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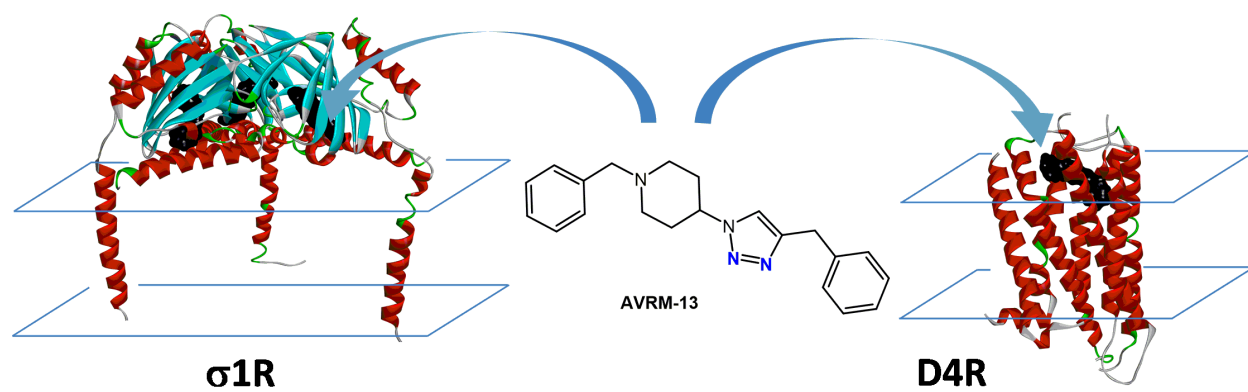
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Discovery of potent dual ligands for Dopamine D4 and σ 1 receptors

Graphical Abstract



Discovery of potent dual ligands for Dopamine D4 and σ 1 receptors

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Keywords: Sigma1; dopamine D4, receptor, piperidine, triazole, hybrids, molecular modelling, molecular dynamics, polypharmacology

Abstract

During our work on exploration of molecules with some piperidine-triazole scaffolds, we realized that our compounds display chemical similarity with some σ , as well as dopaminergic receptor ligands. Here we show that this series of molecules has indeed strong affinity both for σ_1 and dopamine D4 receptors. Moreover, they appear selective towards σ_2 , dopamine paralogues D1, D2, D3 and D5 receptors and hERG channel. Extensive molecular dynamics with our lead compound AVR1013 were carried out on σ_1 , supporting agonist activity of the ligand. Unexpectedly, several observations suggested the existence of a cation binding domain, a probable regulatory site for calcium.

Introduction

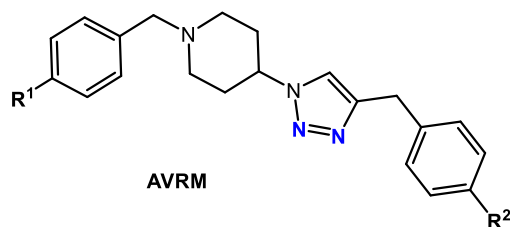
The odd protein σ_1 , so-called “non-opioid σ receptor” is a poorly known protein involved in many cellular functions. Among them, it seems to act as a protein function modulator, amplifying or reducing the signaling initiated by other proteins.^{1,2,3} It was also shown to constitute a chaperone protein, protecting cells from the aggregation of misfolded proteins in the endoplasmic reticulum, such as low-density lipoprotein, insulin or brain-derived neurotrophic factor.⁴

Since its discovery in the mid-1970 as a SKF-10.047 binding protein,^{5,6} σ_1 was shown to be a membrane protein located mainly in the endoplasmic reticulum,⁷ but also in nuclear and mitochondria associated membranes, where it regulates Ca^{2+} fluxes.⁸ Moreover, σ_1 interacts with many proteins such as ion channels and GPCR.^{2,3} It was cloned in 1996,⁹ and its 3D structure was solved recently through X-ray determination.¹⁰ According to the X-ray structure, σ_1 is a homotrimeric protein whom each protomer consists of a single TM helix and a cytosolic globular domain encompassing a ligand binding site. The endogenous ligand is unknown, but several molecules have been suggested to play this role, such as dimethyltryptamine, *D*-erythro-sphingosine, choline and neurosteroids (e.g. progesterone). However, the above molecules have too low affinity to be conclusively considered as natural ligands. The precise consequence of ligand binding to σ_1 are not definitely known, but it is considered that ligand causing the dissociation of high order oligomeric states of the receptor, as well as unbinding of σ_1 protein partners, such as ANK2, ITPR3, BIP, ion channels or receptors¹¹ are agonists.

Apart from the initial opioid ligands, several CNS-targeting molecules in clinical use were shown to bind σ_1 , with strong to moderate affinity, such as pentazocine, haloperidol, pridopidine, blarcamesine, dextromethorphan or antidepressants (e.g. imipramine, fluoxetine). It is currently uncertain if this interaction participates in the mode of action of these drugs. Many biological data and observations suggested that σ_1 could be a promising target in neurodegenerative diseases,¹² such as Huntington's, Alzheimer's or Parkinson's diseases,¹¹ and several selective σ_1 ligands have recently entered clinical trials, such as pridopidine or blarcamesine.

On the other hand, many CNS illnesses, such as neuropsychiatric diseases have been associated with a deregulation of dopamine-releasing neurons. Hence decades ago, there was a great enthusiasm around dopamine D4 receptors when it was discovered that the antipsychotic drug clozapine was a D4 selective ligand, while in the meantime D4 receptor expression was higher in schizophrenic patients than in healthy people. Unfortunately, disappointing clinical trials with a drug candidate from Merck have dampened the enthusiasm. In a new turnaround however, recent advances have renewed the interest of D4 as a promising target,¹³ with the identification of links between D4 and addiction. Genetic association studies in humans, D4 KO mice as well as pharmacological studies, converged on a crucial implication of the receptor in addictive behavior.¹⁴ For example, the D4 selective antagonist L745870 reduced ethanol intake and stress-induced reinstatement *in vivo*.¹⁵ Several links have also been found between D4 and neurodegenerative pathologies. For example, D4 gene methylation is associated with an increased risk of Alzheimer's disease in human.¹⁶ In rats, *in vivo* models showed that D4 ligands altered the non-motion symptoms of Parkinson's diseases.^{17,18}

During the course of our recent work around piperidine-triazole hybrids,^{19,20} we realized that our AVRMs derivatives show significant similarity with a class of dopaminergic, as well as σ_1 receptor ligands (Fig. 1). This appeared to us as a great pharmacological opportunity, because it is nowadays commonly estimated that polypharmacology, or multi-target directed ligands (MTDL) should be a more fruitful approach than classical one-target / one-ligand paradigm.²¹ This seems especially true for complex diseases such as CNS pathologies, as supported by the profile of recent FDA-approved drugs.²²



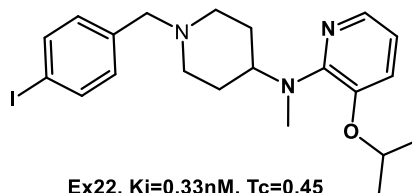
AVRM-13: R¹=R²=H
 AVRM-01: R¹=H; R²=Br
 AVRM-03: R¹=NO₂; R²=Br
 AVRM-04: R¹=F; R²=Br
 AVRM-05: R¹=NH₂; R²=Br

D4R Ligands



Ex1, R=H, K_i=11nM, T_c=0.78

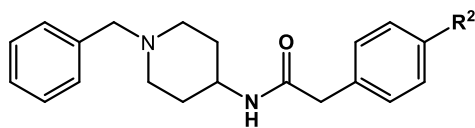
Bioorg. Med. Chem. Lett. 1999, 9, 1285



Ex22, K_i=0.33nM, T_c=0.45

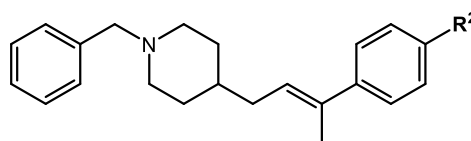
J. Med. Chem. 2017, 60, 7233

σ₁R Ligands



Ex1, R²=H, K_i=3.9nM, T_c=0.81

J. Med. Chem. 1998, 41, 2361



Ex20, R²=H, K_i=0.7nM, T_c=0.77

J Med. Chem. 2020, 63, 15163

Fig. 1 AVRM series and representative examples of ligands for Dopamine D4 or σ₁ receptors. Biological data of one example are given, as well as the Tanimoto coefficient (T_c) as compared to AVRM13 (ECFC_6 fingerprint)

We report in the present work the complete characterization of σ and dopaminergic receptors profile of our lead AVRM-13 and parent molecules, with exploration of their interactions with the σ₁ receptor by use of extensive molecular dynamic studies starting from the crystal structure of this receptor.

Results and discussion

Our lead compound AVR-13 was first tested on a panel of human dopamine and sigma receptors (Fig. 2). It turned out to be very much more potent towards D₄ than the four other dopamine receptors. On the other hand, as concerns σ , it appeared to be a stronger ligand for σ ₁ than σ ₂. We next confirmed these results by determination of the affinity: the K_i of AVR-13 for receptor D_{2s} is 6.3 μ M, for D₃ > 10 μ M, and for D₄ = 260 nM. Further, AVR-13 showed stronger binding to σ ₁ receptor (K_i = 1.4 nM).

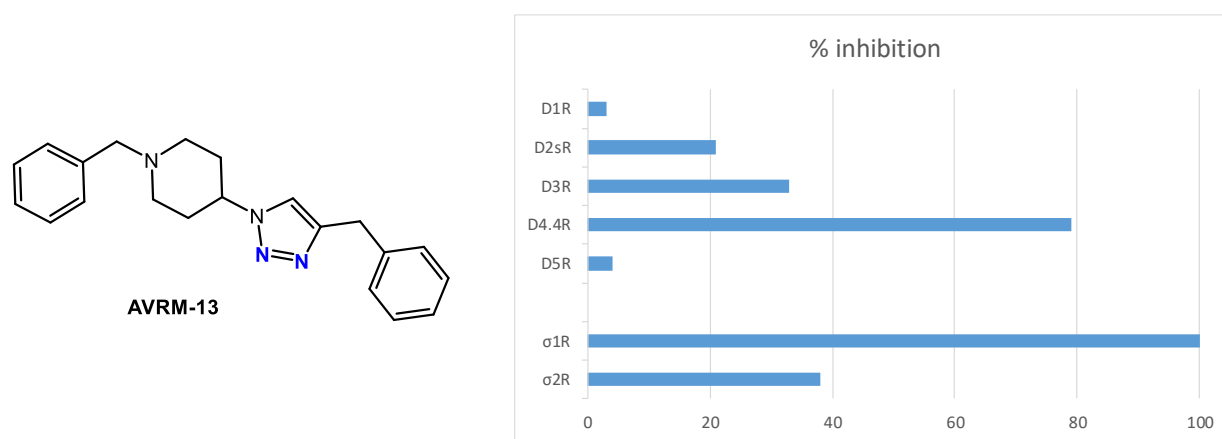


Fig. 2 screening of AVR-13 on a panel of dopamine and σ receptors. The compound was tested at 3 μ M against reference ligand for each receptor.²²

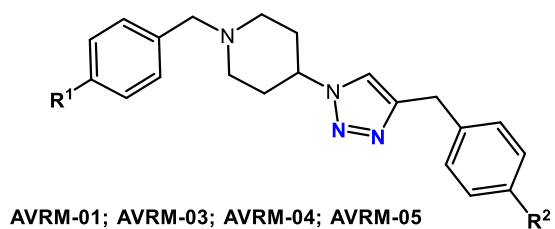
Then we selected several close analogues of AVR-13 to be tested on the most promising targets, namely D₄ and σ ₁ receptors. The results are given in Fig. 3 and some of them are even more potent than our lead, with estimated K_i calculated in Table 1.²³ Particularly, the *para*-bromo substituent of one benzyle is very favorable for D₄R. For the other benzyle, polar substituent is disadvantaged. This structure-activity relationship is in complete agreement with docking experiments. AVR-13 fitted well into the D₄R orthosteric binding site, and docking suggested two binding modes, with head-to-tail orientation (Fig. 4A). In the first, the benzylpiperidine occupied the extracellular end of the binding site. In the second, it is engaged within the TM helices. The *para*-bromo substituent of AVR-01 strongly favored the first, with the *para*-bromo benzyl deeply buried into the intra-TM cavity. The cationic piperidine formed a salt bridge with D115, and the proximal benzyl is in a subcavity located between TM2 and TM3. The triazole formed perpendicular π -stacking with H414, and the distal benzyl with F410 and / or F411 (Fig. 4B).



Fig. 3 Screening of AVRM-13 analogues on dopamine D4 receptors (left, tested at 0.7 μ M) and σ 1 receptor (right, tested at 5 nM).

Table 1.

Estimated K_i for affinity of AVRM molecules against the dopamine D4 and σ 1 receptors based on the above one-dose testing.²³



	R ¹	R ²	D ₄ R	σ 1
			nM	
AVRM-01	H	Br	6	1,5
AVRM-03	NO ₂	Br	11	12
AVRM-04	F	Br	6	2
AVRM-05	NH ₂	Br	144	3,3

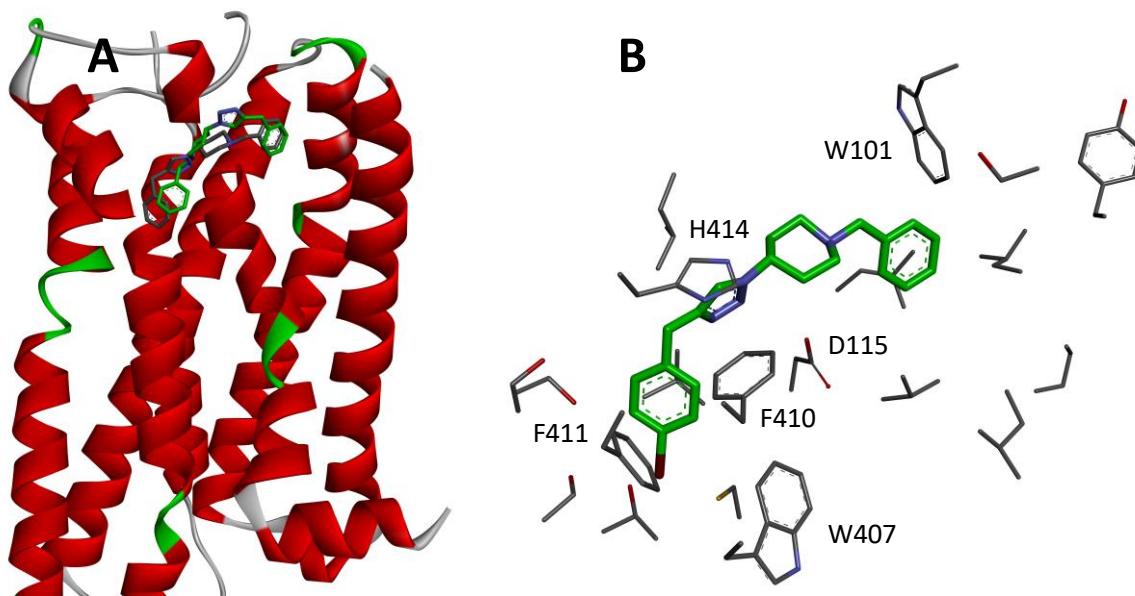


Fig. 4A The docking of AVRMs-13 suggested two possible binding orientation in the D4R. **4B** Details of the AVRMs-01 binding mode, with important amino-acids labelled.

Next pharmacokinetic properties of the compounds were predicted *in silico* using QikProp (Schrödinger LL, NY, 2020). Particularly, we calculated their permeability with Caco-2 (a model of the gut-blood barrier) and MDCK cells (considered to be a good mimic for the blood-brain barrier). Their penetration are in line with drugs of high bioavailability (Table 2),^{24,25} particularly for AVRMs-13, -01 and -04 ($P > 500$ nm/s).²⁶ The cationic and aromatic nature of the series make these molecules possible hERG inhibitors. However, experimental determination of AVRMs-13 activity revealed a very weak inhibitor ($K_i = 4.6$ μ M). Taken together, these results suggest that our compounds might have very favorable properties for further *in vivo* exploration.

Table 2

Predicted parameters and pharmacokinetic properties of the compounds. $P_{\text{Caco-2}}$: apparent Caco-2 cell permeability. P_{MDCK} : apparent MDCK cell permeability. logBB: brain/blood partition coefficient.

	R^1	R^2	MW	clogP	Ro5	$P_{\text{Caco-2}}$	P_{MDCK}	oral absorption	logBB
						(nm/s)		%	
AVRM-13	H	H	332,4	4,4	0	1014	556	100	0,3
AVRM-01	H	Br	411,3	5	0	1014	1473	100	0,5
AVRM-03	NO ₂	Br	456,3	4,1	0	117	143	88	-0,7
AVRM-04	F	Br	429,3	5,2	1	1015	2659	100	0,6
AVRM-05	NH ₂	Br	426,4	4,1	0	244	315	94	-0,3

Although σ_1 is a very singular protein with unknown natural ligand yet, several of its xenobiotic ligands have been described as agonists or antagonists based on the different biological consequences of their binding. At the molecular level, agonists are thought to trigger, or at least facilitate the dissociation of the receptor oligomers. On the contrary, antagonists favor high-molecular-weight species of the receptor.^{27,28,29} Comparison of the crystal structure of σ_1 bound to agonists or antagonists revealed structural differences: (i) a displacement of A185, whom methyl group interacts with phenyl of the agonist, (ii) causing an 1.8 Å increase in the distance between helix α_4 and helix α_5 , (iii) and finally a loss of the inter-chain hydrogen bond between A183 and Q194.³⁰ These modifications in agonist-bound receptor can be seen as early events of the protomer dissociation.

Unfortunately, the experiences needed to show oligomeric shifts in proteins are very tricky and time-consuming. So in order to characterize AVRM-13 activity, we used simulations and undertook molecular dynamics experiments. The compound was first docked in the crystal structure of trimeric σ_1 (PDB: 5HK1).¹⁰ The complex was inserted in a solvated bilayer membrane, assuming that the peptide R₈WAWAALLLLAVAAVLTQVVWLWL₃₀ is a transmembrane helix and that the two segments helix α_4 (R175-F191) and helix α_5 (F196-T216) float just above the membrane (Fig. 5).

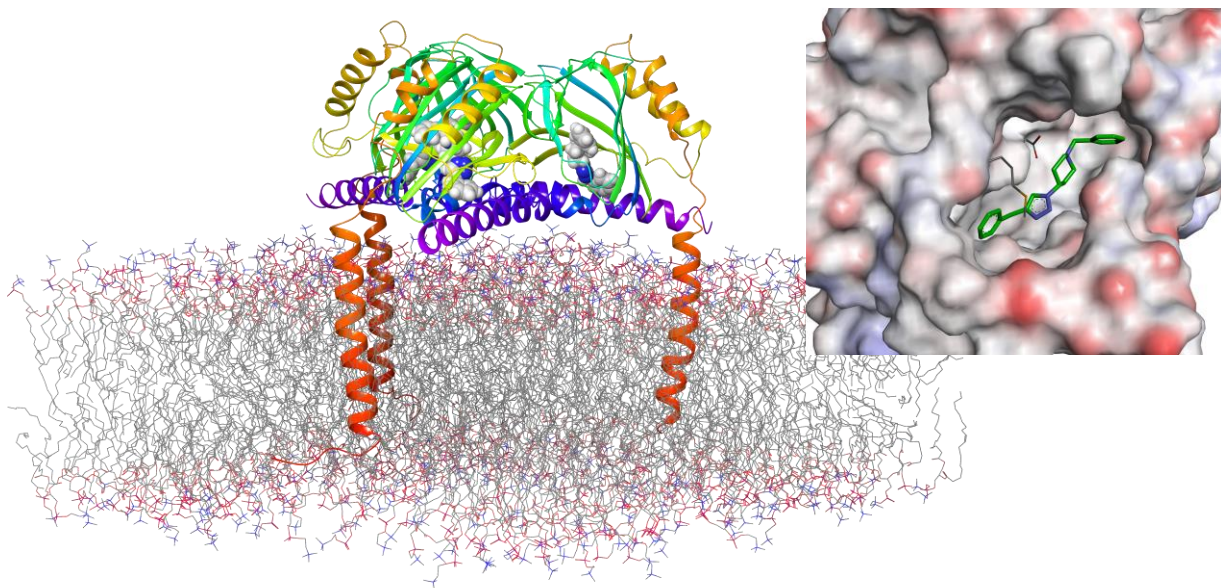


Fig.5 σ_1 complex used for molecular dynamics experiments. AVR-13 (white and blue spheres) was docked into the three protomers of σ_1 receptor (colored ribbons) inserted in a lipid bilayer (water and ions are hidden). In the insert, details of docked AVR-13.

After equilibration, the system was free to evolve during 2 μs . It turned out to be remarkably stable, with the three ligands strongly bound to the binding sites. However, we noticed a general raising of the ligands within their pocket. This seems to be due to the formation of a sulfur/ π interaction,^{31,32,33,34} between M93 and the ligand triazole (Suppl. Fig. 1). The interaction which was absent in the initial conformation of the complex, appeared quickly, within the first 5 ns, and it is maintained till the end of the simulation. We observed that A185 moved away quickly from the ligand, and that distance between helix α_4 and helix α_5 increased gradually (Fig. 6).

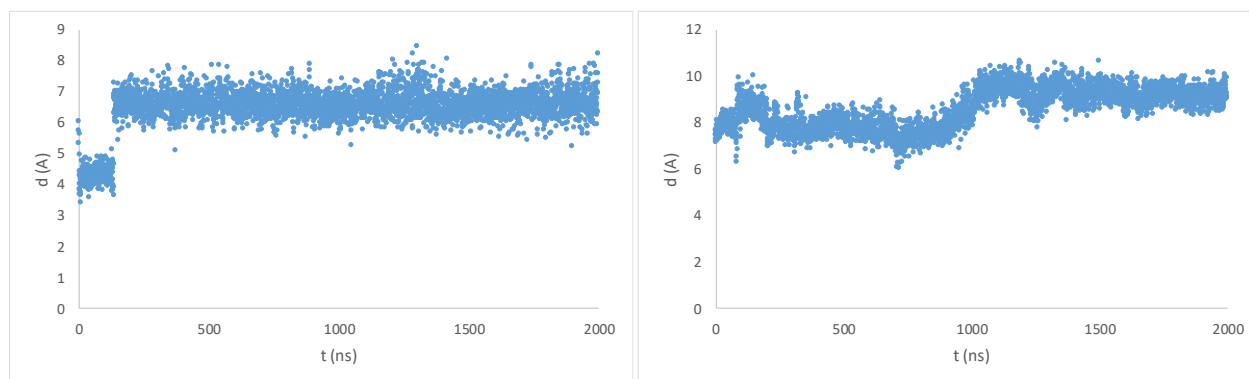


Fig. 6 Conformational rearrangements of the σ_1 receptor after AVR-162 binding, as seen in one protomer. Distance between A185 and the triazole-bound benzyle of AVR-162 (left). Distance between helix α_4 and helix α_5 (T189 / F196 distance, right).

These modifications propagated to the cytosolic end of the binding site and caused a reorganization of the packing of this aromatic-rich region, as well as a slight unfolding of helix α_4 V₁₇₇IPSTLAFALADTVF₁₉₁ (Fig. 7, Suppl. Fig. 2).

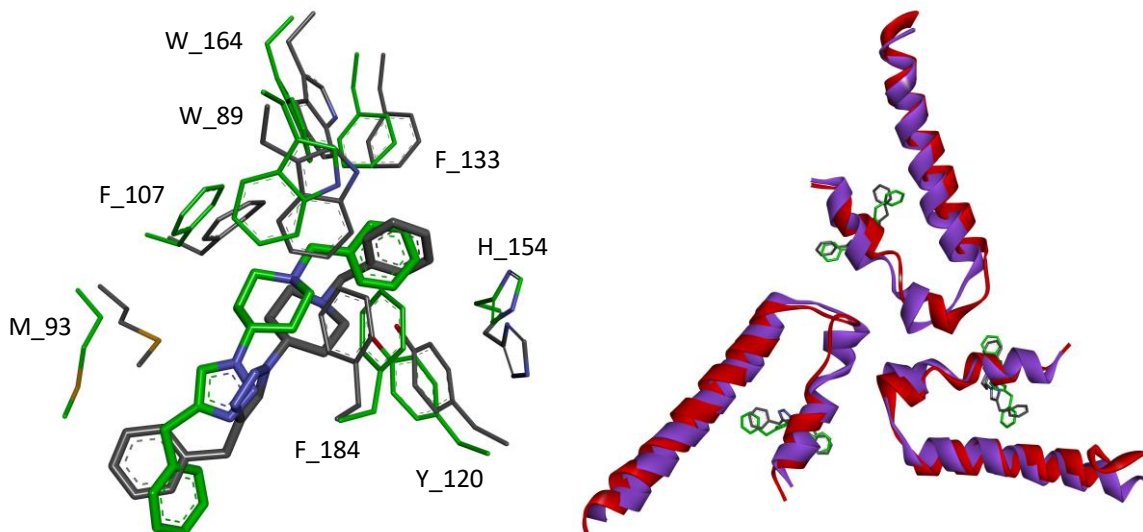


Fig. 7 Superimposition of the σ_1 receptor before and after 2 μ s molecular dynamics. **(A, left)** Details of the ligand binding pocket: AVR-162 is figured with thick bonds. **(B, right)** The two helices α_4 and α_5 forming the floor of σ_1 binding pocket seen from the membrane bilayer, in the trimeric receptor. Note the partial unfolding of the small α -helix. The initial frame is in purple ribbon / green carbon atoms, and final state in red ribbon/ grey carbon atoms.

On the whole, all these movements resulted in an expansion of the binding site, with a nearly 2-fold increase between the initial and final state of our simulation (Fig. 8A, Suppl. Table 1). Inflation of the three ligand binding sites made the protomers moving away from each other, and loss of the initial hydrogen bond between A183 and Q194 (Fig. 8B). Separation of the three protomers is relatively small, but it might constitute the preliminary step of a dissociation event that takes a longer time-scale than our 2 μ s simulation. Since dissociation of σ_1 oligomers is a distinctive feature of agonists, the molecular dynamics experiment suggested that AVR-162 acts as a σ_1 agonist. However, further study will be needed to

increase the duration of the experiment and to compare the behaviour of $\sigma 1$ receptor in presence of reference agonists and antagonists.

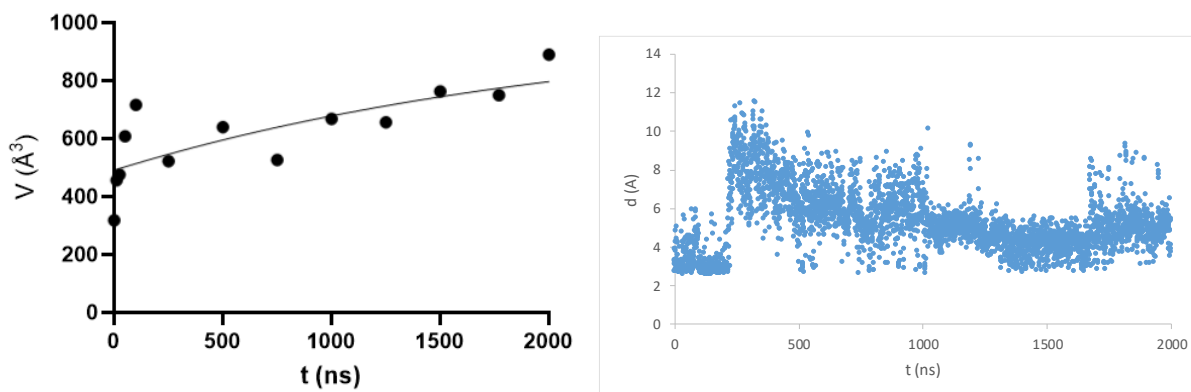


Fig. 8 (A, left) Volume evolution of the interface between the three protomers. A logarithmic function is the best fit to the dots (thin line, $r^2 = 0.6$). (B, right) Distance between A183 and Q194 of two adjacent protomers during molecular dynamics.

Fortuitously, we noticed that the Na^+ ions used to equilibrate the electric charges of the system were sometimes trapped into a small cavity located at the junction between the TM helix and the two helices $\alpha 4$ and $\alpha 5$ forming the floor of the binding site (Fig. 9). This pocket was empty at the beginning of the simulation. For one protomer, a sodium ion bound at 120 ns and remained here for 53 % of the simulation; the site of other protomers were less frequently occupied. It was bound essentially through a salt bridge with E213, and to a lesser extent to E202. Remarkably, the binding and unbinding events seemed correlated with stabilization / destabilization of the protomer structure (Fig. 10). Since the $\sigma 1$ receptor has been described as a calcium fluxes regulator,⁸ this cation pocket could constitute a Ca^{2+} sensor domain, notably because it is present 3 times in the trimeric $\sigma 1$ structure. Hence the response of the receptor, via modification of its conformation, could be different if only one, two or three calcium sites are occupied, proportionally to the Ca^{2+} concentration. Moreover, only the loop A₉₈SLSE₁₀₂ separates the cation and ligand binding sites, so it is easy to understand the convergent effect of ligand binding and calcium depletion.⁴ In a future work, it should be very interesting to investigate experimentally if the cavity we identified constitutes actually an allosteric site for Ca^{2+} ions and to understand its functional effect on the receptor.

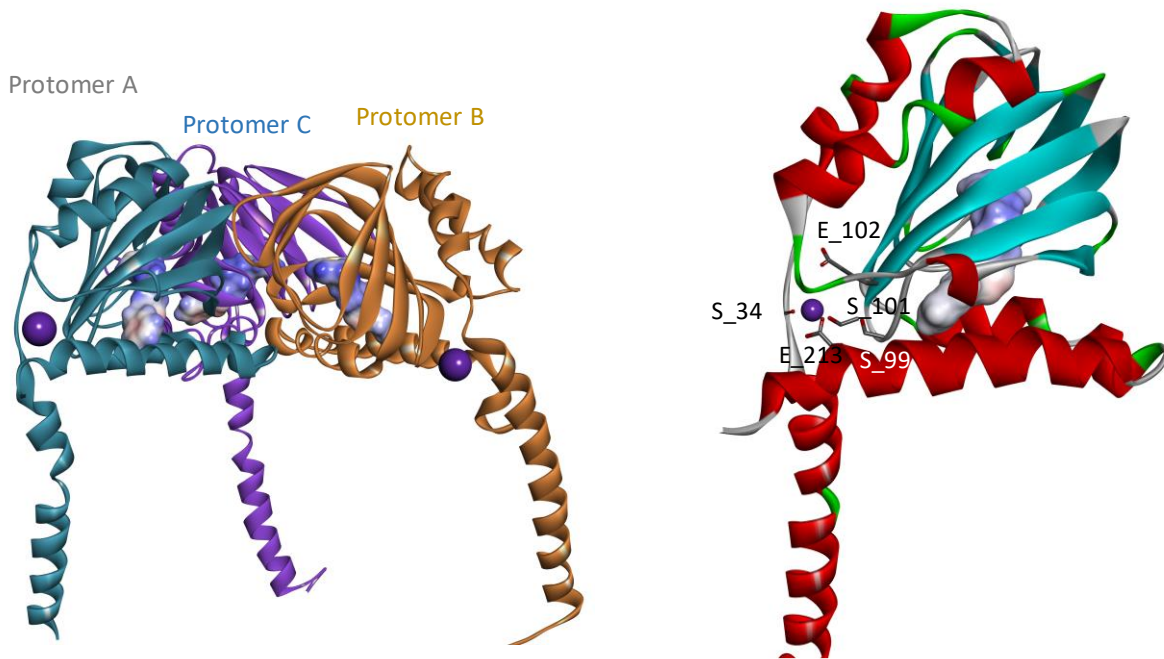


Fig. 9 Identification of a cation binding site in the trimeric $\sigma 1$ structure (left) during molecular dynamics. The Na^+ are shown as purple spheres and AVR-M-13 ligands as colored surface. On the right, details of the ion binding pocket.

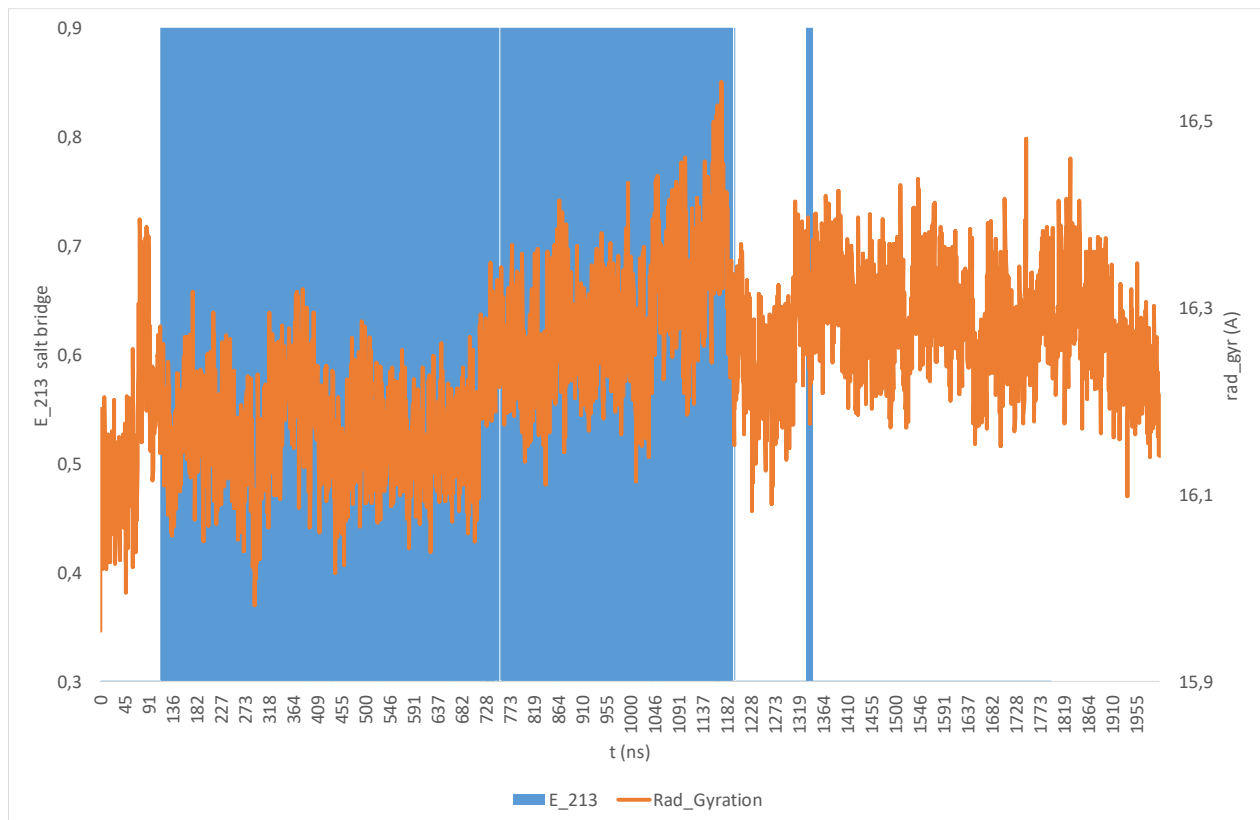


Fig. 10 Na⁺ binding and unbinding to E213 (filled bars: ion bound, empty bars: unbound) superposed to the radius of gyration (calculated for α -carbons of the protomer), as an outward sign of protein flexibility.

Conclusion

During the course of our work on inhibitors of Bcl-xL protein-protein interactions, we noticed that our lead inhibitor AVR-1101 was structurally related to some ligands of dopamine and σ receptors. Here we showed that molecules in this series have indeed a strong affinity for both D4 and σ 1 receptors. They are in the same activity range than several previously reported D4 ligands, also found to have σ 1 affinity.³⁵

There is currently a great hope in medicinal chemistry around multi-target-directed-ligands (MTDL), particularly for CNS disorders. Recently for example, a macromolecular complex involving dopamine D1, ghrelin 1A and σ 1 receptors has been evidenced and suggested to mediate the anorexic effect of cocaine.³⁶ MTDL implicating other dopamine receptors has been recently substantiated by cariprazine, a 5-HT_{2B/D2/D3} receptor ligand,³⁷ which has received a marketing authorization in the US for schizophrenia and bipolar disorders. The MTDL strategy involving σ 1 has also recently associated histamine H₃,³⁸ or μ opioid receptor.³⁹ Further work will be needed to probe the full therapeutic potential of a D4 and σ 1 dual ligand, but it might be very promising, as judged by the prominent role of both receptors in CNS functions.

Chemistry

The synthesis together with the spectral and analytical properties of the molecules used in this paper have been fully reported in the reference 19.

Molecular modeling Material & Method

Molecular docking was performed with Glide, using the standard precision mode (enhanced sampling for ligand conformers, enhanced planarity for conjugated π -group, post-docking minimization and strain correction term for final scoring). The OPLS3e force field was used. The crystal structure PDB: 5W1U was used for D4R,⁴⁰ and PDB: 5HK1 for σ 1R.¹⁰

For molecular dynamics experiment, AVR13 was first docked onto each of the three protomers. Then the complex was inserted in a solvent box constituted of a solvated POPC bilayer membrane of about 120 x 120 x 146 Å. (4 Na⁺ and nearly 49500 water molecules for a total of 216311 atoms). Using periodic boundary conditions, molecular dynamics started after (i) energy minimization for 0.3 ns with restraints for protein and ligand (only ions and water were allowed to move, (ii) energy minimization for 0.3 ns with restraints on protein backbone (iii) 0.1 ns equilibration in NPT conditions (300 K, 1 bar), using the RESPA integrator (2 fs / 6 fs time steps for near or far atoms), and a cut-off radius of 9 Å for Coulombic interactions, keeping protein backbone restraints (iv) production for 2 μs in the same conditions, without any restraint. Statistical parameters were calculated with Desmond, as implemented in Maestro v. 12.4 (Schrödinger LLC, San Diego, CA).

Biochemistry Material & Method

Biological testings were performed by Eurofins (Eurofins-Cerep, Le Bois l'Évêque, 86600 Celle l'Evescault, FRANCE). Percentage inhibition and K_i of the tested compounds were determined by competition with reference tritiated ligands, using the condition reported in the following table:

protein	reference tritiated ligand	concentration (nM)	incubation time (min)	temperature
D ₁ R	SCH 23390	0,3	60	RT
D ₂ R	methyl-spiperone	0,3	60	RT
D ₃ R	methyl-spiperone	0,3	60	RT
D ₄ R	methyl-spiperone	0,3	60	RT
D ₅ R	SCH 23390	0,3	60	RT
σ ₁ R	(+) pentazocine	15	120	RT
σ ₂ R	(+) pentazocine + DTG	1000 + 25	60	RT
hERG	dofetilide	10	60	RT

DTG is 1,3 di-ortho-tolylguanidine

For hERG assay, HEK293 cells were stably transfected with the human hERG receptor gene. Binding assays were performed using 10 µg of cell membranes suspended in 10mM Na-Hepes pH 7.4, 135 mM NaCl, 60 mM DL-Aspartic Acid Potassium, 1 mM EGTA, 0.8 mM MgCl₂, 10 mM (D+)-Glucose.

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

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Supplementary material

Supplementary data to this article can be found online at <https>

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$$K_i = \frac{IC_{50}}{1 + \frac{[ligand]}{K_d}} \quad (1)$$

$$\text{Est IC}_{50} \# \frac{100 - \% \text{ inhibition}}{\% \text{ inhibition}} \times \text{concentration} \quad (2)$$

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