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Synthesis and evaluation of bidentate ligands designed to interact with PDZ domains

Benjamin Boucherle [a], Alexandre Vogrig [a], Hemantkumar Deokar [a], Naoual Bouzidi [a], Isabelle Ripoche [b], Isabelle Thomas [b], Philippe Marin [c], Sylvie Ducki [b,*]

[a] Clermont Université, UBP, EA 987, LCHG, BP 10448, F-63000 Clermont-Ferrand, France
[b] Clermont Université, ENSCCF, EA 987, LCHG, BP 10448, F-63000 Clermont-Ferrand, France
[c] IGF, CNRS UMR5203, INSERM U661,Universités Montpellier 1 & 2, F-34094 Montpellier, France

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ABSTRACT

We designed bidentate ligands to target PDZ domains through two binding sites: site S0, delimited by the GLGF loop, and site S1, a zone situated around loop β_B/β_C. A molecular docking study allowed us to design a generic S0 binder, to which was attached a variable size linker, itself linked to an amino acid aimed to interact with the S1 site of PDZ domains. A series of 15 novel bidentate ligands was prepared in 6–11 steps in good overall yield (24–43%). Some of these ligands showed an inhibitory activity against serotonin 5-HT2A receptor/PSD-95 interaction. This was assessed by pull-down assay using a synthetic decapeptide corresponding to the C-terminal residues of the receptor as a bait.

1. Introduction

Since their discovery two decades ago, 214 human proteins containing Post-synaptic density 95/Disc-Large/Zona ocludens-1 (PDZ) domains have been identified.[1] PDZ domains are important protein–protein interactions domains; they are almost always associated with cell membrane proteins where they play an essential role in the clustering of proteins and signal transduction.[2]

PDZ domains consist of ~90 residues folded into a highly conserved secondary structure which interacts with the C-terminus of a PDZ ligand (Fig. 1). These interactions involve extreme carboxy-terminal of ligand proteins binding to a hydrophobic groove between α_B helix and β_B strand (S0 pocket), ended by GLGF loop of the PDZ domain[3], which is the most conserved pocket of the PDZ domains.

These PDZ ligands belong to one of three classes: Class I (-X-S/T-X-V/I/F) have a Ser or Thr at position (−2) which interacts with a conserved basic residue at α_{B1} of the PDZ domain; those PDZ domains that lack the basic residue at α_{B1} bind ligands belonging to class II (-X-F/Y-X-V/I/F), where a hydrophobic residue, such as Phe or Tyr, at position (−2) was found to interact with hydrophobic

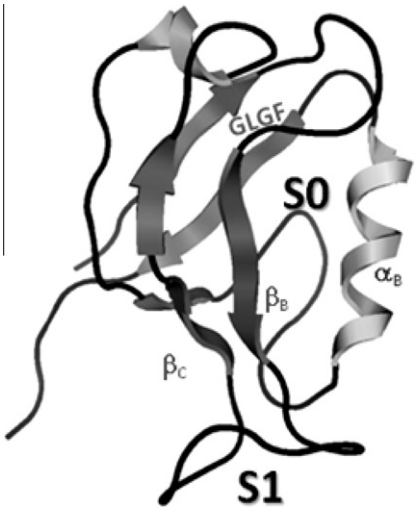

Figure 1. Cartoon representation of the backbone of the 3D structure of PDZ1 domain of PSD95.

Abbreviations: CDI, carbonyldiimidazole; Cyc, cyclohexane; DABCO, diazabicyclooctane; DCM, dichloromethane; DIEA, diisopropylethylamine; DMAP, 4-dimethylaminopyridine; DMF, N,N-dimethylformamide; EDC, 1-ethyl-3-(3-dimethylamino propyl)carbodiimide; EtOAc, ethylacetate; EtOH, ethanol; FC, flash chromatography; HOBT, hydroxybenzotriazole; MeOH, methanol; RP, reverse phase; RT, room temperature; TFA, trifluoroacetic acid; THF, tetrahydrofuran.

* Corresponding author. Tel.: +33 (0)473407132; fax: +33 (0)473407008.
E-mail address: sylvie.ducki@univ-bpclermont.fr (S. Ducki).

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residues at α_{B1}, while the third class of ligands (-X-D/E/K/R-X-V/I/L/F) includes ligands which have a preference for negatively charged residues (−2) and a Tyr at α_{B1} of the PDZ domain.[4]

Structural studies have recently identified a less conserved area involved in interactions between PDZ domains and their ligand proteins, situated upstream of the conserved groove. It seems that binding specificity could be attributed to the β_B/β_C loop, herein named S1 site.[5,6]

Several PDZ domain-containing proteins (PDZ proteins) have been associated with pathological states (cancer,[7–9] cystic fibrosis,[10,11] schizophrenia,[12] Parkinson's disease,[13] Alzheimer's disease,[4] cerebral ischemia,[14] pain[15,16]) and the therapeutic usefulness of inhibiting PDZ-based protein-protein interactions has been clearly demonstrated by using peptide and nonpeptide small molecules .[14,16,17] Recently, reports have emerged on nonpeptide inhibition of PDZ domain-based interactions with small organic molecules. In 2003, Fujii et al. identified indole **A**[18] (Fig. 2) as a selective inhibitor of the PDZ2 domain of MAGI3, an adaptor protein important in the regulation of tissue organisation and differentiation.

Docking experiments revealed that this indole overlapped the C-terminal tetrapeptide of PTEN, a known PDZ ligand of MAGI3, with its hydroxymethyl moiety covalently bound to a His on α_B and the carboxylic acid interacting with the GLGF loop. Fujii later used the indole core to design other PDZ inhibitors.[19] Indole **B** disrupts the interaction between the Frizzled-7 Wnt receptor (Frz-7) and Dvl while indole **C** targets the PDZ domain of NHERF1-PDZ1,[20] an adaptor protein crucial in the recycling and sorting of several receptors including β_2-adrenergic receptor (β2AD-R)[21], cystic fibrosis transmembrane conductance regulator (CFTR),[11,22,23] platelet-derived (PDGFR)[24] and epidermal growth factor receptors (EGFR).[25] The carboxylic acid (COOH) on the side-chain at position 2 was introduced to mimic an aspartate residue Asp(−3) in the PDZ ligand, granting its affinity.

PSD-95 is a three PDZ-domains containing protein identified as a major partner of various membrane receptors including 5-HT2A receptor.[26] Disrupting 5-HT2A receptor/PSD-95 interaction was found to reduce hyperalgesia in a rodent model of neuropathic pain.[16] Thus, inhibiting the interaction between PSD-95 and 5-HT2A receptor could lead to the development of a novel class of analgesic agents.

Our strategy aims at targetting selectively the PDZ domain via the extended groove between β_B and α_B delimited by the GLGF loop, which represents a challenge in terms of selectivity since this is the most conserved pocket of the PDZ domains herein named S0 site.

We now wish to report our recent work in the design of PDZ inhibitors using a bidentate ligand approach which would be able to interact both with the highly conserved (S0) pocket and the less conserved (S1) loop (Fig. 1). Our bidentate ligands were conceived from an indole core **D**, inspired from Fujii's work cited above, to interact with the S0 site of the PDZ domain, and an amino acid, chosen to probe specific interactions with the S1 site (Fig. 3). In order to simplify the synthesis, the aromatic methyl was removed, the carboxylic acid was kept to interact with the GLGF loop (conserved in all PDZ domains), a methyl group was added on position 3 to allow interactions with hydrophobic residues on α_B and a second carboxylic acid function was added at position 2 of the indole to allow H-bonding with residues on α_B of PDZ domains. Both ligands were connected via a linker of variable length to allow spatial exploration.

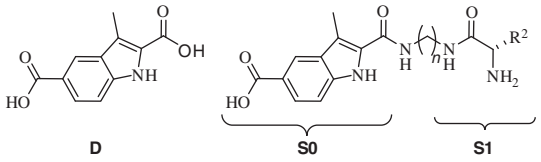


Figure 3. Design of bidentate ligands.

2. Results and discussion

2.1. Design of bidentate ligands

The purpose of the docking was to validate our concept of bidendate ligand, particularly to evaluate the interactions of the indole core into the S0 site and to simulate the possible interactions with the S1 site.

The molecular docking study was carried out using Molecular Operating Environment software.[27] We docked compound **13b** possessing the indole moiety into the active site (Fig. 4). Compound **13b** was found to dock in the same binding site as the cypin peptide ligand. When the best docking pose was visualized, it was observed that this compound fitted in well within the PDZ domain and the whole molecule made interactions with the protein: H-bonds interaction with Phe17, Ser18, Ile19 residues and other interactions (hydrophobic, electrostatic, vdW and columbic interactions) were found with the following amino acid residues Gly14, Leu15, Gly16, Phe17, Ser18, Ile19, His70, Val74 and Leu77. For the best pose, the total interaction energy is −7.73 kcal/mol. Considering the spatial orientation of **13b** within the S0 site, we simulated the interactions of amino acids linked to the indole residue with the S1 loop. Unfortunately due to the high flexibility of the S1 loop, these simulations were considered of low predictability value and are not presented here.

2.2. Synthesis of bidentate ligands

Based on the docking model, we synthesized 15 novel bidentate ligands designed to explore whether it is possible to inhibit interaction between PSD-95 and 5-HT2A. These bidentate ligands were prepared in 6–11 steps from **1**. Iodination of **1** led to ethyl-4-amino-3-iodobenzoate **2**,[28,29] which gave the desired indole **3a** by a

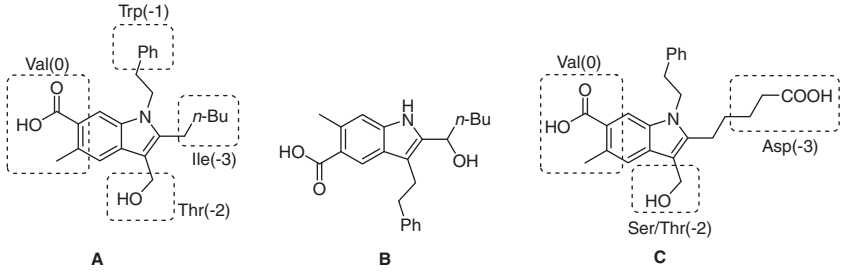


Figure 2. Fujii's designed indoles.

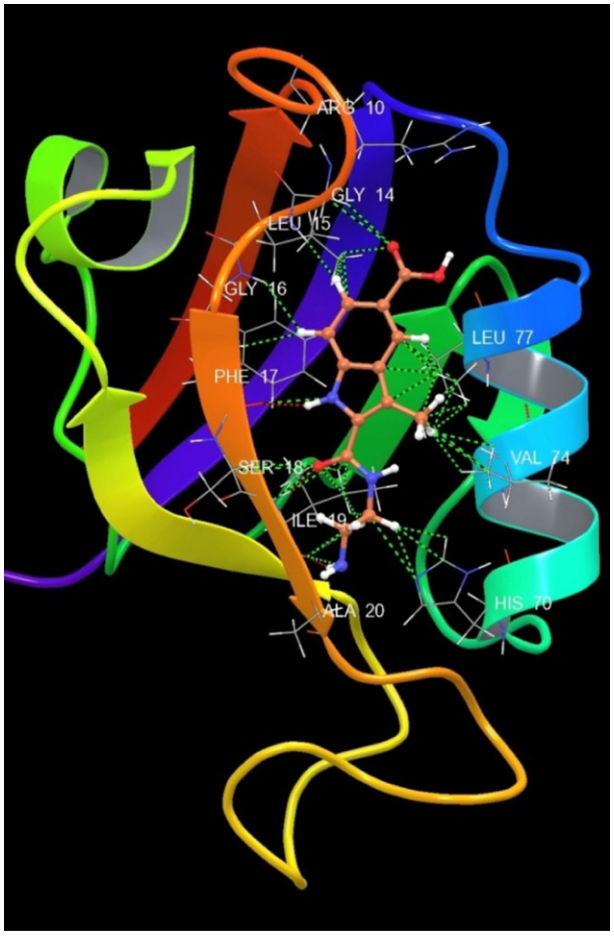


Figure 4. Docking of compound **13b** into PDZ1 domain of PSD-95 showing H-bond interactions (red color) and other interactions (green color).

palladium-catalyzed annulation with an overall yield of 56% for two steps (Scheme 1).[20,30,31]

Next, we use aliphatic diamines with variable chain lengths (number of carbons n = 2, 4, 6 and 8), to allow spatial probing of the S1 site. These diamines N-Boc mono protected[32–34] were linked by amide bond formation to the 2-carboxylic acid moiety of indole **3a**. Optimisation of the coupling conditions led us to use EDC and HOBT to attach the monoprotected diamines to indole **3a** (Scheme 2).[35,36] Boc-deprotection was performed in the presence of trifluoroacetic (TFA)[37] and gave **4a**–**c** which were isolated with good yields of 85–90% (over two steps).

Acid **3a** and ammonium salt **4b** were treated with sodium hydroxide to give the generic ligand **5** and free C6-ligand **6** in good yields. Boc deprotection gave **4a**–**c** in 85–90% yield.

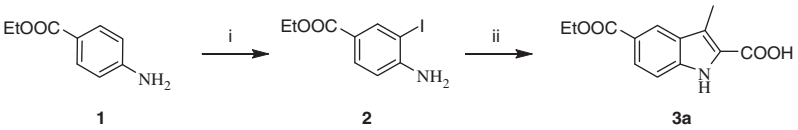
Amino acids with different physicochemical properties were selected to graft onto amines **4b**: Ile **18a** (hydrophobic), Lys **18b** (polar, charged) Trp **18c** (hydrophobic, steric hindrance), Phe **18d** (hydrophobic, steric hindrance), Glu **18e** (polar, uncharged), in order to probe potential interactions with the S1 site.

The protected amino acids **18a**–**e** were coupled to the C6-amine **4b** (Scheme 3).[37,38] Bidentate ligands **7a**–**e** were deprotected to afford the desired deprotected C6-bidentate ligands **8a**–**e** with good yields. When this protocol was applied to the C8 analogue **4a**, we encountered solubility problems of the intermediates which forced us to abandon the synthesis of this serie.

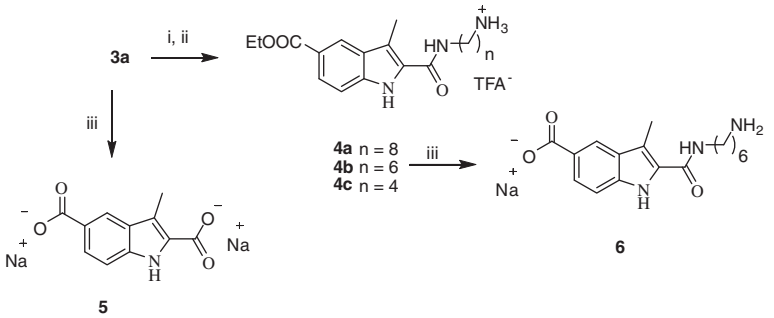
In the same manner, the protected amino acids **18a**–**e** were coupled to the C4-amine **4c** to afford protected C4-bidentate ligands. However, when the deprotection under basic conditions was attempted on the latter, we mainly obtained the amine **4c**. Even under milder conditions (LiOH in THF)[39] we were unable to obtain the desired C4-bidentate ligands.

We therefore envisaged a new synthesis of the indole core with a benzyl ester protection of the acid moiety. Aniline **2** (Scheme 4) was Boc-protected and saponified to afford acid **9**. The latter was Bn-protected and Boc-deprotected to recover the free amine **11**.[40] Finally, amine **11** was reacted with 2-oxobutanoic acid to give Bn-protected indole **3b** with a overall good yield (65% over five steps).

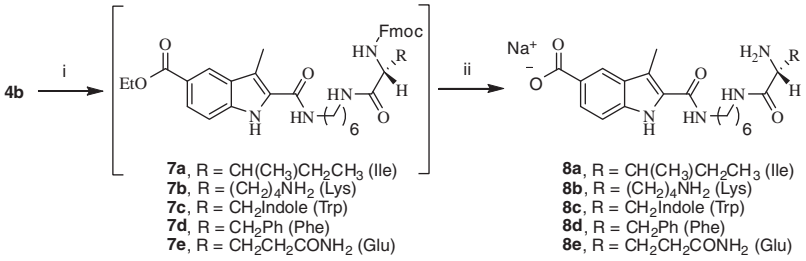
We pursued the synthesis of the C2- and C4-bidentate ligands using the previously optimized coupling conditions (Scheme 5). Indole **3b** was coupled to the N-Boc C2- and C4-diamines, followed by amine deprotection with TFA to afford ammonium **12a** and **12b**. The ammonium **12a**–**b** were then treated with hydrogen over Pd/C catalyst to give C2- and C4-ligands **13a** and **13b**. To avoid basic conditions to remove protecting group of the amino acids, we envisaged to protect the amino acids with a Boc or CBz. To



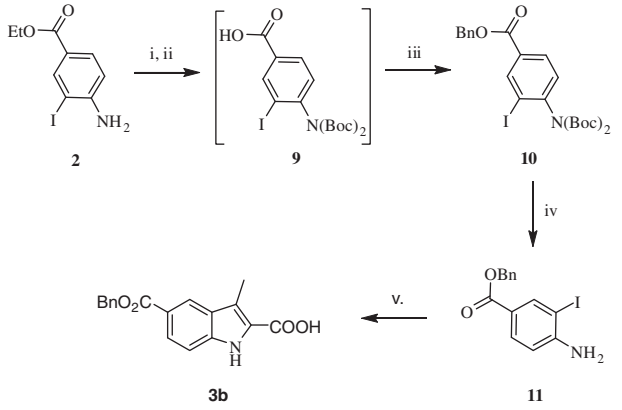
Scheme 1. Reagents and conditions: (a) I_2, Ag_2SO_4, EtOH, RT, 30 min (85%); (b) 2-oxobutanoic acid, $Pd(OAc)_2$, DABCO, DMF, 105 °C, 16 h (65%).



Scheme 2. Reagents and conditions: (a) H_2N–$(CH_2)_n$–NHBoc, EDC, HOBT, DMF, 0 °C to RT, overnight; (b) TFA, DCM, RT, 2 h (**4a** n = 8, 85%; **4b** n = 6, 85%; **4c** n = 4, 90%; over two steps); (c) NaOH, EtOH/H_2O/dioxane (**5**: 80%, **6**: >99%).



Scheme 3. Reagents and conditions: (a) Fmoc-N-protected-amino acid **18a–e**, CDI, DIEA, DMF, 0 °C to RT, 5 h; (b) NaOH, H_2O/EtOH, RT, 24 h (**8a**: 58%, **8b**: 51%, **8c**: 50%, **8d**: 51%, **8e**: 46% over two steps).



Scheme 4. Reagents and conditions: (a) $(Boc)_2O$, Et_3N, DMAP, DCM, RT, 1 h (>99%); (b) NaOH 10 M, EtOH/H_2O, RT, 12 h; (c) CDI, BnOH, DMF, RT, 24 h (95%); (d) TFA, DCM, RT, 4 h (>99%); (e) 2-oxobutanoic acid, $Pd(OAc)_2$, DABCO, DMF, 105 °C, 16 h (75%).

Table 1
Yields for reactions: Indole-derivatives **12a–b** → identate ligands **16a–e** and **17a–e**

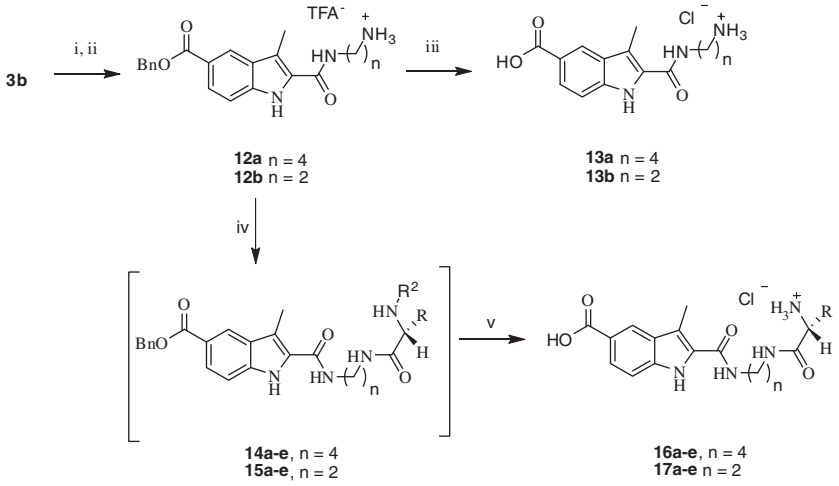
Entry	Indole derivatives	*n*	Amino acids	Bidentate ligands	Yield (%)
1	**12a**	4	Isoleucine (Boc)	**16a**	35
2		4	Isoleucine (CBz)	**16a**	15
3		4	Lysine-*N*′Boc	**16b**	27
4		4	Tryptophane	**16c**	30
5		4	Phenylalanine	**16d**	35
6		4	Glutamine	**16e**	30[a]
7	**12b**	2	Isoleucine	**17a**	45
8		2	Lysine-*N*′Boc	**17b**	41
9		2	Tryptophane	**17c**	40
10		2	Phenylalanine	**17d**	30
11		2	Glutamine	**17e**	[b]

[a] A cyclisation by transamidation of the amide side chain of glutamine was observed.
[b] Unsoluble

optimize the coupling/deprotection sequence, we prepared compound **16a** (Scheme 5) using N-Boc and *N*-CBz isoleucine. As shown in Table 1 (entries 1 and 2), the best results were obtained with the N-Boc protected isoleucine which afford the desired product **16a** in 35% yield. The difference of yield observed for this coupling could be due to the poor solubility or/and stability of the intermediate compound **14a**, as the starting material is totally consumed. The desired free bidentate ligands **16a–d** and **17a–e** were obtained as ammonium chloride salts with a high purity (>95%) and moderate yields after a two-steps synthesis from **12a**,**b** (Table 1, yields 27–45%).

2.3. Biochemical evaluations

We evaluated the ability of compounds **5** (S0 binding motif), **6**, **13a–b** (S0 binding motif and linker), **8a–e**, **16a–e** and **17a–e** (bidentate ligands) to affect the association of the 5-HT2A receptor C-terminus with PSD-95, one of the major PDZ proteins known to



Scheme 5. Reagents and conditions: (a) H_2N–$(CH_2)_n$–NHBoc, EDC, HOBT, DMF, 0 °C to RT, overnight; (b) TFA, DCM, RT, 2 h (**12a**: 81%, **12b**: 76% over two steps); (c) H_2 60 psi, $Pd(OH)_2$/C, MeOH, HCl 1 M, RT, 24 h (**13a**: 30%, **13b**: 40%); (d) CBz- or Boc-N-protected amino acids **a–e**, CDI, DIEA, DMF, RT, 6 h; (e) H_2 (60 psi), $Pd(OH)_2$/C, MeOH, HCl 1 M, RT, 24 h (yields over two steps reported in Table 1).

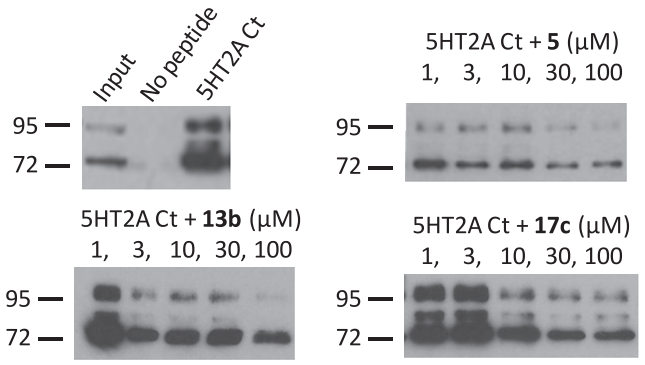


Figure 5. Effects of compounds **5**, **13b** and **17c** on recruitment of PSD-95 by 5-HT2A receptor C-terminal peptide Input represents 1% of the amount of proteins used in pull-downs.

interact with the receptor[26,41] using an in vitro binding assay. A decapeptide comprising the C-terminal residues of the 5-HT2A receptor was coupled N-terminally to beads and incubated with protein extracts from mice brains in the presence of increasing concentrations of tested compounds. Proteins were resolved by SDS–PAGE and PSD-95, on the beads, was assessed by immunoblotting. As previously described,[26] the 5-HT2A receptor C-terminal decapeptide recruited PSD-95 (Fig. 5). This interaction was inhibited in a concentration-dependent manner by compounds **5**, **13b** and **17c** (Fig. 5), consitent with an inhibitory activity towards 5-HT2A receptor/PSD-95 interactions.

3. Conclusion

We have designed an indole which, by molecular docking, was found to interact efficiently with the S0 site of PDZ domains. This generic PDZ binder was used to conceive a series of 15 novel bidentate ligands aimed to explore interactions with the S1 site of PDZ domains. The bidentate ligands were prepared in 6–11 steps in good overall yield (24–43%).

The generic PDZ binder and bidentate ligands were evaluated in pull-down assay and three of them (**5**, **13b** and **17c**) inhibited the interaction between PSD-95 and the 5-HT2A receptor.[42] These preliminary results encourage us to further evaluate binding affinity and specificity of this series of bidentate ligands for PDZ domains.

4. Experimental

4.1. Computational study

The studies were performed using Molecular Operating Environment software (MOE 2009.10).[27]

4.1.1. Protein preparation

The 3D structure of the PDZ1 domain of PSD-95 (PDB 2KA9)[43] was downloaded from the protein database and prepared with MOE: partial charges were assigned using Gasteiger (POPE) method, protein structure was minimized to 0.01 kcal/mol, the cypin ligand was removed, the binding site was identified using the site finder module in MOE. The ligands to dock were built using the molecular builder module in MOE and energy-miniumized to its local minima using the MMF94X forcefield to a constant (0.001 kcal/mol).

4.1.2. Molecular docking

The docking study was carried out using the dock module in MOE. The docking was restricted to the active site pocket located at S0 using the triangle matcher placement module in MOE. Refinement of the docked poses was carried out using the forcefield refinement module and scored using Affinity dG scoring system. Around 30 poses were returned for each docking run. The docked poses were ranked by London dG and Affinity dG scoring functions. All the poses were examined visually for docking accuracy, error and fitness. The best pose was selected and presented in Figure 4 using Maestro.[44]

4.2. Chemical synthesis

4.2.1. Generalities

NMR spectra were recorded on a Brüker 400 MHz apparatus. The chemical shifts are reported in ppm (δ scale) and all J values are in Hz. The following abbreviations are used: singlet (s), doublet (d), doubled doublet (dd), triplet (t), multiplet (m). High resolution mass spectra were performed by CRMP (Clermont-Ferrand, France). Monitoring of the reactions was performed using silica gel TLC plates (silica Merck 60 F_{254}). Spots were visualized by UV light at 254 nm. Flash chromatography columns were performed using silica gel 60 (70–230 mesh) or RP18 (25–40 μM) from Merck Chimie SAS (France) on a Flash II apparatus from Armen instrument (France). All commercial chemical were purchased from Aldrich (Milwaukee, WI) or Alfa Aesar. Anhydrous DMF was purchased from Alfa Aesar. THF was freshly distillated on sodium/benzophenone.

4.2.2. Preparation of indole 3a

4.2.2.1. 5-(Ethoxycarbonyl)-3-methyl-1*H*-indole-2-carboxylic acid (3a). To a solution of iodo compound **2** (1.00 g, 3.44 mmol) in anhydrous DMF (10 mL) under argon, 2-oxobutyric acid (1.75 g, 17.18 mmol) and DABCO (1.16 g, 10.31 mmol) were added. The solution was stirred for 5 min before addition of palladium(II) diacetate (0.04 g, 0.17 mmol). The mixture was warmed to 105 °C for 16 h. After cooling to room temperature, DMF was removed by evaporation and the resulting solid was dissolved in EtOAc (150 mL) and extracted with NaOH (1 M, 4 × 150 mL). The aqueous layers were combined, neutralized with HCl (1 M) and extracted with EtOAc (3 × 150 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo to obtain a brown solid which was purified by trituration in diethyl ether to give the desired indole **3a** as pale brown powder (0.55 g, 2.24 mmol, 65%); mp: 265–266 °C; R_f = 0.32 (SiO_2, Cyc/EtOAc, 7:3); ^{1}H NMR (400 MHz, CD_3OD) δ 8.39 (d, J = 2.0, 1H, H-4), 7.90 (dd, J = 9.0, 2.0, 1H, H-6), 7.43 (d, J = 9.0, 1H, H-7), 4.38 (q, J = 7.0, 2H, CH_2), 2.63 (s, 3H, CH_3), 1.42 (t, J = 7.0, 3H, CH_3); ^{13}C NMR (101 MHz, CD_3OD) δ 169.2 (C═O), 165.1 (C═O), 140.3 (C), 129.2 (C), 126.9 (C), 126.6 (CH), 124.5 (CH), 122.7 (C), 121.8 (C), 112.8 (CH), 61.9 (CH_2), 14.7 (CH_3), 9.9 (CH_3); HR-ESIMS: $(M+H)^+$ m/z calcd for $C_{13}H_{14}NO_4$ 248.0923, found 248.0926.

4.2.3. Preparation of indole 3b

4.2.3.1. Benzyl 4-(bis(tert-butoxycarbonyl)amino)-3-iodobenzoate (10). To a solution of **2** (4.00 g, 13.65 mmol) in DCM (100 mL) DMAP (0.166 g, 1.36 mmol), triethylamine (1.9 mL, 13.65 mmol) and $(Boc)_2O$ (6.260 g, 28.66 mmol) were added. The mixture was stirred at room temperature for 1 h and washed with HCl (0.1 M). The organic layer was concentrated and the resulting solid was directly engaged into the next step with EtOH (200 mL) and NaOH (1 M, 40 mL). The solution was stirred for 12 h at room temperature and then concentrated to remove ethanol. The resulting solid was dissolved in water, acidified to pH 3 and then extracted with EtOAC. The organic layer was concentrated to give the intermediate product **9** which was directly engaged into next step. To a solution of this intermediate in DMF (100 mL), CDI (2.43 g, 15.01 mmol) and benzylic alcohol (1.56 mL, 15.0 mmol) were

added. The mixture was stirred for 24 h, then concentrated and purified by FC (100% Cyc to 100% EtOAc) to give the desired product **10** as pale yellow foam (7.17 g, 12.97 mmol, 95%); R_f = 0.60 (SiO_2, Cyc/EtOAc, 9:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (s, 1H, H-2), 8.06 (d, J = 8.0, 1H, H-6), 7.47–7.25 (m, 6H, H-5, 5 × CH_{ar}), 5.37 (s, 2H, CH_2), 1.42–1.39 (m, 18H, 6 × CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.6 (C═O), 149.8 (2 × C═O), 146.4 (C), 140.4 (CH), 135.7 (C), 130.7 (C), 130.4 (2 × CH), 128.9 (CH), 128.8 (CH), 128.7 (CH), 128.6 (2 × CH), 99.7 (C), 83.51 (2 × C), 67.4 (CH_2), 28.0–27.0 (6 × CH_3); HR-ESIMS: $(M+Na)^+$ m/z calcd for 576.0859, found 576.0879.

4.2.3.2. Benzyl 4-amino-3-iodobenzoate (11). To a solution of carbamate **10** (5.66 g, 10.23 mmol) in DCM (100 mL) TFA (6 mL) was added. The mixture was stirred at room temperature for 4 h and then NaOH (1 M) was added to reach basic pH (10–12). The organic layer was concentrated to give the desired amine **11** as yellow powder in quantitative yield (3.6 g, 10.23 mmol); mp: 80–82 °C; R_f = 0.32 (SiO_2, Cyc/EtOAc, 9:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (s, 1H, H-2), 7.86 (d, J = 8.0, 1H, H-6), 7.44 (m, 1H, H_{ar}), 7.26 (m, 3H, 3 × CH_{ar}), 6.71 (d, J = 8.0, 1H, H-5), 5.31 (s, 2H, CH_2), 4.53 (ls, 2H, NH_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.1 (C═O), 150.8 (C), 141.1 (CH), 136.2 (C), 131.4 (CH), 128.6 (2 × CH), 128.2 (2 × CH), 121.1 (2 × CH), 113.1 (CH), 82.1 (C), 66.4 (CH_2); HR-ESIMS: $(M+H)^+$ m/z calcd for 353.9811, found 353.9811.

4.2.3.3. 5-(Benzyloxycarbonyl)-3-methyl-1*H*-indole-2-carboxylic acid (3b). To a solution of amine **11** (1.00 g, 3.44 mmol, 1) in DMF (10 mL) 2-oxobutyric acid (1.75 g, 17.18 mmol) and DABCO (1.16 g, 10.31 mmol) were added. The solution was stirred for 5 min and then palladium(II) diacetate (0.04 g, 0.17 mmol, 0.05) was added. The mixture was warmed to 105 °C, stirred for 16 h, and then concentrated to remove DMF. The resulting solid was dissolved in EtOAc (150 mL) and washed with water (150 mL). The organic layer was dried over anhydrous Na_2SO_4, then concentrated in vacuo to obtain a dark brown solid which was purified by FC (Silica, 100% Cyc to 100% EtOAc) to afford the desired indole **3b** as a brown powder (0.798 g, 2.58 mmol, 75%); mp: 190–194 °C; R_f = 0.29 (SiO_2, Cyc/EtOAc, 5:5); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (s, 1H, H-4), 7.89 (d, J = 8.5, 1H, H-6), 7.45–7.30 (m, 6H, H-7, 5 × CH_{ar}), 5.34 (s, 2H, CH_2), 2.58 (s, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.2 (C═O), 164.5 (C═O), 139.7 (C), 137.4 (C), 129.0 (2 × CH), 128.6 (CH), 128.6 (2 × CH), 126.1 (CH), 124.1 (CH), 121.9 (C), 121.2 (C), 112.3 (CH), 66.9 (CH_2), 9.3 (CH_3); HR-ESIMS: $(M+Na)^+$ m/z calcd for 331.0820, found 331.0824.

4.2.4. General Procedure for the preparation of protected indole-C_n 4a–c and 12a–b

To a solution of protected indole **3a** or **3b** (1 equiv) in DMF (1 mL for 0.1 mmol of **3a** or **3b**), HOBT (1.1 equiv) and EDC (1.1 equiv) were added at 0 °C. After stirring the solution for 1 h at 0 °C, a solution of N-Boc-diamine (2 equiv) in DMF (1 mL for 0.05 mmol of N-Boc-diamine) was added drop wise to the mixture. The reaction was slowly allowed to warm up to room temperature and stirred overnight at room temperature. After removal of DMF, the resulting residue was dissolved in EtOAc (4 mL for 0.1 mmol of **3a** or **3b**) and washed with water (20 mL for 0.1 mmol of **3a** or **3b**). The organic layer was concentrated in vacuo and the resulting solid was purified by FC (Silica, gradient 100% Cyc to 100% EtOAc) to give the desired product which was directly engaged into the next step in DCM in the presence TFA (5 equiv) for 2 h at RT. The mixture was concentrated in vacuo and the resulting solid was purified by trituration in diethyl ether and filtration to obtain the desired salts **4a–c** and **12a**,**b**.

4.2.4.1. 8-(5-(Ethoxycarbonyl)-3-methyl-1*H*-indole-2-carboxamido)octan-1-aminium2,2,2-trifluoroacetate (4a). Compound **4a** (336 mg, 0.69 mmol, 85% from **3a** 200 mg, 0.81 mmol); ^{1}H NMR (400 MHz, CD_3OD) δ 8.38 (d, J = 2.0, 1H, H-4), 7.92 (dd, J = 9.0, 2.0, 1H, H-6), 7.44 (d, J = 9.0, 1H, H-7), 4.40 (q, J = 7.0, 2H, CH_2), 3.48 (t, J = 8.0, 2H, CH_2), 2.93 (t, J = 7.0, 2H, CH_2), 2.60 (s, 3H, CH_3), 1.70–1.30 (m, 15H, 6 × CH_2, CH_3); ^{13}C NMR (101 MHz, CD_3OD) δ 169.2 (C═O), 164.7 (C═O), 139.9 (C), 130.3 (C), 129.4 (C), 126.0 (CH), 124.0 (CH), 122.8 (C), 116.8 (C), 112.6 (CH), 61.8 (CH_2), 40.7 (CH_2), 40.6 (CH_2), 30.6 (CH_2), 30.1 (CH_2), 28.6 (CH_2), 27.9 (CH_2), 27.4 (CH_2), 14.7 (CH_3), 9.8 (CH_3); HR-ESIMS: $(M\text{-}CF_3COO^-+H)^+$ m/z calcd for $C_{21}H_{32}N_3O_3$ 374.2444, found 374.2443.

4.2.4.2. 6-(5-(Ethoxycarbonyl)-3-methyl-1*H*-indole-2-carboxamido)-hexan-1-aminium 2,2,2-trifluoroacetate (4b). Compound **4b** (317 mg, 0.69 mmol, 85% from **3a** 200 mg, 0.81 mmol); ^{1}H NMR (400 MHz, CD_3OD) δ 8.36 (s, 1H, H-4), 7.90 (d, J = 8.5, 1H, H-6), 7.42 (d, J = 8.5, 1H, H-7), 4.38 (q, J = 7.0, 2H, CH_2), 3.44 (t, J = 7.0, 2H, CH_2), 2.93 (t, J = 7.5, 2H, CH_2), 2.58 (s, 3H, CH_3), 1.74–1.60 (m, 4H, 2 × CH_2), 1.51–1.44 (m, 4H, 2 × CH_2), 1.41 (t, J = 7.0, 3H, CH_3); ^{13}C NMR (101 MHz, CD_3OD) δ 169.2 (C═O), 164.8 (C═O), 139.9 (C), 130.2 (C), 129.4 (C), 126.1 (CH), 124.0 (CH), 122.8 (C), 117.01 (C), 112.6 (CH), 61.9 (CH_2), 40.7 (CH_2), 40.4 (CH_2), 30.4 (CH_2), 28.5 (CH_2), 27.4 (CH_2), 27.0 (CH_2), 14.7 (CH_3), 9.8 (CH_3); HR-ESIMS: $(M+H)^+$ m/z calcd for $C_{19}H_{28}N_3O_3$ 346.2125, found 346.2130.

4.2.4.3. 4-(5-(Ethoxycarbonyl)-3-methyl-1*H*-indole-2-carboxamido)butan-1-aminium 2,2,2-trifluoroacetate (4c). Compound **4c** (314 mg, 0.73 mmol, 90% from **3a** 200 mg, 0.81 mmol); ^{1}H NMR (400 MHz, CD_3OD) δ 8.38 (d, J = 1.0, 1H, H-4), 7.91 (dd, J = 8.0, 1.0, 1H, H-6), 7.43 (d, J = 8.0, 1H, H-7), 4.39 (q, J = 8.0, 2H, CH_2), 3.49 (m, 2H, CH_2), 3.01 (m, 2H, CH_2), 2.60 (s, 3H, CH_3), 1.76–1.74 (m, 4H, 2 × CH_2), 1.42 (t, 2H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.7 (C═O), 156.9 (C═O), 138.1 (C), 134.0 (C), 128.5 (C), 125.7 (CH), 123.9 (CH), 122.8 (C), 111.9 (CH), 61.8 (CH_2), 37.8 (2 × CH_2), 28.8 (2 × CH_2), 14.9 (CH_3) 10.6 (CH_3); HR-ESIMS: $(M\text{-}CF_3COO^-+H)^+$ m/z calcd for $C_{17}H_{24}N_3O_3$ 318.1818, found 318.1826.

4.2.4.4. 4-(5-(Benzyloxycarbonyl)-3-methyl-1*H*-indole-2-carboxamido)-butan-1-aminium 2,2,2-trifluoroacetate (12a). Compound **12a** (648 mg, 1.31 mmol, 81% from **3b** 400 mg, 1.62 mmol); ^{1}H NMR (400 MHz, CD_3OD) δ 8.38 (s, 1H, H-4), 7.91 (d, J = 12.0, 1H, H-6), 7.48–7.32 (m, 6H, H-7, 5 × CH_{ar}), 5.36 (s, 2H, CH_2), 3.47 (m, 2H, CH_2), 2.99 (m, 2H, CH_2), 2.56 (s, 3H, CH_3), 1.75 (m, 4H, 2 × CH_2); ^{13}C NMR (101 MHz, CD_3OD) δ 168.3 (C═O), 163.7 (C═O), 138.7 (C), 131.4 (C), 129.8 (2 × CH), 129.6 (CH), 129.4 (2 × CH), 126.6 (CH), 125.0 (CH), 123.5 (C), 113.1 (CH), 66.5 (CH_2), 40.0 (CH_2), 39.2 (CH_2), 28.0 (2 × CH_2), 10.1 (CH_3); HR-ESIMS: $(M\text{-}CF_3COO^-+H)^+$ m/z calcd for 380.1969, found 380.1972.

4.2.4.5. 2-(5-(Benzyloxycarbonyl)-3-methyl-1*H*-indole-2-carboxamido)-ethanaminium 2,2,2-trifluoroacetate (12b). Compound **12b** (573 mg, 1.23 mmol, 76% from **3b** 400 mg, 1.62 mmol); ^{1}H NMR (400 MHz, CD_3OD) δ 8.42 (s, 1H, H-4), 7.90 (d, J = 8.0, 1H, H-6), 7.49–7.32 (m, 6H, H-7, 5 × CH_{ar}), 5.38 (s, 2H, CH_2), 3.71 (t, J = 8.0, 2H, CH_2), 3.20 (t, J = 8.0, 2H, CH_2), 2.61 (s, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.8 (C═O), 165.7 (C═O), 140.0 (C), 138.0 (C), 129.6 (2 × CH), 129.4 (CH), 129.2 (2 × CH), 126.4 (CH), 124.3 (CH), 122.7 (C), 117.7 (CH), 112.8 (CH), 67.5 (CH_2), 41.2 (CH_2), 38.6 (CH_2) 9.8 (CH_3); HR-ESIMS: $(M\text{-}CF_3COO^-+H)^+$ m/z calcd for 352.1656, found 352.1660.

4.2.5. Preparation of ligands 5 and 6

4.2.5.1. Sodium 3-methyl-1*H*-indole-2,5-dicarboxylate (5). Indole **3a** (100 mg, 0.40 mmol) is engaged with MeOH (10 mL) and NaOH (2 M, 2 mL) and heated under reflux for 24 h. The mixture was concentrated and purified by RPFC (RP18 Silica, 100% H_2O to 100% MeOH) to give the desired salt **5** as a white solid (85 mg, 0.323 mmol, 80%); ^{1}H NMR (400 MHz, CD_3OD) 8.41 (d, J = 1.0, 1H, H-4), 7.92 (dd, J = 8.0, 1.0, 1H, H-6), 7.43 (d, J = 8.0, 1H, H-7), 2.63 (s, 3H, CH_3); ^{13}C NMR (101 MHz, CD_3OD) δ 168.4 (C=O), 165.1 (C=O), 140.2 (C), 128.8 (C), 126.3 (C), 125.7 (CH), 123.5 (CH), 122.9 (C), 121.2 (C), 111.9 (CH), 9.9 (CH_3); HR-ESIMS: $[M-H]^-$ m/z calcd for $C_{11}H_9NO_4$ 218.0459, found 218.0455.

4.2.5.2. Sodium 2-(6-aminohexylcarbamoyl)-3-methyl-1 *H*-indole-5-carboxylate (6). To a solution of ethyl-indole-C6-TFA **4b** (50 mg, 0.109 mmol) was added NaOH (1 M) (0.55 mL, 0.55 mmol) in EtOH (5 mL). The solution was heated to reflux for 24 h. Then the mixture was concentrated in vacuo. The resulting salt was purified by RPFC (RP18 Silica, 100% H_2O to 100% MeOH) to give the desired salt **6** in quantitative yield (37 mg, 0.109 mmol); ^{1}H NMR (400 MHz, CD_3OD) δ 8.38 (s, 1H, H-4), 7.91 (d, J = 12.0, 1H, H-6), 7.41 (d, 1H, J = 12.0, H-7), 3.44 (t, J = 8.0, 2H, CH_2), 2.93 (t, J = 8.0, 2H, CH_2), 2.58 (s, 3H, CH_3), 1.71 (m, 4H, 2 × CH_2), 1.69 (m, 4H, 2 × CH_2); ^{13}C NMR (101 MHz, CD_3OD) δ 140.0 (C), 130.0 (C), 128.7 (C), 126.2 (CH), 124.3 (CH), 117.4 (C), 112.5 (CH), 40.2 (CH_2), 39.6 (CH_2), 33.0 (CH_2), 31.5 (CH_2), 27.5 (CH_2), 25.8 (CH_2), 9.7 (CH_3); HR-ESIMS: $(M+H)^+$ m/z calcd for $C_{17}H_{24}N_3O_3$ 318.1818, found 318.1819.

4.2.6. General Procedure for the preparation of indole-Cn-amino acids 8a–e, 16a–e and 17a–e

To a solution of salt **4a–c**, **12a** or **12b** (1 equiv) in DMF (1 mL for 0.1 mmol of **4a–c**, **12a** or **12b**) was added DIEA (1.1 equiv) at room temperature and the reaction was stirred for 45 min to give solution **A**. To a solution of N-protected amino acid (1.1 equiv) in DMF (1 mL for 0.05 mmol of N-protected amino acid), CDI (1.2 equiv) was added at room temperature and the mixture was stirred for 1 h to give solution **B**. Solution **A** was added drop wise to solution **B** at room temperature and stirred for 4 h. The mixture was concentrated in vacuo to remove DMF and the desired bidentate ligands **7a–e**, **9a**, **14a–e**, **15a–e** were obtained, and directly engaged into the deprotection step (Method A or B below), after verification of crude ^{1}H NMR spectra.

4.2.6.1. Method A for deprotection. To a solution of **7a–e**, (1 equiv) in EtOH (1 mL for 0.1 mmol of **7a–e**) and dioxane (1 mL for 0.1 mmol of **7a–e**), NaOH (5 N, 1 mL for 0.1 mmol of **7a–e**) was added and the reaction was stirred at room temperature for 24 h. The mixture was then concentrated in vacuo, the resulting solid washed with Et_2O (20 mL) and purified by RPFC (RP18 silica, gradient H_2O/MeOH) to give the desired salt **8a–e**.

*4.2.6.1.1. Sodium 2-(((2S,3 S)-2-amino-3-methylpentanamido)-hexyl-carbamoyl)-3-methyl-1H-indole-5-carboxylate (**8a**)* . Compound **8a** 160 mg, 0.35 mmol, 58% from **4b** 280 mg, 0.61 mmol); ^{1}H NMR (400 MHz, CD_3OD) δ 8.33 (d, J = 1.5, 1H, H-4), 7.91 (dd, J = 8.5, 1.5, 1H, H-7), 7.32 (d, J = 8.5, 1H, H-6), 3.42 (t, J = 7.0, 2H, CH_2), 3.29–3.13 (m, 2H, CH_2), 3.09 (d, J = 6.0, 1H, CH), 2.58 (s, 3H, CH_3), 1.70–1.37 (m, 10H, 5 × CH_2), 1.20–1.07 (m, 1H, CH), 0.96–0.85 (m, 6H, 2 × CH_3); ^{13}C NMR (101 MHz, CD_3OD) δ 176.9 (C=O), 176.6 (C=O), 165.1 (C=O), 138.8 (C), 130.4 (C), 129.3 (C), 129.1 (C), 127.1 (CH), 123.0 (CH), 117.1 (C), 111.5 (CH), 61.0 (CH), 40.5 (CH_2), 40.4 (CH), 40.1 (CH_2), 30.6 (CH_2), 30.4 (CH_2), 27.7 (CH_2), 27.7 (CH_2), 25.5 (CH_2), 16.0 (CH_3), 11.9 (CH_3), 10.0 (CH_3); HR-ESIMS: $(M-Na+2H)^+$ m/z calcd for $C_{23}H_{35}N_4O_4$ 431.2653, found 431.2670.

*4.2.6.1.2. (S)-2-((2,6-Diaminohexanamido)buthylcarbamoyl)-3-methyl-1H-indole-5-carboxylic acid (**8b**).* Compound **8b** (100 mg, 0.22 mmol, 51% from **4b** 200 mg, 0.44 mmol). Before the alkaline treatment, the N-Boc of the side chain was removed in presence of TFA (excess) in DCM; ^{1}H NMR (400 MHz, D_2O/CF_3COOD, 95:5) δ 8.00 (s, 1H, H-4), 7.59 (d, J = 8.5, 1H, H-6), 7.14 (d, J = 8.5, 1H, H-7), 3.80 (t, J = 6.7, 1H, CH), 3.24–2.95 (m, 4H, 2 × CH_2), 2.82 (t, J = 7.6, 2H, CH_2), 2.16 (s, 3H, CH_3), 1.81–1.64 (m, 2H, CH_2), 1.59–1.48 (m, 2H, CH_2), 1.44–1.23 (m, 6H, 3 × CH_2), 1.21–1.08 (m, 4H, 2 × CH_2); ^{13}C NMR low solubility; HR-ESIMS: $(M+H)^+$ m/z calcd for $C_{23}H_{36}N_5O_6$ 446.2762, found 446.2786.

*4.2.6.1.3. Sodium (S)-2-((2-amino-3-(1H-indol-2-yl)propanamido)-hexylcarbamoyl)-3-methyl-1H-indole-5-carboxylate (**8c**).* Compound **8c** (160 mg, 0.31 mmol, 50% from **4b** 280 mg, 0.61 mmol); ^{1}H NMR (400 MHz, CD_3OD) δ 8.34 (s, 1H, H-4), 7.92 (d, J = 8.5, 1H, H-6), 7.57 (d, J = 8.5, 1H, H-7), 7.36–7.27 (m, 2H, 2 × CH_{ar}), 7.09–7.01 (m, 2H, 2 × CH_{ar}), 6.98 (t, J = 7.5, 1H, CH_{ar}), 3.60 (t, J = 6.5, 1H, CH), 3.37 (t, J = 7.0, 2H, CH_2), 3.18–3.07 (m, 2H, CH_2), 3.07–2.94 (m, 2H, CH_2), 2.58 (s, 3H, CH_3), 1.63–1.49 (m, 2H, CH_2), 1.36–1.24 (m, 4H, 2 × CH_2), 1.19–1.07 (m, 2H, CH_2); ^{13}C NMR (101 MHz, CD_3OD) δ 176.9 (C=O), 176.6 (C=O), 165.1 (C=O), 138.8 (C), 138.1 (C), 130.3 (C), 129.3 (C), 129.1 (C), 128.8 (C), 127.1 (CH), 124.6 (CH), 123.0 (CH), 122.4 (CH), 119.8 (CH), 119.5 (CH), 117.2 (C), 112.3 (CH), 111.6 (CH), 111.2 (C), 57.1 (CH), 40.5 (CH_2), 40.2 (CH_2), 32.3 (CH_2), 30.5 (CH_2), 30.0 (CH_2), 27.6 (CH_2), 27.4 (CH_2), 10.0 (CH_3); HR-ESIMS: $(M-Na+2H)^+$ m/z calcd for $C_{28}H_{34}N_5O_4$ 504.2611, found 504.2628.

*4.2.6.1.4. Sodium (S)-2-((2-amino-3-phenylpropanamido)hexylcarbamoyl)-3-methyl-1H-indole-5-carboxylate (**8d**).* Compound **8d** (124 mg, 0.26 mmol, 51% from **4b** 230 mg, 0.50 mmol); 1H NMR (400 MHz, CD_3OD) δ 8.35 (s, 1H, H-4), 7.93 (d, J = 8.5, 1H, H-6), 7.33 (d, J = 8.5, 1H, H-7), 7.28–7.15 (m, 5H, 5 × CH_{ar}), 3.51 (t, J = 6.5, 1H, CH), 3.38 (t, J = 7.0, 2H, CH_2), 3.22–3.08 (m, 1H, CH_2), 3.08–2.97 (m, 1H, CH_2), 2.97–2.87 (m, 1H, CH_2), 2.87–2.73 (m, 1H, CH_2), 2.59 (s, 3H, CH_3), 1.64–1.51 (m, 2H, CH_2), 1.39–1.26 (m, 4H, 2 × CH_2), 1.24–1.13 (m, 2H, CH_2); ^{13}C NMR (101 MHz, CD_3OD) δ 176.7 (C=O), 176.3 (C=O), 165.0 (C=O), 138.7 (C), 138.6 (C), 130.4 (CH), 130.2 (C), 129.5 (CH), 129.3 (C), 129.1 (C), 127.7 (CH), 127.0 (CH), 123.0 (CH), 117.3 (C), 111.6 (CH), 57.8 (CH), 42.6 (CH_2), 40.5 (CH_2), 40.1 (CH_2), 30.5 (CH_2), 30.2 (CH_2), 27.7 (CH_2), 27.5 (CH_2), 10.1 (CH_3); HR-ESIMS: $(M-Na+2H)^+$ m/z calcd for $C_{26}H_{32}N_4O_4$ 465.2496, found 465.2511.

*4.2.6.1.5. Sodium (S)-2-((2-amino-4-carboxybutanamido)hexylcarbamoyl)-3-methyl-1H-indole-5-carboxylate (**8e**).* Compound **8e** (134 mg, 0.29 mmol, 46% from **4b** 286 mg, 0.62 mmol); ^{1}H NMR (400 MHz, CD_3OD) δ 8.36 (d, J = 1.0, 1H, H-4), 7.95 (dd, J = 8.5, 1.0, 1H, H-6), 7.35 (d, J = 8.5, 1H, H-7), 3.42–3.16 (m, 5H, 2 × CH_2 + CH), 2.61 (s, 3H, CH_3), 2.27 (t, J = 8.0, 2H, CH_2), 2.02–1.93 (m, 1H, CH_2), 1.88–1.81 (m, 1H, CH_2), 1.69–1.62 (m, 2H, CH_2), 1.60–1.50 (m, 2H, CH_2), 1.46–1.39 (m, 4H, 2 × CH_2); ^{13}C NMR (101 MHz, CD_3OD) δ 181.8 (C=O), 177.1 (C=O), 176.7 (C=O), 165.0 (C=O), 138.8 (C), 130.2 (C), 129.3 (C), 129.0 (C), 127.0 (CH), 122.9 (CH), 117.4 (C), 111.6 (CH), 55.9 (CH), 40.5 (CH_2), 40.2 (CH_2), 35.4 (CH_2), 33.6 (CH_2), 30.5 (CH_2), 30.3 (CH_2), 27.7 (CH_2), 27.6 (CH_2), 10.0 (CH_3); HR-ESIMS: $(M-Na+2H)^+$ m/z calcd for $C_{22}H_{31}N_4O_6$ 447.2238, found 447.2264.

4.2.6.2. Method B for deprotection. To a solution of **12a** and **b**, **14a–e** or **15a–e** (1 equiv) in MeOH (2 mL for 0.1 mmol of **12a** and **b**, **14a–e** or **15a–e**), $Pd(OH)_2/C$ (1 mg for 0.01 mmol) was added. The mixture was stirred under H_2 (60 psi). After 24 h, the solution was filtrated over Celite® and an aqueous solution of HCl (1 N) is added. The mixture was concentrated and purified by RPFC (RP18 Silica, gradient 100% H_2O to 100% MeOH) to allow isolation of the desired salt **13a** and **13b**, **16a–e**, **17a-d**.

*4.2.6.2.1. 4-(5-Carboxy-3-methyl-1 H-indole-2-carboxamido)butan-1-aminium chloride (**13a**).* Compound **13a** (19 mg, 0.06 mmol, 30% from **12a** 100 mg, 0.20 mmol); ^{1}H NMR (400 MHz, CD_3OD) δ 8.37 (s, 1H, H-4), 7.90 (d, J = 12.0, 1H, H-6), 7.40 (d, 1H, J = 12.0, H-7), 3.50 (t, J = 8.0, 2H, CH_2), 3.00 (t, J = 8.0, 2H, CH_2), 2.58 (s, 3H, CH_3), 1.75 (m, 4H, 2 × CH_2); ^{13}C NMR (101 MHz, CD_3OD) δ 170.3 (C=O), 165.0 (C=O), 139.7 (C), 129.7 (C), 129.2 (C), 126.3 (CH), 124.1 (CH), 117.0 (C), 112.3 (CH), 40.2 (CH_2), 39.6 (CH_2), 27.5 (CH_2), 25.8 (CH_2), 9.7 (CH_3); HR-ESIMS: $(M+H)^+$ m/z calcd for $C_{15}H_{20}N_3O_3$ 290.1505, found 290.1512.

*4.2.6.2.2. 2-(5-Carboxy-3-methyl-1H-indole-2-carboxamido)ethan aminium chloride (**13b**).* Compound **13b** 25 mg, 0.08 mmol, 40% from **12b** 100 mg, 0.21 mmol); ^{1}H NMR (400 MHz, CD_3OD) δ 8.41 (s, 1H, H-4), 7.93 (d, J = 12.0, 1H, H-6), 7.44 (d, 1H, J = 12.0, H-7), 3.72 (t, J = 8.0, 2H, CH_2), 3.19 (t, J = 8.0, 2H, CH_2), 2.62 (s, 3H, CH_3); ^{13}C NMR (101 MHz, CD_3OD) δ 139.4 (C), 129.4 (C), 128.2 (C), 125.9 (CH), 123.7 (CH), 116.4 (C), 111.9 (CH), 41.5 (CH_2), 40.8 (CH_2), 9.2 (CH_3); HR-ESIMS: M^+ m/z calcd for $C_{13}H_{16}N_3O_3$ 262.1192, found 262.1202.

*4.2.6.2.3. (2S,3 S)-1-((5-Carboxy-3-methyl-1H-indole-2-carboxamido)-butylamino)-3-methyl-1-oxopentan-2-aminium chloride (**16a**).* Compound **16a** (99 mg, 0.22 mmol, 35% from **12a** 316 mg, 0.64 mmol); ^{1}H NMR (400 MHz, CD_3OD) δ 8.28 (s, 1H, H-4), 7.85 (d, J = 12.0, 1H, H-6), 7.28 (d, 1H, J = 12.0, H-7), 3.48 (m, 1H, CH), 3.36 (t, J = 8.0, 2H, CH_2), 3.22 (t, J = 8.0, 2H, CH_2), 2.51 (s, 3H, CH_3), 1.60–1.57 (m, 7H, 3 × CH_2, CH), 0.93–0.85 (m, 6H, 2 × CH_3); ^{13}C NMR (101 MHz, CD_3OD) δ 171.0 (C=O), 164.3 (C=O), 138.5 (C), 128.6 (C), 126.2 (CH), 122.7 (CH), 116.6 (C), 111.3 (CH), 58.9 (CH), 39.5 (2 × CH_2), 38.0 (CH), 27.4 (CH_2), 27.1 (CH_2), 25.0 (CH_2), 14.7 (CH_3), 11.0 (CH_3), 9.4 (CH_3); HR-ESIMS: M^+ m/z calcd for $C_{21}H_{31}N_4O_4$ 403.2345, found 403.2328.

*4.2.6.2.4. (S)-6-(4-(5-Carboxy-3-methyl-1H-indole-2-carboxamido)-butylamino)-6-oxohexane-1,5-diaminium chloride (**16b**).* Compound **16b** (85 mg, 0.17 mmol, 27% from **12a** 316 mg, 0.64 mmol); ^{1}H NMR (400 MHz, CD_3OD) δ 8.20 (d, J = 1.0, 1H, H-4), 7.79 (dd, J = 8.0, 1.0, 1H, H-6), 7.19 (d, J = 8.0, 1H, H-7), 3.29 (m, 2H, CH_2), 3.11 (m, 3H, CH_2, CH), 2.45 (s, 3H, CH_3), 2.41 (m, 2H, CH_2), 1.52–1.16 (m, 10H, 5 × CH_2); ^{13}C NMR (101 MHz, CD_3OD) δ 177.6 (C=O), 176.7 (C=O), 165.1 (C=O), 138.8 (C), 130.3 (C), 129.8 (C), 129.1 (C), 127.0 (CH), 122.9 (CH), 117.4 (C), 111.7 (CH), 56.1 (CH), 42.3 (CH_2), 40.2 (CH_2), 39.9 (CH_2), 36.3 (CH_2), 33.7 (CH_2), 28.0 (CH_2), 27.9 (CH_2), 24.1 (CH_2), 10.1 (CH_3); HR-ESIMS: $(M+H)^+$ m/z calcd for $C_{21}H_{32}N_5O_4$ 418.2449, found 418.2446.

*4.2.6.2.5. (S)-1-(4-(5-Carboxy-3-methyl-1H-indole-2-carboxamido)-butylamino)-3-(1H-indol-3-yl)-1-oxopropan-2-aminium chloride (**16c**).* Compound **16c** (89 mg, 0.17 mmol, 30% from **12a** 286 mg, 0.58 mmol); ^{1}H NMR (400 MHz, CD_3OD) δ 8.37 (s, 1H, H-4), 7.91 (d, J = 12.0, 1H, H-6), 7.40 (d, 1H, J = 12.0, H-7), 7.40–7.00 (m, 5H, 5 × CH_{ar}), 4.06 (m, 1H, CH), 3.63–3.57 (m, 3H, 2 × CH_2), 3.38–3.14 (m, 3H, 2 × CH_2), 2.57 (s, 3H, CH_3), 1.15–1.40 (m, 4H, 2 × CH_2); ^{13}C NMR (101 MHz, CD_3OD) δ 170.7 (C=O), 165.2 (C=O), 140.1 (C), 138.6 (C), 136.4 (C), 130.3 (C), 129.8 (C), 128.8 (C), 127.0 (CH), 125.9 (CH), 123.3 (CH), 120.7 (CH), 119.6 (CH), 117.6 (CH), 113.0 (CH), 112.8 (CH), 108.7 (C), 55.8 (CH), 40.6 (CH_2), 40.7 (CH_2), 29.4 (CH_2), 28.3 (CH_2), 27.8 (CH_2), 10.33 (CH_3); HR-ESIMS: $(M+H)^+$ m/z calcd for $C_{26}H_{30}N_5O_4$ 476.2298, found 476.2314.

*4.2.6.2.6. (S)-1-((5-carboxy-3-methyl-1 H-indole-2-carboxamido)-buthylamino)-1-oxo-3-phenylpropan-2-aminium chloride (**16d**).* Compound **16d** (96 mg, 0.20 mmol, 35% from **12a** 286 mg, 0.58 mmol); ^{1}H NMR (400 MHz, CD_3OD) δ 8.36 (s, 1H, H-4), 7.90 (d, J = 12.0, 1H, H-6), 7.40 (d, 1H, J = 12.0, H-7), 7.31–7.13 (m, 5H, 5 × CH_{ar}), 4.06 (m, 1H, CH), 3.37–3.09 (m, 6H, 3 × CH_2), 2.56 (s, 3H, CH_3), 1.52–1.63 (m, 4H, 2 × CH_2); ^{13}C NMR (101 MHz, CD_3OD) δ 169.2 (C=O), 164.2 (C=O), 139.2 (C), 135.3 (C), 130.1 (2 × CH), 129.5 (2 × CH), 129.3 (C), 128.9 (C), 128.2 (CH), 126.1 (CH), 123.7 (CH), 116.9 (C), 112.0 (CH), 55.5 (CH), 39.7 (2 × CH_2), 38.4 (CH_2), 27.4 (CH_2), 27.0 (CH_2), 9.5 (CH_3); HR-ESIMS: $(M+H)^+$ m/z calcd for $C_{24}H_{29}N_4O_3$ 437.2189, found 437.2178.

*4.2.6.2.7. 3-Methyl-2-(4-(5-oxopyrrolidine-2-carboxamido)-butylcarbamoyl)-1 H-indole-5-carboxylic acid (**16e**).* Compound **16e** (70 mg, 0.17 mmol, 30% from **12a** 286 mg, 0.58 mmol); ^{1}H NMR (400 MHz, CD_3OD) δ 8.36 (s, 1H, H-4), 7.89 (d, J = 12.0, 1H, H-6), 7.40 (d, 1H, J = 12.0, H-7), 4.16 (m, 1H, CH), 3.43 (t, J = 8.0, 2H, CH_2), 3.27 (t, J = 8.0, 2H, CH_2), 2.57 (s, 3H, CH_3), 2.35–2.40 (m, 4H, 2 × CH_2), 1.68–1.62 (m, 4H, 2 × CH_2); ^{13}C NMR (101 MHz, CD_3OD) δ 175.2 (C=O), 140.0 (C), 130.1(C), 129.6 (C), 126.8 (CH), 124.3 (CH), 117.3 (C), 112.5 (CH), 58.5 (CH), 40.4 (CH_2), 40.3 (CH_2), 30.7 (CH_2), 28.1 (CH_2), 28.0 (CH_2), 27.0 (CH_2), 10.1 (CH_3); HR-ESIMS: $(M+Na)^+$ m/z calcd for $C_{20}H_{24}N_4O_5Na$ 423.1644, found 423.1643.

*4.2.6.2.8. (2S,3S)-1-(2-(5-Carboxy-3-methyl-1H-indole-2-carboxamido)-ethylamino)-3-methyl-1-oxopentan-2-aminium chloride (**17a**).* Compound **17a** (118 mg, 0.29 mmol, 45% from **12b** 300 mg, 0.64 mmol); ^{1}H NMR (400 MHz, CD_3OD) δ 8.33 (s, 1H, H-4), 7.90 (d, J = 8.0, 1H, H-6), 7.34 (d, 1H, J = 8.0, H-7), 3.67 (m, 1H, CH), 3.58 (m, 4H, 2 × CH_2), 2.58 (s, 3H, CH_3), 1.85 (m, 1H, CH), 1.52 (m, 1H, CH_2), 1.16 (m, 1H, CH_2), 0.88–0.84 (m, 6H, 2 × CH_3); ^{13}C NMR (101 MHz, CD_3OD) δ 165.2 (C=O), 164.9 (C=O), 139.4 (C), 129.3 (C), 126.8 (CH), 125.5 (C), 125.8 (C), 123.9 (CH), 117.4 (C), 112.2 (CH), 59.62 (CH), 40.5 (CH_2), 40.2 (CH_2), 38.3 (CH), 25.5 (CH_2), 15.2 (CH_3), 11.6 (CH_3), 10.0 (CH_3); HR-ESIMS: $(M+H)^+$ m/z calcd for $C_{19}H_{27}N_4O_4$ 375.2032, found 375.2030.

*4.2.6.2.9. ((S)-6-(2-(5-(Benzyloxycarbonyl)-3-methyl-1H-indole-2-carboxamido)-ethylamino)-6-oxohexane-1,5-diaminium chloride (**17b**).* Compound **17b** (104 mg, 0.23 mmol, 41% from **12b** 256 mg, 0.55 mmol); ^{1}H NMR (400 MHz, CD_3OD) δ 8.37 (s, 1H, H-4), 7.90 (d, J = 8 Hz, 1H, H-6), 7.46 (d, J = 8 Hz, 1H, H-7), 3.95 (m, 1H, CH), 3.65–3.48 (m, 4H, 2 × CH_2), 2.89 (m, 2H, CH_2), 2.60 (s, 3H, CH_3), 1.93–1.86 (m, 2H, CH_2), 1.67 (m, 2H, CH_2), 1.50 (m, 2H, CH_2); ^{13}C NMR (101 MHz, CD_3OD) 171.2 (C=O), 170.6 (C=O), 165.1(C=O), 140.0 (C), 129.8 (C), 129.5 (C), 126.7 (CH), 124.5 (CH), 123.2 (C), 117.9 (C) 112.8 (CH), 54.5 (CH), 40.6 (CH_2), 40.4 (CH_2), 40.3 (CH_2), 32.2 (CH_2), 28.2 (CH_2), 23.1 (CH_2), 10.2 (CH_3); HR-ESIMS: $(M+H)^+$ m/z calcd for $C_{19}H_{27}N_5O_4$ 390.2141, found 390.2122.

*4.2.6.3.0. (S)-1-(2-(5-(Benzyloxycarbonyl)-3-methyl-1 H-indole-2-carboxamido)ethylamino)-3-(1H-indol-3-yl)-1-oxopropan-2-aminium chloride (**17c**).* Compound **17c** (82 mg, 0.13 mmol, 40% from **12b** 149 mg, 0.32 mmol); ^{1}H NMR (400 MHz, CD_3OD) δ 8.35 (s, 1H, H-4), 7.89 (d, J = 8.0, 1H, H-6), 7.60 (d, 1H, J = 8.0, H-7), 7.37–7.00 (m, 5H, 5 × CH_{ar}), 4.00 (m, 1H, CH), 3.41–3.12 (m, 6H, 3 × CH_2), 2.46 (s, 3H, CH_3); ^{13}C NMR (101 MHz, CD_3OD) δ 171.4 (C=O), 164.8 (C=O), 139.3 (C), 137.9 (C), 129.1 (C), 129.0 (C), 128 (C), 126.4 (C), 125.0 (CH), 123.8 (CH), 122.6 (CH), 119.9 (CH), 118.8 (CH), 117.1 (CH), 112.3 (CH), 112.1 (CH), 108.3 (C), 55.2 (CH), 40.3 (CH_2), 39.9 (CH_2), 29.1 (CH_2), 9.7 (CH_3); HR-ESIMS: M^+ m/z calcd for $C_{24}H_{26}N_5O_4$ 448.1985, found 448.1974.

*4.2.6.3.1. (S)-1-(2-(5-carboxy-3-methyl-1 H-indole-2-carboxamido)-ethylamino)-1-oxo-3-phenylpropan-2-aminium chloride (**17d**).* Compound **17d** (43 mg, 0.10 mmol, 30% from **12b** 149 mg, 0.32 mmol); ^{1}H NMR (400 MHz, CD_3OD) δ 8.27 (s, 1H, H-4), 7.82 (d, J = 8.0, 1H, H-6), 7.38–7.07 (m, 6H, H-7, 5 × CH_{ar}), 3.98 (m, 1H, CH), 3.46–2.93 (m, 6H, 3 × CH_2), 2.46 (s, 3H, CH_3); ^{13}C NMR (101 MHz, CD_3OD) δ 170.4 (C=O), 164.8 (C=O), 139.5 (C), 135.7 (C), 130.3 (2 × CH), 129.8 (2 × CH), 129.4 (C), 129.2 (C), 128.5 (CH), 126.4 (CH), 124.0 (CH), 117.5 (C), 112.3 (CH), 55.9 (CH), 40.2 (CH_2), 40.0 (CH_2), 38.6 (CH_2), 9.8 (CH_3); HR-ESIMS: M^+ m/z calcd for $C_{22}H_{25}N_4O_4$ 409.1876, found 409.1859.

4.3. Biochemical evaluation

4.3.1. Pull-down assay

Synthetic peptide (>95% purity, Eurogentec, Seraing, Belgium) encompassing the 10 C-terminal amino acids (ETVNEKVSCV) of mouse 5-HT2A receptor was coupled via its N-terminal extremity to activated CH-Sepharose 4B (GE Healthcare) according to the manufacturer's instructions. MALDI–TOF mass spectrometry analysis indicated that the coupling efficacy was higher than 90%. Immobilized peptides were stored at 4 °C in Tris–HCl (50 mM, pH 7.4) and dithiothreitol (DTT, 10 mM) to prevent cysteine oxidation at the (−1) position in the peptide sequence. Brains of Swiss mice (obtained from Janvier, Le Genest-St. Isle, France) were thoroughly washed in phosphate-buffered saline (PBS), homogenized with a Polytron homogenizer, and centrifuged at 200×*g* for 3 min. Pellets were resuspended in ice-cold lysis buffer containing Tris–HCl (50 mM, pH 7.4), EDTA (1 mM), and a mixture of protease inhibitors (Roche Applied Science), homogenized 20 times on ice with a glass Teflon homogenizer, and centrifuged at 10,000×*g* for 30 min. The membrane pellets were resuspended in CHAPS extraction buffer (50 mM Tris–HCl, pH 7.4, 0.05 mM EDTA, 10 mM CHAPS, and protease inhibitors) for 3 h in rotation at 4 °C. Then samples were centrifuged for 1 h at 10,000×*g*. Solubilized proteins (5 mg/condition) were incubated overnight at 4 °C with 20 μg of immobilized peptide in absence or presence of increasing concentrations of synthesized compounds. Samples were washed twice with 1 ml of extraction buffer and then four times with PBS. Proteins retained by affinity were eluted with 50 μl of SDS sample buffer (50 mM Tris–HCl, pH 6.8, 2% SDS, 10% glycerol, 100 mM DTT, and bromophenol blue).

4.3.2. Immunoblotting

Proteins, resolved on 8% polyacrylamide gels, were transferred electrophoretically onto nitrocellulose membranes (Hybond-C, GE-Healthcare). Membranes were incubated in blocking buffer (Tris–HCl, 50 mM, pH 7.5, 200 mM NaCl, Tween 20, 0.1% and 5% skimmed dried milk) for 1 h at room temperature and incubated overnight with primary antibodies (anti-PSD-95, clone C7E3, SC32290 mouse monoclonal IgG_1, Santa Cruz Biotechnology) 1:500 in blocking buffer at 4 °C. Blots were washed three times with blocking buffer and incubated with a horseradish peroxidase-conjugated anti-mouse antibody (1:2000 in blocking buffer) for 1 h at room temperature. Immunoreactivity was detected with an enhanced chemiluminescence method (Renaissance Plus, PerkinElmer Life Sciences).

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