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The 5-HT$_{1A}$ Agonism Potential of Substituted Piperazine-Ethyl-Amide Derivatives Is Conserved in the Hexyl Homologues: Molecular Modeling and Pharmacological Evaluation

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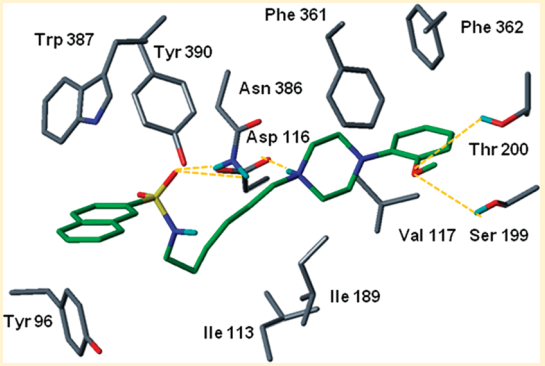
ABSTRACT: In a series of carboxamide and sulphonamide alkyl (ethyl to hexyl) piperazine analogues, although the size of the linker is very different, ethyl and hexyl derivatives possess a high affinity for 5-HT$_{1A}$ receptors. Docking studies clearly show that hexyl and ethyl compounds favorably interact with the binding site of the active conformation of 5-HT$_{1A}$ receptors, thus confirming a possible agonist profile. This activity is effectively detected in electrophysiological experiments in which all four compounds inhibit the activity of rat dorsal raphe serotonergic neurons.



INTRODUCTION

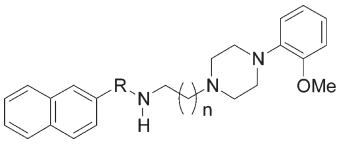
In a recent study, we reported that, in a series of carboxamide and sulphonamide alkyl (ethyl to hexyl) piperazine analogues (see Table 1), most compounds possess a high affinity for 5-HT$_{1A}$ receptors whatever the size of the linker.[1] Therefore, it seems interesting to know if the impact of homology regarding the interaction with 5-HT$_{1A}$ receptors has also an impact in terms of activity. Arylpiperazine-ethyl-naphthamide derivatives, such as *N*-((2-aminoethyl-(4-phenylpiperazin-1-yl) 2-naphthyl carboxamide (**5**) (Figure 1), are claimed to be 5-HT$_{1A}$ ligands with an agonist potential.[2] In our hands, this compound possesses indeed a high affinity for 5-HT$_{1A}$ receptors (K_i = 14 ± 4 nM) (unpublished results). Although it is well-known that bioisosteric replacement of the carboxamide by a sulphonamide strongly modifies the spatial orientation of the side chain all affinities were found in a close range. Thus it could be possible that hexyl compounds (**3**,**4**) (See Table 1) due to different degrees of freedom of this long chain adopt a conformation that could get closer pharmacophore elements to those of the ethyl analogues (**1**,**2**) (See Table 1). Therefore, the binding mode of these compounds was examined by molecular docking studies performed on a homology model of 5-HT$_{1A}$ receptors. Moreover, ethyl (**1**,**2**) and hexyl (**3**,**4**) analogues have been tested to determine their activity on the 5-HT$_{1A}$ receptors in an electrophysiological procedure by recording the activity of serotonergic neurons in rat brain slices.

MATERIALS AND METHODS

Molecular Modeling. *5-HT$_{1A}$ Receptor Modeling.* The receptor model was generated by homology modeling using the SYBYL 8.0 molecular modeling package (SYBYL 8.0, Tripos Inc., St. Louis, MO) and consisted of several steps.

First, the human sequence of the 5-HT$_{1A}$ receptor extracted from the Universal Protein Resource[3] (code entry P08908)[4] was aligned with the sequence of the turkey β1 adrenergic G-protein-coupled receptor (GPCR) (code entry P07700)[5] by the use of the FUGUE sequence alignment module.[6] Great attention was

Table 1. Pharmacological Evaluations of Hexyl Analogues (3,4) in Comparison with the Ethyl Derivatives (1,2)



compound	R	*n*	K_i[a]	IC$_{50}$[b]
1	CO	1	1.69 ± 0.18	179 ± 68
2	SO_2	1	5.19 ± 0.49	804 ± 328
3	CO	5	6.15 ± 1.92	1734 ± 336
4	SO_2	5	2.87 ± 0.52	760 ± 398

[a] Affinity in nM, mean ± SD; $n \geq 2$ if unspecified.[1] [b] Concentration in nM producing a 50% inhibition of the firing rate of DR serotonergic neurons; mean ± SEM; n = 3.

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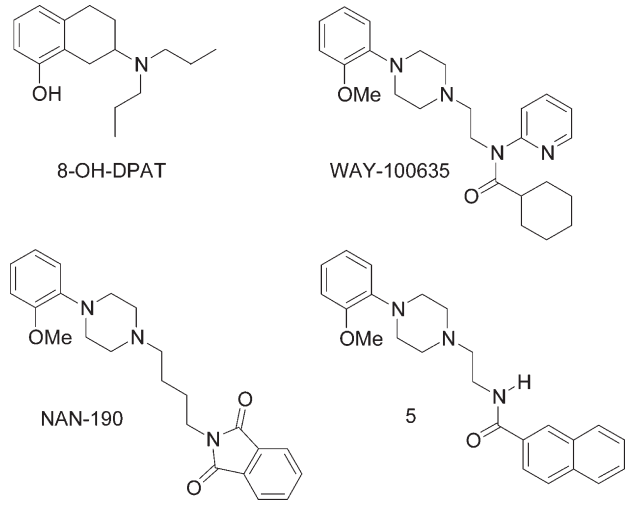


Figure 1. Chemical structure of 8-OH-DPAT, WAY-100635, NAN-190, and a naphthamide derivative **5**.

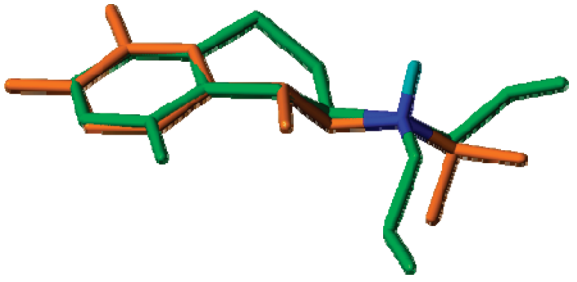


Figure 2. Alignment of R-8-OH-DPAT (green) on the bioactive conformation of isoprenaline (orange). The protonated nitrogen is indicated in blue and cyan.

paid to a correct alignment of the highly conserved residues of the GPCR superfamily according to Baldwin et al.[7] The sequence alignment revealed homology rates of 41 and 80%, respectively, with the full-length sequence and the transmembrane (TM) regions of the 5-HT_{1A} sequence.

The second step consisted in the transfer of a set of constraints derived from the ligand-biased crystal structure of the turkey β1 adrenergic GPCR (PDB entry 2Y03)[8] to the corresponding amino acids of the sequence to be modeled using the ORCHESTRAR protein structure modeling module.[9] Indeed, in the model-building protocol, the cocrystallized agonist isoprenaline in the β1 crystal structure was replaced by R-8-OH-DPAT, a full and potent agonist of the 5-HT_{1A} receptor (Figure 1).[10,11] For this purpose, a complete coverage of conformational space (308 conformations) for R-8-OH-DPAT was generated using the program Random Search[12] of SYBYL. All the conformations were then aligned on the basis of two common structural features for the ligand binding in monoamine receptors by the use of an "in-house" developed method written in SYBYL programming language (SPL). The first key binding feature is the protonated amine which forms hydrogen bonds with Asp 3.32 (according to the Ballesteros and Weinstein numbering),[13] a specific residue for ligand binding among all mammalian biogenic amine receptors.[14] The second key binding feature is the aromatic ring interacting with an aromatic cluster in helices V and VI.[15−17] The best alignment is presented in Figure 2. In the transfer process,

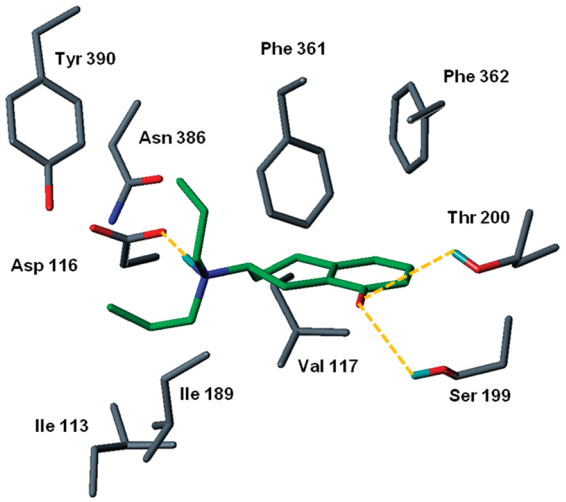


Figure 3. Binding mode of R-8-OH-DPAT (C, N, O, and H atoms in green, blue, red, and cyan, respectively) in a human 5-HT_{1A} receptor model. The hydrogen and ionic bonds are indicated by yellow dashed lines.

R-8-OH-DPAT was specified as a block residue where ORCHESTRAR treats it as a rigid body by copying it from the template into the model protein. N- and C-terminal overhangs that are not aligned with the template structure as well as the very variable IL3 region were cut and not considered since they are not involved in the ligand binding site. The conserved disulfide bond between the cysteine Cys 3.25 of helix 3 and the residue cysteine in the middle of extracellular loop ECL2 was also created and was kept as a constraint in the model refinement.

The resulting R-8-OH-DPAT−5-HT_{1A} complex was then iteratively energy minimized using the Powell method available in Maximin2 procedure[18] with the Tripos force field[19] including the electrostatic term and a nonbond distance cutoff of 12 Å. Solvent effects were implicitly included in the energy minimization by using a distance-dependent dielectric model and a dielectric constant set to 4.0. Energy of the system was minimized in three stages in which the ligand was allowed to be flexible. First, clashes in the side chains were softened by 1000 steps of conjugated gradients, followed by 2000 iterations with the backbone of the loops being free to move. Lastly, the whole protein was allowed to move until the gradient value was smaller than 0.001 kcal mol^{-1} $Å^{-1}$. Hydrogen-bond energies are included in the evaluation of the Tripos force field by scaling the van der Waals interactions between N, O, and hydrogens bonded to N or O. The MMFF94 force field[20] known as reliable in describing the geometries and energies of intermolecular complexes[21] was also used and compared to the Tripos force field. The resulting minimized complexes were found to be very close to each other with a root mean square deviation (rmsd) score of 0.15 Å. For these reasons, the selection of the Tripos force field was maintained for other energy calculations.

Finally, the accuracy of the complex model was evaluated by checking its structural geometry and the binding mode of R-8-OH-DPAT. The PROCHECK software[22] was used to assess the stereochemical quality of the model, and this resulted in high-quality parameters with a very good distribution of φ and ψ angles. More than 97% of the residues were found in the most favored regions. As shown in Figure 3, the binding mode of R-8-OH-DPAT was found to be consistent with that published by Dabrowska et al.[11] Indeed, in addition to the expected ionic

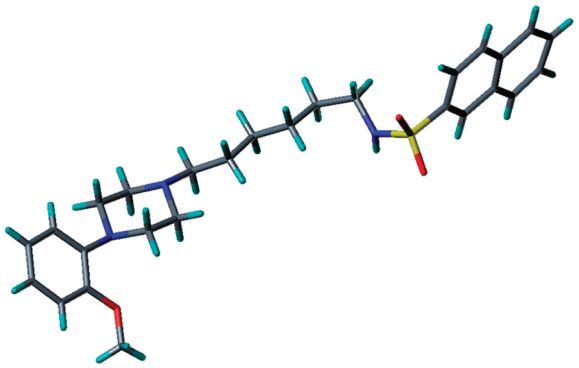


Figure 4. Crystal structure of compound **4**. Atoms are are color coded: carbon (gray), nitrogen (blue), oxygen (red), and sulfur (yellow).

interaction between the protonated nitrogen atom and the residue Asp 116 (Asp 3.32) and the edge-to-face aromatic stacking between the aromatic ring and the residues Phe 361 (Phe 6.51) and Phe 362 (Phe 6.52), a bifurcated hydrogen bond (HB) was observed between the hydroxyl group and the side chains of Ser 199 (Ser 5.42) with O−H···O HB lengths and angles of 3.2 Å and 138° and Thr 200 (Thr 5.43) with O−H···O HB lengths and angles of 3.3 Å and 134°, thus supporting the importance of these residues in the binding process.[23,24]

Ligands Modeling. Three-dimensional structures of carboxamide (**1**,**3**) and sulphonamide (**2**,**4**) alkyl-piperazine analogues were built from the crystal structure of compound **4** (Figure 4). In this structure, long alkyl chain adopted an extended conformation. Every structure was then protonated and refined by energy minimization using the Tripos force field[19] of SYBYL 8.0, including the electrostatic term calculated from Gasteiger and Hückel atomic charges.[25,26] The Powell method available in Maximin2 procedure[18] was used for energy minimization until the gradient value was smaller than 0.001 kcal mol^{-1} $Å^{-1}$.

Binding Mode of the Ligands. The binding mode of the ligands for the human 5-HT_{1A} receptor was studied by flexible ligand docking simulations using the GOLD 4.0 program.[27] The receptor binding pocket was defined as a 15 Å sphere centered on the center of mass of R-8-OH-DPAT. Flexibility of the ligands was considered by using torsion angle distributions extracted from the Cambridge Structural Database (CSD) (http://www.ccdc.cam.ac.uk/products/csd/). These distributions improve the chances of GOLD finding the correct answer by biasing the search toward ligand torsion-angle values that are commonly observed in crystal structures. Thirty docking runs were performed. The most stable docking models were selected according to the best-scoring conformation predicted by GoldScore.[27] The docking protocol was validated by redocking R-8-OH-DPAT into the rigid 5-HT_{1A} model. The resulting position of the redocked R-8-OH-DPAT was found to be very close to the initial position with a rmsd score of 0.18 Å (Figure 5). The ligand−5-HT_{1A} receptor complexes derived from docking were further refined by energy using the Powell method available in Maximin2 procedure[18] with the Tripos force field,[19] a distance-dependent dielectric function ($\varepsilon_r = 4r$), and a nonbond distance cutoff of 12 Å until the gradient value was smaller than 0.01 kcal mol^{-1} $Å^{-1}$. The receptor and the ligands were allowed to move.

Electrophysiological Experiments. Electrophysiological experiments were performed in order to investigate the effect of compounds **1**−**4** (see Table 1) on the firing rate of serotonergic

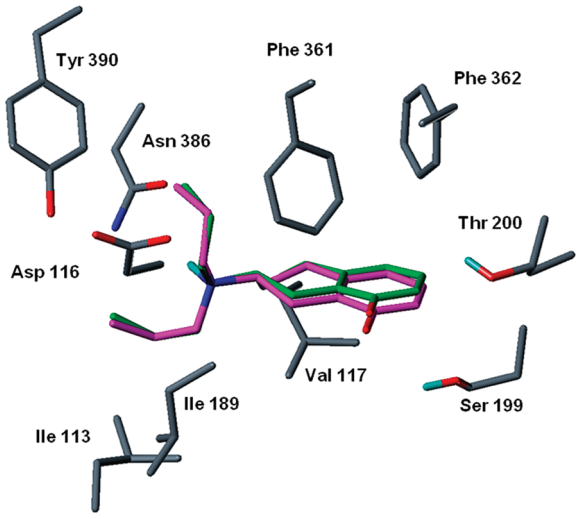


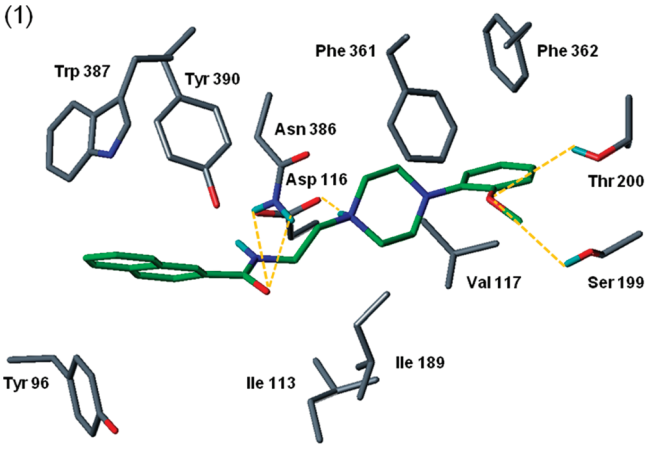
Figure 5. Superimposition of the 5-HT_{1A}−R-8-OH-DPAT docking model (magenta, blue, red, and cyan) and the homology model of 5-HT_{1A}−R-8-OH-DPAT complex (green, blue, red, and cyan).

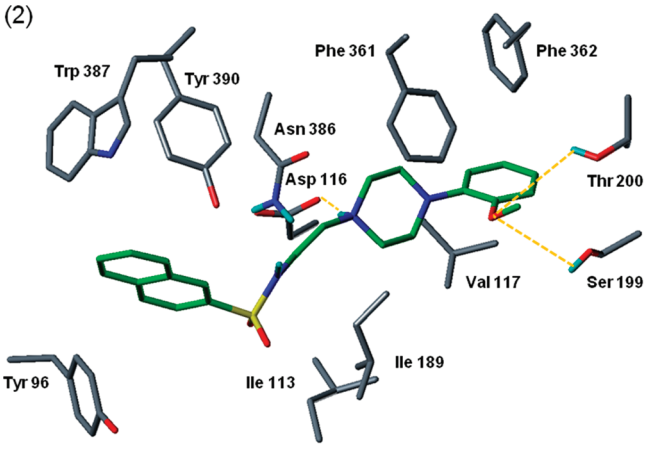
neurons of the dorsal raphe nucleus (DR) recorded on rat brain slices. Indeed, several studies have shown that the firing of these neurons is inhibited by 5-HT_{1A} agonists such as 8-OH-DPAT (Figure 1),[28] an effect that is blocked or reversed by 5-HT_{1A} antagonists, such as WAY-100635 (Figure 1).[29]

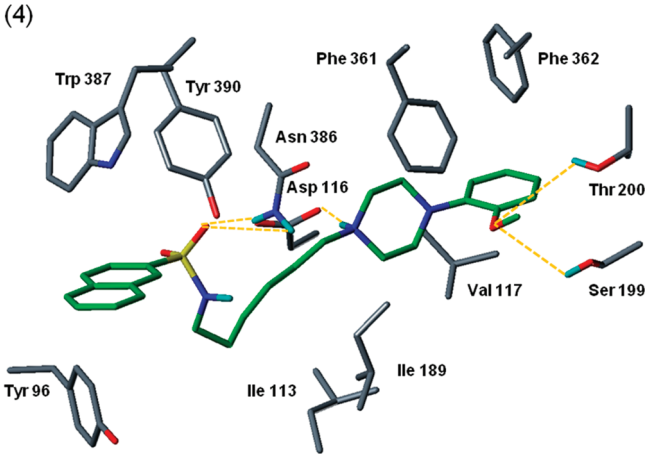
The method used was previously described.[30] Briefly, male Wistar rats (150−200 g) were housed and handled in accordance with the guidelines of the National Institute of Health (NIH publication no. 85−23, revised 1985). Animals were anaesthetized with chloral hydrate (400 mg/kg, i.p.). After decapitation, the brain was quickly removed and put in ice-cold artificial cerebrospinal fluid (aCSF) of the following composition (in mM): NaCl, 130; KCl, 3.5; $NaHCO_3$, 24; NaH_2PO_4, 1.25; glucose, 10; $CaCl_2$, 2; and $MgSO_4$, 1.25 saturated with 95% O_2 and 5% CO_2. A block of tissue containing the brainstem was cut in transverse slices (thickness 400 μm). A slice containing the DR area was placed in a recording chamber (volume 0.5 mL). The slice was completely immersed in a continuously flowing aCSF, heated (±34 °C), and oxygenated (flow rate ±2 mL/min).

Extracellular recordings were made using glass micropipets filled with aCSF (impedance: 5−10 MΩ). Action potentials were amplified 1000× by a homemade amplifier and displayed on a Tektronix oscilloscope. The signals were also introduced into an amplitude discriminator and counted every 10 s. Signals were also recorded with Spike2 software (Cambridge Electronic Design). Unlike the situation in vivo in which the majority of DR serotonergic neurons are spontaneously active, most DR presumed serotonergic neurons are silent in the slice preparation.[31,32] In the presence of 10 μM phenylephrine, they fire at a rate of 0.5−3 spikes/s and are characterized by long duration (>2 ms), often triphasic action potentials.

In all experiments, a 5 min control period was used in order to assess the stability of the firing rate. Drugs were superfused using three-way taps so that the flow remained constant. Increasing concentrations of the compound under study were applied. Each concentration was superfused until equilibrium was obtained (usually 10 min). For each concentration, the percent inhibition relative to the mean control period firing rate was calculated, and the concentration producing a 50% inhibition (IC_{50}) was graphically







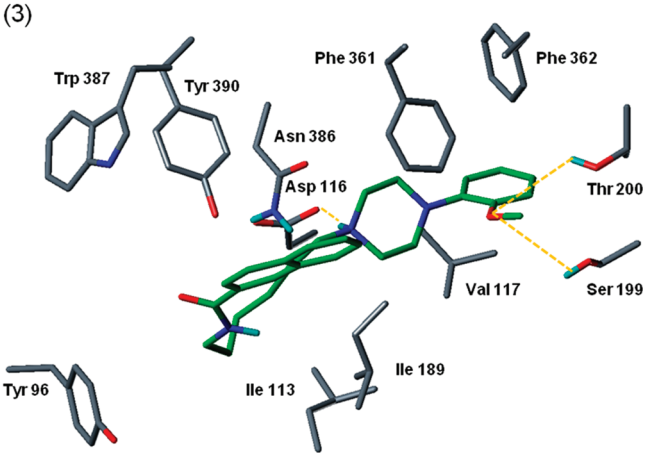


Figure 6. Binding mode of compounds **1** and **4** (C, N, S, O, and H atoms in green, blue, yellow, red, and cyan, respectively) in a human 5-HT_{1A} receptor agonist model. The hydrogen and ionic bonds are indicated by yellow dashed lines.

Figure 7. Binding mode of compounds **2** and **3** (C, N, S, O, and H atoms in green, blue, yellow, red, and cyan, respectively) in a human 5-HT_{1A} receptor agonist model. The hydrogen and ionic bonds are indicated by yellow dashed lines.

determined for each cell by extrapolation on a semilogarithmic graph. Values are expressed as means ± SEM.

WAY-100635 (Sigma-Aldrich, St. Louis, MO) was dissolved in water. Compounds **1**−**4** were dissolved in DMSO. The final concentration of DMSO in the assay is less than 1%.

RESULTS AND DISCUSSION

The binding mode of the hexyl homologues (**3**,**4**) was explored by molecular docking analysis on a 5-HT_{1A} receptor agonist model and compared to that of the ethyl compounds (**1**,**2**). Some ethyl analogues are effectively reported 5-HT_{1A} agonists.[2] First of all, as shown in Figures 6 and 7, the interactions found for the full and potent agonist R-8-OH-DPAT (Figure 3) are observed for the ethyl molecules (**1**,**2**) as well as their hexyl analogues (**3**,**4**). Indeed, the ionic interaction with Asp 116 and the aromatic interactions with Phe 361 and Phe 362 are maintained with the phenol-piperazine part of the four compounds. The bifurcated HB with Ser 199 and Thr 200 is also kept for each compound. The O−H···O HB lengths (from 3.4 to 3.5 Å for Ser 199 and from 3.6 to 3.7 Å for Thr 200) and angles (from 130 to 132° for Ser 199 and from 128 to 129° for Thr 200) are found to be very close. However, compared to those of R-8-OH-DPAT, the HB lengths and angles are, respectively, slightly longer and shorter, likely resulting in HB strengths somewhat weaker. Regarding the naphthalene ring of each compound, it is shown to lie in the vicinity of the aromatic network of the binding site formed by the residues Tyr 96, Trp 387 and Tyr 390. The position of the naphthalene ring is quite similar except for compound **3**, which is found to be in the opposite direction. The only noticeable difference is related to the carboxamide or sulphonamide groups. Indeed, interestingly, a bifurcated HB with the amide group of Asn 386 is only found for the compound **1** with N−H···O HB lengths of 3 Å and N−H···O HB angles from 100° to 110°, and the compound **4** with N−H···O HB lengths from 2.4 Å to 2.9 Å and N−H···O HB angles from 98° to 119° (Figure 6). The absence or the presence of this HB interaction could explain the tendency of the carboxamide ethyl (**1**) and sulphonamide hexyl (**4**) to display a better affinity than their homologues with a ratio of ∼3 and ∼2, respectively (see Table 1).[1] Given the flexibility of the ligands, especially for compounds **3** and **4**, entropy could be also expected to play a role in the binding processes. Indeed, the reduced mobility of the ligands in the complex should result in an entropy penalty and therefore an unfavorable contribution to the binding strength. However, the very flexible compound **4** displayed a better affinity than its ethyl analogue **2**, thus showing that the loss of conformational energy is not critical for the binding affinity. Consequently, in this case, enthalpy seems to mainly drive the binding

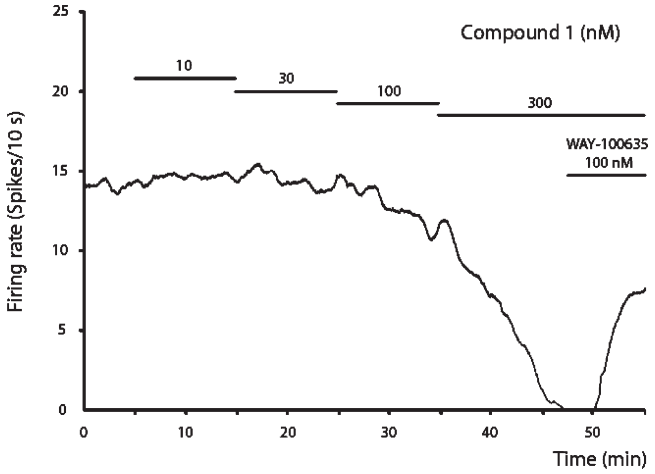


Figure 8. In vitro extracellular recording of a DR neuron. Increasing concentrations of compound **1** induce a progressive inhibition of the firing rate, and the effect is reversed by application of the 5-HT$_{1A}$ antagonist WAY-100635.

of these ligands whose capacity to favorably interact with the binding site of the active conformation of 5-HT$_{1A}$ receptors suggests an agonist profile. In order to support this assumption, the docking of antagonists, such as WAY-100635 and NAN-190,[33] (Figure 1) on the 5-HT$_{1A}$ receptor agonist model was also performed in the same protocol previously described. Interestingly, these molecules were unable to find favorable interactions with the binding site. Indeed the ligands had steric clashes with the protein side chains and were not able to form a HB with Ser 199 and Thr 200.

The agonist profile of the compounds **1**−**4** detected by the docking studies was further examined in electrophysiological experiments. In the electrophysiological evaluation, all four compounds induced a concentration-dependent decrease in the firing rate of DR serotonergic neurons. The IC$_{50}$s were respectively 179 ± 68 nM for compound **1** (n = 3), 804 ± 328 nM for compound **2** (n = 4), 1734 ± 336 nM for compound **3** (n = 5), and 760 ± 398 nM for compound **4** (n = 3). The inhibitory effect of all four compounds was reversed by the simultaneous application of WAY-100635 (100 nM). One experiment performed with compound **1** is illustrated in Figure 8. These electrophysiological experiments show that they all behave as 5-HT$_{1A}$ agonists. Moreover, the order of potency is in agreement with binding affinities obtained on cloned receptors. At a chemical point of view, the increase in the size of the linker does not modify the intrinsic activity of the compounds on 5-HT$_{1A}$ receptors.

CONCLUSION

The docking studies clearly show that hexyl and ethyl compounds favorably interact with the binding site of the active conformation of 5-HT$_{1A}$ receptors, thus confirming a possible agonist profile of these ligands. This activity is effectively detected in electrophysiological experiments in which all four compounds inhibited the activity of DR serotonergic neurons. Although the sp^3 hybridization with a tetrahedral configuration of the sulphonamide differentially orientates the side chain in comparison with the carboxamide and the sp^2 hybridization, the high flexibility of the hexyl side chain makes possible an interaction in the binding pocket of the 5-HT$_{1A}$ receptors. Moreover, some differences found in the interaction modes could explain the difference in affinity observed in binding experiments.

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