# PET and SPECT Imaging of the NMDA Receptor System: An Overview of Radiotracer Development

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**Abstract:** Imaging the *N*-methyl-D-aspartate receptors (NMDARs) in the living human brain by positron emission tomography (PET) or single photon emission computed tomography (SPECT) would provide useful information on the role of these receptors in ischemia and in various neurological disorders such as degenerative diseases, epilepsy or schizophrenia. To assess NMDAR radiotracer development and to propose perspectives, we overviewed the PET and SPECT candidate radioligands developed until now. Labelled molecules of interest were classified in three groups according to their binding site: intrachannel pore site blockers, glycine site inhibitors and NR2B selective subunit antagonists.

**Keywords:** Imaging, NMDA receptors, PET, radiotracer, radiopharmaceutical, SPECT.

## INTRODUCTION

The *N*-methyl-D-aspartate receptor (NMDAR) complex is one family of ligand-gated ion channels (kainate, AMPA, and NMDA) and constitutes a subclass of the excitatory ionotropic glutamatergic neurotransmitter receptor system [1, 2]. Widely expressed in the central nervous system, NMDARs are characterized by specific molecular composition and unique pharmacological and functional properties [3, 4]. Once activated, they permit the passage of calcium ($Ca^{2+}$) ions as well as sodium ($Na^{+}$) and potassium ($K^{+}$) ions. The high permeability to calcium ions confers on NMDARs a central role in both synaptic plasticity under physiological conditions and neuronal death under excitotoxic pathological conditions. NMDARs are involved in brain development and cognitive processes such as learning and memory and also in numerous neurological disorders [5-9]. The latter include brain dysfunctions: i) due to chronic neurodegeneration including Alzheimer's, Parkinson's or Huntington's diseases, ii) resulting from acute excitotoxic insults such as ischemic stroke or traumatic brain injury, iii) arising from sensitisation of neurons in case of epilepsy or neuropathic pain, iv) corresponding to an hypofunction of the NMDAR in schizophrenia, v) and during drug abuse [1, 4]. In consequence, NMDARs are targets of high therapeutic interest.

NMDARs occur as multiple subtypes endowed with distinctive functional properties and expression patterns [10]. They consist in a heteromeric assembly of a relatively large pool of subunits which contain a diversity of endogenous ligands binding sites. At the present, NMDARs are characterized by the binding sites for glutamate and glycine, the ion-channel pore and recently identified allosteric sites on the N-terminal domain. Some other potential sites are currently considered, in particular at the interface between subunits. Since the cloning of the different subunit isoforms, a continuous challenge stays to relate particular functions and NMDAR subtypes. Works to identify the molecular determinants for the subunit selectivity from structural and functional studies are still in progress [2].

As diverse brain disorders implicate obviously specific NMDAR subtypes, the development of specific NMDAR probes for *in vivo* imaging studies became of a growing interest. In particular, non invasive molecular imaging techniques i.e. positron emission tomography (PET) and single photon emission computed tomography (SPECT) would be helpful to map and functionally assess the NMDARs. Those imaging techniques would allow the study of the biochemical changes associated with neurologic disorders in living human brain and the diagnosis of NMDAR dysfunctions with evaluation of therapeutic strategies. Considering these multiple objectives, numerous NMDARs ligands have been synthesized, radiolabelled and tested *in vitro* and/or *in vivo* for twenty years to attempt the visualization and quantification of NMDARs. This review reports the actual statement of the PET and SPECT candidate tracers developed for *in vivo* NMDAR investigation.

## 1. NMDARS: CONSTITUTION, PHARMACOLOGY AND BIODISTRIBUTION

### Constitution

NMDARs are heteromeric complexes incorporating different subunits within a repertoire of three subtypes, NR1, NR2 and NR3 [2]. There are eight different NR1 subunits generated by alternative splicing from a single gene, four different NR2 subunits (A, B, C and D) and two NR3 subunits (A and B) encoded by six separate genes [1]. Expression of functional recombinant NMDARs in mammalian cells requires the co-expression of at least one NR1 and one NR2 subunits. The stoichiometry of NMDARs has not yet been established definitely but from a common consensus, the NMDAR is considered as a tetramer mostly incorporating two NR1 and two NR2 subunits. In NR3 subunit ex-

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pressing cells, these NR3 subunits are co-assembled with NR1 and NR2 subunits to form ternary NR1/NR2/NR3 tetrameric complexes.

All functional domains in NMDAR subunits share a common membrane topology characterized by: i) a large extracellular N-terminus, ii) a membrane region comprising three transmembrane segments (domains 1, 3 and 4) plus a re-entrant pore loop (domain 2), iii) an extracellular loop between the transmembrane domains 3 and 4, and iv) a cytoplasmic C-terminus [2] (Fig. (**1**)). Three-dimensional structures of the extracellular N-terminal domain have been proposed based on the X-ray coordinates [11-14].

**Pharmacology**

NMDARs possess a variety of regulatory extracellular binding sites for endogenous ligands [2] (Fig. (**1**)). The opening of the channel pore requires the binding of two agonists, the glutamate and the glycine (or D-serine), to their own binding extracellular site located on the NR2 subunit for the glutamate and on the NR1 and NR3 subunits for the glycine. NR2 subunits also possess polyamines and zinc ion binding sites. The zinc binding site on NR2B subunit presents an allosteric binding site called ifenprodil-like site. $Mg^{2+}$ ion binds to a site located within the ion channel and blocks the ion flow through the channel at resting membrane potential. At the opposite $Mg^{2+}$ allows greatly enhanced passage of ions when it is expelled from the channel by depolarisation [5]. NMDARs mediate long-term potentiation by allowing prolonged influx of $Ca^{2+}$ ions, as well as $Na^{+}$ and $K^{+}$, into the synapse. But excess of activation of the NMDA receptor leads to excessive accumulation of intracellular $Ca^{2+}$ inducing apoptotic cascade cell death.

**Exogenous Ligands**

The main exogenous ligands were developed as neuroprotective drugs. They act at one of the three target sites and could be classified as follows: i) competitive antagonists at the glutamate or glycine sites, ii) channel blockers, iii) and non competitive allosteric inhibitors of the other extracellular sites particularly the zinc binding site [2, 12].

The organic compounds that inhibit NMDARs by occluding the ion channel pore are uncompetitive antagonists and their action requires prior activation of the receptor (i.e. pore opening) [15, 16]. Although they present diverse structures, they are all positively charged and act in a voltage-dependent manner. NMDAR pore blockers usually discriminate poorly between NMDAR subtypes. This is the case for the dissociative anaesthetics phencyclidine (PCP), thienylcyclohexylpiperidine (TCP) and ketamine, and for the clinical drugs memantine, amantadine and dizolcipine (MK-801).

The ligands of glycine site act by binding at a recognition site on the NR1 subunit (commonly referred as the $glycine_B$ binding site) [9]. Only a few agonists have been reported and

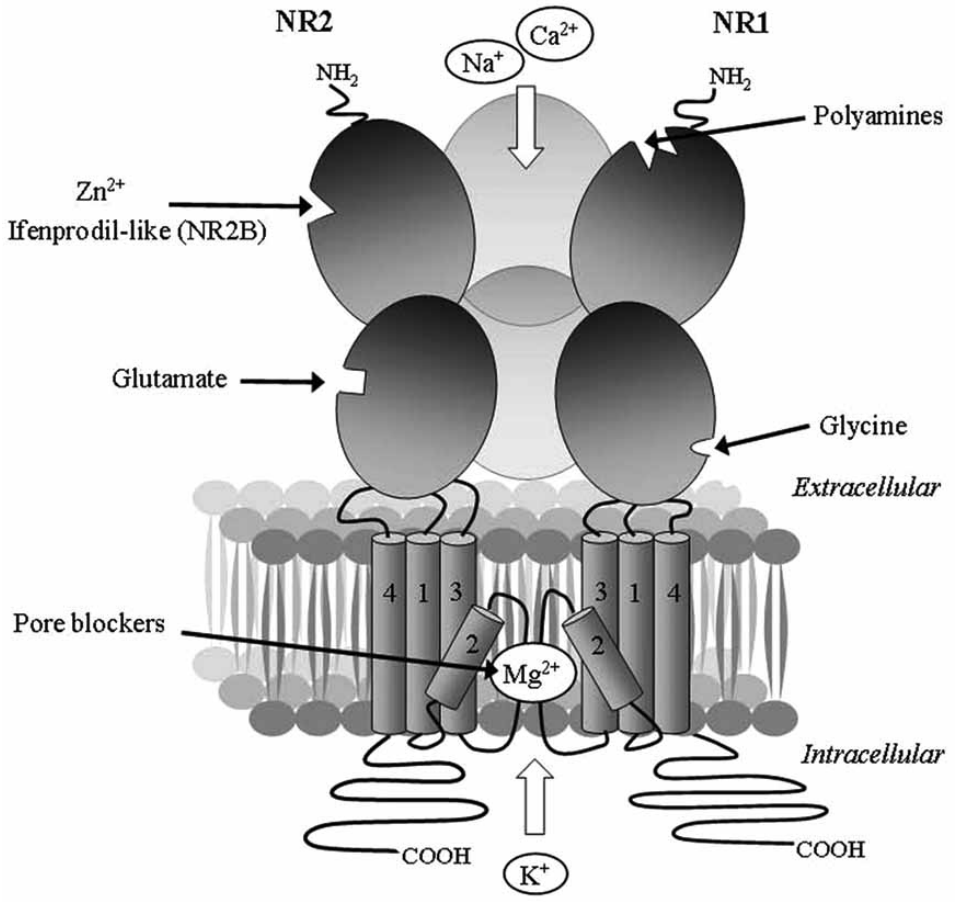



**Fig. (1).** Structure of the NMDA receptor.

consisted of neutral α-animo acids. Several antagonists have been developed containing the quinoline moiety [17]. The prototypical 5,7-dichlorokinurenic acid (5,7-DCK) has shown efficacy in animal neuropathic pain models [18]. However this compound, as well as most of the developed glycine$_B$ antagonists, appeared to have differing effects on the kinetics of NMDA regulation that could not be only related with a glycine site binding. They also displayed several side effects such as sedation.

The NMDAR competitive antagonists acting at the glutamate binding site are usually amino acid derivatives containing an ω-phosphonic group such as (R)-2-amino-5-phosphonopentanoate ((R)-AP5) [15]. These compounds show some selectivity between the different NR2 subunits (affinity ranking typically NR2A > NR2B > NR2C > NR2D) but the variations of affinity are modest. The nanomolar NR2A ligand, $Zn^{2+}$, and ifenprodil-like compounds, which selectively inhibit NR2B-containing receptors, are the only known NMDAR antagonists that display strong subunit selectivity [19-23]. Several highly potent NR2B-selective antagonists, analogues to ifenprodil, show good efficacy as neuroprotective and Parkinson's disease drugs as well as pain killers in a variety of animal models [24, 25]. In humans, NR2B-selective antagonists do not induce the side effects usually seen with non-selective NMDAR antagonists, even at highest neuroprotective doses. Whereas the first NR2B-selective antagonists displayed off-target activity (particularly at adrenergica1 receptors) and therefore presented side-effects, the second-generation compounds showed substantially improved safety profiles [26].

### Biodistribution

It was generally assumed that NMDAR expression in the central nervous system was restricted to neurons, with low expression in glial cells. The anatomic distribution of NMDAR was investigated by receptor autoradiography with tritiated specific ligands or *in situ* hybridization. NMDARs cerebral distribution does not present important differences between mammalian species. The NR1 subunits are widely distributed throughout the brain while NR2 or NR3 have discrete patterns: NR2A receptors are present in cerebral cortex and hippocampus and in a weaker manner in cerebellum; NR2B receptors are localized in forebrain essentially in hippocampus, cerebral cortex, striatum and thalamus; NR2C subunits are found in cerebellum and at a lower level in human brain stem; NR2D subunits are distributed in diencephalic lower brainstem; NR3A are located in cortex and brainstem essentially and NR3B are present in forebrain and in cerebellum [2, 27-31].

## 2. INTRACHANNEL SITE RADIOTRACERS

The NMDAR ion channel is permeable to $Na^+$, $K^+$ and $Ca^{2+}$. The ion flow through the NMDAR channel is inhibited by the binding of $Mg^{2+}$ ion within the pore. Phencyclidine (PCP), thienylcyclohexyl piperidine (TCP), ketamine, me-

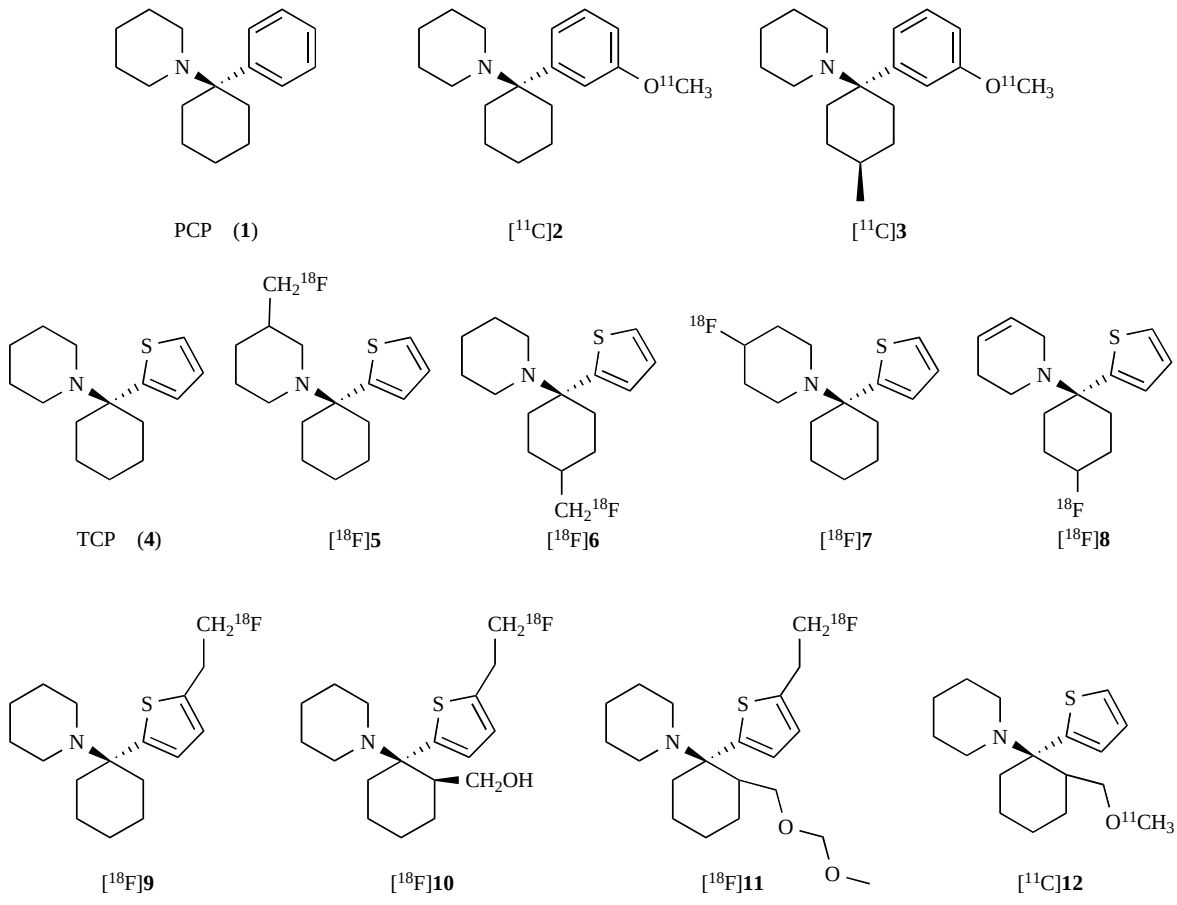



**Fig. (2).** PCP and TCP radiolabelled analogues.

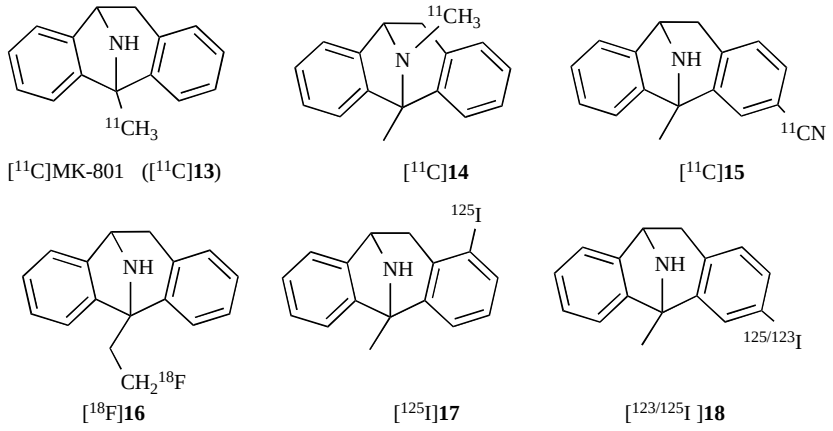



**Fig. (3).** MK-801 radiolabelled analogues.

mantine or MK-801 could bind to this site and blocked the channel pore. These channel blockers were the first compounds used as drugs and therefore were labelled to develop PET or SPECT imaging probes for the NMDARs. Their radiopharmacological behaviours were reviewed by Waterhouse [32].

### 2.1. Radiolabelled PCP or TCP Analogues for PET

PCP (**1**), a potent analgesic and anaesthetic, was formerly used in veterinary medicine as an immobilising agent (Fig. (**2**)). evere adverse effects, especially postoperative psychoses, precluded its clinical use. Two methoxy derivatives [$^{11}$C]**2** and [$^{11}$C]**3** were labelled with carbon-11 by *O*-[$^{11}$C]-methylation of the corresponding phenols with a specific radioactivity higher than 41 GBq/µmol [33]. [$^{11}$C]**2** and [$^{11}$C]**3** freely entered into the rat brain and showed similar regional cerebral distributions with a slightly higher uptake in the hippocampus and striatum than for the other regions studied. The NMDAR-rich regions (hippocampus, striatum, cerebral cortex) to cerebellum ratio for [$^{11}$C]**2** and [$^{11}$C]**3** did not exceed 1.2 and suggested high non specific interactions with other brain regions [33].

The thienyl analogue of PCP (TCP, **4**) did not present easily accessible labelling position and investigations on TCP have been carried out to introduce fluorine-18 atom on one of its three structural domains: the cyclohexyl, the aromatic and the piperidine rings (Fig. (**2**)). First, TCP analogues bearing a fluoromethyl group on the piperidine ring ([$^{18}$F]**5**) or on the cyclohexyl moiety ([$^{18}$F]**6**) were radiolabelled by nucleophilic substitution with fluoride-18 anion from the mesylate precursor [34, 35]. [$^{18}$F]**5** was obtained with a specific radioactivity of 27 GBq/µmol. The radiotracer readily crossed the blood brain barrier (BBB) in baboon. Its *in vivo* brain distribution did not match the *in vitro* distribution of TCP binding sites [36]. The radiolabelling of cis- and trans-[$^{18}$F]**6** was developped by nucleophilic fluorination from the tosylate or mesylate precursor, but no biological data were reported for those radioligands [35]. The 4-fluoropiperidine derivative **7** demonstrated a $K_i$ of 26 nM for NMDARs in competitive *in vitro* binding assay versus [$^3$H]TCP [37]. [$^{18}$F]**7** has been synthesized from the mesylate precursor with a specific radioactivity of 70 GBq/µmol [38]. In preliminary PET studies in monkey, activation of the receptors with NMDA indicated an increased binding of [$^{18}$F]**7** compared to control condition, suggesting that this tracer would reflect the activation state of receptor more than its change in density [39]. [$^3$H]**7** demonstrated a $K_d$ between 43 and 58 nM for rat brain membranes and $B_{max}$ varied from 0.5 pmol/mg of protein in cerebellum membrane up to 8.5 pmol/mg of protein in hippocampus membrane from rat brain [39]. The low $B_{max}/K_d$ ratio[1] comprised between 1 and 15 depending on the brain region, could explain the lack of PET image contrastobtained with [$^{18}$F]**7** [40]. Later, [$^{18}$F]**8** was labelled at the para-position of the cyclohexyl cycle and the specific radioactivity obtained was 18-37 GBq/µmol [41]. In preliminary rat studies, brain uptake of [$^{18}$F]**8** was low 30 min after injection (approximately 0.1 % ID/g). However, little retention of [$^{18}$F]**8** was observed in the known receptor rich regions compared to cerebellum. Rhesus monkey PET scans demonstrated a rapid clearance and no difference between cerebral regions. The authors related these disappointing results to the moderate affinity of **8** ($IC_{50}$ = 96 nM vs [$^3$H]MK-801) [41]. The introduction of a fluoroethyl group at the 3-position of the thienyl cycle in **9** also affected the binding affinity ($K_i$ = 38 nM vs [$^3$H]TCP) compared with TCP (Ki = 5 nM) but the level of binding was considered sufficient to deserve the preparation of [$^{18}$F]**9**. [$^{18}$F]**9** was obtained with a specific radioactivity of 2.2-3.3 GBq/µmol. Unfortunately, despite a heterogeneous distribution of radioactivity, [$^{18}$F]**9** demonstrated a relatively high non-specific binding [42]. When a hydroxymethyl group ([$^{18}$F]**10**) or a MOM-hydroxymethyl group ([$^{18}$F]**11**) was added to the cyclohexyl cycle, the affinities decreased that could explain the homogenous brain distribution in rats [43]. Besides these numerous [$^{18}$F]fluorinated tracers, only one [$^{11}$C]labelled TCP analogue has been synthesized ([$^{11}$C]**12**). The introduction of a cis-methoxymethyl group on the cyclohexyl ring of TCP displayed *in vitro* a five fold higher inhibition concentration than for the PCP [37]. [$^{11}$C]**12** was obtained by *O*-methylation with [$^{11}$C]iodomethane leading to a specific radioactivity of 41 GBq/µmol. Initial mouse brain uptake of [$^{11}$C]**12** was high (about 2.7 % ID/g) but the radio-

[1] $B_{max}/K_d$ ratio could be used as one *in vitro* criterion in the radiotracers development guideline. Values ranging from 20 to 900 are usually observed for successful radiotracers. For details see reference [40].

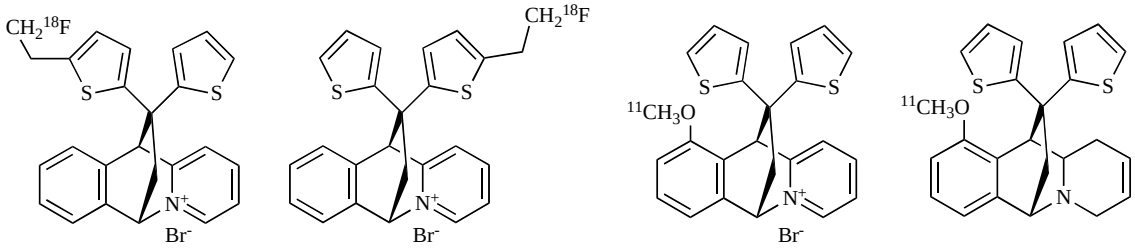



**Fig. (4).** Radiolabelled 9,10-ethanobenzo [b]quinolizinium derivatives.

activity decreased rapidly with time from all brain regions without showing any selective localization [37].

### 2.2. Radiolabelled MK-801 Analogues

MK-801 (dizocilpine (**13**), Fig. (**3**)) interacts with a high selectivity and affinity with PCP/NMDA binding site both in radioligand binding assays and electrophysiological experiments [44]. In animals, MK-801 showed anticonvulsant, dissociative anaesthetic and neuroprotective properties but also side effects including brain lesions, neurotoxic and psychotogenic reactions. [$^{11}$C]MK-801 ([$^{11}$C]**13**) was synthesized by an original method involving the deprotonation at the 5-position followed by alkylation with $^{11}CH_3I$ [45]. Unfortunately, [$^{11}$C]**13** was obtained with a very low specific radioactivity that prevented any use as PET radiotracer.

Several MK-801 analogues (Fig. (**3**)) were developed and radiolabelled with carbon-11 [45-47], fluorine-18 [48, 49], iodine-125 or iodine-123 [48, 50-52] for assessment of NMDAR [32]. The *N*-[$^{11}$C]methyl-MK-801 ([$^{11}$C]**14**) displayed an affinity two fold lower than the one for MK-801 in [$^3$H]TCP binding assay [21]. The cyano derivative [$^{11}$C]**15** (specific radioactivity of 220-600 GBq/μmol) [53] presented *in vitro* $K_D$ of 8.2 nM and a $B_{max}$ of 1.62 pmol/mg of protein in rat forebrain membranes giving a moderate $B_{max}/K_D$ ratio about 20 [47]. Autoradiography study using [$^{11}$C]**15** on rat brain sections demonstrated the highest specific binding in hippocampus, cerebral cortex and striatum with a non specific fraction representing 25% of the total binding. PET studies in rhesus monkey showed a rapid and high uptake of [$^{11}$C]**15** in cortices, striatum, and thalamic regions but the specific binding was not established after MK-801 or ketamine administration [47]. [$^{18}$F]Fluoromethyl-MK-801 ([$^{18}$F]**16**) was synthesized with a specific radioactivity of 40 GBq/μmol [49]. PET scans in baboon showed slight differences for retentions in the cerebral cortex and striatum compared to the cerebellum, but those uptakes were not clearly altered by pretreatment with MK-801 or PCP [54]. In order to develop a SPECT agent, radioactive iodo-MK-801 analogues were synthesized. While the (±)-1-[$^{125}$I]iodo-MK-801 ([$^{125}$I]**17**) was labelled by isotopic exchange leading to a low specific radioactivity of 2.2 GBq/μmol, the (±)-3-[$^{125}$I]iodo-MK-801 ([$^{125}$I]**18**) was obtained by halogen exchange from the bromo analogue with a specific radioactivity more than 50 GBq/μmol [48]. *In vitro* affinity ($K_i$) of **17** was only of 790 nM compared to 79 nM for (±)-MK-801 and *in vivo* blocking experiments failed to uniformly displace [$^{125}$I]**17** binding in rat brain [51]. [$^{125}$I]**18** demonstrated a high $K_D$ of 383 pM and a $B_{max}/K_D$ ratio of 28 [55]. The (+)-enantiomer [$^{123}$I]**18** was labelled with iodine-123 with a specific radioactivity of 75-110 GBq/μmol [52]. *In vitro* experiments on cerebral cortical membranes indicated that [$^{123}$I]**18** bound the NMDAR with a specific binding higher than 95% of the total binding, preferentially under the activated state of the receptor [52]. After administration of [$^{123}$I]**18** to normal human subjects, a rapid uptake was observed in the whole brain, especially in cerebellum and white matter, areas with a low NMDARs concentration. In five patients with cerebral ischemia, an initial uptake reduction compared to healthy subjects was observed and was consistent with a reduced level of cerebral blood flow. Only in two cases, the increased retention of [$^{123}$I]**18** in cortical areas adjacent to the site of the haemorrhage was consistent with activated NMDARs [56]. After injection to Alzheimer's disease suffering patients, the regional distribution of [$^{123}$I]**18** was quite uniform and similar to control scans [57]. These disappointing studies in human were explained by the *in vivo* high non-specific binding attributed to the lipophilicity of the molecule.

### 2.3. Radiolabelled 9,10-ethanobenzo [*b*]quinozilinium Derivatives

9,10-Ethanobenzo[*b*]quinolizinium derivatives have been reported to be specific to the open state of the NMDA ion channel. *In vitro* affinities ($IC_{50}$) for the NMDARs of **19** and **20** (Fig. (**4**)) were 47 and 89 nM respectively in binding assays using [$^3$H]TCP on rat nerve cell membranes [58]. The radiosynthesis of [$^{18}$F]**19** and [$^{18}$F]**20** was achieved in two steps. The labelling of the thiophene moiety was first achieved in 70% RCY and was followed by a Diels-Alder reaction. Then, both isomers [$^{18}$F]**19** and [$^{18}$F]**20** (1/1 ratio) were obtained after HPLC separation in 20% RCY with a low specific radioactivity about 260 MBq/μmol [58]. These [$^{18}$F]-labelled quinolizinium exhibited rapid blood clearance, poor ability to penetrate the BBB and no specific accumulation in any brain tissues.

The [$^{11}$C]methoxy-substituted derivative [$^{11}$C]**21** was obtained with a poor specific radioactivity of about 63 MBq/μmol. Biodistribution of radioactivity in mice showed a high accumulation of [$^{11}$C]**21** in kidney, liver and heart but no brain penetration that could be explained by the cationic charge [58]. To avoid this problem, a prodrug of quinolizinium, the reduced derivative quinolizine [$^{11}$C]**22**, was developed. The radiolabelling was similar to the synthesis of [$^{11}$C]**21** [59]. Following intravenous administration in mice, this neutral form penetrated the brain where it was oxidized into the quinolizinium analogue [$^{11}$C]**21**. Although brain/blood ratio of radioactivity was close to 2 at 30 min,

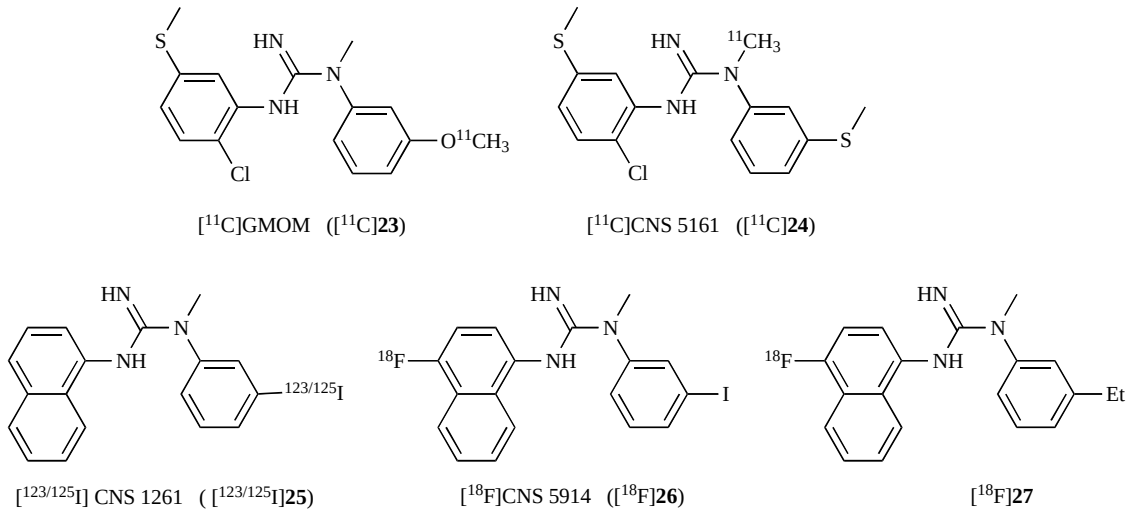



**Fig. (5).** Structures of radiolabelled *N*,*N*'-diaryl-*N*-methylguanidines**.**

only 0.5 % ID/g of radioactivity was still present in the brain at this time due to a rapid clearance. Nevertheless, the cerebral regional uptakes were about ten fold higher than those obtained after injection of [$^{11}$C]**21**, demonstrating the ability of [$^{11}$C]**22** to be a prodrug of [$^{11}$C]**21**. A pre-treatment with MK-801 or with **22** did not supply clear information on the specificity of the radioactive distribution.

### 2.4. Radiolabelled *N*,*N*'-diaryl-*N*-methyl Guanidines

Starting from structure–activity relationship studies on *N*-(2,5-disubstituted phenyl)-*N*'-(3-substituted phenyl)-*N*'-methylguanidines [60], Waterhouse *et al.* [61] have characterized **23** (GMOM**,** Fig. (**5**)) demonstrating a $K_i$ of 5.2 nM. [$^{11}$C]**23** was prepared by [$^{11}$C]methylation and the specific radioactivity was 44 GBq/µmol. In rats, the regional brain uptake of [$^{11}$C]**23** ranged from 0.75 ± 0.13 % ID/g in the medulla and pons to 1.15 ± 0.17 % ID/g in the occipital cortex. Pretreatment with antagonist MK-801 in awake rat did not change radioactivity distribution in brain while pre-administration of the glycine site co-agonist D-serine increased the accessibility of [$^{11}$C]**23** to the channel sites in all regions (10-24%). The NR2B subunit antagonist Ro-25-698 reduced the uptake of [$^{11}$C]**23** (24-68%). In baboon, a fairly uniform regional brain distribution of the tracer was not significantly altered by administration of MK-801 indicating a low degree of saturable binding [62]. In the same series, CNS 5161 (**24**) presenting a $K_i$ of 1.87 nM, was evaluated in clinical phase I trial [63, 64]. [$^{11}$C]Methylation on the desmethyl guanidine precursor led to a mixture of two *N*- and *N*'-[$^{11}$C]methylguanidines. In consequence [$^{11}$C]**24** was obtained with a low RCY and with a specific radioactivity of 41 GBq/µmol [65]. In humans, [$^{11}$C]**24** demonstrated differential uptakes in brain tissues with lowest uptake in cerebellum and highest in putamen and thalamus. The specific binding was not demonstrated and plasmatic metabolism was rapid [66, 67]. A naphtyl- iodophenyl-methylguanidine, CNS 1261 (**25**) was developed by Owens *et al.* [68]. In a [$^{3}$H]MK-801 binding assay using rat brain synaptic membranes, $K_i$ value was 4.2 nM (compared to 1.65 nM for MK-801) and complementary binding assays for 41 other receptors systems conferred to **25** selective properties for the MK-801 binding site on the NMDARs. *In vivo* evaluation of [$^{125}$I]**25** was promising with an hippocampal uptake 2.4 fold superior than in cerebellum, 2 h after administration to anesthetized rats. [$^{125}$I]**25** was metabolized rapidly in plasma (half-life = 2.2 min), the radioactivity detected 2 h post-injection in brain was due to authentic radioligand [68]. For administration in humans, [$^{123}$I]**25** was prepared with a high specific radioactivity up to 185 GBq/µmol [69]. A rigorous pharmacokinetic and pharmacological study permitted to define the total distribution volume ($V_T$)[2] in brain regions as the pertinent parameter to obtain reliable clinical data [70, 71]. Administered to healthy volunteers, [$^{123}$I]**25** gave higher $V_T$ values in the following regions: thalamus > striatum > cortical regions > white matter [70, 71]. The displacement of [$^{123}$I]**25** from the PCP site by ketamine confirmed the selectivity of the radiotracer for NMDARs [72]. [$^{123}$I]**25** demonstrated also a decline $V_T$ in case of treatment of schizophrenia using clozapine, an attenuator of NMDAR function [73]. A direct clinical application of this property was the exploration in SPECT of the relationship between ketamine-induced blockade of NMDAR and psychoactive effects of (S)-ketamine in 10 healthy human volunteers [74]. This study showed that ketamine might primarily induce negative symptoms through its direct inhibition of the NMDAR. The clinical use of [$^{123}$I]**25** in schizophrenia was reviewed since [75]. Recent preclinical works gave limits to the uses of [$^{123}$I]**25** and specified that: i) the optimal time point of post injection for the assessment of tracer distribution in rat brain was 2 h, ii) facilitation of NMDAR activation by D-serine did not result in an enhancement of binding of [$^{123}$I]**25**, iii) a high non specific binding limited the detection of small changes in receptor availability [69].

Our group developed [$^{18}$F]fluorinated derivatives of CNS 1261, [$^{18}$F]**26** and [$^{18}$F]**27**. The multistep radiolabelling occurred by nucleophilic substitution with fluoride-18 from the

[2]The total distribution volume ($V_T$) is defined as the ratio between the radiotracer concentration in the brain region of interest and the radioactive metabolite corrected concentration in arterial plasma [71]. Thus, the total volume of distribution of a brain region could be considered as proportional to density of receptors ($B_{max}$) multiplied by the affinity of the receptor for the ligand ($1/K_d$) and independent of regional blood flow.

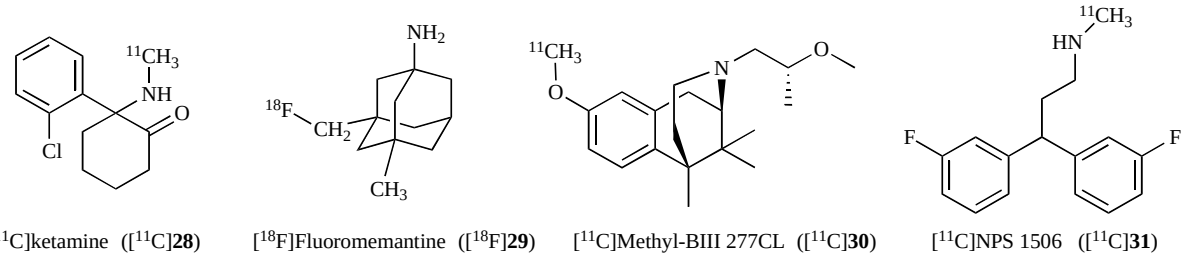



**Fig. (6).** Structure radiolabelled channel inhibitors.

4-nitro-1-trimethylammonium-naphthalene precursor followed by reduction of the nitro group. The last step was a coupling reaction with the corresponding arylcyanamide leading to the final compound [$^{18}$F]**26** or [$^{18}$F]**27**. The decay corrected RCYs were between 40 and 70% with a specific radioactivity of 75-370 GBq/µmol. *Ex vivo* autoradiographic studies on rat brain showed homogenous brain distribution and the development of these compounds was not pursued [76].

### 2.5. Miscellaneous Radiotracers

#### *[$^{11}$C]Ketamine*

Racemic ketamine is a short-acting parenteral agent used as PCP-like dissociative anaesthetic in human and veterinary medicine. Ketamine binds weakly but specifically the PCP-site and blocks the receptor in a non-competitive manner [77]. The affinity of the (*S*)-enantiomer occurs in the micromolar range ($K_i$ = 1.2 µM in guinea pig forebrain) and is about two to three fold higher than for the (*R*)-enantiomer. At anaesthetic doses, it also interacts weakly with opioid receptors especially the µ sub-type [78]. (±)-[$^{11}$C]Ketamine racemate ([$^{11}$C]**28**) (Fig. (**6**)) and both enantiomers were synthesized by [$^{11}$C]methylation with a specific radioactivity of 11-16 GBq/µmol [79, 80]. In mice, the brain uptake of (±)-[$^{11}$C]ketamine was high at 5 min (2.8% ID/g) then declined to 1.2 % ID/g at 60 min [80]. Preliminary PET studies in rhesus monkeys also revealed an appropriate BBB passage of (*R*)- and (*S*)-[$^{11}$C]**28**. The radioactivity in the primate brain was maximal 5-8 min after administration of the tracer (3% ID/g) and declined to about 1% ID/g with a terminal half-life of more than 60 min [79]. Most of the radioactivity was located in striatum, thalamus and cortical areas with a brain distribution quite similar for the two enantiomers of [$^{11}$C]**28** [79]. Metabolism study in baboon plasma indicated a rapid degradation of [$^{11}$C]**28** (remaining of 29 and 45% of unchanged [$^{11}$C]**28** for respectively (+)- or (-)-enantiomer at 10 min) [80]. Nevertheless, administration of (*S*)-[$^{11}$C]**28** in five healthy volunteers demonstrated a fast (maximal after 6 min) heterogeneous radioactive uptake into the brain, with a concentration 2.5 fold higher in thalamus than in white matter. The radioactivity declined rapidly to lead to a homogenous distribution in all brain regions after 20 min. Doses of (*S*)-ketamine injected together with the radiotracer resulted in a statistically significant and dose-dependent reduction of binding potential[3] (up to 20 %) in the regions of highest uptake i.e. thalamus, caudate putamen, temporal and parietal cortices [81]. In eight patients with medial temporal lobe epilepsy, Kumlien *et al.* [82] did not detect any (*S*)-[$^{11}$C]**28** binding potential changes *in vivo* compared to healthy volunteers. Considering the low contrast between regions of interest and reference regions, the rapid plasma metabolism, the high dissociation binding rate and the affinity for opiate receptors, [$^{11}$C]**28** was not considered a suitable tracer for studying NMDAR with PET.

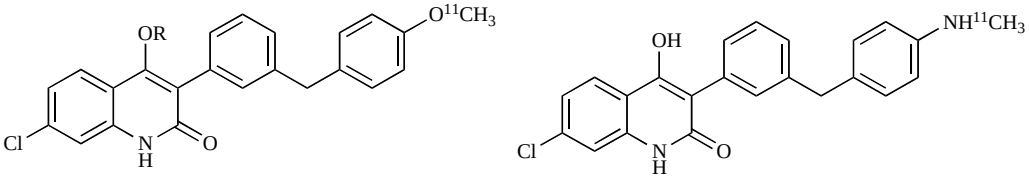
#### *[$^{18}$F]Fluoromemantine*

Memantine is actually known to be an uncompetitive NMDAR antagonist with a moderate affinity. It has been approved by numerous agencies for treatment of moderate-to-severe Alzheimer's disease. Its ready ability to penetrate the BBB and its poor metabolism in human [83] conferred to the memantine the characteristics of a candidate PET tracer. A fluorinated memantine analogue ([$^{18}$F]**29**) was synthesized (Fig. (**6**)) [84, 85]. In mouse, the brain uptake was high (up to 3.7 % ID/g, 30 min post injection) and the regional accumulation of radioactivity was consistent with the known distribution of the PCP sites. Specific binding was attested by the reduction of the activity concentration after co-injection of [$^{18}$F]**29** with MK-801 [85]. Unfortunately, in two different PET evaluations in rhesus monkey [85, 86], the regional brain distribution of [$^{18}$F]**29** was changed by memantine or MK-801 administration, but also by haloperidol, a dopamine $D_2$ and sigma receptor antagonist suggesting the existence of more than one recognition site. In human, [$^{18}$F]**29** demonstrated i) a considerable inter-subject variability, ii) a high perfusion dependent uptake and iii) a brain distribution that did not reflect the specific NMDAR compartment. [$^{18}$F]**29** was not considered as a suitable radioligand for the PET imaging of NMDARs [87]. Recent studies have demonstrated that memantine possesses affinities for 5-$HT_3$ and nicotinic acetylcholine receptors which could explain the lack of specificity of memantine [88].

#### *[$^{11}$C]Methyl-BIII 277CL*

BIII 277CL was described as a potent anticonvulsant and neuroprotective agent that binds selectively the PCP-binding site with a high affinity [89]. [$^{11}$C]Methyl-BIII 277CL ([$^{11}$C]**30**, Fig. (**6**)) was obtained by *O*-methylation of BIII 277CL with a specific radioactivity of 35 to 70 GBq/µmol. **30** displayed a 3-fold lower affinity (Ki=49 nM) than BIII 277CL in binding assays using [$^{3}$H]MK 801 in rat cortical

[3]The binding potential is defined as function of the interaction between endogenous ligand(s) and the neuroreceptors to which the radioligand binds (concentration of available receptor), corrected for the ratio(s) of the affinity constants (Kd) of all involved receptors towards their ligands. The binding potential is usually estimated using ratio of the distribution volume of the region of interest and the distribution volume of the reference region minus one.



R=H [$^{11}$C]L-703,717 ([$^{11}$C]**32**)
R=$COCH_3$ [$^{11}$C]AcL703 ([$^{11}$C]**33**)

[$^{11}$C]**34**

**Fig. (7).** [$^{11}$C]L-703,717 derivatives.

membrane. *In vitro* saturation experiments using [$^{11}$C]**30** demonstrated a high affinity ($K_D$=6 nM) but a weak $B_{max}/K_D$ ratio of approximately 10 ($B_{max}$=670 fmol/mg of protein). PET imaging in pig revealed for [$^{11}$C]**30** a fast uptake with similar time activity curves in cortex and cerebellum and a homogenous distribution in the whole brain [90].

### *[$^{11}$C]NPS 1506*

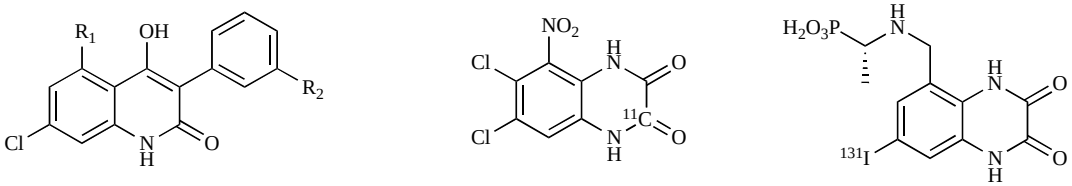
NPS 1506 (**31**, Fig. (**6**)) possesses neuroprotective properties in animal models of stroke and head injury without characteristic PCP-like side-effect in rodents. These effects were attributed to the weak affinity ($IC_{50}$=664 nM) for the PCP-site in [$^3$H]MK-801 binding assay. While NPS 1506 was under human clinical trials for the treatment of acute ischemic stroke, this compound was labelled using [$^{11}$C]methyl iodide with a specific radioactivity of 50 GBq/μmol [91]. The radioactive brain uptake after intravenous administration of [$^{11}$C]**31** in rodents was high (about 3.5 % ID/g) and increased slightly with time. However, the uptake was fairly uniform and inconsistent with the known localization of NMDARs. Co-injection of [$^{11}$C]**31** with NPS 1506 to rats did not cause any change in the hippocampus and striatum kinetics compared to the control group. Pretreatment with an activator of NMDAR (3-nitropropionic acid) gave unmodified data confirming high non-specific binding of [$^{11}$C]**31** [91].

## 3. GLYCINE SITE RADIOTRACERS

The glycine binding site is located on the extracellular part of the NR1 subunit. Glycine and/or D-serine act as co-agonists of the NMDAR and their presence is required to allow channel activation by glutamate or NMDA.

### 3.1. [$^{11}$C]Quinolone Derivatives

L-703,717 (**32**) is an antagonist of the NMDA glycine site ($IC_{50}$ = 4.5 nM in rat membranes displacement assay using [$^3$H]L-689,560). [$^{11}$C]**32** (Fig. (**7**)) was synthesized by *O*-[$^{11}$C]-methylation with a specific radioactivity of 47-53 GBq/μmol [92, 93]. *In vitro* binding of [$^{11}$C]**32** had been markedly located in hippocampus and cortex (twice more than in cerebellum) and significantly inhibited by glycine-site agonists (glycine and D-serine) or antagonist (MDL-105,519). The poor rat brain uptake of [$^{11}$C]**32** was attributed to a high fixation on the plasmatic albumin [94]. This hypothesis was confirmed by the inhibition of the serum albumin binding with warfarin pretreatment. Warfarin is an anticoagulant with structural similarity with **32**. Thus, the brain penetration of [$^{11}$C]**32** increased up to five-fold with co-injection of warfarin [95]. Although *in vivo* brain uptake of [$^{11}$C]**32** was inhibited by the co-injection of non radioactive L-703,717, the radioactivity concentration was one-half more in cerebellum than in hippocampus and cortex [95]. According to Haradahira's opinion, this unusual *in vivo* cerebellar localization of [$^{11}$C]**32** might be caused by preferential binding to NMDA NR2C subunits predominantly expressed in the cerebellum [96] and by regional variations in endogenous agonist concentrations [97]. A pro-drug, the 4-acetoxy derivative of [$^{11}$C]**32** ([$^{11}$C]**33**) was obtained by acetylation of previously radiolabelled [$^{11}$C]**32** with a specific radioactivity of 51-73 GBq/μmol [98]. The brain uptake of [$^{11}$C]**33** increased of two-fold in mouse, rat and monkey, compared to [$^{11}$C]**32**. After a 20 min incubation with rat brain homogenates, 80% of [$^{11}$C]**33** was hydrolyzed into [$^{11}$C]**32** [98]. [$^{11}$C]**33** was administered to six healthy volunteers and maximal whole brain uptake was 1.30 ± 0.16 %ID at 1.5 min after injection [99]. After 40 min, radioactivity in human brain reached a steady state with a region/white matter ratio of 2.32 for cerebellum, 1.78 for cerebral cortices and 1.35 for striatum. This regional distribution and the low brain uptake would be not suitable for the clinical use of [$^{11}$C]**33**. The replacement of the methoxy group by the *N*-methylamino ([$^{11}$C]**34,** $K_i$=11.7 nM) led to a decreased



$R_1$=H, $R_2$=$O^{11}CH_3$ [$^{11}$C]**35**
$R_1$=Et, $R_2$=$O^{11}CH_3$ [$^{11}$C]**36**
$R_1$=I, $R_2$=$O^{11}CH_3$ [$^{11}$C]**37**

$R_1$=Et, $R_2$=$NH^{11}CH_3$ [$^{11}$C]**38**

[$^{11}$C]ACEA 1021 ([$^{11}$C]**39**)

[$^{131}$I]I-PAMQX ([$^{131}$I]**40**)

**Fig. (8).** Radiolabelled glycine site ligands.

plasma protein binding but no BBB passage in mice [100]. A more extensive development of this series seems limited by: i) the low BBB penetration, ii) a high binding for plasma albumin, iii) a possible affinity for efflux transporter P- glycoprotein, iv) a specificity for glycine receptor altered by an affinity for glutamate receptor [99]. More recently, a series of quinolones with a substituent at the 5-position (Fig. (**8**)) was described [100-101]. [$^{11}$C]**35** and derivatives ([$^{11}$C]**36**, [$^{11}$C]**37,** [$^{11}$C]**38**) were labelled by the same procedure as for [$^{11}$C]L-703,717 with a specific radioactivity between 58 to 63 GBq/µmol. *In vitro*, the $K_i$ values for the binding of typical glycine antagonist [$^{3}$H]MDL-105,519 were 170, 7.2, 10.3 and 11.8 nM for **35**, **36**, **37** and **38** respectively. *In vitro* autoradiography showed the highest accumulation of [$^{11}$C]**36** in the hippocampus and the lowest in cerebellum whereas [$^{11}$C]**37** showed homogeneous accumulation throughout the brain. [$^{11}$C]**38** showed high specific binding in rat brain slices displaced by agonist or antagonist competition [100]. In mice, initial brain uptakes (cerebellum higher than cerebrum) of [$^{11}$C]**35**, [$^{11}$C]**36** and [$^{11}$C]**37** were similar and were only slightly enhanced by warfarin co-administration contrary to [$^{11}$C]**32**. BBB passage of [$^{11}$C]**38** was very low in mice. Pre-treatment with nonradioactive **35** did not significantly inhibit [$^{11}$C]**36** and [$^{11}$C]**37** binding [101]. Monkey PET studies with [$^{11}$C]**35** indicated that this tracer displayed higher localization in the cortex regions than in the cerebellum but also insufficient BBB permeability and high non-specific binding [102].

### 3.2. Radiolabelled 1,4-dihydroquinoxaline-2,3-diones

ACEA 1021 (**39,** Fig. (**8**)) is a potent NMDA antagonist (apparent dissociation constants $K_b$ = 6 nM) in electrophysiological assay with NMDA receptors in rat cortical neurons [103] with a selectivity for glycine-site/non-NMDA receptors about 250 [104]. Sufficient amounts of [$^{11}$C]**39** for preliminary animal screening studies were obtained by a multistep synthesis in 70-80 min from [$^{11}$C]$CN^-$ leading to the [$^{11}$C]ACEA 1021 in a low RCY and a low specific radioactivity of 15-20 GBq/µmol [105]. No biological evaluation was reported.

A iodinated derivative (**40**) of the PAMQX has demonstrated also a high binding affinity ($IC_{50}$ = 8 nM in [$^{3}$H]MDL-105519 binding assay). [$^{131}$I]**40** was prepared with a high specific radioactivity of 54 MBq/µmol by iododestannylation followed by acidic deprotections. The radioactivity uptake in mouse brain was low, decreasing from 0.03 % ID at 0.5 h to 0.01 % ID at 2.5 h. These unfavourable findings were related to the high polarity due to the phosphonic acid function [106].

### 3.3. Radiolabelled Substituted Dichloroindole Carboxylic Acids and Analogues

[$^{11}$C]GV150526A ([$^{11}$C]**41**, Fig. (**9**)), as a potent and selective inhibitor of the glycine site receptor ($K_i$ = 1 nM in [$^{3}$H]glycine binding assay) was prepared from [$^{11}$C]iodomethane according to a complex five steps synthesis achieved in 60 min with a very poor specific radioactivity of 26-30 GBq/µmol [107]. No biological evaluation was subsequently published. Waterhouse *et al*. [108] prepared methoxyphenyl derivatives of **41** and the meta-methoxy compound (3MPICA, **42**) retained an *in vitro* affinity ($K_i$ = 4.8 ± 0.9 nM) in [$^{3}$H]MDL-105,519 binding assay with rat cortical membranes. Radiolabelling was achieved by *O*-[$^{11}$C]-methylation with a specific radioactivity about 81 GBq/µmol. Biodistribution of [$^{11}$C]**42** in rat revealed a high blood concentration and a low brain penetration that was not significantly increased by co-administration with warfarin. The regional uptake at 2 min was highest in the cerebellum (0.19 % ID/g) and lower in hippocampus (0.13 % ID/g) and frontal cortex (0.11 % ID/g). A blocking experiment with unlabelled 3MPICA did not demonstrate evident saturable binding of [$^{11}$C]**42** in the rodent brain at 60 min [108]. Three analogues ([$^{76}$Br]**43**, [$^{18}$F]**44),** ([$^{123}$I]**45)** with α-amino carboxylic acid function and dihalogenated benzyl structure were synthesized. A two step synthesis was performed to

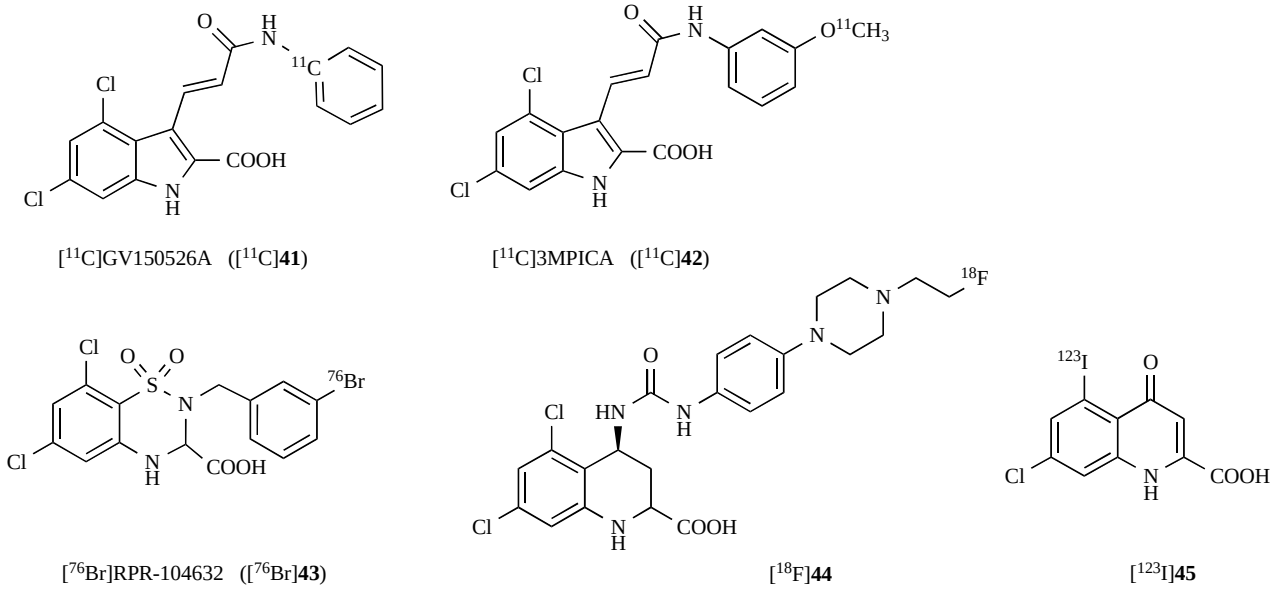



**Fig. (9).** Carboxylic acid containing glycine inhibitors.

obtain [$^{76}$Br]RPR 104632 ([$^{76}$Br]**43**) using the condensation of 3-[$^{76}$Br]bromobenzylbromide with the adequate benzothiadiazine-1,1-dioxide-3-carboxylic acid. Brain uptake of [$^{76}$Br]**43** was homogenous and low (< 0.1% ID/g at 20 min post injection) [109]. The synthesis of the radioligand [$^{18}$F]**44** was carried out by coupling of the 2- [$^{18}$F]fluoroethyltosylate with the piperazinyl precursor [110]. The specific radioactivity obtained was between 50 and 75 GBq/µmol. The high affinity of the ligand **44** for the glycine binding site ($K_i$ =12 nM) was initially determined in [$^3$H]MDL-105,519 binding assays. The biodistribution kinetics in rats showed an ubiquitous low amount of [$^{18}$F]**44** accumulation in all brain structures [110]. Compound [$^{123}$I]**45** was synthesized by halogen-exchange with a specific radioactivity of 37 GBq/µmol. The radioactivity reached a level of 0.13% ID/g in mice brain 20 min after i.v. injection of [$^{123}$I]**45**. The low passage into the brain was explained by the rapid deiodination (85% at 5 min) of the radiotracer and the high polarity of the carboxylic acid function [111].

## 4. NR2B SITE RADIOTRACERS

The clinical usefulness of the first generation of non selective NMDA channel-blockers was limited by severe side effects. The second generation of drugs was NR1/NR2B selective antagonists such as ifenprodil and derivatives, CP-101,606 or Ro-25-6981. These compounds displayed improved neuroprotective potential with reduced side-effects [112]. Ifenprodil (**46**, Fig. (**10**)) was the first NR2B antagonist described and was consequently used as a model for pharmacomodulation studies. Polyamines binding sites are specifically located on the NR2B subunit.

### 4.1. Carbon-11 Labelled Ifenprodil-Like Antagonists

The compound **47** that displayed high selectivity properties ($IC_{50}$ NR2B = 5.3 nM; NR2A= 35 µM; NR2C > 1 mM) was considered as a good candidate for NR2B imaging. Radiosynthesis of [$^{11}$C]**47** was achieved by a cyclization reaction using [$^{11}$C]phosgene with a specific radioactivity of 37-75 GBq/µmol [113]. *In vivo* evaluation of [$^{11}$C]**47** in rat gave the following results: low and rather uniform uptake in the whole brain (0.07 % ID /mL), the cerebellum having the highest uptake (0.1% ID /mL) and striata the lowest (0.04% ID /mL).

This *in vivo* brain distribution did not match with the known distribution of the NR2B subtype. A high affinity ligand of the NR2B subunit ($IC_{50}$= 3.9 nM), EMD-95885 (**48**), was labelled by cyclization of the benzoxazolone ring with [$^{11}$C]phosgene. The specific radioactivity obtained was of 37-75 GBq/µmol [114]. The rat brain uptake of [$^{11}$C]**48** was uniform (0.4-0.6 % ID/mL) among the different selected brain structures. The ratio of the hippocampus/cerebellum close to 0.8 supported the lack of specificity. Competition studies with **46** and **48** and also with sigma ligand (DTG) or $D_2$/sigma antagonist (haloperidol) displayed reduction of the cerebral concentration of [$^{11}$C]**48** (40-60%). Unfortunately, these inhibitions were homogeneous and did not reflect any specific binding to receptors. Glycine site antagonist (MDL-105519) and ion channel blocker (MK-801) had no displacement effect. **49** was described as a potent ($IC_{50}$=5 nM in [$^3$H]Ro-25,6981 binding assay) and selective NR2B antagonist. [$^{11}$C]**49** was developed in our group with a specific radioactivity ranged from 75 to 125 GBq/µmol [115]. µPET studies in rats displayed an absence of BBB passage of [$^{11}$C]**49**. A carbon-11 labelled methoxy analogue of CP-101,606 ([$^{11}$C]**50**) was prepared with a specific radioactivity of 55-85 GBq/µmol [116]. *In vitro* study using [$^{11}$C]**50** ($IC_{50}$ = 14 nM) showed a high specific localization in the forebrain regions and a non specific binding inferior to 5%. In contrast, the *in vivo* binding in mouse showed i) a moderate brain uptake (0.46 to 1.09 %ID/g); ii) no apparent specific localization of the radioactivity in any brain regions, iii) a target/non target ratio less than 1.2; iv) an absence of competitive effect by pre-administration of **50**. Monkey PET images led to similar conclusions [116].

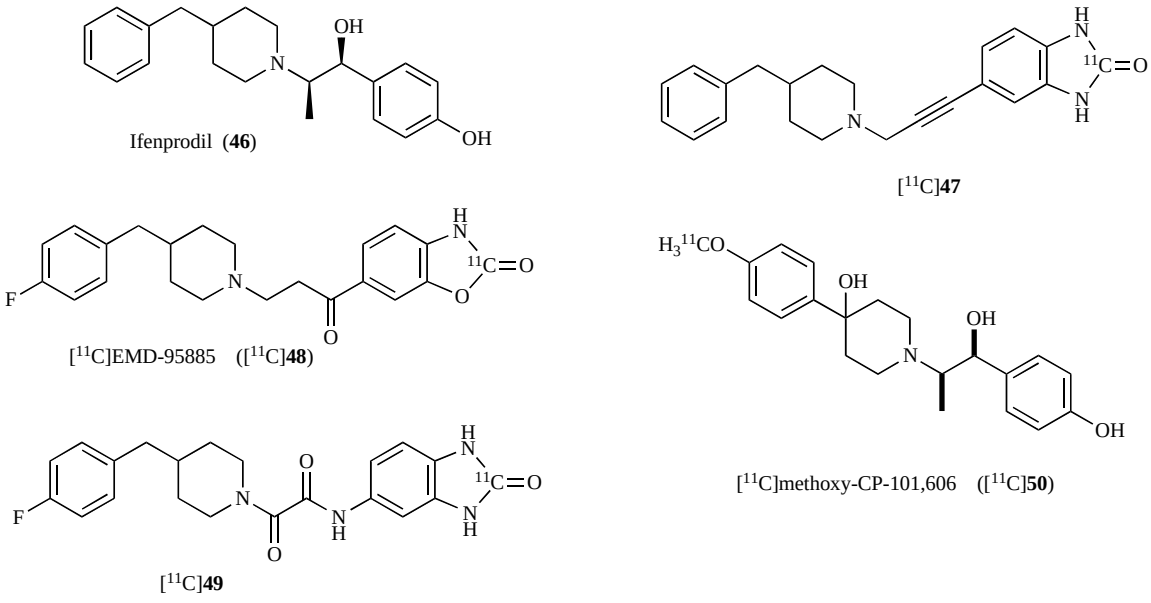



**Fig. (10).** Ifenprodil and radiolabelled derivatives.

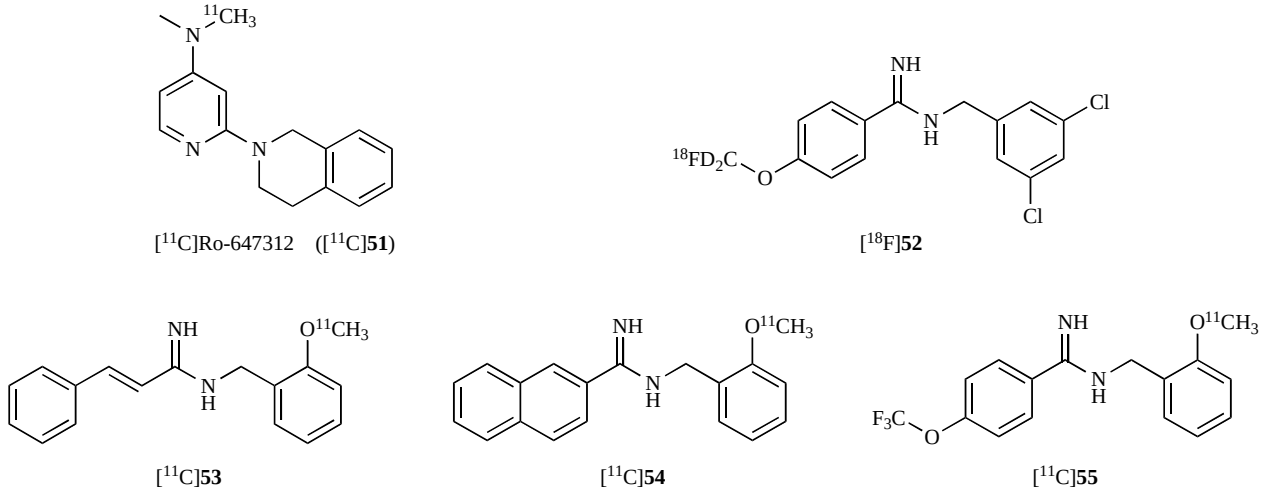



**Fig. (11).** Radiolabelled amidine derivatives.

### 4.2. [$^{11}$C]Ro-647312

The pyridine [$^{11}$C]Ro-647312 ([$^{11}$C]**51,** Fig. (**11**)) that belongs to an another class of NR2B subtype NMDA antagonists (Ki = 8 nM vs [$^{3}$H]Ro-256981), was radiolabelled with [$^{11}$C]methyl triflate. The specific radioactivity ranged from 37 to 130 GBq/µmol. The *in vivo* regional brain distribution of [$^{11}$C]**51** did not correlate with the known distribution of NR2B subunits and the radioactive uptake ratio in hippocampus versus cerebellum was between 1 and 0.7 throughout the time course of experiment [117].

### 4.3. Radiolabelled Amidines

A series of benzamidines (Fig. (**11**)) was reported as potent NR2B antagonists [118]. One of these antagonists (*N*-(3,5-dichloro)benzyl-4-([$^{18}$F]fluoromethoxy-$d_2$)benzamidine, [$^{18}$F]**52**) demonstrated a $K_i$ of 1.5 nM and was labelled with fluorine-18 from the deuterated [$^{18}$F]fluorobromomethane-$d_2$. The presence of deuterium might prevent the *in vivo* defluorination of the fluoromethyl group [119]. The specific radioactivity was 107 GBq/µmol and until now, no biological evaluation was published. Compound [$^{11}$C]**53** ($K_i$ = 0.7 nM) was prepared by *O*-[$^{11}$C]-methylation with a specific radioactivity ranged from 30 to 45 GBq/µmol [120]. Simultaneously, Årtsad *et al.* [121] published the synthesis and evaluation of [$^{11}$C]**53** and also of two other ligands of this series i.e. [$^{11}$C]**54** ($K_i$= 1.3 nM) and [$^{11}$C]**55** ($K_i$= 5.7 nM) (specific radioactivity > 74 GBq/µmol for the three compounds).

Autoradiography of rat brain slices showed *in vitro* specific binding of [$^{11}$C]**55** about 75% and a comparable binding pattern of [$^{11}$C]**55** to that obtained with [$^{3}$H]ifenprodil with some discrepancies like higher binding in caudate putamen and thalamus or lower binding in cerebellum.The initial *in vivo* brain uptake of [$^{11}$C]**53** and [$^{11}$C]**54** was moderate (1% ID/g at 5 min in rat) and was superior for [$^{11}$C]**55** (1.8% ID/g). [$^{11}$C]**53** and [$^{11}$C]**54** were rapidly metabolized **(**31-37% of intact radiotracer at 5 min) while intact [$^{11}$C]**55** represented 91% at 5 min and 49% at 40 min. Metabolites were substantially present in the brain. Although favourable uptake and *in vitro* specific binding in accordance with the distribution of NR2B-containing NMDAR were found, the use of [$^{11}$C]**55** for PET imaging was limited due to a rapid metabolism.

### 4.4. [$^{11}$C]Bis(phenyl)alkylamines

The bis(phenylalkyl)amines have been shown to bind the glycine-independent polyamine modulatory site, the NR2B subunit controlling the stimulatory effect [122]. The bis(phenylpropyl)amine [$^{11}$C]**56** and bis(phenylbutyl)amine [$^{11}$C]**57** (Fig. (**12**)) were labelled by a *O*-[$^{11}$C]methylation of the corresponding diphenol precursors (specific radioactivity around 75 GBq/µmol). *In vitro* binding in rat brain slices gave a ratio between regions of interest versus cerebellum, almost similar for all brain regions tested for the two radioligands. The non specific bindings in the presence of 100 µM of the non radioactive ligands were between 50 to 60% of the total binding. [$^{11}$C]**56** contrary to [$^{11}$C]**57** demonstrated a high binding for serotonin receptors. [$^{11}$C]**57** was chosen for further *in vivo* evaluation because of its greater selectivity for the spermine- and ifenprodil-binding sites. After injection of [$^{11}$C]**57** to mice, the brain uptake was moderate (0.8-0.9% ID/g) and the brain regional distribution was similar to the *in vitro* brain distribution. The pre-treatment with spermine or with **57** caused a significant increase of radioactivity in cortex and hippocampus suggesting that the uptake was non specific [123]. In a conscious rhesus monkey, PET scans demonstrated no significant regional differences in radioactivity uptake but a slight decrease after co-injection with **52** indicated the presence of a small fraction of displaceable binding [123].

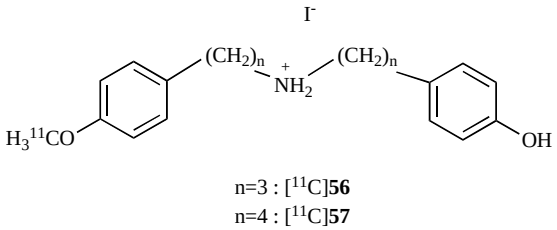



**Fig. (12).** [$^{11}$C]bis(phenylalkyl)amines.

## CONCLUSION

Since 1990, about sixty molecules were successfully radiolabelled for *in vivo* imaging the NMDAR system. The majority of them displayed the common main handicaps encountered during *in vivo* evaluation of radioligands: poor brain penetration, extensive metabolism, high non specific and/or poor specific bindings, homogeneous brain distribution and/or distribution inconsistent with the known distribution of NMDARs.

Many radiotracers for the labelling of intrachannel site inhibitors demonstrated promising *in vitro* properties (high selectivity and affinity). Unfortunately, *in vivo* experiments were not encouraging. Indeed, numerous brain penetrating radiotracers displayed a homogenous distribution in the whole brain with no significant uptake in the regions rich in NMDARs and no clear density change after competitive blockage of the channel receptor. These poor results could be related to classical causes: i) the highly lipophilic or hydrophilic nature of some ligands, ii) undesirable non-specific interactions with brain components, iii) and a rapid metabolism. The disprenancy between *in vitro* and *in vivo* pharmacological results was extensively discussed by Waterhouse [32]. Nevertheless, among the seven radiotracers tested in humans, six were channel blockers: [$^{11}$C]ketamine, [$^{18}$F]fluoromemantine, [$^{123}$I]iodo-MK-801, [$^{11}$C ]CNS 5161, [$^{123}$I]CNS 1261 and [$^{11}$C]L-703,717. Until now, the [$^{123}$I]CNS 1261 is the sole radiotracer used for clinical studies on patients affected by schizophrenia [75]. A recent article based on pre-clinical data concluded that [$^{123}$I]CNS 1261 would not allow the quantification of small changes in NMDAR distribution [69]. Excepted for a few examples [124], no major efforts in the development of channel-blockers have been made since this first generation of drugs presented important side-effects. The *N*,*N*'-diaryl-*N*-methylguanidine series development should be continued to obtain imaging agents with improved pharmacological properties. But the access to the channel pore binding site needs receptor activation to be in the open state [32] and competition experiments are quite difficult to analyze. Moreover, the channel maximal open channel probability differs between NR2 subunits with a 50-fold range [125]. This kind of tracer would image the activated receptors and thus study the activation state differences between pathological versus control conditions.

Although the labelling of several compounds with promising *in vitro* properties has been effective, no ligand was successfully retained for imaging the glycine-site of the NMDAR. The main blocking issues were: i) a poor brain penetration due to high polarity of molecules (acidic function) ii) some discrepancies between *in vitro* and *in vivo* bindings, iii) an unexpected higher uptake in cerebellum than in area of interest, iv) a high non specific binding, v) some difficulties to interpret the inhibition experiments. Although [$^{11}$C]AcL-703 was an efficacious prodrug in humans for the brain penetration and delivery of [$^{11}$C]L-703,717, the clinical data were disappointing. The imaging of the glycine binding site could be difficult due to the presence of large amount of endogenous agonist as glycine or D-serine.

The outcomes of the development of radiotracers for imaging the NR1/NR2B subunit containing MNDAR are limited in spite of very promising *in vitro* specific binding on rat brain slices for several radioligands. Biological applications clearly indicated that the most part did not have the required properties for imaging NMDAR using PET. The majority of them displayed a low BBB penetration, a non specific distribution in the whole brain or an extensive metabolism. However, potential interaction of NR2B NMDARs with anaesthetics could be an explanation of the disappointing preclinical pharmacological evaluations of these radiotracers [126, 127]. The only expectation comes from the series of amidines in which one compound was promising in term of brain uptake and *in vitro* specificity. A further optimization of this series might lead to successful PET tracers if the metabolic stability could be achieved by pharmacomodulation.

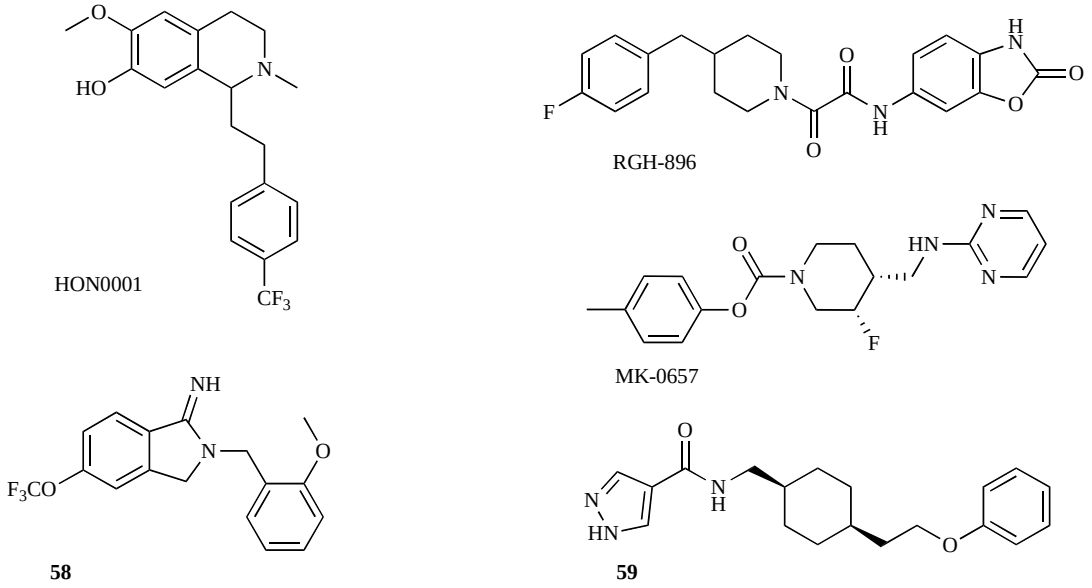



**Fig. (13).** Examples of new NR2B ligands with promising *in vivo* pharmacological properties.

The NMDAR system remains of very high therapeutic interest by its potential applications: stoke, brain injury, neurodegenerative diseases, schizophrenia, epilepsy or chronic pain. A lot of clinical trials are still under current investigation but essentially using first generation molecules as memantine, ketamine or amantadine [1]. Those drugs have been labelled and evaluated as radiotracers for nuclear medicine imaging without success as we showed in this review.

For several years, the major effort in drug development has focused on the NR2B subunit containing NMDAR [26, 128-130] since *in vitro* highly selective ligands for the other NR2 subunits are still lacking. Medicinal chemistry research led recently to new NR2B antagonists with low nanomolar affinities ($K_i$ <5 nM) and no binding to the others NMDA subunits and to the hERG receptors contrary to the early NR2B antagonists (Fig. (**13**)). For example, RGH-896 presented *in vitro* a minimal 100-fold affinity ratio towards 149 other receptor types and no cardiac effects due to potential hERG activation [131]. An isoinolin-1-imine (**58**) described by Nguyen *et al*. showed a good oral efficacy in a rat hyperalgesia model [132]. In a series of arylamides, Kawai *et al*. realized a structure-activity relationship study to avoid reactive metabolites formation leading to a new pyrazolecarboxiamide compound (**59**) with high bioavailability in rats (43%) and a high metabolic stability (44 min) [133]. HON0001 demonstrated a weaker *in vitro* potency but a good brain penetration with a brain/plasma ratio of 34 at 1 hour post-administration in rats [134]. The MK-0657 comes from a series presenting bioavailability of 45% in rats and 26% in rhesus monkeys and a brain/plasma ratio of 1.4 [135]. Those compounds presented an orally pharmacological efficacious dose ($ED_{50}$) usually less than 10 mg/kg in diverse animal models. Among these recent NR2B antagonists, the MK-0657 and RGH-896 are under current clinical evaluation [136, 137]. The radiolabelling of those new promising series of antagonists would provide new pharmacological tools for PET and SPECT imaging to study *in vivo* the NMDARs and particularly the NR2B subunit.

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