale brown solid. Mp.: 88–90 °C.; . ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 8.13 (s, 1 H, NH_{Ind}), 7.57 (m, 1 H, Ar_{benz}), 7.40–7.19 (m, 5 H, Ar_{Ind}, Ar_{benz}), 7.00 (bs, 1 H, Ar_{Ind}), 6.92 (dt, 1 H, $J_{H,H}$ = 9.0 Hz $J_{H,H}$, = 2.5 Hz, Ar_{ind}), 3.84 (t, 2 H, $J_{H,H}$ = 5.2 Hz CH_2Pip), 3.62 (m, 4 H, CH_2Pip), 3.28 (m, 2 H, CH_2Pip), 3.22 (s, 2 H, COCH_2N), 2.74 (t, 2 H, $J_{H,H}$ = 7.5 Hz, ArCH$_2$CH$_2$CH_2N), 2.61 (m, 4 H, CH_2Pip), 2.43 (m, 6 H, CH_2Pip, ArCH_2CH$_2$CH$_2$N), 1.88 (q, 2 H, $J_{H,H}$ = 7.5 Hz, CH$_2$CH_2CH$_2$). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 167.6 (NCOAr), 167.3 (NCOCH$_2$), 157.7 (d, $^1J_{C,F}$ = 236 Hz, CqF$_{Ind}$), 137.8 (COC$_q$), 132.8 (C_qNH$_{Ind}$), 130.3 (2C, CHAr$_{benz}$), 127.9, (2C, CHAr$_{benz}$), 127.8 (C$_{qInd}$), 123.0 (CHAr$_{ind}$), 119.0 (CqBr), 116.2 (d, $^4J_{C,F}$ = 5.0 Hz, CAr$_{Ind}$), 111.7 (d, $^3J_{C,F}$ = 9.6 Hz CHAr$_{Ind}$), 110.2 (d, $^2J_{C,F}$ = 26.0 Hz, C_{qInd}CH$_2$CH$_2$CH$_2$), 103.7 (d, $^2J_{C,F}$ = 23.0 Hz, CHAr$_{Ind}$), 60.7 (COCH$_2$N), 57.9 (CH$_2$CH$_2$CH$_2$N), 53.6 (2C, CH_2Pip), 53.0 (2C, CH_2Pip), 45.4 (2C, CH_2Pip), 41.5 (2C, CH_2Pip), 27.0 (ArCH$_2$CH$_2$CH$_2$N), 22.7 (ArCH$_2$CH$_2$CH$_2$N). HRMS: (ESI-TOF) *m/z* calcd. for $C_{28}H_{33}FBrN_5O_2$: 569.1802. Found: 569.1815.

*4.1.2.23. 2-(4-benzoylpiperazin-1-yl)-1-(4-(3-(5-methoxy-1H-indol-3-yl)propyl)piperazin-1-yl)ethan-1-one **(25)**.* From 0.150 g (0.36 mmol) of compound **2a** and tosylate **C** were obtained 0.125 g (68%) of compound **25** as a pale brown oil. ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 8.00 (s, 1 H, NH_{Ind}), 7.39 (bs, 5 H, Ar_{benz}), 7.22 (d, 1 H, $J_{H,H}$ = 8.8 Hz, Ar_{Ind}), 7.02 (d, 1 H, $J_{H,H}$ = 2.3 Hz, Ar_{Ind}), 6.94 (bs, 1 H, Ar_{ind}), 6.84 (dd, 1 H, $J_{H,H}$ = 2.3 Hz, $J_{H,H}$ = 8.8 Hz, Ar_{Ind}), 3.85 (s, 3 H, OCH_3), 3.79 (bs, 2 H, CH_2Pip), 3.61 (m, 4 H, CH_2Pip), 3.44 (bs, 2 H, CH_2Pip), 3.22 (s, 2 H, COCH_2N), 2.75 (t, 2 H, $J_{H,H}$ = 7.7 Hz, ArCH$_2$CH$_2$CH_2N), 2.57 (bs, 2 H, CH_2Pip), 2.49 (bs, 2 H, CH_2Pip), 2.43 (m, 6 H, CH_2Pip, ArCH_2CH$_2$CH$_2$N), 1.90 (q, 2 H, $J_{H,H}$ = 7.7 Hz, CH$_2$CH_2CH$_2$). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 170.4 (NCOAr), 167.4 (NCOCH$_2$), 153.8 (C_qOCH$_3$), 135.6 (COC$_q$), 131.5 (C_qNH$_{Ind}$), 129.7 (CHAr$_{Benz}$), 128.5 (2C, CHAr$_{benz}$), 127.8 (C$_{qInd}$), 127.0 (2C, CHAr$_{benz}$), 122.0 (CHAr$_{ind}$), 115.7 (CHAr$_{ind}$), 111.9 (CHAr$_{ind}$), 111.8 (C_{qInd}CH$_2$CH$_2$CH$_2$), 101.0 (CHAr$_{Ind}$), 60.7 (COCH$_2$N), 58.2 (CH$_2$CH$_2$CH$_2$N), 56.0 (OCH_3), 53.6 (2C, CH_2Pip), 53.0 (2C, CH_2Pip), 45.5 (2C, CH_2Pip), 41.8 (2C, CH_2Pip), 27.0 (ArCH$_2$CH$_2$CH$_2$N), 22.8 (ArCH$_2$CH$_2$CH$_2$N). HRMS: (ESI-TOF) *m/z* calcd. for $C_{29}H_{37}N_5O_3$: 503.2896. Found: 503.2890.

*4.1.2.24. 2-(4-(4-chlorobenzoyl)piperazin-1-yl)-1-(4-(3-(5-methoxy-1H-indol-3-yl)propyl) piperazin-1-yl)ethan-1-one **(26)**.* From 0.10 g (0.22 mmol) of compound **2b** and tosylate **C** were obtained 0.09 g (73%) of compound **26** as a white solid. Mp.: 172–173 °C.; ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 8.14 (s, 1 H, NH_{Ind}), 7.38 (d, 2 H, $J_{H,H}$ = 8.7 Hz, Ar_{benz}), 7.33 (d, 2 H, $J_{H,H}$ = 8.7 Hz, Ar_{benz}), 7.25 (d, 1 H, $J_{H,H}$ = 7.2 Hz, Ar_{Ind}), 7.02 (d, 1 H, $J_{H,H}$ = 2.3 Hz, Ar_{ind}), 6.94 (bs, 1 H, Ar_{Ind}), 6.84 (dt, 1 H, $J_{H,H}$ = 8.7 Hz, $J_{H,H}$, = 2.5 Hz, Ar_{ind}), 3.86 (s, 3 H, OCH_3), 3.78 (bs, 2 H, CH_2Pip), 3.60 (m, 4 H, CH_2Pip), 3.43 (bs, 2 H, CH_2Pip), 3.22 (s, 2 H, COCH_2N), 2.75 (t, 2 H, $J_{H,H}$ = 7.6 Hz, $ArCH_2CH_2CH_2N$), 2.59 (bs, 2 H, CH_2Pip), 2.50 (bs, 2 H, CH_2Pip), 2.44 (m, 6 H, CH_2Pip, $ArCH_2CH_2CH_2N$), 1.90 (q, 2 H, $J_{H,H}$ = 7.6 Hz, $CH_2CH_2CH_2$). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 169.3 (NCOAr), 167.3 ($NCOCH_2$), 153.7 (C_qOCH_3), 135.9 (C_qCl), 133.9 (COC_q), 131.5 (C_qNH_{Ind}), 128.8 (2C, $CHAr_{benz}$), 128.6 (2C, $CHAr_{benz}$), 127.8 (C_{qInd}), 122.1 ($CHAr_{ind}$), 115.7 ($CHAr_{ind}$), 111.9 ($CHAr_{Ind}$), 118.8 ($C_{qInd}CH_2CH_2CH_2$), 100.9 ($CHAr_{Ind}$), 60.6 (COCH_2N), 58.2 ($CH_2CH_2CH_2N$), 56.0 (OCH_3), 53.6 (2C, CH_2Pip), 53.0 (2C, CH_2Pip), 45.5 (2C, CH_2Pip), 41.8 (2C, CH_2Pip), 27.1 ($ArCH_2CH_2CH_2N$), 22.9 ($ArCH_2CH_2CH_2N$). HRMS: (ESI-TOF) *m*/*z* calcd. for $C_{29}H_{36}ClN_5O_3$: 537.2507. Found: 537.2518.

*4.1.2.25. 1-(4-(3-(5-methoxy-1H-indol-3-yl)propyl)piperazin-1-yl)-2-(4-(4-methoxybenzoyl) piperazin-1-yl)ethan-1-one **(27)**.* From 0.140 g (0.31 mmol) of compound **2c** and tosylate **C** were obtained 0.142 g (84%) of compound **27** as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 8.31 (s, 1 H, NH_{Ind}), 7.37 (d, 2 H, $J_{H,H}$ = 8.7 Hz, Ar_{benz}), 7.31 (d, 1 H, $J_{H,H}$ = 8.7 Hz, Ar_{Ind}), 7.00 (bs, 1 H, Ar_{Ind}), 6.93 (bs, 1 H, Ar_{ind}), 6.90 (d, 2 H, $J_{H,H}$ = 8.7 Hz, Ar_{benz}), 6.83 (dd, 1 H, $J_{H,H}$ = 8.7 Hz, $J_{H,H}$, = 2.3 Hz, Ar_{ind}), 3.85 (s, 3 H, OCH_{3Ind}), 3.81 (s, 3 H, OCH_{3benz}), 3.72–3.52 (m, 8 H, CH_2Pip), 3.22 (s, 2 H, COCH_2N), 2.74 (t, 2 H, $J_{H,H}$ = 7.7 Hz, $ArCH_2CH_2CH_2N$), 2.53 (bs, 4 H, CH_2Pip), 2.43 (m, 6 H, CH_2Pip, $ArCH_2CH_2CH_2N$), 1.90 (q, 2 H, $J_{H,H}$ = 7.7 Hz, $CH_2CH_2CH_2$). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 170.4 (NCOAr), 167.4 ($NCOCH_2$), 161.6 (C_qOCH_{3Benz}), 153.7 (C_qOCH_{3Ind}), 131.6 (C_qNH_{Ind}), 129.1 (2C, $CHAr_{benz}$), 127.8 (C_qInd), 127.6 (COC_q), 122.2 ($CHAr_{Ind}$), 115.6 ($CHAr_{ind}$), 113.7 (2C, $CHAr_{benz}$), 111.9 ($CHAr_{ind}$), 111.8 ($C_{qInd}CH_2CH_2CH_2$), 100.8 ($CHAr_{Ind}$), 60.7 ($COCH_2N$), 58.2 ($CH_2CH_2CH_2N$), 56.0 (OCH_{3Ind}), 55.4 (OCH_{3Benz}), 53.6 (2C, CH_2Pip), 53.0 (2C, CH_2Pip), 45.5 (2C, CH_2Pip), 41.7 (2C, CH_2Pip), 27.0 ($ArCH_2CH_2CH_2N$), 22.9 ($ArCH_2CH_2CH_2N$). HRMS: (ESI-TOF) *m*/*z* calcd. for $C_{30}H_{39}N_5O_4$: 533.3002. Found: 533.3010.

*4.1.2.26. 2-(4-(4-bromobenzoyl)piperazin-1-yl)-1-(4-(3-(5-methoxy-1H-indol-3-yl)propyl) piperazin-1-yl)ethan-1-one **(28)**.* From 0.14 g (0.28 mmol) of compound **2d** and tosylate **C** were obtained 0.144 g (89%) of compound **28** as a pale brown solid. Mp.: 205–208 °C.; ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): = 8.10 (s, 1 H, NH_{Ind}), 7.53 (d, 2 H, $J_{H,H}$ = 8.5 Hz, Ar_{benz}), 7.27 (d, 2 H, $J_{H,H}$ = 8.5 Hz, Ar_{benz}), 7.22 (d, 1 H, $J_{H,H}$ = 8.9 Hz, Ar_{Ind}), 7.02 (d, 1 H, $J_{H,H}$ = 2.3 Hz, Ar_{ind}), 6.94 (bs, 1 H, Ar_{Ind}), 6.84 (dt, 1 H, $J_{H,H}$ = 8.9 Hz $J_{H,H}$, = 2.4 Hz, Ar_{ind}), 3.86 (s, 3 H, OCH_3), 3.78 (bs, 2 H, CH_2Pip), 3.59 (m, 4 H, CH_2Pip), 3.43 (bs, 2 H, CH_2Pip), 3.22 (s, 2 H, COCH_2N), 2.77 (t, 2 H, $J_{H,H}$ = 7.1 Hz, $ArCH_2CH_2CH_2N$), 2.57 (bs, 2 H, CH_2Pip), 2.49 (bs, 2 H, CH_2Pip), 2.43 (m, 6 H, CH_2Pip, $ArCH_2CH_2CH_2N$), 1.90 (q, 2 H, $J_{H,H}$ = 7.1 Hz, $CH_2CH_2CH_2$). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 169.3 (NCOAr), 167.3 ($NCOCH_2$), 153.8 (C_qOCH_3), 134.4 (COC_q), 131.7 (2C, $CHAr_{benz}$), 131.5 (C_qNH_{Ind}), 128.8 (2C, $CHAr_{benz}$), 127.8 (Cq_{ind}), 124.1 (C_qBr), 122.1 ($CHAr_{Ind}$), 115.8 ($CHAr_{Ind}$), 111.9 ($CHAr_{Ind}$), 111.8 ($C_{qInd}CH_2CH_2CH_2$), 100.9 ($CHAr_{Ind}$), 60.6 ($COCH_2N$), 58.2 ($CH_2CH_2CH_2N$), 56.0 (OCH_3), 53.6 (2C, CH_2Pip), 53.0 (2C, CH_2Pip), 45.5 (2C, CH_2Pip), 41.8 (2C, CH_2Pip), 27.0 ($ArCH_2CH_2CH_2N$), 22.7 ($ArCH_2CH_2CH_2N$). HRMS: (ESI-TOF) *m*/*z* calcd. for $C_{29}H_{36}BrN_5O_3$: 581.2002. Found: 581.2015.

*4.1.2.27. 1-(4-(3-(5-methoxy-1H-indol-3-yl)propyl)piperazin-1-yl)-2-(4-(4-methylbenzoyl) piperazin-1-yl)ethan-1-one **(29)**.* From 0.170 g (0.39 mmol) of compound **2e** and tosylate **C** were obtained 0.124 g (61%) of compound **29** as a pale yellow solid. Mp.: 84–86 °C.; ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): = 8.10 (s, 1 H, NH_{Ind}), 7.30 (d, 2 H, $J_{H,H}$ = 8.0 Hz, Ar_{benz}), 7.25 (m, 1 H, $CHAr_{Ind}$), 8.00 (d, 2 H, $J_{H,H}$ = 8.0 Hz, Ar_{benz}), 7.04 (d, 1 H, $J_{H,H}$ = 2.3 Hz, Ar_{ind}), 6.97 (d, 1 H, $J_{H,H}$ = 2.1 Hz, Ar_{Ind}), 6.84 (dd, 1 H, $J_{H,H}$ = 8.7 Hz $J_{H,H}$, = 2.4 Hz, Ar_{ind}), 3.88 (s, 3 H, OCH_3), 3.78 (bs, 2 H, CH_2Pip), 3.64 (m, 4 H, CH_2Pip), 3.52 (bs, 2 H, CH_2Pip), 3.24 (s, 2 H, COCH_2N), 2.77 (t, 2 H, $J_{H,H}$ = 7.1 Hz, $ArCH_2CH_2CH_2N$), 2.55 (bs, 4 H, CH_2Pip), 2.47 (m, 6 H, CH_2Pip, $ArCH_2CH_2CH_2N$), 2.39 (s, 3 H, $ArCH_3$), 1.93 (q, 2 H, $J_{H,H}$ = 7.1 Hz, $CH_2CH_2CH_2$). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 170.5 (NCOAr), 167.4 ($NCOCH_2$), 153.8 (C_qOCH_3), 134.4 (CH_3C_q), 132.7 (COC_q), 131.6 (C_qNH_{Ind}), 129.0 (2C, $CHAr_{benz}$), 127.9 (Cq_{ind}), 127.9 (2C, $CHAr_{benz}$), 122.1 ($CHAr_{Ind}$), 115.7 ($C_{qInd}CH_2CH_2CH_2$), 111.9 ($CHAr_{Ind}$), 111.8 ($CHAr_{Ind}$), 101.0 ($CHAr_{Ind}$), 60.7 ($COCH_2N$), 58.1 ($CH_2CH_2CH_2N$), 56.0 (OCH_3), 53.6 (2C, CH_2Pip), 53.0 (2C, CH_2Pip), 45.5 (2C, CH_2Pip), 41.7 (CH_2Pip), 27.0 ($ArCH_2CH_2CH_2N$), 22.8 ($ArCH_2CH_2CH_2N$), 21.3 (C_qCH_3). HRMS: (ESI-TOF) *m*/*z* calcd. for $C_{30}H_{39}N_5O_3$: 517.3053. Found: 517.3065.

*4.1.2.28. 2-(4-(4-fluorobenzoyl)piperazin-1-yl)-1-(4-(3-(5-methoxy-1H-indol-3-yl)propyl) piperazin-1-yl)ethan-1-one **(30)**.* From 0.20 g (0.46 mmol) of compound **2f** and tosylate **C** were obtained 0.155 g (65%) of compound **30** as a pale yellow solid. Mp.: 82–83 °C.; ^{1}H NMR (300 MHz, CD_3OD, 298 K): = 7.46 (m, 2 H, $CHAr_{benz}$), 7.26–7.14 (m, 3 H, $CHAr_{benz}$, $CHAr_{Ind}$), 7.00 (m, 2 H, $CHAr_{Ind}$), 6.76 (dt, 1 H, $J_{H,H}$ = 8.7 Hz $J_{H,H}$, = 2.4 Hz, Ar_{ind}), 3.82 (s, 3 H, OCH_3), 3.76 (bs, 2 H, CH_2Pip), 3.59 (m, 4 H, CH_2Pip), 3.46 (bs, 2 H, CH_2Pip), 3.25 (s, 2 H, COCH_2N), 2.77 (t, 2 H, $J_{H,H}$ = 7.1 Hz, $ArCH_2CH_2CH_2N$), 2.55 (bs, 4 H, CH_2Pip), 2.45 (m, 6 H, CH_2Pip, $ArCH_2CH_2CH_2N$), 1.92 (q, 2 H, $J_{H,H}$ = 7.1 Hz, $CH_2CH_2CH_2$). ^{13}C NMR (75 MHz, CD_3OD, 298 K): δ = 170.0 (NCOAr), 168.4 ($NCOCH_2$), 169.2 (d, $^1J_{C,F}$ = 249 Hz, CqF_{Benz}), 153.4 (C_qOCH_3), 132.0 (C_qNH_{Ind}), 131.4 (d, $^4J_{C,F}$ = 3.5 Hz, COC_q), 129.3 (d, 2C, $^3J_{C,F}$ = 8.8 Hz, $CHAr_{benz}$), 127.6 (Cq_{ind}), 122.3 ($CHAr_{Ind}$), 115.3 (d, 2C, $^2J_{C,F}$ = 22.2 Hz, $CHAr_{benz}$), 114.0 ($CHAr_{Ind}$), 111.4 ($CHAr_{Ind}$), 110.0 ($C_{qInd}CH_2CH_2CH_2$), 100.1 ($CHAr_{Ind}$), 59.5 ($COCH_2N$), 57.9 ($CH_2CH_2CH_2N$), 54.9 (OCH_3), 53.1 (2C, CH_2Pip), 52.6 (2C, CH_2Pip), 44.9 (2C, CH_2Pip), 41.2 (2C, CH_2Pip), 26.7 ($ArCH_2CH_2CH_2N$), 22.5 ($ArCH_2CH_2CH_2N$). HRMS: (ESI-TOF) *m*/*z* calcd. for $C_{29}H_{36}FN_5O_3$: 521.2802. Found: 521.2811.

*4.1.2.29. 2-(4-(3-chlorobenzoyl)piperazin-1-yl)-1-(4-(3-(5-methoxy-1H-indol-3-yl)propyl) piperazin-1-yl)ethan-1-one **(31)**.* From 0.20 g (0.44 mmol) of compound **2g** and tosylate **C** were obtained 0.166 g (70%) of compound **31** as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): = 8.10 (bs, 1 H, NH_{Ind}), 7.42–7.19 (m, 5 H, $CHAr_{Ind}$, $CHAr_{benz}$), 7.02 (d, 1 H, $J_{H,H}$ = 2.3 Hz, Ar_{ind}), 6.94 (bs, 1 H, $CHAr_{Ind}$), 6.84 (dd, 1 H, $J_{H,H}$ = 8.7 Hz, $J_{H,H}$, = 2.4 Hz, Ar_{ind}), 3.86 (s, 3 H, OCH_3), 3.77 (bs, 2 H, CH_2Pip), 3.60 (m, 4 H, CH_2Pip), 3.42 (bs, 2 H, CH_2Pip), 3.22 (s, 2 H, COCH_2N), 2.75 (t, 2 H, $J_{H,H}$ = 7.6 Hz, $ArCH_2CH_2CH_2N$), 2.54 (bs, 4 H, CH_2Pip), 2.43 (m, 6 H, CH_2Pip, $ArCH_2CH_2CH_2N$), 1.90 (q, 2 H, $J_{H,H}$ = 7.6 Hz, $CH_2CH_2CH_2$). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 168.8 (NCOAr), 167.3 ($NCOCH_2$), 153.8 (C_qOCH_3), 137.4 (COC_q), 134.6 (C_qCl), 131.5 (C_qNH_{Ind}), 129.9 (2C, $CHAr_{benz}$), 127.9 (Cq_{ind}), 127.2 ($CHAr_{Benz}$), 125.1 ($CHAr_{benz}$), 122.1 ($CHAr_{Ind}$), 115.7 ($CHAr_{Ind}$), 111.9 ($C_{qInd}CH_2CH_2CH_2$), 111.8 ($CHAr_{Ind}$), 100.1 ($CHAr_{Ind}$), 60.6 ($COCH_2N$), 58.1 ($CH_2CH_2CH_2N$), 56.0 (OCH_3), 53.6 (2C, CH_2Pip), 53.0 (2C, CH_2Pip), 45.5 (2C, CH_2Pip), 41.8 (2C, CH_2Pip), 27.0 ($ArCH_2CH_2CH_2N$), 22.9 ($ArCH_2CH_2CH_2N$). HRMS: (ESI-TOF) *m*/*z* calcd. for $C_{29}H_{36}ClN_5O_3$: 537.2507. Found: 537.2520.

*4.1.2.30. 1-(4-(3-(5-methoxy-1H-indol-3-yl)propyl)piperazin-1-yl)-2-(4-(3-methoxybenzoyl) piperazin-1-yl)ethan-1-one **(32)**.*

From 0.180 g (0.40 mmol) of compound **2h** and tosylate **C** were obtained 0.173 g (80%) of compound **32** pale brown oil. ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 8.15 (bs, 1 H, NH_{Ind}), 7.29 (t, 1 H, $J_{H,H}$ = 7.6 Hz, Ar_{ind}), 7.21 (d, 1 H, $J_{H,H}$ = 8.8 Hz, $CHAr_{benz}$), 7.01 (d, 1 H, $J_{H,H}$ = 2.3 Hz, Ar_{ind}), 6.96–6.90 (m, 4 H, $CHAr_{benz}$, $CHAr_{Ind}$), 6.84 (dd, 1 H, $J_{H,H}$ = 8.8 Hz $J_{H,H}$, = 2.3 Hz, Ar_{ind}), 3.85 (s, 3 H, OCH_{3Ind}), 3.80 (s, 3 H, OCH_{3Benz}), 3.77 (bs, 2 H, CH_2Pip), 3.61 (m, 4 H, CH_2Pip), 3.43 (bs, 2 H, CH_2Pip), 3.21 (s, 2 H, $COCH_2$N), 2.74 (t, 2 H, $J_{H,H}$ = 7.6 Hz, $ArCH_2CH_2CH_2N$), 2.56 (bs, 4 H, CH_2Pip), 2.44 (m, 6 H, CH_2Pip, $ArCH_2CH_2CH_2N$), 1.90 (q, 2 H, $J_{H,H}$ = 7.6 Hz, $CH_2CH_2CH_2$). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 170.1 (NCOAr), 167.4 ($NCOCH_2$), 159.7 (C_qOCH_{3Benz}), 153.8 (C_qOCH_{3Ind}), 136.9 (COC_q), 131.6 (C_qNH_{Ind}), 129.6 ($CHAr_{benz}$), 127.8 (Cq_{ind}), 122.1 ($CHAr_{Ind}$), 119.0 ($CHAr_{benz}$), 115.7 ($CHAr_{Ind}$), 115.6 ($CHAr_{benz}$), 112.4 ($CHAr_{benz}$), 111.9 ($C_{qInd}CH_2CH_2CH_2$), 111.8 ($CHAr_{Ind}$), 100.1 ($CHAr_{Ind}$), 60.7 ($COCH_2N$), 58.1 ($CH_2CH_2CH_2N$), 56.0 (OCH_{3Ind}), 55.3 (OCH_{3Benz}), 53.5 (2C, CH_2Pip), 53.0 (2C, CH_2Pip), 45.4 (2C, CH_2Pip), 41.7 (2C, CH_2Pip), 27.0 ($ArCH_2CH_2CH_2N$), 22.8 ($ArCH_2CH_2CH_2N$). HRMS: (ESI-TOF) *m/z* calcd. for $C_{30}H_{39}N_5O_4$: 533.3002. Found: 533.3007.

*4.1.2.31. 2-(4-(3-bromobenzoyl)piperazin-1-yl)-1-(4-(3-(5-methoxy-1H-indol-3-yl)propyl) piperazin-1-yl)ethan-1-one **(33)**.* From 0.140 g (0.28 mmol) of compound **2i** and tosylate **C** were obtained 0.123 g (75%) of compound **33** as a pale orange oil. ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): = 8.02 (bs, 1 H, NH_{Ind}), 7.55 (m, 1 H, Ar_{benz}), 7.53 (m, 1 H, $CHAr_{benz}$), 7.30 (m, 1 H, Ar_{benz}), 7.28–7.21 (m, 2 H, $CHAr_{benz}$, $CHAr_{Ind}$), 7.02 (d, 1 H, $J_{H,H}$ = 2.4 Hz, Ar_{ind}), 6.95 (d, 1 H, $J_{H,H}$ = 2.1 Hz, Ar_{ind}), 6.85 (dd, 1 H, $J_{H,H}$ = 8.8 Hz $J_{H,H}$, = 2.4 Hz, Ar_{ind}), 3.86 (s, 3 H, OCH_3), 3.79 (m, 2 H, CH_2Pip), 3.61 (m, 4 H, CH_2Pip), 3.43 (bs, 2 H, CH_2Pip), 3.22 (s, 2 H, $COCH_2$N), 2.75 (t, 2 H, $J_{H,H}$ = 7.6 Hz, $ArCH_2CH_2CH_2N$), 2.59 (bs, 4 H, CH_2Pip), 2.45 (m, 6 H, CH_2Pip, $ArCH_2CH_2CH_2N$), 1.91 (q, 2 H, $J_{H,H}$ = 7.6 Hz, $CH_2CH_2CH_2$). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 168.6 (NCOAr), 167.3 ($NCOCH_2$), 153.8 (C_qOCH_3), 137.6 (COC_q), 132.8 (CAr_{benz}), 131.6 (C_qNH_{Ind}), 130.2 ($CHAr_{benz}$), 130.1 ($CHAr_{benz}$), 127.5 (Cq_{ind}), 125.5 ($CHAr_{benz}$), 122.6 (CqBr), 122.1 ($CHAr_{Ind}$), 115.8 ($CHAr_{Ind}$), 111.9 ($C_{qInd}CH_2CH_2CH_2$), 111.8 ($CHAr_{Ind}$), 100.1 ($CHAr_{Ind}$), 60.6 ($COCH_2N$), 58.1 ($CH_2CH_2CH_2N$), 56.0 (OCH_3), 53.5 (2C, CH_2Pip), 53.0 (2C, CH_2Pip), 45.4 (2C, CH_2Pip), 41.7 (2C, CH_2Pip), 27.0 ($ArCH_2CH_2CH_2N$), 22.8 ($ArCH_2CH_2CH_2N$). HRMS: (ESI-TOF) *m/z* calcd. for $C_{29}H_{36}BrN_5O_3$: 581.2002. Found: 581.2796.

*4.1.2.32. 2-(4-(3-fluorobenzoyl)piperazin-1-yl)-1-(4-(3-(5-methoxy-1H-indol-3-yl)propyl) piperazin-1-yl)ethan-1-one **(34)**.* From 0.20 g (0.46 mmol) of compound **2j** and tosylate **C** were obtained 0.159 g (67%) of compound **34** as a pale brown oil. ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): = 8.16 (bs, 1 H, NH_{Ind}), 7.36 (m, 1 H, $CHAr_{benz}$), 7.21 (d, 1 H, $J_{H,H}$ = 8.9 Hz, $CHAr_{benz}$),7.15 (m, 1 H, $CHAr_{benz}$), 7.10 (m, 2 H, $CHAr_{benz}$, Ar_{ind}), 7.01 (d, 1 H, $J_{H,H}$ = 2.3 Hz, $CHAr_{Ind}$), 6.93 (bs, 1 H, $CHAr_{ind}$), 6.83 (dd, 1 H, $J_{H,H}$ = 2.3 Hz, $J_{H,H}$ = 8.8 Hz, $CHAr_{Ind}$), 3.84 (s, 3 H, OCH_3), 3.78 (bs, 2 H, CH_2Pip), 3.59 (m, 4 H, CH_2Pip), 3.43 (bs, 2 H, CH_2Pip), 3.22 (s, 2 H, $COCH_2$N), 2.74 (t, 2 H, $J_{H,H}$ = 7.3 Hz, $ArCH_2CH_2CH_2N$), 2.57 (bs, 2 H, CH_2Pip), 2.48 (bs, 2 H, CH_2Pip), 2.43 (m, 6 H, CH_2Pip, $ArCH_2CH_2CH_2N$), 1.89 (q, 2 H, $J_{H,H}$ = 7.6 Hz, $CH_2CH_2CH_2$). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 168.8 (NCOAr), 167.3 ($NCOCH_2$), 162.6 (d, $^1J_{C,F}$ = 243 Hz, CqF), 153.8 (C_qOCH_3), 137.9 (d, $^3J_{C,F}$ = 7.3 Hz, COC_q), 131.6 (C_qNH_{Ind}), 130.4 (d, $^3J_{C,F}$ = 7.8 Hz, $CHAr_{benz}$), 127.8 (Cq_{ind}), 122.7 (d, $^4J_{C,F}$ = 3.0 Hz, $CHAr_{benz}$), 122.1 ($CHAr_{Ind}$), 116.8 (d, $^2J_{C,F}$ = 22.3 Hz, $CHAr_{benz}$), 115.6 ($CHAr_{Ind}$), 114.3 (d, $^2J_{C,F}$ = 28.0 Hz, $CHAr_{benz}$), 111.8 ($C_{qInd}CH_2CH_2CH_2$), 111.8 ($CHAr_{Ind}$), 100.9 ($CHAr_{Ind}$), 60.6 ($COCH_2N$), 58.1 ($CH_2CH_2CH_2N$), 56.0 (OCH_3), 53.5 (2C, CH_2Pip), 53.0 (2C, CH_2Pip), 45.5 (2C, CH_2Pip), 41.8 (2C, CH_2Pip), 27.0 ($ArCH_2CH_2CH_2N$), 22.8 ($ArCH_2CH_2CH_2N$). HRMS: (ESI-TOF) *m/z* calcd. for $C_{29}H_{36}FN_5O_3$: 521.2802. Found: 521.2609.

*4.1.2.33. 2-(4-(2-bromobenzoyl)piperazin-1-yl)-1-(4-(3-(5-methoxy-1H-indol-3-yl)propyl) piperazin-1-yl)ethan-1-one **(35)**.* From 0.140 g (0.28 mmol) of compound **2k** and tosylate **C** were obtained 0.11 g (67%) of compound **35** as a pale brown solid. Mp.: 84–85 °C.; ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): = 7.97 (bs, 1 H, NH_{Ind}), 7.57 (m, 1 H, $CHAr_{benz}$), 7.35 (m, 1 H, $CHAr_{benz}$), 7.25 (m, 3 H, $CHAr_{ind}$, $CHAr_{benz}$), 7.02 (d, 1 H, $J_{H,H}$ = 2.3 Hz, Ar_{ind}), 6.95 (bs, 1 H, $CHAr_{Ind}$), 6.85 (dd, 1 H, $J_{H,H}$ = 8.8 Hz $J_{H,H}$, = 2.3 Hz, $CHAr_{ind}$), 3.86 (s, 3 H, OCH_3), 3.85 (m, 2 H, CH_2Pip), 3.62 (m, 4 H, CH_2Pip), 3.27 (m, 2 H, CH_2Pip), 3.22 (s, 2 H, $COCH_2$N), 2.75 (t, 2 H, $J_{H,H}$ = 7.5 Hz, $ArCH_2CH_2CH_2N$), 2.60 (m, 4 H, CH_2Pip), 2.44 (m, 6 H, CH_2Pip, $ArCH_2CH_2CH_2N$), 1.91 (q, 2 H, $J_{H,H}$ = 7.5 Hz, $CH_2CH_2CH_2$). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 167.6 (NCOAr), 167.3 ($NCOCH_2$), 154.0 (C_qOCH_{3Ind}), 137.8 (COC_q), 132.8 (CAr_{Benz}), 131.6 (C_qNH_{Ind}), 130.3 ($CHAr_{benz}$), 127.8 (Cq_{ind}), 127.7 ($CHAr_{benz}$), 127.0 ($CHAr_{benz}$), 122.1 ($CHAr_{Ind}$), 119.1 (CqBr), 115.7 ($CHAr_{Ind}$), 111.9 ($C_{qInd}CH_2CH_2CH_2$), 111.8 ($CHAr_{Ind}$), 100.1 ($CHAr_{Ind}$), 60.7 ($COCH_2N$), 58.1 ($CH_2CH_2CH_2N$), 56.0 (OCH_3), 53.5 (2C, CH_2Pip), 53.0 (2C, CH_2Pip), 45.4 (2C, CH_2Pip), 41.7 (2C, CH_2Pip), 27.0 ($ArCH_2CH_2CH_2N$), 22.8 ($ArCH_2CH_2CH_2N$). HRMS: (ESI-TOF) *m/z* calcd. for $C_{29}H_{36}BrN_5O_3$: 581.2002. Found: 581.2011.

4.2. Biological assays

4.2.1. Acetylcholinesterase inhibition assay

The inhibition of AChE by synthesized compounds was evaluated according to literature procedures [55]. Briefly, the assays were carried out in a total volume of 200 μL per well containing human blood plasma 180 μg protein/mL, DTNB 500 μM, synthesized compound in concentrations from 1 to 100 μM or DMSO 1% v/v (control), ATC 500 μM in Phosphate Buffer 50 mM pH 7.4. The general procedure was as follows: Human blood plasma, DTNB, and the synthesized compounds (dissolved in DMSO 1% v/v) in phosphate buffer 50 mM pH 7.4 were mixed and incubated at 37 °C for 15 min. After incubation, substrate ATC was added into the mixture. The reaction was recorded for 10 min measuring the absorbance at 412 nm using a Biotek Cytation5 multiplate reader. Data were plotted using GraphPad Prism 8.0 software (GraphPad Software Inc.) to obtain IC_{50} values. All values were expressed as the mean ± SD of three independent assays each in triplicate.

4.2.2. Radioligand binding assay at hSERT

Binding for hSERT was determined using [^{3}H]-paroxetine as radioligand and the clonal cell line HEK-293 that overexpresses SERT (PerkinElmer), as previously described [56]. Briefly, assays were carried out in a total volume of 250 μL Tris–HCl buffer, pH 7.4, 120 mM NaCl, 5 mM KCl, membrane solution (100 μL; 9.0 μg/per tube assay) and (50 μL, 2 nM) of [^{3}H]paroxetine and 100 μL of a compound solution in buffer at different concentrations (10^{-12}–10^{-5} M). After 30 min at 27 °C, incubations were terminated by rapid filtration, with three 3-mL washes of cold buffer, through Whatman GF/C filters that were presoaked with buffer containing 0.5% of polyethyleneimine, using a cell harvester (Brandel Instruments, Gaithersburg, MD). The radioactivity was measured by liquid scintillation spectrometry (MicroBeta 2450 microplate counter, PerkinElmer) with an efficiency of approximately 50%. Nonspecific binding for [^{3}H]-paroxetine was defined in the presence of 3 μM citalopram. Data were plotted using GraphPad Prism 8.0 software (GraphPad Software Inc.) to obtain IC_{50} values. All values were expressed as the mean ± SD of three independent assays each in triplicate.

4.2.3. Cell culture

Neuroblastoma (N2a) cells stably transfected with human APP695 were maintained in a medium containing 50% DMEM, 50%

Opti-MEM, supplemented with 5% fetal bovine serum, 200 mg/mL of G418, and Pen/Strep antibiotics (GIBCO). Two hundred thousand N2A/APP695 cells were seeded in 6 well plates and incubated for 16 h with 10 μM of selected compounds. Next, the conditioned media was analyzed by ELISA to measure Aβ40 levels.

4.2.4. ELISA

Supernatants of conditioned media from N2A/APP695 cells were diluted in buffer and incubated in a 96-well plate with Ab40 antibody for 3 h at room temperature. After washes of unbound material, a secondary antibody conjugated to horseradish peroxidase (HRP) was added for 30 min. Unbound secondary antibody was washed, and subsequently samples were incubated with a developing reagent for 30 min. Stop solution was added to block further reaction between HRP and the colorimetric substrate. An absorbance multiplate reader was used to quantify the colorimetric reaction at 450 nm.

4.2.5. Western blot

Cultured cells were lysed in a buffer composed of 50 mM Tris•HCl, pH 7.5, 150 mM NaCl, supplemented with 1% Triton X-100 and a protease inhibitor mixture (Complete-EDTA free; Roche). The cell lysates were disrupted with a probe-type sonicator for 10 s twice, centrifuged, and the protein levels in the supernatant measured by the BCA method. The samples were boiled in standard protein sample buffer, and subjected to SDS/PAGE followed by protein transfer onto a PVDF membrane and incubated overnight at 4 °C with the following antibodies: anti-APP (6E10, mouse monoclonal, 1:1000; Covance) for total APP and C99 and anti-actin (rabbit polyclonal, 1:1000; Santa Cruz).

4.2.6. MTT cell viability assay

The effect of synthesized compounds on HEK293 and SH-SY5Y cell lines was analyzed using MTT viability assay. Briefly, 1×10^5 cells/ml were seeded in a 96-well flat bottom plate and incubated overnight in Dulbecco's Modified Eagle Medium supplemented with 5% fetal bovine serum. After incubation, synthesized compounds were added in concentration from 0.1 to 100 μM or DMSO 1% v/v (control) and plates were incubated for 72 h. After incubation, medium was removed and 100 μl of 5 μg/ml MTT solution in non-supplemented medium. Plates were incubated for 4 h and then 100 μl of an acidic solution of SDS 10% was added to dissolve the formazan crystals. Absorbance was measured at a wavelength of 590, using a reference at 690 nm in a Cytation5 multiplate reader (Biotek). Data were plotted using GraphPad Prism 7.0 software (GraphPad Software Inc.) to obtain IC_{50} values. All values were expressed in mean ± SD of three independent assays in triplicate.

4.3. Molecular docking studies

4.3.1. Acetylcholinesterase

Docking simulations were carried out for the two most active derivatives **18** and **19** in their diprotonated states. Docking of other selected compounds in their diprotonated state and compounds **18** and **19** in their monoprotonated states was also performed (Supporting Information). The crystal structure of the recombinant human Acetylcholinesterase enzyme (rhAChE, PDB ID: 4EY7) protonated at pH 7.4 was used. The energetic minimization and protonation of the proposed compounds were carried out using the LigPrep tool in the program Maestro Schrödinger suite v.11.8 (Schrödinger, LLC). Docking studies were performed using AutoDock Vina following the standard docking procedure for rigid protein [74]. A grid of 26 × 26 × 26 points in the x, y, and z directions was built centered on the coordinates −14.86, −43.744, 29.5 (x,y,z) near the putative ligand-binding site with grid spacing of 0.1 Å. Default settings were used for all other parameters. Protein-ligand complexes were built using the lowest free-energy binding positions. The resulting structure files were analyzed using the Visual Molecular Dynamic (VMD) visualization program [75]. Validation of the docking protocol was preformed using the co-crystallized ligand Donepezil.

4.3.2. SERT

The energetic minimization and protonation of the proposed compounds were carried out using the LigPrep tool in the program Maestro Schrödinger suite v.11.8 (Schrödinger, LLC) For the induced molecular docking the crystallized serotonin transporter complex (hSERT) was used. The structure was obtained from the Protein Data Bank database (PDB: 5I73) [73]. The optimization was carried out using the Protein Preparation Wizard available in the Maestro program. The water molecules were removed and the hydrogen bonds corresponding to pH 7.0 were added. Appropriate ionization states for acid and basic amino acid residues were considered. The OPLS3e force field was used to minimize protein energy. The active site of h-SERT was defined as a 14 Å radius grid around the co-crystal (S-Citalopram) and the only restriction was the formation of a hydrogen bond with the COO group of the residue Glu494. This interaction is highly conserved in almost all known complexes between hSERT and related ligands [76]. The Glide Induced Fit Docking protocol [77] was used for the final coupling. This takes into account the flexibility of the compounds and the protein. In the first stage, a preliminary induced coupling was performed using the ligand coupling algorithm based on the energy grid (Glide v7.0, Schrödinger v.11.2). In the second stage, the iterative combination of rigid receptor couplings was carried out, as well as the remodeling of proteins by the lateral chain. Search and minimization techniques were applied using the Prime module [78]. The ligands were then re-coupled to the refined receptor structure using Glide. The molecules were punctuated by the Glide scoring function in the extra-precision mode (Glide XP; Schrödinger, LLC) [79]. Finally, the molecules were filtered on the basis of the best scores and best RMSD values (less than 1 unit as a cutting criterion).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work has been funded by FONDECYT Grant 1170269 (H.Pessoa-Mahana), Grant 1170662 (M. Reyes-Parada), FONDECYT Postdoctoral Grant 3170264 (J.Rodríguez-Lavado), FONDEQUIP EQM 160042, Conicyt-PFCHA/Doctorado Nacional/2018−21180427 and the CBIB-UNAB for the provision of software license (M. Lorca), and Fisher Foundation for Alzheimer's Research (V. Bustos). We also acknowledge Roberto Cighetti for graphical abstract design.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2020.112368.

References

[1] Alzheimer's Disease International, World Alzheimer Report 2019: Attitudes to Dementia, Alzheimer's Disease International, London, 2019.
[2] A.L. Gross, R.N. Jones, D.A. Habtemariam, T.G. Fong, D. Tommet, L. Quach,

E. Schmitt, L. Yap, S.K. Inouye, Delirium and long-term cognitive trajectory among persons with dementia, Arch. Intern. Med. 172 (2012) 1324–1331, https://doi.org/10.1001/archinternmed.2012.3203.
[3] M.W. Bondi, E.C. Edmonds, D.P. Salmon, L. Jolla, Alzheimer's disease: past, present, and future, J. Int. Neuropsychol. Soc. 23 (2017) 818–831, https://doi.org/10.1017/S135561771700100X.
[4] P. Scheltens, K. Blennow, M.M.B. Breteler, B. de Strooper, G.B. Frisoni, S. Salloway, W.M. Van der Flier, Alzheimer's disease, Lancet 388 (2016) 505–517, https://doi.org/10.1016/S0140-6736(15)01124-1.
[5] D. Mehta, R. Jackson, G. Paul, J. Shi, M. Sabbagh, Expet Opin. Invest. Drugs 26 (2017) 735–739, https://doi.org/10.1080/13543784.2017.1323868.
[6] E. Uliassi, A. Gandini, R.C. Perone, M.L. Bolognesi, Neuroregeneration versus neurodegeneration: toward a paradigm shift in Alzheimer's disease drug discovery, Future Med. Chem. 9 (2017) 995–1013, https://doi.org/10.4155/fmc-2017-0038.
[7] J. Cummings, C. Reiber, P. Kumar, The price of progress: funding and financing Alzheimer's disease drug development, Alzheimer's Dement. Transl. Res. Clin. Interv. 4 (2018) 330–343, https://doi.org/10.1016/j.trci.2018.04.008.
[8] K. McKeage, Memantine: a review of its use in moderate to severe alzheimers disease, CNS Drugs 23 (2009) 881–897, https://doi.org/10.2165/11201020-000000000-00000.
[9] R. Howard, R. McShane, J. Lindesay, C. Ritchie, A. Baldwin, R. Barber, A. Burns, T. Dening, D. Findlay, C. Holmes, A. Hughes, R. Jacoby, R. Jones, R. Jones, I. McKeith, A. Macharouthu, J. O'Brien, P. Passmore, B. Sheehan, E. Juszczak, C. Katona, R. Hills, M. Knapp, C. Ballard, R. Brown, S. Banerjee, C. Onions, M. Griffin, J. Adams, R. Gray, T. Johnson, P. Bentham, P. Phillips, Donepezil and memantine for moderate-to-severe Alzheimer's disease, N. Engl. J. Med. 366 (2012) 893–903, https://doi.org/10.1056/NEJMoa1106668.
[10] E. Koseoglu, New treatment modalities in Alzheimer's disease, World J. Clin. Cases 7 (2019) 1764–1774, https://doi.org/10.12998/wjcc.v7.i14.1764.
[11] M.S. Shilpa Parmar, Garima Verma, Mohemmed Faraz Khan, Wasim Akhtar, Syed Rashiduddin Haiderb, M. Mumtaz Alama, Razia Khatoon, Mymoona Akhter, Tarique Anwer, Ensuing new derivatives in developing therapeutics for alzheimer's disease - a review, Int. J. Pharmaceut. Sci. Rev. Res. 45 (2017) 113–125.
[12] M. Carreiras, E. Mendes, M. Perry, A. Francisco, J. Marco-Contelles, The multifactorial nature of Alzheimer's disease for developing potential therapeutics, Curr. Top. Med. Chem. 13 (2014) 1745–1770, https://doi.org/10.2174/1568026613139990135.
[13] A. Tarditi, A. Caricasole, G.C. Terstappen, Therapeutic targets for Alzheimer's disease, Expert Opin. Ther. Targets 13 (2009) 551–567, https://doi.org/10.1517/14728220902865614.
[14] S. Morales-navarro, L. Prent-peñaloza, Y.A. Rodr, S. Laura, O. Forero-doria, W. Gonz, N.E. Campilllo, Theoretical and Experimental Approaches Aimed at Drug Design Targeting Neurodegenerative Diseases, 2019.
[15] X. Du, X. Wang, M. Geng, Alzheimer's disease hypothesis and related therapies, Transl. Neurodegener. 7 (2018) 1–7, https://doi.org/10.1186/s40035-018-0107-y.
[16] J. Cummings, P.S. Aisen, B. Dubois, L. Frölich, C.R.J. Jr, R.W. Jones, J.C. Morris, J. Raskin, S.A. Dowsett, P. Scheltens, Drug development in Alzheimer ' s disease : the path to 2025, Alzheimer's Res. Ther. (2016) 1–12, https://doi.org/10.1186/s13195-016-0207-9.
[17] R. Vassar, M. Citron, Minireview A-generating enzymes: recent advances in β- and γ-secretase research is higher in neurons of the brain, Neuron 27 (2000) 419–422. https://www.cell.com/neuron/pdf/S0896-6273(00)00051-9.pdf.
[18] M.R. Brier, B. Gordon, K. Friedrichsen, J. McCarthy, A. Stern, J. Christensen, C. Owen, P. Aldea, Y. Su, J. Hassenstab, N.J. Cairns, D.M. Holtzman, A.M. Fagan, J.C. Morris, T.L.S. Benzinger, B.M. Ances, Tau and Ab imaging, CSF measures, and cognition in Alzheimer's disease, Sci. Transl. Med. 8 (2016) 1–10, https://doi.org/10.1126/scitranslmed.aaf2362.
[19] S. Gauthier, H.H. Feldman, L.S. Schneider, G.K. Wilcock, G.B. Frisoni, J.H. Hardlund, H.J. Moebius, P. Bentham, K.A. Kook, D.J. Wischik, B.O. Schelter, C.S. Davis, R.T. Staff, L. Bracoud, K. Shamsi, J.M.D. Storey, C.R. Harrington, C.M. Wischik, Efficacy and safety of tau-aggregation inhibitor therapy in patients with mild or moderate Alzheimer's disease: a randomised, controlled, double-blind, parallel-arm, phase 3 trial, Lancet 388 (2016) 2873–2884, https://doi.org/10.1016/S0140-6736(16)31275-2.
[20] T. Persson, B.O. Popescu, A. Cedazo-Minguez, Oxidative stress in alzheimer's disease: why did antioxidant therapy fail? Oxid. Med. Cell. Longev. 2014 (2014) https://doi.org/10.1155/2014/427318.
[21] M. Saresella, E. Calabrese, I. Marventano, F. Piancone, A. Gatti, M.G. Calvo, R. Nemni, M. Clerici, PD1 negative and PD1 positive CD4+ T regulatory cells in mild cognitive impairment and Alzheimer's disease, J. Alzheim. Dis. 21 (2010) 927–938, https://doi.org/10.3233/JAD-2010-091696.
[22] E.K. Perry, R.H. Perry, G. Blessed, B.E. Tomlinson, Lancet 22 (1997).
[23] P.T. Francis, A.M. Palmer, M. Snape, G.K. Wilcock, The cholinergic hypothesis of Alzheimer's disease: a review of progress, J. Neurol. Neurosurg. Psychiatry 66 (1999) 137–147, https://doi.org/10.1136/jnnp.66.2.137.
[24] J.R. Cirrito, B.M. Disabato, J.L. Restivo, D.K. Verges, W.D. Goebel, A. Sathyan, D. Hayreh, G. D'Angelo, T. Benzinger, H. Yoon, J. Kim, J.C. Morris, M.A. Mintun, Y.I. Sheline, Serotonin signaling is associated with lower amyloid-βlevels and plaques in transgenic mice and humans, Proc. Natl. Acad. Sci. U. S. A 108 (2011) 14968–14973, https://doi.org/10.1073/pnas.1107411108.
[25] C. Vakalopoulos, U. Rüb, Alzheimer's disease: the alternative serotonergic hypothesis of cognitive decline, J. Alzheim. Dis. 60 (2017) 859–866, https://doi.org/10.3233/JAD-170364.
[26] C. Haass, Take five - BACE and the γ-secretase quartet conduct Alzheimer's amyloid β-peptide generation, EMBO J. 23 (2004) 483–488, https://doi.org/10.1038/sj.emboj.7600061.
[27] J.M. Long, B. Maloney, J.T. Rogers, D.K. Lahiri, Novel upregulation of amyloid-β precursor protein (APP) by microRNA-346 via targeting of APP mRNA 5′-untranslated region: implications in Alzheimer's disease, Mol. Psychiatr. 24 (2019) 345–363, https://doi.org/10.1038/s41380-018-0266-3.
[28] M.S. Brown, J. Ye, R.B. Rawson, J.L. Goldstein, Regulated Intramembrane Proteolysis: review A Control Mechanism Conserved from Bacteria to Humans includes the SREBPs (sterol regulatory element-binding proteins), transmembrane proteins of the ER whose cy-tosolic transcription factor domains are liberate, Cell 100 (2000) 391–398.
[29] S. Krishnaswamy, G. Verdile, D. Groth, L. Kaneyenda, The structure and function of Alzheimer's gamma secretase enzyme complex, Crit. Rev. Clin. Lab Sci. 46 (2009) 282–301, https://doi.org/10.3109/10408360903335821.
[30] Z. Wu, Z. Guo, M. Gearing, G. Chen, model, Nat. Commun. 5 (2014) 1–12, https://doi.org/10.1038/ncomms5159.
[31] O. Ambrée, H. Richter, N. Sachser, L. Lewejohann, E. Dere, M.A. de Souza Silva, A. Herring, K. Keyvani, W. Paulus, W.R. Schäbitz, Levodopa ameliorates learning and memory deficits in a murine model of Alzheimer's disease, Neurobiol. Aging 30 (2009) 1192–1204, https://doi.org/10.1016/j.neurobiolaging.2007.11.010.
[32] P.S. Goldman-rakic, P. Rakic, Dopamine D2 receptor, Sci. Exch. 6 (2013) 39, https://doi.org/10.1038/scibx.2013.39.
[33] F. Lezoualc'h, 5-HT4 receptor and Alzheimer's disease: the amyloid connection, Exp. Neurol. 205 (2007) 325–329, https://doi.org/10.1016/j.expneurol.2007.02.001.
[34] B. Lasheras, Differential Involvement of 5-HT 1B/1D and 5-HT 6 Receptors in Cognitive and Non-cognitive Symptoms in Alzheimer ' S Disease, 2004, pp. 410–416, https://doi.org/10.1038/sj.npp.1300330.
[35] J. Lalut, D. Karila, P. Dallemagne, C. Rochais, Modulating 5-HT4 and 5-HT6 receptors in Alzheimer's disease treatment, Future Med. Chem. 9 (2017) 781–795, https://doi.org/10.4155/fmc-2017-0031.
[36] R. Mössner, A. Schmitt, Y. Syagailo, M. Gerlach, P. Riederer, K.P. Lesch, The serotonin transporter in Alzheimer's and Parkinson's disease, J. Neural. Transm. Suppl. (2000) 345–350.
[37] P. Esteve, J. Rueda-carrasco, M.I. Mateo, M.J. Martin-bermejo, J. Draffin, G. Pereyra, Á. Sandonís, I. Crespo, I. Moreno, E. Aso, P. Garcia-esparcia, E. Gomez-tortosa, A. Rábano, J. Fortea, D. Alcolea, A. Lleo, M.T. Heneka, J.M. Valpuesta, J.A. Esteban, I. Ferrer, M. Domínguez, P. Bovolenta, Elevated levels of Secreted-Frizzled-Related- pathogenesis, Nat. Neurosci. 22 (2019), https://doi.org/10.1038/s41593-019-0432-1.
[38] M.V. Pulina, M. Hopkins, V. Haroutunian, P. Greengard, V. Bustos, C99 selectively accumulates in vulnerable neurons in Alzheimer's disease, Alzheimer's Dementia (2019) 1–11, https://doi.org/10.1016/j.jalz.2019.09.002.
[39] S. Kwak, J.H. Weiss, Calcium-permeable AMPA channels in neurodegenerative disease and ischemia, Curr. Opin. Neurobiol. 16 (2006) 281–287, https://doi.org/10.1016/j.conb.2006.05.004.
[40] H. Fukumoto, B.S. Cheung, B.T. Hyman, M.C. Irizarry, β-secretase protein and activity are increased in the neocortex in Alzheimer disease, Arch. Neurol. 59 (2002) 1381–1389, https://doi.org/10.1001/archneur.59.9.1381.
[41] C. Dingwall, Spotlight on BACE: the secretases as targets for treatment in Alzheimer disease, J. Clin. Invest. 108 (2001) 1243–1246, https://doi.org/10.1172/JCI14402.
[42] A. Talevi, C.L. Bellera, M. Di Ianni, M. Gantner, E.A. Castro, CNS drug development – lost in translation? Mini Rev. Med. Chem. 12 (2012) 959–970, https://doi.org/10.2174/138955712802762356.
[43] A. Koeberle, O. Werz, Multi-target approach for natural products in inflammation, Drug Discov. Today 19 (2014) 1871–1882, https://doi.org/10.1016/j.drudis.2014.08.006.
[44] A. Talevi, Multi-target pharmacology: possibilities and limitations of the "skeleton key approach" from a medicinal chemist perspective, Front. Pharmacol. 6 (2015) 1–7, https://doi.org/10.3389/fphar.2015.00205.
[45] F. Mesiti, D. Chavarria, A. Gaspar, S. Alcaro, F. Borges, The chemistry toolbox of multitarget-directed ligands for Alzheimer's disease, Eur. J. Med. Chem. 181 (2019) 111572, https://doi.org/10.1016/j.ejmech.2019.111572.
[46] C. Rochais, C. Lecoutey, F. Gaven, P. Giannoni, K. Hamidouche, D. Hedou, E. Dubost, D. Genest, S. Yahiaoui, T. Freret, V. Bouet, F. Dauphin, J.S. De Oliveira Santos, C. Ballandonne, S. Corvaisier, A. Malzert-Fréon, R. Legay, M. Boulouard, S. Claeysen, P. Dallemagne, Novel multitarget-directed ligands (MTDLs) with acetylcholinesterase (AChE) inhibitory and serotonergic subtype 4 receptor (5-HT_4R) agonist activities as potential agents against alzheimer's disease: the design of donecopride, J. Med. Chem. 58 (2015) 3172–3187, https://doi.org/10.1021/acs.jmedchem.5b00115.
[47] H. Zheng, M. Fridkin, M. Youdim, From single target to multitarget/network therapeutics in Alzheimer's therapy, Pharmaceuticals 7 (2014) 113–135, https://doi.org/10.3390/ph7020113.
[48] Y. Wang, H. Wang, H. Chen, AChE inhibition-based multi-target-directed ligands, a novel pharmacological approach for the symptomatic and disease-modifying therapy of alzheimer's disease, Curr. Neuropharmacol. 14 (2016) 364–375, https://doi.org/10.2174/1570159x14666160119094820.
[49] T. Heinrich, H. Böttcher, R. Gericke, G.D. Bartoszyk, S. Anzali, C.A. Seyfried, H.E. Greiner, C. Van Amsterdam, Synthesis and structure-activity relationship in a class of indolebutylpiperazines as dual 5-HT1A receptor agonists and

serotonin reuptake inhibitors, J. Med. Chem. 47 (2004) 4684–4692, https://doi.org/10.1021/jm040793q.
[50] M.N. Modica, S. Intagliata, V. Pittalà, L. Salerno, M.A. Siracusa, A. Cagnotto, M. Salmona, G. Romeo, Synthesis and binding properties of new long-chain 4-substituted piperazine derivatives as 5-HT1A and 5-HT7 receptor ligands, Bioorg. Med. Chem. Lett 25 (2015) 1427–1430, https://doi.org/10.1016/j.bmcl.2015.02.042.
[51] J. Reis, F. Cagide, M.E. Valencia, J. Teixeira, D. Bagetta, C. Pérez, E. Uriarte, P.J. Oliveira, F. Ortuso, S. Alcaro, M.I. Rodríguez-Franco, F. Borges, Multi-target-directed ligands for Alzheimer's disease: discovery of chromone-based monoamine oxidase/cholinesterase inhibitors, Eur. J. Med. Chem. 158 (2018) 781–800, https://doi.org/10.1016/j.ejmech.2018.07.056.
[52] C. Oliveira, D. Bagetta, F. Cagide, J. Teixeira, R. Amorim, T. Silva, J. Garrido, F. Remião, E. Uriarte, P.J. Oliveira, S. Alcaro, F. Ortuso, F. Borges, Benzoic acid-derived nitrones: a new class of potential acetylcholinesterase inhibitors and neuroprotective agents, Eur. J. Med. Chem. 174 (2019) 116–129, https://doi.org/10.1016/j.ejmech.2019.04.026.
[53] S. Ghanei-Nasab, M. Khoobi, F. Hadizadeh, A. Marjani, A. Moradi, H. Nadri, S. Emami, A. Foroumadi, A. Shafiee, Synthesis and anticholinesterase activity of coumarin-3-carboxamides bearing tryptamine moiety, Eur. J. Med. Chem. 121 (2016) 40–46, https://doi.org/10.1016/j.ejmech.2016.05.014.
[54] D. Osmaniye, B.N. Saglık, U.A. Çevik, S. Levent, B.K. Çavusoglu, Y. Özkay, Z.A. Kaplancıklı, G. Turan, Synthesis and AChE inhibitory activity of novel thiazolylhydrazone derivatives, Molecules 24 (2019) 2932, https://doi.org/10.3390/molecules24132392.
[55] C. Méndez-Rojas, G. Quiroz, M. Faúndez, C. Gallardo-Garrido, C.D. Pessoa-Mahana, H. Chung, E. Gallardo-Toledo, C. Saitz-Barría, R. Araya-Maturana, M.J. Kogan, M.C. Zúñiga-López, P. Iturriaga-Vásquez, C. Valenzuela-Gutiérrez, H. Pessoa-Mahana, Synthesis and biological evaluation of potential acetylcholinesterase inhibitors based on a benzoxazine core, Arch. Pharm. (Weinheim) 351 (2018) 1–17, https://doi.org/10.1002/ardp.201800024.
[56] H. Pessoa-Mahana, U. Núñez, R. Araya-Maturana, C. Saitz, G. Zapata-torres, C.D. Pessoa-Mahana, P. Iturriaga-Vasquez, J. Mella-Raipán, M. Reyes-Parada, C. Celis-Barros, Studies of 3- [3- (4-Aryl-1-piperazinyl) -propyl] -1 H -indole derivatives, Chem. Pharm. Bull. 60 (2012) 632–638, https://doi.org/10.1248/cpb.60.632.
[57] C. Ojeda-gómez, H. Pessoa-mahana, P. Iturriaga-Vásquez, C.D. Pessoa-Mahana, G. Recabarren-Gajardo, C. Méndez-Rojas, Synthesis and biological screening of novel indolalkyl arenes targeting the serotonine transporter, Arch. Pharm. Chem. Life Sci. 347 (2014) 174–184, https://doi.org/10.1002/ardp.201300321.
[58] H. Pessoa-Mahana, C. González-Lira, A. Fierro, G. Zapata-Torres, C.D. Pessoa-Mahana, J. Ortiz-Severin, P. Iturriaga-Vásquez, M. Reyes-Parada, P. Silva-Matus, C. Saitz-Barría, R. Araya-Maturana, Synthesis, docking and pharmacological evaluation of novel homo- and hetero-bis 3-piperazinylpropylindole derivatives at SERT and 5-HT1A receptor, Bioorg. Med. Chem. 21 (2013) 7604–7611, https://doi.org/10.1016/j.bmc.2013.10.036.
[59] H. Pessoa-Mahana, P. Silva-Matus, C.D. Pessoa-Mahana, H. Chung, P. Iturriaga-Vásquez, G. Quiroz, P. Möller-Acuña, G. Zapata-Torres, C. Saitz-Barría, R. Araya-Maturana, M. Reyes-Parada, Synthesis and docking of novel 3-indolylpropyl derivatives as new polypharmacological agents displaying affinity for 5-HT 1A R/SERT, Arch. Pharm. (Weinheim) 350 (2017) 1–15, https://doi.org/10.1002/ardp.201600271.
[60] F.J. Pérez-Areales, A.L. Turcu, M. Barniol-Xicota, C. Pont, D. Pivetta, A. Espargaró, M. Bartolini, A. De Simone, V. Andrisano, B. Pérez, R. Sabate, F.X. Sureda, S. Vázquez, D. Muñoz-Torrero, A novel class of multitarget anti-Alzheimer benzohomoadamantane–chlorotacrine hybrids modulating cholinesterases and glutamate NMDA receptors, Eur. J. Med. Chem. 180 (2019) 613–626, https://doi.org/10.1016/j.ejmech.2019.07.051.
[61] J. Jiang, C.J. Seel, A. Temirak, V. Namasivayam, A. Arridu, J. Schabikowski, Y. Baqi, S. Hinz, J. Hockemeyer, C.E. Müller, A 2B adenosine receptor antagonists with picomolar potency, J. Med. Chem. 62 (2019) 4032–4055, https://doi.org/10.1021/acs.jmedchem.9b00071.
[62] Y. Shan, J. Dong, X. Pan, L. Zhang, J. Zhang, Y. Dong, M. Wang, Expanding the structural diversity of Bcr-Abl inhibitors: dibenzoylpiperazin incorporated with 1 H -indazol-3-amine, Eur. J. Med. Chem. 104 (2015) 139–147, https://doi.org/10.1016/j.ejmech.2015.09.034.
[63] F. Liu, S. Dawadi, K.M. Maize, R. Dai, S.W. Park, D. Schnappinger, B.C. Finzel, C.C. Aldrich, Structure-based optimization of pyridoxal 5′-phosphate-dependent transaminase enzyme (BioA) inhibitors that target biotin biosynthesis in Mycobacterium tuberculosis, J. Med. Chem. 60 (2017) 5507–5520, https://doi.org/10.1021/acs.jmedchem.7b00189.
[64] R. Wodtke, J. Steinberg, M. Köckerling, R. Löser, C. Mamat, NMR-based investigations of acyl-functionalized piperazines concerning their conformational behavior in solution, RSC Adv. 8 (2018) 40921–40933, https://doi.org/10.1039/C8RA09152H.
[65] X. Pan, J. Dong, Y. Shi, R. Shao, F. Wei, J. Wang, J. Zhang, Discovery of novel Bcr-Abl inhibitors with diacylated piperazine as the flexible linker, Org. Biomol. Chem. 13 (2015) 7050–7066, https://doi.org/10.1039/c5ob00430f.
[66] D.A. Evrard, P. Zhou, S.Y. Yi, D. Zhou, D.L. Smith, K.M. Sullivan, G.A. Hornby, L.E. Schechter, T.H. Andree, R.E. Mewshaw, Studies towards the next generation of antidepressants . Part 4 : Deriv. 4- (5-fluoro-1 H -indol-3-yl) cyclohexylamine with affinity for the serotonin transporter and the 5-HT 1A receptor, 15, 911-914, https://doi.org/10.1016/j.bmcl.2004.12.064, 2005.
[67] G. Kryger, I. Silman, J.L. Sussman, Structure of acetylcholinesterase complexed with E2020 (Aricept®): implications for the design of new anti-Alzheimer drugs, Structure 7 (1999) 297–307, https://doi.org/10.1016/S0969-2126(99)80040-9.
[68] J. Cheung, M.J. Rudolph, F. Burshteyn, M.S. Cassidy, E.N. Gary, J. Love, M.C. Franklin, J.J. Height, Structures of human acetylcholinesterase in complex with pharmacologically important ligands, J. Med. Chem. 55 (2012) 10282–10286, https://doi.org/10.1021/jm300871x.
[69] C. Bartolucci, J. Stojan, Q.S. Yu, N.H. Greig, D. Lamba, Kinetics of Torpedo californica acetylcholinesterase inhibition by bisnorcymserine and crystal structure of the complex with its leaving group, Biochem. J. 444 (2012) 269–277, https://doi.org/10.1042/BJ20111675.
[70] A.S. Mahadevi, G.N. Sastry, Cation-π interaction: its role and relevance in chemistry, biology, and material science, Chem. Rev. 113 (2013) 2100–2138, https://doi.org/10.1021/cr300222d.
[71] L.K. Tsou, C.D. Tatko, M.L. Waters, Simple cation-π interaction between a phenyl ring and a protonated amine stabilizes an α-helix in water, J. Am. Chem. Soc. 124 (2002) 14917–14921, https://doi.org/10.1021/ja026721a.
[72] Y. Bourne, P. Taylor, Z. Radić, P. Marchot, Structural insights into ligand interactions at the acetylcholinesterase peripheral anionic site, EMBO J. 22 (2003) 1–12, https://doi.org/10.1093/emboj/cdg005.
[73] J.A. Coleman, E.M. Green, E. Gouaux, X-ray structures and mechanism of the human serotonin transporter, Nature 532 (2016) 334–339, https://doi.org/10.1038/nature17629.
[74] O. Trott, A.J. Olson, AutoDock Vina, Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading, J. Comput. Chem. 31 (2009), https://doi.org/10.1002/jcc.21334. NA-NA.
[75] W. Humphrey, A. Dalke, K. Schulten, VMD: visual molecular dynamics, J. Mol. Graph. 14 (1996) 33–38, https://doi.org/10.1016/0263-7855(96)00018-5.
[76] C.L. Morales-Perez, C.M. Noviello, R.E. Hibbs, X-ray structure of the human α4β2 nicotinic receptor, Nature 538 (2016) 411–415, https://doi.org/10.1038/nature19785.
[77] W. Sherman, T. Day, M.P. Jacobson, R.A. Friesner, R. Farid, Novel procedure for modeling ligand/receptor induced fit effects, J. Med. Chem. 49 (2006) 534–553, https://doi.org/10.1021/jm050540c.
[78] M.P. Jacobson, R.A. Friesner, Z. Xiang, B. Honig, On the role of the crystal environment in determining protein side-chain conformations, J. Mol. Biol. 320 (2002) 597–608, https://doi.org/10.1016/S0022-2836(02)00470-9.
[79] R.A. Friesner, R.B. Murphy, M.P. Repasky, L.L. Frye, J.R. Greenwood, T.A. Halgren, P.C. Sanschagrin, D.T. Mainz, Extra precision glide: docking and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes, J. Med. Chem. 49 (2006) 6177–6196, https://doi.org/10.1021/jm051256o.