Bioorganic & Medicinal Chemistry Letters 20 (2010) 5552–5558

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Identification of a novel NR2B-selective NMDA receptor antagonist using a virtual screening approach

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ARTICLE INFO

Article history:
Received 2 June 2010
Revised 9 July 2010
Accepted 9 July 2010
Available online 15 July 2010

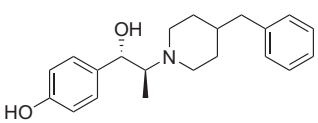
Keywords:
Glutamate
NMDA
NR2B-selective antagonist
Pharmacophore
Virtual screening

ABSTRACT

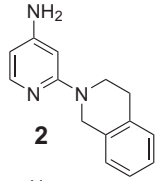
We report the identification of a novel NR2B-selective NMDAR antagonist with an original scaffold, LSP10-0500. This compound was identified by a virtual high-throughput screening approach on the basis of a quantitative pharmacophore model of NR2B-specific NMDAR antagonists. A SAR study around LSP10-0500 is also described.

NMDA receptors (NMDARs) form glutamate-gated ion channels highly permeable to calcium and widely distributed in the vertebrate central nervous system. They play major roles in physiological processes such as memory formation and synaptic plasticity but their overactivation, resulting in an excess of intracellular calcium, triggers neuronal injury and is involved in numerous pathologies such as stroke, epilepsy and chronic pain.[1] NMDARs are tetrameric assemblies usually composed of two NR1 and two NR2 subunits, these latter occurring as four subtypes (NR2A-D) and conferring distinct biophysical and pharmacological properties.[2,3] The first compounds developed to encounter the deleterious effects of NMDAR overactivation, either competitive antagonists or pore (ion channel) blockers, while neuroprotective, failed in clinical trials because of unacceptable side effects.[4] More recently, a large family of compounds selectively inhibiting NMDARs containing the NR2B subunit was developed and, encouragingly, such compounds display a much improved side effect profile compared to first-generation broad-spectrum NMDAR antagonists.[5–7] At the level of the receptor, NR2B-selective antagonists act as allosteric (non-competitive) inhibitors and their binding site has been mapped to the NR2B N-terminal domain (NTD), an extracellular clamshell-like domain preceding the glutamate-binding domain on the NR2B subunit.[8–12] So far, however, none of these NR2B-selective antagonists have turned into approvable drugs, due to low oral bioavailability and poor pharmacokinetic profile.[4] Developing new NR2B-selective NMDARs with original chemical scaffolds is thus of interest.

Virtual high-throughput screening is a convenient approach to find original ligand structures among a chemical database.[13–16] In this work, we built a pharmacophore model of NR2B-specific NMDAR antagonists that was suitable for ligand-based virtual screening. This allowed us to identify one compound with an original central core, LSP10-0500 (see below), which acts as selective inhibitor of NMDARs containing the NR2B subunit. A structure–activity relationship (SAR) study around this compound is also described.



1 (ifenprodil)



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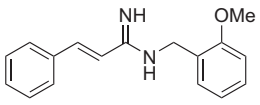

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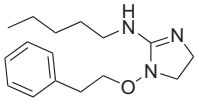

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Compounds that selectively inhibit NR2B-containing receptors are diverse in their structural and chemical features, as exemplified by compounds **1**–**4**.[17–20] There is therefore a good chance that

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doi:10.1016/j.bmcl.2010.07.043

Table 1
Experimental and estimated activities together with the error values of the training set compounds **5**–**20**

N	Experimental activity[a]	Estimated activity[b]	Error[c]	Fit value	Reference
5	0.03	0.0087	−3.5	10.64	26
6	0.03	0.12	+4	9.50	32
7	0.048	0.33	+6.8	9.06	33
8	0.3	0.29	−1	9.11	34
9	0.45	1.9	+4.1	8.31	33
10	0.45	0.49	+1.1	8.89	35
11	2.5	4.1	+1.6	7.96	26
12	4	71	+18	6.73	36
13	5	2.8	−1.8	8.12	35
14	11	7.3	−1.5	7.71	37
15	43	180	+4.1	6.33	36
16	58	11	−5	7.52	38
17	140	55	−2.5	6.84	33
18	250	160	−1.6	6.38	39
19	440	63	−7.1	6.78	39
20	560	49	−11	6.88	33

[a] Experimental activity (IC_{50} or K_i values) normalised to the activity of ifenprodil[8,21] (IC_{50} = 0.1 μM).
[b] Activity predicted by the pharmacophore model.
[c] Error values are defined as the ratio between experimental and estimated activities. A negative value indicates that the experimental activity is higher than the predicted activity. The estimated activity is considered to be satisfactorily predicted by the model when the error is in the range of ±3 compared to the experimental activity.

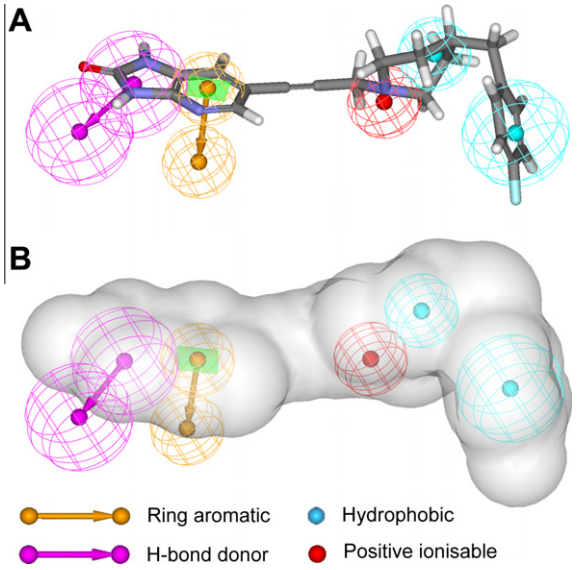


Figure 2. Pharmacophore model of NR2B-specific, 'ifenprodil-like', NMDAR antagonists. (A) Pharmacophore model with molecule **5** fitted into it. The spheres represent the tolerances of the features; (B) pharmacophore model with the shape of molecule **5**. The shape represents the van der Waals volume occupied by a molecule.

different structural series adopt different binding modes or even interact with different binding pockets. We thus decided to build a quantitative pharmacophore model that was restricted to compounds with a structure similar to ifenprodil (**1**), the prototypical NR2B-selective antagonist, for which we can reasonably assume a common binding model.[10,11] Such compounds share the following chemical features[21]: one central positively charged group, one H-bond donor and two aromatic cycles. Biological activities of 'ifenprodil-like' compounds in the literature are either IC_{50}, or inhibition constants (K_i) that have been determined by different means (electrophysiology, [^{3}H] ifenprodil or [^{3}H] Ro 25–6981 displacement). In order to work with a consistent dataset, we normalised all activities to that of ifenprodil[8,21–23] (IC_{50} = 0.1 μM; Table 1).

The pharmacophore modelling calculations were carried out in the Discovery Studio 2.0 environment (Accelrys, San Diego CA). We first selected from the literature a training set of 16 compounds (Fig. 1) with normalised activities ranging from 0.03 to 560 (Table 1). The error of the experimental values was set to one log of activity. The conformational enumeration of the training set was performed in Discovery Studio using the 'Best' algorithm of Catalyst/Catconf[24] (Energy range of 20 kcal mol^{-1} and maximum number of conformers of 250). We then used Catalyst/Hypogen[25] to build a quantitative pharmacophore model of NR2B-selective, 'ifenprodil-like', NMDAR antagonists. The minimum distance between two features was set to 2 Å, and the weight and the tolerance of each feature were allowed to vary. We chose a

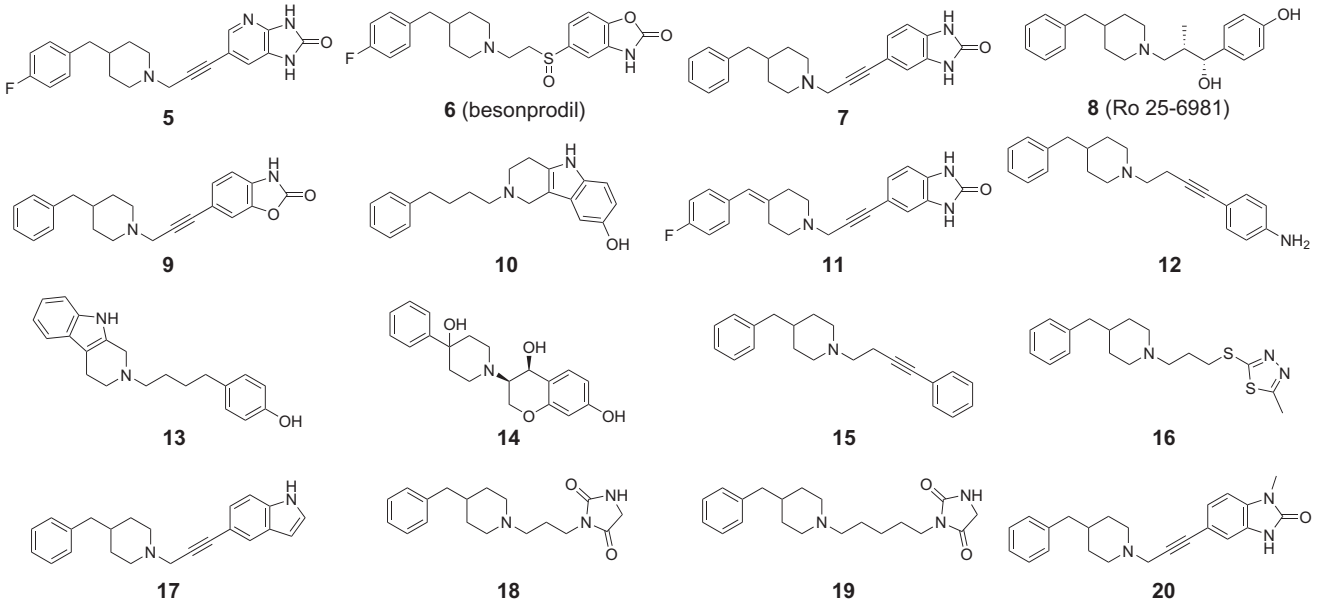


Figure 1. Chemical structures of the NR2B-specific NMDAR antagonists used in the training set.

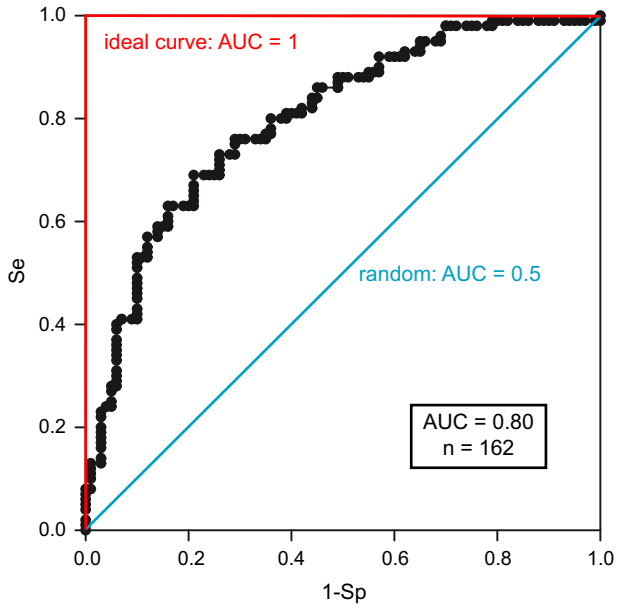


Figure 3. ROC curve analysis of the pharmacophore model of NR2B-specific NMDAR antagonists. The random curve, where a score is randomly attributed to each molecule, is represented in blue. The ideal curve is represented in red. Se (sensitivity) represents the ability of the model to select truly active molecules; Sp (specificity) represents the ability of the model to discard inactive molecules. AUC, area under the curve; *n*, number of molecules in the test set used to perform the ROC curve analysis.

pharmacophore model that contained the following five features: two hydrophobic, one ring aromatic, one positive ionisable and one H-bond donor (Fig. 2A). As expected, the nature of the features was in agreement with previously described qualitative pharmacophores.[9,21,26] Although the activities of only half of the molecules in the training set were correctly predicted by the model (Table 1), our pharmacophore model had acceptable statistical parameters for virtual screening purposes, with a correlation coefficient (r^2) between 'estimated' and 'experimental' activities of 0.90 and a RMSD (root mean square deviation) value of 1.32 log of activity.

To be suitable for virtual screening, a pharmacophore model must lead to the identification of the maximum of true positive, that is, experimentally active molecules, while avoiding the identification of false positive compounds. Accordingly, to test the quality of our pharmacophore model, we performed a ROC (receiver operating characteristics) curve analysis[27,28] of a test-set of 162 molecules of known experimental activity. The 162 molecules were mapped onto the pharmacophore model, and a score, called fit value, was attributed to each molecule depending on the best geometric fit of the molecule with the pharmacophore model. Compounds were then ranked according to their fit values. A ROC curve represents, for each threshold of fit value, the selectivity (Se), that is, the proportion of true positive compounds, as a function of 1-Sp (specificity), that is, the proportion of false positive compounds. Calculation of the area under the curve (AUC) gives a good indication of the discriminatory power of the pharmacophore model.[27,28] Indeed, an ideal pharmacophore model would lead to the identification of all and only true positive hits, thus leading to an AUC of 1. On the contrary, attributing molecule scores randomly would lead to an AUC of 0.5. In our case, we found an AUC of 0.80 (Fig. 3), indicating that the retained pharmacophore model is discriminant enough to perform a virtual screening campaign.

We next exploited our pharmacophore model to screen the commercial database of InterBioScreen Ltd (IBS STOCK, synthetic compounds, March 2007; Moscow, Russia) for new NR2B-selective NMDAR antagonists. Figure 4 summarizes the strategy used to screen this database and to filter the results. The chosen IBS database contains 403,273 compounds. As our pharmacophore model contains a 'positive ionisable' feature, a feature which is less frequently found in 'drug-like' chemical compounds than hydrophobic or H-bond donor and acceptor features,[28] we first screened the IBS database with a single 'positive ionisable' criterion to decrease the number of candidates. The resulting 82,302 molecules (bearing a 'positive ionisable' group) were then adjusted to the pharmacophore model, and 754 molecules were retained. To further refine the retrieved compounds, we finally applied a steric filter to our set of molecules. For that purpose, the shape of the experimentally most active molecule of the training set (molecule **5**; Fig. 2B), defined as the van der Waals volume occupied by this bioactive conformation, was taken into account, in order to discard compounds that are too large to fit in the ifenprodil binding site. Of the 754 compounds adjusted to the combination of pharmacophore model and shape, 58 molecules satisfied both the pharmacophoric and steric criteria. These molecules were then regrouped by structural similarity and 10 compounds, named LSP10-0100–LSP10-1000, were purchased for functional studies (see Table 2).

Functional activities of the selected compounds were determined by two-electrode voltage-clamp recordings on *Xenopus* oocytes expressing wild-type NR1/NR2B receptors.[9] Application of 10 μM LSP10-0100–LSP10-0400 or LSP10-0600–LSP10-1000 induced little (<20%) inhibition of NR1/NR2B receptors. In contrast 10 μM LSP10-0500 strongly inhibited NR1/NR2B receptors (78 ± 5% inhibition, $n = 10$; Table 2). To verify that the inhibitory effect seen with LSP10-0500 was specific for the NR2B subunit and, like ifenprodil, engaged the NR2B NTD, we tested the effects of LSP10-0500 on NR1/NR2A receptors and on NR1/NR2B receptors truncated for the entire NR2B NTD[29] (NR1/NR2B-ΔNTD). Both receptor subtypes were almost completely insensitive to LSP10-0500 (4–6% inhibition; Table 2), indicating that this compound, like ifenprodil, selectively inhibits NMDARs containing the NR2B subunit and most likely binds to NR2B NTD. Similarly to ifenprodil, LSP10-0500 contains two aromatic cycles, one benzyl (called ring A, see Fig. 5) and one phenol group (called ring B). However its cen-

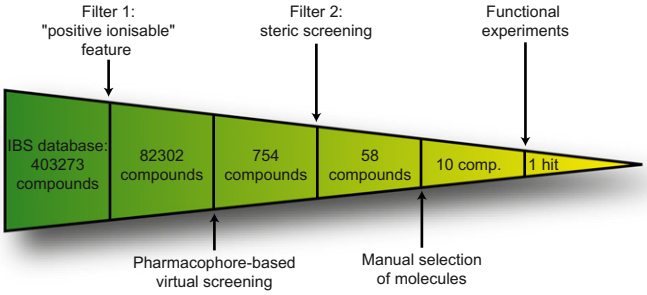
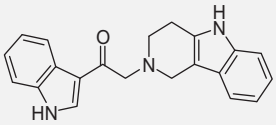
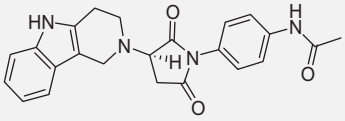
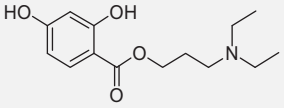
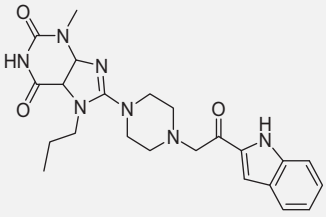
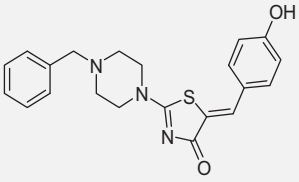
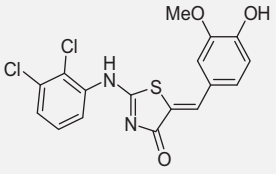
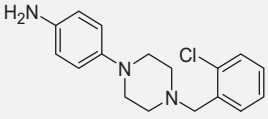
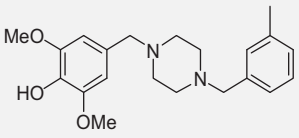
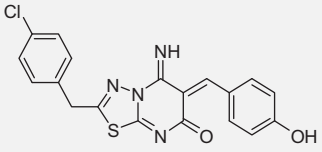
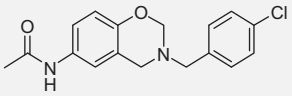


Figure 4. Summary of the virtual screening workflow.

Table 2
Inhibitory effect of compounds LSP10-0100–LSP10-1000 (applied each at 10 μM) on NMDARs containing the NR1a and various NR2 subunits

Name	Structure	Residual current[a]		
		NR2B	NR2B-ΔNTD	NR2A
Ifenprodil		0.06 ± 0.03	0.87 ± 0.01	0.77 ± 0.06
LSP10-0100		0.90	0.94	0.96
LSP10-0200		0.97	0.98	0.98
LSP10-0300		0.94	0.97	0.97
LSP10-0400		0.95 ± 0.01	0.97	0.98
LSP10-0500		0.22 ± 0.05	0.96 ± 0.02	0.94 ± 0.05
LSP10-0600		0.925 ± 0.005	0.96	0.97
LSP10-0700		0.98	0.98	0.97
LSP10-0800		0.96	0.97	0.98
LSP10-0900		0.90 ± 0.01	0.97	0.99
LSP10-1000		0.83 ± 0.01	0.88	0.77

[a] The residual current is defined as the ratio of the current elicited by agonists (100 μM each) plus 10 μM of compound on the current elicited by agonists alone. Each value is the mean value obtained from 1 to 10 cells. Errors represent the standard deviations.

tral core, composed of the association of a piperazine and a thiazolone moiety, is original compared to all the previously published NR2B-specific antagonists.

LSP10-0500 inhibited wild-type NR1/NR2B receptors with an IC_{50} of 2.7 ± 0.2 μM, a value ~10-fold higher than that of ifenprodil (IC_{50} = 0.19 ± 0.01 μM in parallel experiments; Fig. 5). Despite this

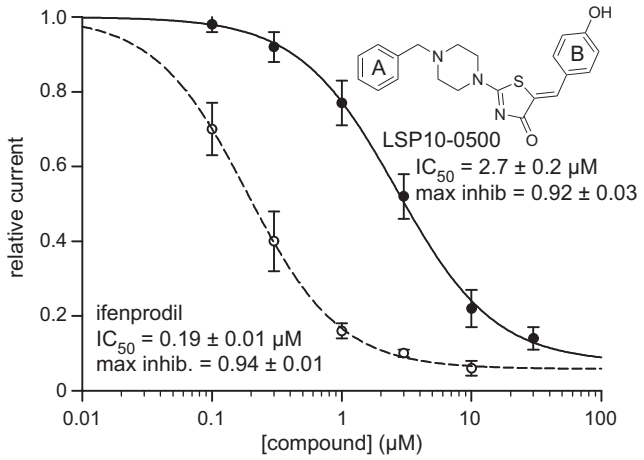


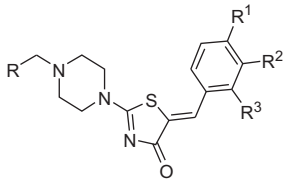
Figure 5. Dose–response curves of IBS LSP10-0500 and ifenprodil on wild-type NR1/NR2B receptors. Each point is the mean value obtained from at least three independent experiments. Error bars represent standard deviation. Dose–response curves were fitted according to the Hill equation: $I_{compound}/I_{control} = 1 - a/(1 + (IC_{50}/[compound])^n$, where $I_{compound}/I_{control}$ is the mean relative current, [compound] is the concentration of the considered compound, IC_{50} is the concentration of compound producing 50% of the maximal inhibition, n is the Hill coefficient and a is the maximal inhibition at saturating compound concentration. The IC_{50}, maximal inhibition and Hill coefficient are, respectively, 0.19 ± 0.01 μM, 0.94 ± 0.01 and 1.22 ± 0.05 for ifenprodil, and 2.7 ± 0.2 μM, 0.92 ± 0.03 and 1.1 ± 0.1 for LSP10-0500.

lower potency, the original structure of LSP10-0500 renders this molecule a promising lead for the discovery of novel NR2B-selective NMDAR antagonists. To study the molecular determinants responsible for LSP10-0500 activity on NR2B-containing NMDARs, and in an attempt to improve the activity of this compound, we performed a structure–activity relationship (SAR) study around LSP10-0500. Analogues of LSP10-0500 were pulled from two different databases, IBS and CAP (Chemicals available for purchase, Accelrys, San Diego CA, CAP reagents and CAP screening 2006), on the basis of the structure of the central core, that is, the piperidine and thiazolone moieties. The retrieved compounds were then filtered against the shape of LSP10-0500 to remove the compounds that are too bulky. Next, using the C-DOCKER program,[30] we docked 59 derivatives of LSP10-0500 in a homology model of NR2B NTD based on the crystallographic structure of the agonist-binding domain of mGluR1[9] (pdb: 1EWK:A). It is worth noting that although a crystallographic structure of NR2B NTD was recently solved[31] (pdb: 3JPW), we were able to use it neither for ifenprodil, nor for any derivative of LSP10-0500, due to a lack of space in the (closed) interlobe cleft. The NR2B NTD, upon binding of ifenprodil, may thus adopt a degree of closure that is different from the zinc-bound NR2B NTD. Based on our docking, LSP10-0500 adopted a similar binding mode as ifenprodil,[9] with its phenol group (ring B) contacting polar residues lining the border of the NTD cleft and its benzyl group (ring A) interacting more deeply with the hinge of the NTD. We finally selected 13 compounds differing by three criteria: nature of the group connected to the piperazine moiety; nature of the substitutions of the aromatic cycles A and B; and configuration of the C–C double bond of the benzylidene moiety.

As predicted by our pharmacophore model, removal of the hydrogen-bond donor in derivatives of LSP10-0500 induced a loss in activity (LSP10-0503, Table 3). The same effect was seen when ring A was replaced by a methyl group (LSP10-0506), as previously demonstrated for other ifenprodil derivatives.[23] Moving the phenolic hydroxyl group from the para to the ortho position, in molecule LSP10-0501, also strongly decreased the activity for NR2B-containing NMDARs. On the contrary, compound LSP10-0502, which contained hydroxyl groups at both *para* and *ortho* positions of ring B had a slightly increased activity compared to LSP10-0500, with an IC_{50} of 1.65 ± 0.06 μM. Changing the configuration of the benzylidene moiety from Z to E hardly modified the activity, with compound LSP10-0504 inhibiting NR1/NR2B receptors with an IC_{50} of 1.8 ± 0.1 μM. This phenotype was not expected, as modifying the configuration of the benzylidene double bond would change the orientation of ring B, leading to different interactions of LSP10-0500 and LSP10-0504 with the residues of their binding pocket. It is therefore possible that NR2B interlobe cleft can allow two different binding modes that lead to the same IC_{50}. LSP10-0502 and LSP10-0504 induced almost no inhibition on NMDARs containing NR2Awt and NR2B-ΔNTD subunits (maximum 6% inhibition at a concentration of 10 μM), suggesting that these compounds, like LSP10-0500, selectively bind NR2B NTD. Adding on LSP10-0504 a methoxy group at the *meta* position of ring B decreased the activity fivefold (LSP10-0505, IC_{50} = 11 ± 4 μM; see Table 3), probably because of a steric hindrance between the –OMe group and residues located at the entrance of the NTD cleft.

We next investigated the activity of the phenyl–piperazinyl–thiazolone derivatives of LSP10-0500 (Table 4). These compounds,

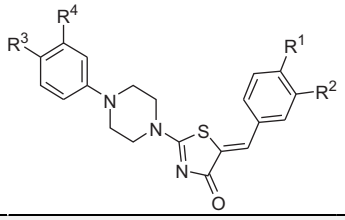
Table 3
Structure–activity relationship study of LSP10-0500 derivatives: benzyl derivatives



LSP10-	R	C=C config.[a]	R^1	R^2	R^3	Residual current[b]			IC_{50} (μM)[c]
						NR2B	NR2B-ΔNTD	NR2Awt	
0500	Ph	Z	OH	H	H	0.22 ± 0.05	0.96 ± 0.02	0.94 ± 0.05	2.7 ± 0.2
0501	Ph	Z	H	H	OH	0.96 ± 0.01	0.96 ± 0.00	n.d.	n.d.
0502	Ph	Z	OH	H	OH	0.18 ± 0.02	0.92 ± 0.01	0.92 ± 0.03	1.65 ± 0.06
0503	Ph	Z	H	H	H	0.95 ± 0.01	0.97 ± 0.00	n.d.	n.d.
0504	Ph	E	OH	H	H	0.15 ± 0.01	0.96 ± 0.00	0.90 ± 0.04	1.8 ± 0.1
0505	Ph	E	OH	OMe	H	0.61 ± 0.04	0.97 ± 0.00	n.d.	11 ± 4
0506	Me	Z	OH	H	H	0.93 ± 0.02	0.96 ± 0.01	n.d.	n.d.

Errors represent the standard deviations.
[a] Configuration of the C–C double bond of the benzilidene moiety.
[b] Residual current after application of 10 μM compound. Each value is the mean value obtained from at least two cells.
[c] Each value is the mean value obtained from at least three cells (n.d. = not determined).

Table 4
Structure–activity relationship study of LSP10-0500 derivatives: phenyl derivatives



LSP10-	C=C config.[a]	R^1	R^2	R^3	R^4	Residual current[b]			IC_{50}[c] (μM)
						NR2B	NR2B-ΔNTD	NR2Awt	
0507	Z	OH	H	H	H	0.75 ± 0.06	0.89 ± 0.01	0.88 ± 0.04	n.d.
0508	Z	OH	H	F	H	0.75 ± 0.01	0.93 ± 0.01	n.d.	13 ± 6
0509	Z	OH	H	H	Cl	0.94 ± 0.03	0.95 ± 0.00	0.90 ± 0.04	n.d.
0510	Z	OH	OMe	Me	H	0.95 ± 0.03	0.98 ± 0.01	n.d.	n.d.
0511	Z	OH	OMe	H	Me	0.95 ± 0.03	0.98 ± 0.01	n.d.	n.d.
0512	Z	OH	OMe	Me	Cl	0.90 ± 0.02	0.98 ± 0.01	n.d.	n.d.
0513	E	OH	H	H	H	0.74 ± 0.01	0.95 ± 0.00	n.d.	n.d.

Errors represent the standard deviations.
[a] Configuration of the C–C double bond of the benzilidene moiety.
[b] Residual current after application of 10 μM compound. Each value is the mean value obtained from at least two cells.
[c] Each value is the mean value obtained from at least three cells (n.d. = not determined).

shorter and less flexible than LSP10-0500, were predicted by our pharmacophore and docking models to have some activity on NMDARs. Unfortunately, this series of compounds appeared poorly active. LSP10-507, the analogue of LSP10-500 but with a phenyl instead of a benzyl moiety, produced only 25% inhibition when applied at a concentration of 10 μM (Table 4). Changing the configuration of the benzylidene group (LSP10-513) or adding a fluoride on ring A did not modify the activity of these compounds (see Table 4). Furthermore, adding bulkier groups on ring A to increase the molecule length induced a complete loss of activity. Apart from the difference of length, the main difference between the benzyl and the phenyl derivatives resides in the flexibility of ring A, this aromatic ring being much less flexible in the phenyl derivatives due to a conjugation with a nitrogen of the piperazine group. Based on the docking of LSP10-0500, ring A would interact with the hinge region of NR2B NTD, a region likely to be structurally constrained.[7] It is thus possible that the phenyl derivatives are not able to trigger the closure of NR2B NTD that induces inhibition of NMDARs.[8,31]

In summary, using a virtual screening approach based on a quantitative pharmacophore model, we found a new molecule, the hit compound LSP10-0500, which selectively inhibits NMDARs containing the NR2B subunit. This compound is 10-fold less potent than ifenprodil, but its original central core makes of LSP10-0500 a promising starting point for the development of new NR2B-selective antagonists. The preliminary SAR around LSP10-0500 gives directions on where to conduct a subsequent optimisation. Replacing the benzyl group (ring A) of LSP10-0500 by a phenyl group strongly reduced compound activity. However, changing the configuration of the double bond of the benzylidene moiety (LSP10-0504), or adding a second hydroxyl group (LSP10-0502) slightly increases the potency on NR1/NR2B receptors. It would therefore be interesting to conduct new SAR studies on the basis of these latter compounds.

Acknowledgments

This work was supported by Agence Nationale de la Recherche (ANR to P.P. and F.A.), Fondation pour la Recherche Médicale (FRM to L.M. and 'Equipe FRM grant' to P.P.), Ministère de la recherche (L.M.).

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