Full Paper

Targeting GluN2B-Containing *N*-Methyl-D-aspartate Receptors: Design, Synthesis, and Binding Affinity Evaluation of Novel 3-Substituted Indoles

Maria Rosa Buemi, Laura De Luca, Stefania Ferro, and Rosaria Gitto

Dipartimento di Scienze del Farmaco e Prodotti per la Salute, Università di Messina, Messina, Italy

In an effort to improve our knowledge about structure–affinity relationships (SARs) for a class of 3-substituted-indole derivatives as GluN2B-containing *N*-methyl-D-aspartate-type receptor (NMDAR) ligands, we herein describe the design, synthesis, and preliminary screening of a new series of molecules. The *in vitro* determination of binding affinities suggested that 5-hydroxy- and 6-hydroxyindole derivatives **12** and **13** were active ligands. Generally, the novel compounds proved to be less potent than their homologs previously reported as promising neuroprotective agents. In fact, our lead compound 3-(4-benzylpiperidin-1-yl)-1-(5-hydroxy-1*H*-indol-3-yl)ethan-1-one (**2**) was about 10-fold more active than the new propan-1-one derivative (**12**). To rationalize the low potency of the new analog **12**, docking studies were also performed and the *in silico* results were consistent with the *in vitro* data.

Keywords: Glutamate / GluN2B / Ifenprodil / Indoles / Molecular docking

Received: February 19, 2014; Revised: April 1, 2014; Accepted: April 2, 2014

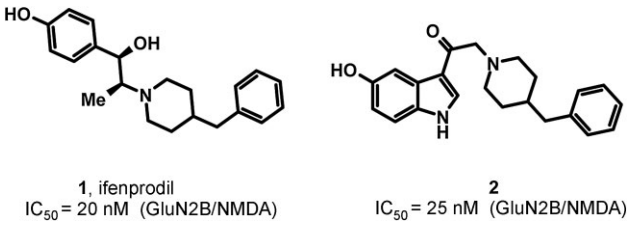
DOI 10.1002/ardp.201400061

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Introduction

Experimental evidences demonstrated that glutamate (Glu) is a critical transmitter for signaling neurons to degenerate during the excitotoxic process. Glu exerts its neurotoxic function through the activation of ionotropic receptors (iGluRs) such as *N*-methyl-D-aspartate (NMDA)-type receptors (NMDARs) [1], and the resulting elevation of intracellular calcium can produce neuronal death [2]. So NMDARs are involved in various neurodegenerative pathologies [3, 4], and drugs antagonizing Glu-mediated neuronal excitation could prevent the associated neurotoxicity. NMDA receptors are heterotetrameric complexes composed of subunits from seven homologous genes, GluN1, GluN2A–GluN2D, and GluN3A–GluN3B [5, 6]. The majority of NMDARs are composed of two GluN1 and two GluN2 subunits. It is known that different subpopulations of NMDARs may generate different functional outputs. In particular, the different composition of the subunits GluN2A–D and the subcellular localization of NMDARs contribute to induce signal transduction cascade and to promote neuronal plasticity and neuronal survival or death [7]. While the synaptic NMDAR activation is implicated in neuroprotection, the stimulation of extrasynaptic NMDARs, composed of GluN2B subunits, may play a key role in the Glu-induced excitotoxicity [8]. Thereby antagonists targeting GluN2B-containing NMDARs are expected to show neuroprotective effects [9]. The prototype of noncompetitive antagonists of GluN2B-containing NMDARs is the ifenprodil (**1**) [10], which binds the interface of the extracellular amino-terminal domain of GluN1/GluN2B functional dimer thus impairing the opening of NMDARs through the stabilization of the twisted closed-clamshell configuration [11]. In our efforts to identify selective GluN2B-containing NMDAR ligands we have discovered a series of potent 3-substituted indoles [12–19] showing high potency (IC_{50} values ranging from 5.4 to 51 nM) in a [^{3}H]ifenprodil competition binding assay. Among them the 3-(4-benzylpiperidin-1-yl)-1-(5-hydroxy-1*H*-indol-3-yl)ethan-1-one (**2**, Fig. 1) reduced NMDA receptor-mediated current in patch clamp experiments and showed

Correspondence: Prof. Rosaria Gitto, Dipartimento di Scienze del Farmaco e Prodotti per la Salute, Università degli Studi di Messina, Viale Annunziata, 98168 Messina, Italy.
E-mail: rgitto@unime.it
Fax: +39 090 6766402



1, ifenprodil
IC_{50} = 20 nM (GluN2B/NMDA)

2
IC_{50} = 25 nM (GluN2B/NMDA)

Figure 1. Chemical structures and IC_{50} values of two prototypes of GluN2B-containing NMDAR ligands: ifenprodil (**1**) and 3-(4-benzylpiperidin-1-yl)-1-(5-hydroxy-1*H*-indol-3-yl)ethan-1-one (**2**).

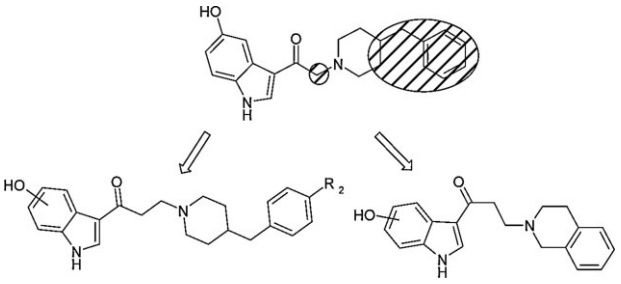


Figure 2. Designed hydroxyindole derivatives as GluN2B-containing NMDAR ligands.

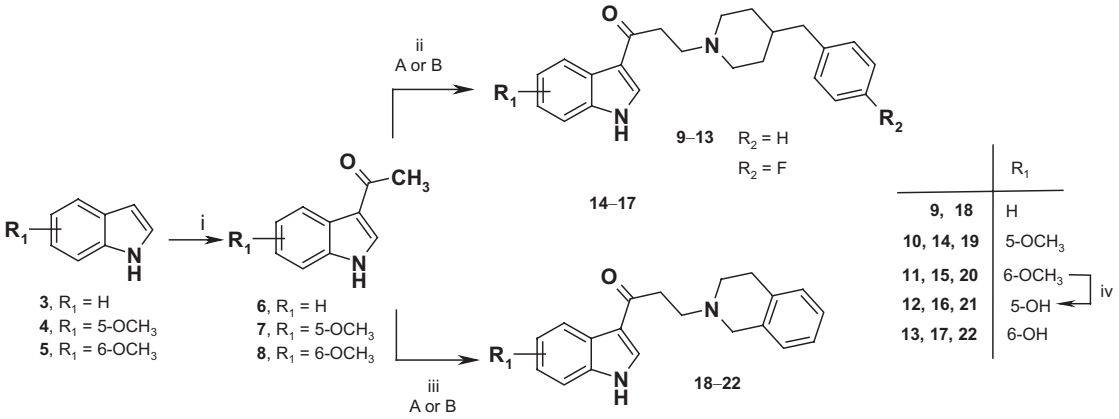
in vivo efficacy as anticonvulsant [14]. Starting from crystallographic data (PDB code 3QEL) we performed a docking study suggesting that the compound **2** establishes crucial hydrogen bond interactions and hydrophobic contacts within the interface of the functional amino-terminal dimer composed of GluN1/GluN2B subunits. In more detail the 5-hydroxyindole derivative **2** engages profitable contacts both with residues from GluN1 (Y109, G112, L135) and GluN2B (Q110, I111, F176, P177, E236) subunits [15].

To gain further information about SARs for this class of GluN2B-containing receptor ligands, we herein report the synthesis of new hydroxyindole derivatives (Fig. 2) as homologs of previously reported active ligands bearing ethanone linker. So through an extra methylene bridge, we lengthened the linker between the indole ring and benzylpiperidine fragment and designed a series of 3-(4-benzylpiperidin-1-yl)-1-(1*H*-indol-3-yl)propan-1-one derivatives. Because it is well known that the presence of a fluorine atom could increase the metabolic stability of xenobiotic bearing a phenyl substituent, we also replaced the benzylpiperidine fragment with the 4-fluorobenzylpiperidine one. Finally, in order to reduce the distance between the nitrogen atom and aromatic ring, the isoquinoline nucleus replaced the benzylpiperidine fragment thus designing the corresponding 3-(3,4-dihydroisoquinolin-2(1*H*)-yl)-1-(1*H*-indol-3-yl)propan-1-ones.

The binding affinity for displacement of [^{3}H]ifenprodil of the new hydroxyindole derivatives was studied. Moreover, docking simulations were carried out to investigate the plausible binding mode of the title ligands.

Results and discussion

The synthesis of 3-(4-benzylpiperidinyl)-1-(1*H*-indol-3-yl)-propan-1-one derivatives (**9–17**) and 3-(3,4-dihydroisoquinolin-2(1*H*)-yl)-1-(1*H*-indol-3-yl)propan-1-one derivatives (**18–22**) was performed as outlined in Scheme 1. Initially, the appropriate indoles **3–5** were 3-acetylated by a Vilsmeier Haack reaction in the presence of phosphoryl chloride and dimethylacetamide to give intermediates **6–8** [20–23]. By



Scheme 1. Synthetic pathway of 3-substituted indole derivatives **9–22**. Reagents and conditions: (i) $POCl_3$, *N,N*-dimethylacetamide, r.t. 24 h; (ii) route A (R_1 and R_2 = H): 4-benzylpiperidine hydrochloride, 1,3-dioxolane, HCl 37%, reflux 5 h, then r.t. 20 h; route B (R_1 = H, OMe, and R_2 = H, F): DMF, paraformaldehyde, 4-benzylpiperidine hydrochloride or 4-fluorobenzylpiperidine hydrochloride, HCl 37%, MW 3 min 80°C; (iii) route A (R_1 = H): 1,2,3,4-tetrahydroisoquinoline hydrochloride, 1,3-dioxolane, HCl 37%, reflux 5 h, then r.t. 20 h; route B (R_1 = H, OMe): DMF, paraformaldehyde, 1,2,3,4-tetrahydroisoquinoline hydrochloride, HCl 37%, MW 3 min 80°C; (iv) BBr_3 (1.0 M DCM), r.t., 10 h.

reaction of ketone **6** with a suitable secondary amine derivative and 1,3-dioxolane, we set up the synthetic approach through the Mannich reaction conditions thus obtaining unsubstituted indoles **9** and **18** (R_1 and $R_2 = H$) (route A) as prototypes. In an attempt to improve the yield as well as to reduce reaction time and side products, the indoles **9** and **18** were also synthesized following an alternative synthetic procedure that has been performed using paraformaldehyde under micro-assisted irradiation conditions (route B). Therefore, this optimized synthetic route B was used to obtain the six methoxyindoles **10–11**, **14–15** and **19–20** in good yields. Lastly, these precursors were demethylated to give the expected hydroxyindoles **12–13**, **16–17** and **21–22**.

Although we carried out synthesis of a series of 14 new indole derivatives, we chose to evaluate the affinity for GluN2B-containing NMDA receptors only for six selected derivatives **12–13**, **16–17**, and **21–22** bearing the hydroxyl group on the indole nucleus. These selection criteria were based on our knowledge of the highest affinity of 3-(4-benzylpiperidin-1-yl)-1-(5-hydroxy-1*H*-indol-3-yl)ethan-1-one (**2**, $IC_{50} = 25$ nM) and its 6-hydroxy analog ($IC_{50} = 17$ nM) [15] that could establish a fruitful hydrogen bonding interaction with the crucial residue E236 of GluN2B subunit. In addition, the unsubstituted and 5/6-methoxy-indoles were generally inactive GluN2B ligands. As reported in Table 1, a preliminary binding assay using [^{3}H]ifenprodil has been carried out to measure the percentage of inhibition at fixed dose of 0.1 μM concentration. Then, three concentrations (10 μM, 0.1 μM, 0.001 μM, in duplicate) of test compound were used to calculate the corresponding IC_{50} values (see Table 1) in the same displacement assay. The ifenprodil (**1**) and previously reported indole **2** served as reference compounds to compare the binding affinity potencies of the new indole derivatives.

Table 1. GluN2B/NMDA binding affinities of indole derivatives and reference compounds ifenprodil (**1**) and **2**.

	R_1	R_2	Inhibition % (@ 0.1 μM)[a)]	IC_{50} (nM)[a)]
12	5-OH	H	47%	230
13	6-OH	H	57%	80
16	5-OH	F	19%	1130
17	6-OH	F	39%	360
21	5-OH	H	<10	ND
22	6-OH	H	<10	ND
2a[b)]	5-OH	H	75%	25.0
Ifenprodil (**1**)[b)]			77%	20.0

ND, not detectable.

[a)] Displacement of [^{3}H]ifenprodil, three concentrations (10 μM, 0.1 μM, 0.001 μM, in duplicate) of test compounds were used in displacement assay.

[b)] Data from reference [14].

The results of the *in vitro* assay evidenced that the new indole derivatives were lower active ligands in comparison with the prototype **1** and the parent compound **2**. The fluorine atom at *para* position of benzylpiperidine moiety decreased the binding affinity at NMDA receptor. Moreover, compounds **21** and **22** showed no displacement of [^{3}H]ifenprodil at 0.1 μM concentration, thus suggesting that the rigid and bulky 1,2,3,4-tetrahydroisoquinoline system induces a remarkable loss of affinity. Only compounds **12** and **13** were able to produce about 50% [^{3}H]ifenprodil displacement at 0.1 μM concentration and consequently displayed IC_{50} values of 230 and 80 nM, respectively. These data could suggest that the elongation of the linker between carbonyl group and piperidine nitrogen atom negatively influences the binding affinity. A noticeable reduction of affinity has been especially observed for compound **12** ($IC_{50} = 230$ nM) if compared to parent compound **2** ($IC_{50} = 25$ nM). The 6-hydroxy derivative **13** was only fourfold less active than corresponding parent compound ($IC_{50} = 80$ nM versus $IC_{50} = 17$ nM).

By means of a computational approach, we attempted to explain this significant reduction of potency due to the presence of the extra methylene bridge for compound **12**. So, we performed a docking study using GOLD program and the docking protocol applied in our previous work [15]. The indole **12** was docked into the dimer GluN1/GluN2B interface retrieved from the RCSB Protein Data Bank (entry code 3QEL) [11]. As shown in Fig. 3 the benzylpiperidine portion of ligand **12** is surrounded by hydrophobic GluN1 (Y109 and Y114) and GluN2B (P78, F114 and I111) residues. This ligand makes an unexpected bidentate H bond interaction with crucial residue Q110 of GluN2B subunit. This network involves two pivotal functional groups of indole derivative that is both the hydrogen atom bound to the positive ionizable piperidine nitrogen and the oxygen atom of propanone linker. Moreover, the ligand is also able to form a further hydrogen bonding interaction between the hydroxyl group and E106 residue of GluN2B. This multiple H-bond interaction forces the indole ring to occupy a novel position thus losing the expected key interaction between hydroxyl group and E236 residue.

This molecular modeling investigation confirms that the lengthening of the linker between the indole ring and benzylpiperidine fragment determines the shift of hydroxy-indole position that accounts for the higher IC_{50} value of **12** when compared to parent 5-hydroxy analog **2** (230 nM vs. 25 nM). In addition, our computational studies suggested that there are no striking differences in the proposed binding modes of 4-fluorobenzylpiperidine derivatives in comparison to benzylpiperidine analogs. Thus, these experiments failed to explain the reduction of ligand affinity measured for 4-fluorosubstituted derivative **16** ($IC_{50} = 1130$ nM) in comparison to unsubstituted compound **12** ($IC_{50} = 230$ nM). Finally,

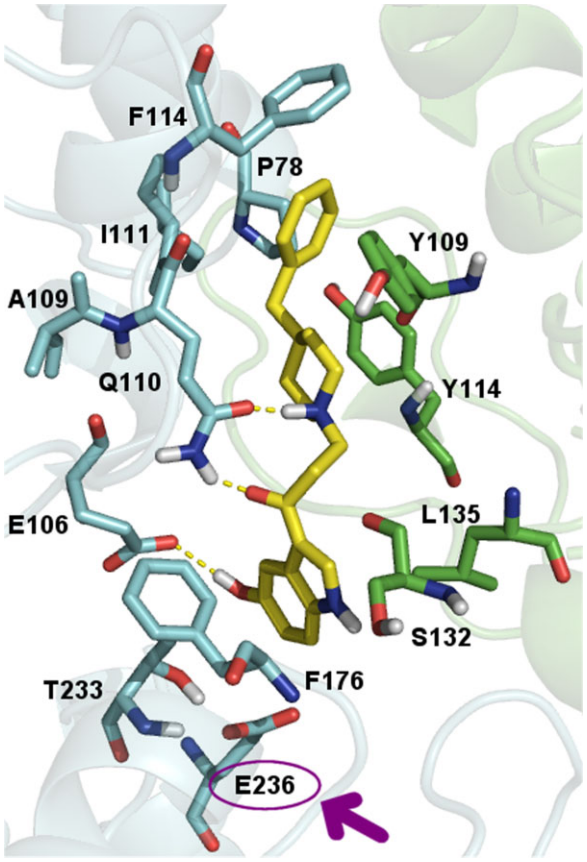


Figure 3. Docking pose of compound **12** at the GluN1-GluN2B subunit interface (PDB code 3QEL). Crucial residues are drawn in stick and colored in cyan (GluN2B) and in green (GluN1). Hydrogen bonds are shown as dashed yellow lines. The figure was prepared in PyMOL software.

we performed docking studies of inactive indoles **21** and **22** (see Supporting Information). It appears that they do not fill well the binding pocket within dimeric GluN1/GluN2B interface. This matter of fact could be attributed to the shorter distance between piperidine nitrogen atom and aromatic region of 1,2,3,4-tetrahydroisoquinoline system, which failed to map hydrophobic region of GluN1/GluN2B interface.

In conclusion, by structural modifications on the 3-substituted indole moiety, new ligands targeting the GluN2B-containing NMDA receptor were designed and synthesized. The most potent ligands of this series exhibited about 50% [^{3}H]ifenprodil displacement at 0.1 μM concentration and IC_{50} values in the nanomolar range. This work furnished further SAR information about the recognition process to the GluN2B-containing NMDA receptor subtype. Taking these findings into account, the design of new hydroxyindole derivatives should be addressed to restore the profitable contact between hydroxyl group and E236 residue, thus improving the interaction within the GluN1/GluN2B interface.

Experimental

Chemistry

All starting materials and reagents commercially available (Sigma–Aldrich Milan, Italy; Alfa Aesar Karlsruhe, Germany) were used without further purification. Microwave-assisted reactions were carried out in a focused Microwave Synthesis System (CEM Technology Ltd Buckingham, UK). Melting points were determined on a Buchi B-545 apparatus (BUCHI Labortechnik AG Flawil, Switzerland) and are uncorrected. Elemental analyses (C, H, N) were carried out on a Carlo Erba Model 1106 Elemental Analyzer (Carlo Erba Milano, Italy); the results confirmed a ≥95% purity. Merck silica gel 60 F254 plates were used for analytical TLC (Merck KGaA, Darmstadt, Germany). Flash Chromatography (FC) was carried out on a Biotage SP1 EXP (Biotage AB, Uppsala, Sweden). R_f values were determined on TLC plates using a mixture of DCM/MeOH (90:10) as eluent. ^{1}H NMR spectra were measured in dimethylsulfoxide-d_6 (DMSO-d_6) with a Gemini 300 spectrometer (Varian Inc., Palo Alto, CA, USA); chemical shifts are expressed in δ (ppm) and coupling constants (J) in Hz. By using a Varian Gemini 300 spectrometer the ^{13}C NMR spectra were measured for two selected compounds as prototypes of this series of indoles. All exchangeable protons were confirmed by addition of D_2O.

*General procedure for the synthesis of 1-(1H-indol-3-yl) ethanone derivatives **6–8***

Compounds **6–8** were prepared according to the previously reported procedure [20–23]. In particular, phosphoryl chloride (0.92 mL, 10 mmol) was added to ice cold dimethylacetamide (2.79 mL, 30 mmol) and this mixture was stirred. A suitable indole (**3**, **4**, or **5**) (1 mmol) was added and the reaction mixture was stirred at room temperature for 24 h, then poured and basified with a sodium hydroxide solution (4 N). The mixture was extracted with EtOAc (3 × 10 mL) and dried over Na_2SO_4. After the removal of the solvent under reduced pressure, the residue was poured with a mixture of diethyl ether and dichloromethane to give the desired 1-(1*H*-indol-3-yl)ethanone derivatives (**6**, **7**, or **8**). The spectral data of obtained compounds **6–8** were in accordance with literature [24, 25].

*General procedures for the synthesis of 3-(4-benzylpiperidinyl)-1-(1H-indol-3-yl)propan-1-one derivatives (**9–17**) and 3-(3,4-dihydroisoquinolin-2(1H)-yl)-1-(1H-indol-3-yl)propan-1-one derivatives (**18–22**)*

*Route A for the synthesis of prototypes **9** and **18***: The appropriate secondary amine (4-benzylpiperidine or 1,2,3,4-tetrahydroisoquinoline) hydrochloride (1 mmol) was dissolved in the mixture of 1,3-dioxalane (0.22 mL, 3 mmol) and 1-(1*H*-indol-3-yl)ethanone **6** (1.2 mmol) in the presence of a catalytic amount of hydrochloric acid (37%). The mixture was heated at 90 °C for 5 h and then it was stirred at room temperature for 20 h. Then, the cooled reaction mixture was diluted with water and washed with EtOAc (3 × 10 mL). The aqueous layer was made alkaline with sodium hydroxide (2 N) and extracted with EtOAc (3 × 10 mL). The organic

layer was washed with water (3 × 5 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The crude final indole derivative **9** or **18** was purified by flash chromatography (FC, DCM/CH_3OH 90:10) and recrystallized by treatment with Et_2O.

Route B for the synthesis of ***9–11, 14–15*** *and* ***18–20***: To a stirred mixture of the appropriate *1*-(1*H*-indol-3-yl)ethanone derivatives **6–8** (1 mmol) in DMF (3 mL), paraformaldehyde (39.0 mg, 1.3 mmol), and corresponding secondary amine (4-benzylpiperidine, 4-fluorobenzylpiperidine or 1,2,3,4-tetrahydroisoquinoline) hydrochloride (1.1 mmol), a catalitic amount of hydrochloric acid (37%) was added. Then, the mixture was capped in closed vessel and irradiated in a microwave oven at 80°C for 3 min using a CEM focused Microwave Synthesis System. The progress of the reaction was monitored by TLC using a mixture of DCM/CH_3OH (90:10) as eluent. The mixture was quenched with water (10 mL) and extracted with EtOAc (3 × 10 mL). The aqueous layer was made alkaline with sodium hydroxide (2 N) and extracted with EtOAc (3 × 10 mL). The combined extracts were dried with dry Na_2SO_4 and concentrated *in vacuo*. The crude desired compound was purified by flash chromatography (FC, DCM/CH_3OH 90:10) and recrystallized by treatment with Et_2O.

General procedure for the synthesis of hydroxyl derivatives ***12–13, 16–17****, and* ***21–22***

The appropriate methoxy derivative (**10–11**, **14–15**, and **19–20**) was dissolved in DCM (5 mL), treated with BBr_3 (1M in DCM) (6 mL, 6 mmol) under nitrogen atmosphere and stirred overnight at room temperature. Successively, MeOH (7 mL) was carefully added at 0°C and the solvent removed under reduced pressure. The residue was dissolved in EtOAc (10 mL) and washed with H_2O (3 × 10 mL) and with $NaHCO_3$ saturated aqueous solution (2 × 10 mL). The organic layer was dried over dry Na_2SO_4 and after the solvent was removed under reduced pressure. The crude compound was purified by flash chromatography (FC, DCM/MeOH, 90:10) and recrystallized by treatment with EtOH and Et_2O to give the desired final products.

*3-(4-Benzylpiperidin-1-yl)-1-(1H-indol-3-yl)propan-1-one (****9****)*

Colorless solid. Yield 16% (route A). Yield 40% (route B). M.p.: 191–192°C. $R_f = 0.17$. ^{1}H NMR (DMSO-d_6) (δ) 1.12–2.96 (m, 15H), 7.07–7.23 (m, 9H, ArH, H-6, H-7), 7.41 (m, 1H, H-5), 8.14 (m, 1H, H-4), 8.33 (d, 1H, H-2, $J = 3.7$), 11.88 (bs, 1H, NH). Anal. calcd. for $C_{23}H_{26}N_2O$: C, 79.73; H, 7.56; N, 8.09. Found C, 79.71; H, 7.54, N 8.07.

*3-(4-Benzylpiperidin-1-yl)-1-(5-methoxy-1H-indol-3-yl) propan-1-one (****10****)*

Colorless solid. Yield 40%. M.p.: 171–172°C. $R_f = 0.17$. ^{1}H NMR (DMSO-d_6) δ: 1.20–3.02 (m, 15H), 3.77 (s, 3H, OCH_3), 6.83 (dd, 1H, H-6, $J = 2.4$, $J = 8.8$), 7.14–7.30 (m, 5H, ArH), 7.35 (d, 1H, H-7, $J = 8.8$), 7.69 (s, 1H, H-4), 8.29 (d, 1H, H-2, $J = 2.4$), 11.85 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 31.39, 37.13, 42.26, 53.21, 53.86, 55.27, 57.58, 103.1, 109.6, 112.7, 116.3, 126.2, 127.0, 128.2, 129.0, 131.5, 134.2, 140.3, 155.4, 193.9. Anal. calcd. for $C_{24}H_{28}N_2O_2$: C, 76.56; H, 7.50; N, 7.44. Found C, 76.66; H, 7.54; N, 7.34.

*3-(4-Benzylpiperidin-1-yl)-1-(6-methoxy-1H-indol-3-yl) propan-1-one (****11****)*

Colorless solid. Yield 42%. M.p.: 187–188°C. $R_f = 0.18$. ^{1}H NMR (DMSO-d_6) δ: 1.13–2.96 (m, 15H), 3.77 (s, 3H, OCH_3), 6.81 (d, 1H, H-5, $J = 8.8$), 6.93 (s, 1H, H-7), 7.13–7.26 (m, 5H, ArH), 8.02 (d, 1H, H-4, $J = 8.8$), 8.21 (bs, 1H, H-2), 11.70 (bs, 1H, NH). Anal. calcd. for $C_{24}H_{28}N_2O_2$: C, 76.56; H, 7.50; N, 7.44. Found C, 76.59; H, 7.53; N, 7.47.

*3-(4-Benzylpiperidin-1-yl)-1-(5-hydroxy-1H-indol-3-yl) propan-1-one (****12****)*

Colorless solid. Yield 54%. M.p.: 216–217°C. $R_f = 0.09$. ^{1}H NMR (DMSO-d_6) δ: 1.16–2.95 (m, 15H), 6.67–7.30 (m, 7H, ArH, H-6, H-7), 7.59 (d, 1H, H-4, $J = 3.6$), 8.22 (d, 1H, H-2, $J = 3.2$), 8.96 (s, 1H, OH), 11.67 (bs, 1H, NH). Anal. calcd. for $C_{23}H_{26}N_2O_2$: C, 76.21; H, 7.23; N, 7.73. Found C, 76.22; H, 7.24; N, 7.74.

*3-(4-Benzylpiperidin-1-yl)-1-(6-hydroxy-1H-indol-3-yl) propan-1-one (****13****)*

Colorless solid. Yield 80%. M.p.: 121–122°C. $R_f = 0.09$. ^{1}H NMR (DMSO-d_6) δ: 1.12–2.93 (m, 15H), 6.66 (dd, 1H, H-5, $J = 8.5$, $J = 1.8$), 6.77 (d, 1H, H-7, $J = 1.8$), 7.19–7.29 (m, 5H, ArH), 7.91 (d, 1H, H-4, $J = 8.5$), 8.13 (d, 1H, H-2, $J = 2.7$), 9.17 (s, 1H, OH), 11.52 (bs, 1H, NH). Anal. calcd. for $C_{23}H_{26}N_2O_2$: C, 76.21; H, 7.23; N, 7.73. Found C, 76.19; H, 7.21; N, 7.71.

*2-(4-(4-Fluorobenzyl)piperidin-1-yl)-1-(5-methoxy-1H-indol-3-yl)propan-1-one (****14****)*

Colorless solid. Yield 47%. M.p.: 164–165°C. $R_f = 0.20$. ^{1}H NMR (DMSO-d_6) δ: 1.11–2.98 (m, 15H), 3.76 (s, 3H, OCH_3), 6.83 (dd, 1H, H-6, $J = 8.8$, $J = 2.3$), 7.04–7.17 (m, 4H, ArH), 7.33 (d, 1H, H-7, $J = 8.8$), 7.69 (d, 1H, H-4, $J = 2.3$), 8.28 (d, 1H, H-2, $J = 2.9$), 11.81 (bs, 1H, NH). Anal. calcd. for $C_{24}H_{27}FN_2O_2$: C, 73.07; H, 6.90; N, 7.10. Found C, 73.17; H, 6.88; N, 7.15.

*2-(4-(4-Fluorobenzyl)piperidin-1-yl)-1-(6-methoxy-1H-indol-3-yl)propan-1-one (****15****)*

Colorless solid. Yield 20%. M.p.: 207–208°C. $R_f = 0.20$. ^{1}H NMR (DMSO-d_6) δ: 1.11–2.96 (m, 15H), 3.77 (s, 3H, OCH_3), 6.80 (dd, 1H, H-5, $J = 8.8$, $J = 2.3$), 6.92 (d, 1H, H-7, $J = 2.3$), 7.04–7.20 (m, 4H, ArH), 8.02 (d, 1H, H-4, $J = 8.8$), 8.22 (s, 1H, H-2), 11.71 (bs, 1H, NH). Anal. calcd. for $C_{24}H_{27}FN_2O_2$: C, 73.07; H, 6.90; N, 7.10. Found C, 73.15; H, 6.85; N, 7.16.

*2-(4-(4-Fluorobenzyl)piperidin-1-yl)-1-(5-hydroxy-1H-indol-3-yl)propan-1-one (****16****)*

Colorless solid. Yield 66%. M.p.: 210–211°C. $R_f = 0.13$. ^{1}H NMR (DMSO-d_6) δ: 1.11–2.94 (m, 15H), 6.67 (dd, 1H, H-6, $J = 8.8$, $J = 2.3$), 6.77 (d, 1H, H-7, $J = 1.8$), 7.07–7.20 (m, 5H, ArH), 7.57 (d, 1H, H-4, $J = 2.4$), 8.21 (s, 1H, H-2), 8.96 (s, 1H, OH), 11.66 (bs, 1H, NH). Anal. calcd. for $C_{23}H_{25}FN_2O_2$: C, 72.61; H, 6.62; N, 7.36. Found C, 72.71; H, 6.50; N, 7.40.

*2-(4-(4-Fluorobenzyl)piperidin-1-yl)-1-(6-hydroxy-1H-indol-3-yl)propan-1-one (****17****)*

Colorless solid. Yield 40%. M.p.: 161–162°C. $R_f = 0.07$. ^{1}H NMR (DMSO-d_6) δ: 1.11–2.93 (m, 15H), 6.65 (dd, 1H, H-5, $J = 8.5$, $J = 2.1$), 6.78 (s, 1H, H-7), 7.04–7.20 (m, 4H, ArH), 7.91 (d, 1H, H-4, $J = 8.5$), 8.13 (s, 1H, H-2, $J = 3.2$), 9.71 (s, 1H, OH), 11.51 (bs, 1H, NH). Anal. calcd. for $C_{23}H_{25}FN_2O_2$: C, 72.61; H, 6.62; N, 7.36. Found C, 72.72; H, 6.51; N, 7.42.

*3-(3,4-Dihydroisoquinolin-2(1H)-yl)-1-(1H-indol-3-yl) propan-1-one (**18**)*

Brown solid. Yield 20% (route A). Yield 50% (route B). M.p.: 165–166°C. $R_f = 0.55$. ^{1}H NMR (DMSO-d_6) δ: 2.73–3.14 (m, 8H), 3.63 (s, 2H), 7.05–7.23 (m, 6H, ArH), 7.46 (m, 1H, H-5), 8.21 (m, 1H, H-4), 8.43 (s, 1H, H-2), 11.95 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 28.77, 36.78, 50.50, 53.72, 55.49, 111.4, 112.2, 116.6, 121.5, 122.8, 125.5, 126.0, 126.4, 126.6, 128.4, 129.0, 134.1, 135.0, 136.7, 194.33. Anal. calcd. for $C_{20}H_{20}N_2O$: C, 78.92; H, 6.62; N, 9.20. Found C, 78.91; H, 6.61; N, 9.19.

*3-(3,4-Dihydroisoquinolin-2(1H)-yl)-1-(5-methoxy-1H-indol-3-yl)propan-1-one (**19**)*

Brown solid. Yield 48%. M.p.: 160–161°C. $R_f = 0.57$. ^{1}H NMR (DMSO-d_6) δ: 2.73–3.11 (m, 8H), 3.63 (s, 2H), 3.77 (s, 3H, OCH_3), 6.83 (dd, 1H, H-6, $J = 2.2$, $J = 8.8$), 7.04–7.10 (m, 4H, ArH), 7.35 (d, 1H, H-7, $J = 8.8$), 7.72 (d, 1H, H-4, $J = 2.2$), 8.36 (d, 1H, H-2, $J = 3.0$), 11.82 (bs, 1H, NH). Anal. calcd. for $C_{21}H_{22}N_2O_2$: C, 75.42; H, 6.63; N, 8.38. Found C, 75.46; H, 6.67; N, 8.42.

*3-(3,4-Dihydroisoquinolin-2(1H)-yl)-1-(6-methoxy-1H-indol-3-yl)propan-1-one (**20**)*

Brown solid. Yield 40%. M.p.: 176–177°C. $R_f = 0.53$. ^{1}H NMR (DMSO-d_6) δ: 2.72–3.28 (m, 8H), 3.62 (s, 2H), 3.78 (s, 3H, OCH_3), 6.82 (dd, 1H, H-5, $J = 8.8$, $J = 2.2$), 6.93 (s, 1H, H-7, $J = 2.2$), 7.02–7.12 (m, 4H, ArH), 8.04 (d, 1H, H-4, $J = 8.8$), 8.29 (d, 1H, H-2, $J = 2.2$), 11.74 (bs, 1H, NH). Anal. calcd. for $C_{21}H_{22}N_2O_2$: C, 75.42; H, 6.63; N, 8.38. Found C, 75.48; H, 6.69; N, 8.45.

*3-(3,4-Dihydroisoquinolin-2(1H)-yl)-1-(5-hydroxy-1H-indol-3-yl)propan-1-one (**21**)*

Brown solid. Yield 30%. M.p.: 222–223°C. $R_f = 0.29$. ^{1}H NMR (DMSO-d_6) δ: 2.72–3.07 (m, 8H), 3.61 (s, 2H), 6.66 (dd, 1H, H-6, $J = 2.7$, $J = 8.5$), 7.02–7.10 (m, 4H, ArH), 7.22 (d, 1H, H-7, $J = 8.5$), 7.58 (d, 1H, H-4, $J = 2.7$), 8.25 (d, 1H, H-2, $J = 3.2$), 8.94 (s, 1H, OH), 11.67 (bs, 1H, NH). Anal. calcd. for $C_{20}H_{20}N_2O_2$: C, 74.98; H, 6.29; N, 8.74. Found C, 74.95; H, 6.26; N, 8.71.

*3-(3,4-Dihydroisoquinolin-2(1H)-yl)-1-(6-hydroxy-1H-indol-3-yl)propan-1-one (**22**)*

Brown solid. Yield 37%. M.p.: 196–197°C. $R_f = 0.20$. ^{1}H NMR (DMSO-d_6) δ: 2.73–3.05 (m, 8H), 3.61 (s, 2H), 6.67 (d, 1H, H-5, $J = 8.8$), 6.78 (bs, 1H, H-7), 7.01–7.20 (m, 4H, ArH), 7.95 (d, 1H, H-4, $J = 8.8$), 8.21 (bs, 1H. H-2), 9.17 (s, 1H, OH), 11.54 (bs, 1H, NH). Anal. calcd. for $C_{20}H_{20}N_2O_2$: C, 74.98; H, 6.29; N, 8.74. Found C, 74.88; H, 6.33; N, 8.78.

Computational studies

The crystal structure of amino terminal domains of the NMDA receptor subunit GluN1 and GluN2B in complex with ifenprodil (**1**) was retrieved from the RCSB Protein Data Bank (entry code 3QEL) [11]. The ligands and water molecules were discarded and the hydrogen atoms were added to protein by Discovery Studio 2.5 [26]. The structures of the ligands were constructed using Discovery Studio 2.5 [26]. The conformational behavior of simulated compounds was investigated by a MonteCarlo procedure (as implemented in the VEGA suite of programs which generated 1000 conformers by randomly rotating the rotors) [27]. All geometries obtained were stored and optimized to avoid high-energy rotamers. The 1000 conformers were clustered by similarity to discard redundancies; in this analysis, two geometries were considered non-redundant when they differed by more than 60° in at least one torsion angle. For each derivative, the lowest energy structure was then submitted to docking simulations The ligands minimized in this way were docked in their corresponding proteins by means of Gold Suite 5.0.1. The region of interest used by Gold was defined in order to contain the residues within 15 Å from the original position of the ligand in the X-ray structure. The side chain of residue Leu135 was allowed to rotate according to the internal rotamer libraries in GOLD Suite 5.0.1. GoldScore [28] was chosen as a fitness function and the standard default settings were used in all the calculations and the ligands were submitted to 100 genetic algorithm runs. The "allow early termination" command was deactivated. Results differing by <0.75 Å in ligand-all atom RMSD, were clustered together. The best ranked GOLD-calculated conformation was used for analysis and representation [15].

Receptor binding studies

The radioligand binding assays against NMDA receptor containing GluN2B-subunit were carried out using [^{3}H]ifenprodil (Custom Screen by Eurofin Panlab, LCC, USA) [29, 30]. Cerebral cortices of male Wistar derived rats weighing 175 ± 25 g are used to prepare glutamate NMDA receptors in Tris–HCl buffer pH 7.4. A 5 mg aliquot is incubated with 2 nM [^{3}H]Ifenprodil (plus 5 μM GBR-12909 to block non-polyamine sensitive sites) for 120 min at 4°C. Non-specific binding is estimated in the presence of 10 μM ifenprodil (**1**). Membranes are filtered and washed, the filters are then counted to determine [^{3}H]Ifenprodil specifically bound. Three concentrations (10 μM, 0.1 μM, 0.001 μM, in duplicate) of test compounds were used in displacement assay.

Financial support for this research by Fondo di Ateneo per la Ricerca (PRA 2009 – grant number ORME09SPNC – Università di Messina).

Author contributions

M.R.B. and S.F. performed the synthesis, purification, and chemical characterization of new molecules under the supervision of R.G., L.D.L. carried out computational studies. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

The authors have declared no conflict of interest.

References

[1] M. J. Niciu, B. Kelmendi, G. Sanacora, *Pharmacol. Biochem. Behav.* **2012**, *100*, 656–664.

[2] T. W. Lai, S. Zhang, Y. T. Wang, *Prog. Neurobiol.* **2014**, *115*, 157–188.

[3] A. Mehta, M. Prabhakar, P. Kumar, R. Deshmukh, P. L. Sharma, *Eur. J. Pharmacol.* **2013**, *698*, 6–18.

[4] P. Paoletti, *Eur. J. Neurosci.* **2011**, *33*, 1351–1365.

[5] R. Gitto, L. De Luca, S. De Grazia, A. Chimirri, *Curr. Top. Med. Chem.* **2012**, *12*, 971–993.

[6] P. Paoletti, J. Neyton, *Curr. Opin. Pharmacol.* **2007**, *7*, 39–47.

[7] O. A. Shipton, O. Paulsen, *Philos. Trans. R Soc. Lond. B Biol. Sci.* **2014**, *369*, 20130163.

[8] E. S. Vizi, M. Kisfali, T. Lorincz, *Brain Res. Bull.* **2013**, *93*, 32–38.

[9] J. M. Lima-Ojeda, M. A. Vogt, N. Pfeiffer, C. Dormann, G. Kohr, R. Sprengel, P. Gass, D. Inta, *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2013**, *45*, 28–33.

[10] C. Carter, J. Benavides, P. Legendre, J. D. Vincent, F. Noel, F. Thuret, K. G. Lloyd, S. Arbilla, B. Zivkovic, E. T. MacKenzie, *J. Pharmacol. Exp. Ther.* **1988**, *247*, 1222–1232.

[11] E. Karakas, N. Simorowski, H. Furukawa, *Nature* **2011**, *475*, 249–253.

[12] R. Gitto, L. De Luca, S. Ferro, F. Occhiuto, S. Samperi, G. De Sarro, E. Russo, L. Ciranna, L. Costa, A. Chimirri, *ChemMedChem* **2008**, *3*, 1539–1548.

[13] A. Chimirri, L. De Luca, S. Ferro, G. De Sarro, L. Ciranna, R. Gitto, *ChemMedChem* **2009**, *4*, 917–922.

[14] R. Gitto, L. De Luca, S. Ferro, R. Citraro, G. De Sarro, L. Costa, L. Ciranna, A. Chimirri, *Bioorg. Med. Chem.* **2009**, *17*, 1640–1647.

[15] R. Gitto, L. De Luca, S. Ferro, M. R. Buemi, E. Russo, G. De Sarro, L. Costa, L. Ciranna, O. Prezzavento, E. Arena, S. Ronsisvalle, G. Bruno, A. Chimirri, *J. Med. Chem.* **2011**, *54*, 8702–8706.

[16] R. Gitto, L. De Luca, S. Ferro, M. R. Buemi, E. Russo, G. De Sarro, M. Chisari, L. Ciranna, A. Chimirri, *J. Med. Chem.* **2012**, *55*, 10532–10539.

[17] M. R. Buemi, L. De Luca, A. Chimirri, S. Ferro, R. Gitto, J. Alvarez-Builla, R. Alajarin, *Bioorg. Med. Chem.* **2013**, *21*, 4575–4580.

[18] R. Gitto, L. De Luca, S. Ferro, E. Russo, G. De Sarro, M. Chisari, L. Ciranna, J. Alvarez-Builla, R. Alajarin, M. R. Buemi, A. Chimirri, *Bioorg. Med. Chem.* **2014**, *22*, 1040–1048.

[19] R. Gitto, L. De Luca, S. Ferro, A. Scala, S. Ronsisvalle, C. Parenti, O. Prezzavento, M. R. Buemi, A. Chimirri, *Bioorg. Med. Chem.* **2014**, *22*, 393–397.

[20] L. De Luca, S. Ferro, R. Gitto, M. L. Barreca, S. Agnello, F. Christ, Z. Debyser, A. Chimirri, *Bioorg. Med. Chem.* **2010**, *18*, 7515–7521.

[21] L. De Luca, S. De Grazia, S. Ferro, R. Gitto, F. Christ, Z. Debyser, A. Chimirri, *Eur. J. Med. Chem.* **2011**, *46*, 756–764.

[22] S. Ferro, L. De Luca, M. L. Barreca, S. De Grazia, F. Christ, Z. Debyser, A. Chimirri, *Bioorg. Med. Chem.* **2010**, *18*, 5510–5518.

[23] B. R. R. Kesteleyn, P. J.-M. B. Raboisson, D. L. N. G. Surleraux, G. Y. P. Hache, S. M. H. Vendeville, A. A. Peeters, P. T. B. P. Wigerinck, PCT Int. Appl. (2005), WO 2005111047 A1 20051124. CAPLUS: 2005:1241144 CAN144: 6779.

[24] T. S. Jiang, G. W. Wang, *Org. Lett.* **2013**, *15*, 788–791.

[25] M. Venkatanarayana, P. K. Dubey, *Lett. Org. Chem.* **2011**, *8*, 656–662.

[26] Discovery, Studio, Modeling, Environment, R. 2.5. 2009, Accelrys Software Inc, San Diego.

[27] A. Pedretti, L. Villa, G. Vistoli, *J. Mol. Graph. Model.* **2002**, *21*, 47–49.

[28] G. Jones, P. Willett, R. C. Glen, A. R. Leach, R. Taylor, *J. Mol. Biol.* **1997**, *267*, 727–748.

[29] Eurofins Panlabs Inc. http://www.eurofinspanlabs.com.

[30] H. Schoemaker, J. Allen, S. Z. Langer, *Eur. J. Pharmacol.* **1990**, *176*, 249–250.

