Bioorganic & Medicinal Chemistry Letters 21 (2011) 3349–3353

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

NMR evaluation of interactions between substituted-indole and PDZ1 domain of PSD-95

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ARTICLE INFO

Article history:
Received 23 February 2011
Revised 29 March 2011
Accepted 3 April 2011
Available online 9 April 2011

Keywords:
PDZ domain
Protein–protein interaction
PSD-95
Analgesia

ABSTRACT

We synthesized small organic molecules designed as PDZ ligands. These indole-based compounds were evaluated for their interaction with the PDZ1 domain of the post-synaptic density 95 (PSD-95) protein. Three molecules were found to interact with the targeted PDZ protein by NMR. One of them showed chemical shift perturbations closely related to the natural ligands.

Post-synaptic density 95/Disc-Large/Zona occludens-1 (PDZ) domains (~90 amino acids) are highly conserved structural modules which are found in signaling proteins of bacteria, yeast or animals and mediate protein–protein interactions.[1] These interactions involve extreme carboxy-terminal of ligand proteins binding to a hydrophobic groove between α_B helix and β_B strand, delimited by the GLGF loop of the PDZ domain.[2]

PSD-95 is a three PDZ-domain containing protein. The first PDZ domain (PDZ1) (Fig. 1) was found to interact with the C-terminal extremity of the serotonin receptor 5HT2a.[3] A disruption of the interaction between these two proteins was found to reduce hyperalgesia in rodent models.[4] Thus, inhibiting the interaction between PSD-95 PDZ1 and 5-HT2a could lead to the development of a novel class of analgesic agents.

Small molecules such as indoles **A**–**C** (Fig. 2) interrupting PDZ interactions, were reported by Fujii and colleagues.[5–8] Although these small molecules were designed as peptido-mimetics of C-terminus of PDZ ligands, little information is available about their interactions with specific PDZ domains.

Here, we describe the design and the synthesis of novel indolic analogues based on these previously reported indole-based PDZ inhibitors. The objective is to carry out an in-depth study of the chemical space within the ligand binding site and to establish the relative importance of the different types of interactions within this binding site. These compounds were evaluated by Nuclear Magnetic Resonance (NMR) spectrometry in order to identify their interactions with the PDZ1 domain of PSD-95. A docking study was subsequently carried out using a previously developed model to explain the results.[9]

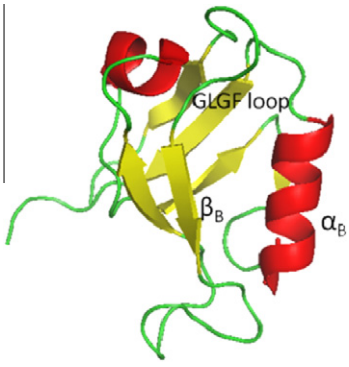

Figure 1. Cartoon representation of the backbone of the 3D structure of PDZ1 domain of PSD95 (PDB 2KA9).

The substituted indoles were synthesized via *o*-iodo-anilines **2** and **4** which are easily obtained in three steps from the commercially available nitro-benzoic acids **1** and **3** (Scheme 1).[5,6]

Palladium-catalyzed annulation[10] between *o*-iodo-anilines **2** or **4** and 2-oxobutanoic acid (R^3 = Me) or 2-oxopropanoic acid (R^3 = H)

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doi:10.1016/j.bmcl.2011.04.011

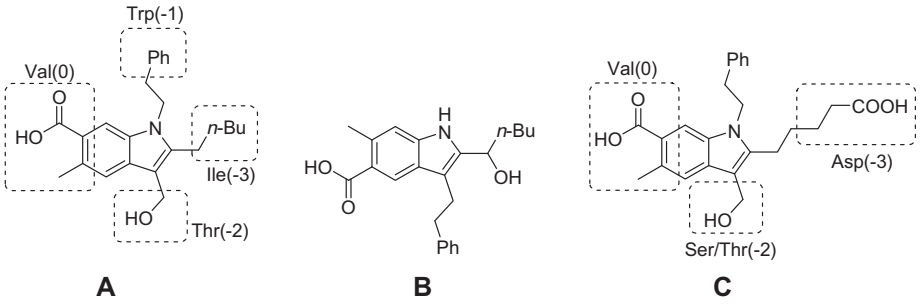
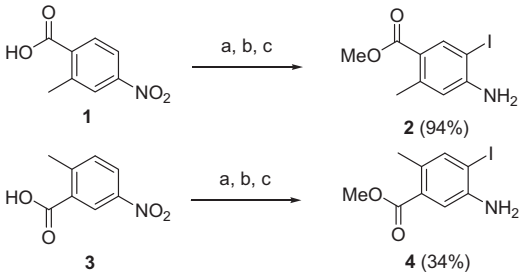


Figure 2. Fujii's indoles **A**–**C**.

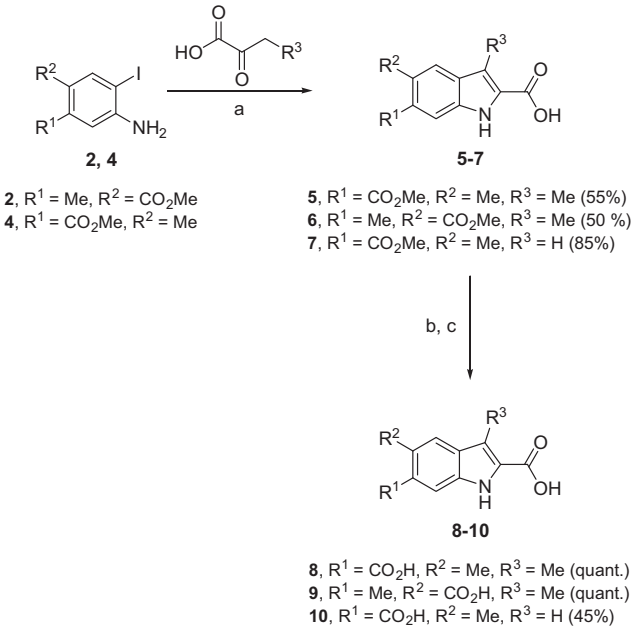


Scheme 1. Synthesis of *o*-iodoanilines **2** and **4**. Reagents and conditions: (a) $SOCl_2$, MeOH, 70 °C, 4 h; (b) Pd/C, H_2 (1 atm), MeOH, rt, 24 h; (c) ICl, $CaCO_3$, MeOH, H_2O, rt, 24 h.

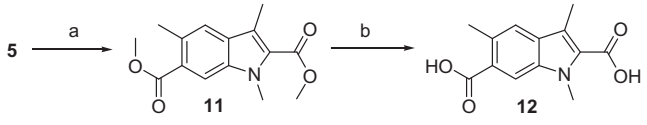
Table 1
Chemical shift perturbations observed by ^{15}N-^{1}H HSQC for compounds **5**, **6**, **8**–**10** and **12**–**14**

Entry	Cpds	R^1	R^2	R^3	R^4	Chemical shift perturbation observed
1	**5**	CO_2Me	Me	Me	H	No
2	**6**	Me	CO_2Me	Me	H	No
3	**8**	CO_2H	Me	Me	H	Yes
4	**9**	Me	CO_2H	Me	H	Yes
5	**10**	CO_2H	Me	H	H	No
6	**12**	CO_2H	Me	Me	Me	No
7	**13**[*]	H	CO_2Et	Me	H	No
8	**14**[*]	H	CO_2H	Me	H	Yes

[*] The synthesis of compounds **13** and **14** is described elsewhere.[9]



Scheme 2. Synthesis of substituted indoles **8**–**10**. Reagents and conditions: (a) $Pd(OAc)_2$, DABCO, DMF, 105 °C, 16 h; (b) KOH (2 M), reflux, 24 h; (c) HCl (1 M).



Scheme 3. Synthesis of substituted indole **12**. Reagents and conditions: (a) K_2CO_3, CH_3I, DMF, RT, 12 h (90%); (b) KOH (5 M), reflux, 24 h (quant.).

gave the substituted indoles **5**–**7** in moderate to good yields (Scheme 2). After saponification, carboxylic acids **8**–**10** were obtained.

Indole **5** was *N*-methylated[5] to afford ester **11** (Scheme 3) which was quantitatively saponified to give carboxylic acid **12**.

To evaluate the interaction between compounds **5**, **6**, **8**–**10** and **12** and PDZ domains, ^{15}N labeled PDZ1 domain of PSD-95 was produced and purified after cloning and expression in BL21 *E. coli*.[11] $^{1}H/^{15}N$ heteronuclear single-quantum coherence NMR experiments were recorded using labeled protein with potential ligands. Chemical shifts assignment of the ^{15}N-^{1}H HSQC spectrum was based on Ref. 12. Chemical shift changes in the ^{15}N-^{1}H HSQC were then used to identify the amino acids whose chemical environments were perturbed in the presence of the compounds. These variations may be due to direct interactions with the ligand or could be induced by conformational changes in the protein tertiary structure.

The data (Tables 1 and 2) show that while addition of acids **8**, **9** and **14** results in several chemical shifts in the protein NMR spectrum, none of the esters **5**, **6** and **13** led to a chemical shift perturbation of the NMR spectrum. These data conclude on the importance of the carboxylic acid moiety on the six-membered ring of the indole, suggesting it is important for the ligands' interactions with the protein.

Acids **10** and **12** did not induce variations of chemical shifts. These inactive analogues of indole **8** demonstrate the importance of substituents at positions 1 and 3 of the indole. Indeed, addition of a methyl group at position 1 or suppression of the methyl group at position 3 led to non-interacting molecules.

Specific chemical shift perturbations were observed upon addition of indole **14** to the protein: signals for amino acids G20, I27, H69 and L76 shifted (Fig. 4a). The first two amino acids (G20, I27) are located on the loop between β_B- and β_C-sheets while the other two (H69, L76) are on α_B-helix (Table 2, entry 1). Although close, none of these perturbations are consistent with

Table 2
Localization of amino acids showing chemical shift perturbation due to ligand (s.c. = side chain)

Entry	Compounds	β_A/β_B loop	GLGF	β_B	β_B/β_C loop	β_E/α_B loop	α_B
1	**14**				G20, I27		H69, L76
2	**9**	N11		S17, I18	G21, N24 (s.c.)*, H26, I27		H69, V73
3	**8**	N11	G15, F16	S17	G21, N24, N24 (s.c.)*, H26, I27,	V67	H69, V73, L76

* s.c. = side chain.

the ligand interacting within the defined groove.[12] Thus, indole **14** is probably interacting with PDZ domain at the generic binding site but not as deep as natural ligands. Perturbations of chemical shifts observed were moderate, meaning that **14** is a weak binder.

Indole **9** was designed as a more hydrophobic derivative of **14**, by addition of a methyl group at position 6. This methyl moiety was introduced to mimic hydrophobic residues at position 0 (first C-term residue) of the natural ligands. Indole **9** was found to perturb the chemical environment of more amino acids than **14**. Furthermore, many of these amino acids are located within the targeted pocket (Table 2, entry 2; Fig. 3b). Two amino acids of β_B-sheet (S17, I18) and two of α_B-helix are perturbed by interactions between **9** and the PDZ protein. Perturbations induced by 6-methyl indole **9** are related to perturbations described for the natural ligand. Indeed, due to the additional methyl group, this compound may be inserting deeper into the groove delimitated by β_B and α_B. However, amino acids of the GLGF loop, which are involved in the interaction with terminal acid function of natural ligands, did not shift upon addition of compound **9**. Furthermore, the binding is still weak because perturbations of chemical shift are moderate.

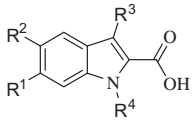


Figure 3. General structure of evaluated compounds.

Chemical shifts, measured during $^1H/^{15}N$ HSQC experiments with PDZ1 of PSD-95 and compound **8**, are represented on Fig. 5. Binding of compound **8** and its structural isomer **9** to the PDZ protein generated quite similar perturbations (Table 2, entries 2 and 3; Fig. 4c and d). However, new amino acids located in the GLGF loop (G15 and F16) showed chemical shift changes upon binding of indole **8**.

Indole **8** was found to interact in a very similar way to natural ligands because all parts of the binding pocket were involved. Perturbations for amino acids located in GLGF loop (G15 and F16), β_B sheet (S17), β_B/β_C loop (G21, N24, H26 and I27) and α_B helix (H69, V73 and L76) were noted. Despite moderate binding, compound **8**

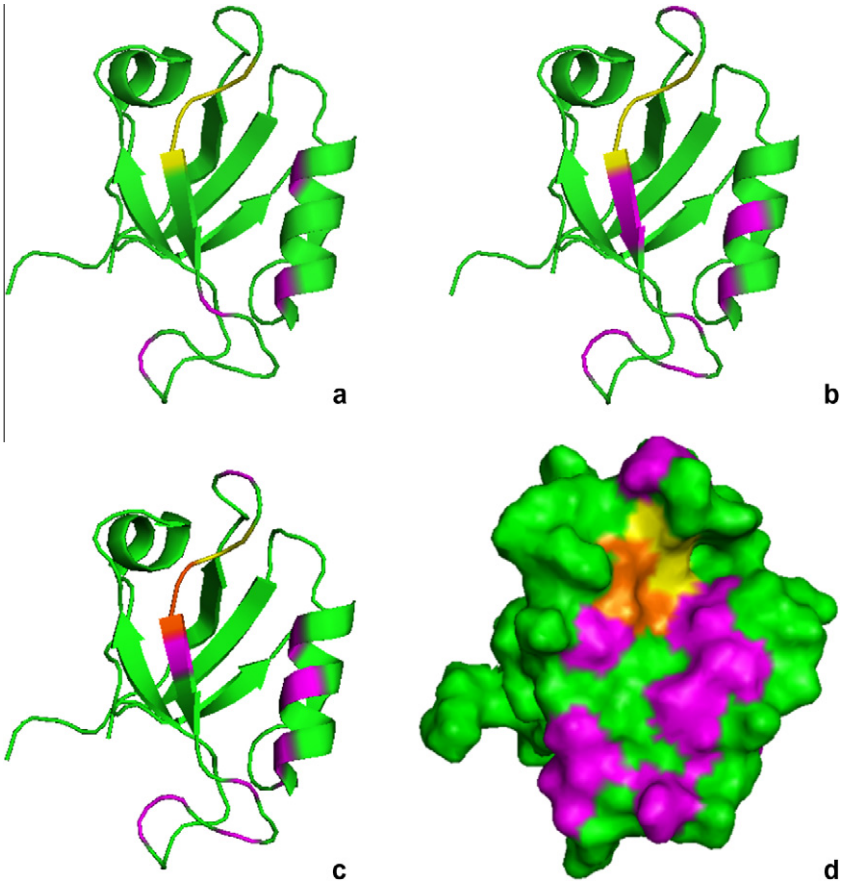


Figure 4. 3D structure of PDZ1 domain of PSD95 (PDB 2KA9). Yellow indicates GLGF loop; purple indicates amino acids with chemical shift perturbation in presence of: (a) **14**, (b) **9**, (c) and (d) **8**; orange indicates amino acids with chemical shift perturbation in the GLGF loop (purple + yellow).

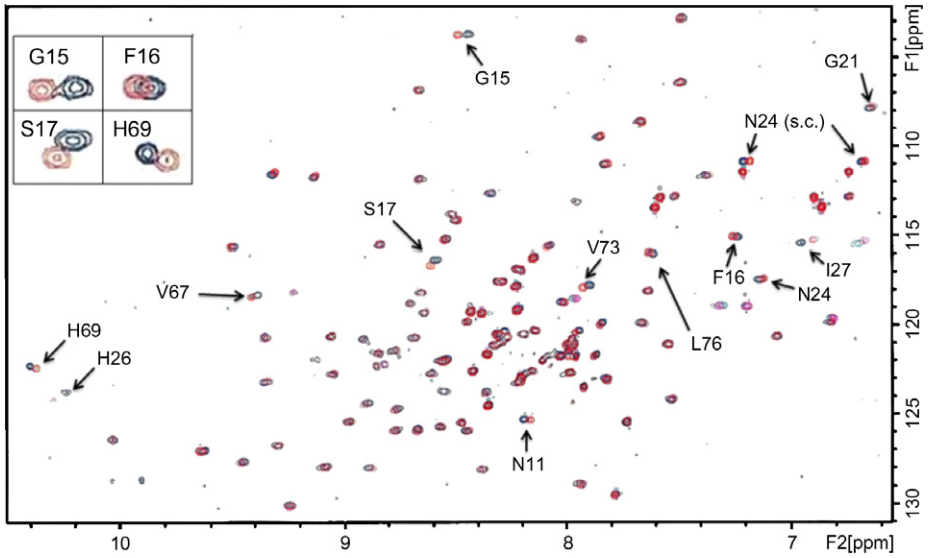


Figure 5. Chemical shift perturbation of PDZ1domain of PSD95. Blue dots correspond to $^{1}H/^{15}N$ HSQC of the free protein; red dots correspond to the protein + 10 equiv of indole **8**.

appeared to interact within the targeted groove permitting its evaluation as a basis to build competitive inhibitor of natural ligands.

The observation that H69 is involved in binding of the three compounds **8**, **9**, and **14** is consistent with an interaction in the same way as natural ligands. Indeed this histidine is known to be responsible for class selectivity of PDZ domain by hydrogen bonding to hydroxyl group of amino acid at position -2 (thirs C-term residue) of the natural ligand. H69 is probably interacting with the carboxylic function of the 5-membered ring of the indole.

Molecular docking is one of the most important and useful method for predicting and investigating protein–ligand interactions. We performed molecular docking study using Molecular Operating Environment software (MOE 2010.10),[13] to access binding affinity between PDZ-1 domain of PSD-95 and substituted indoles. The purpose of the docking study was to compare the interactions determined by NMR for the best interacting compound with the docking results. Previously we had carried out docking study on indole moiety,[9] the same protocol was used for the present docking study. The indole moiety has been modified at various positions in order to optimize lead molecule.

The best interacting indole **8** was docked into the PDZ1 domain of PSD-95 in order to try to explain the NMR results. Several docking poses were found, and the best pose showed indole **8** bound to the PDZ domain as anticipated (Fig. 6) with total interaction energy of 7.41 kcal/mol. When the docking pose was analyzed more closely, it was found that the 6-COOH made hydrogen bond (H-bond) interactions with G15, P16 of GLGF loop, and made several other interactions vdW and columbic interaction with G13, L14, G15, P16, S17, I18, A19, H69, V73, L76. The other carboxylic group (2-COOH) made H-bond interaction with I18 and vdW and columbic interaction with H69, I18. The 3-methyl group interacted with V73 and L76 through vdW and columbic interactions. The indole nucleus also showed vdW and columbic interactions with P16, I18 and L76.

The docking pose is in agreement with the NMR results obtained. Indeed the acid analogues were found to interact while the esters did not, which is explained by the fact that both carboxylic acid moieties are involved in several interactions with the protein. The methyl group at position 3 was found to interact with the protein, which is consistent with the fact that 3-demethylated analogue **10** did not show interactions with the protein. Although, *N*1 is not involved in any interaction according to the docking pose, an addition of a methyl group at this position (compound **12**) proved detrimental to interaction witht eh PDZ protein. This is probably explained by the steric hindrance of the *N*-Me too close to the β_B-sheet of the PDZ domain. Finally, the 5-Me was found important by NMR but no interaction was detected on the docked pose. This could be explained by an electronic effect of this group more than a hydrophobic or steric effect.

Figure 6. Docking of compound **8** into PDZ1 domain showing H-bond interactions (red) and other interactions (green).

Overall, interactions observed by docking and NMR data were consistent with each other. We identified three ligands of the PSD-95 PDZ1 domain. One of them, compound **8**, led to perturbations in chemical shift very closely related to changes induced by natural ligands. This observation was confirmed by molecular docking.

Acknowledgements

We thank Robert Gibson and Mark Tully for their assistance during protein production/purification and Marie Phelan for her expertise in NMR. We also would like to thank the French National Research Agency (ANR) for funding of the project PDZ-CANPAIN and a postdoctoral fellowship for BB; the Regional Council of Auvergne (Conseil Régional d'Auvergne) and the European Fund for Regional Economical Development (FEDER) for a doctoral scholarship for AV; and the PRES Clermont Université for a postdoctoral fellowship for HD.

Supplementary data

Supplementary data (Synthetic procedure, analysis data, protocols for protein production and purification and protocols for NMR experiments are available.) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.04.011.

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