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Research Article

Synthesis and evaluation of 5,7-dichloro-4-(3-{4-[4-(2-[^{18}F]fluoroethyl)-piperazin-1-yl]-phenyl}-ureido)-1,2,3,4-tetrahydroquinoline-2-carboxylic acid as a potential NMDA ligand to study glutamatergic neurotransmission in vivo

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Summary

The neurotransmitter glutamate is thought to be crucially involved in a huge number of neurological and psychiatric disorders, such as Morbus Parkinson, Alzheimer's disease and schizophrenia. Aiming at an improved diagnostic tool for PET a new [^{18}F]fluorine labelled NMDA receptor ligand was developed that may potentially allow the *in vivo* visualization of glutamatergic neurotransmission. The ^{19}F-analogue *trans*-5,7-dichloro-4-(3-{4-[4-(2-fluoroethyl)-piperazin-1-yl]-phenyl}-ureido)-1,2,3,4-tetrahydro quinoline-2-carboxylic acid was synthesised to determine the binding affinity, lipophilicity and biodistribution of the ligand. This substance exhibits a K_i of 12 nM for the glycine binding site using [^{3}H]MDL-105,519 assays on pig cortical membranes. A logD of 1.3 was determined for this compound according to the OECD

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guidelines employing the HPLC method. Radiosynthesis of this ligand was achieved by labelling the precursor *trans*-5,7-dichloro-4-[3-(4-piperazin-1-yl-phenyl)-ureido]-1,2,3,4-tetrahydroquinoline-2-carboxylic acid methyl ester with 2-[^{18}F]fluoroethyltosylate and subsequent cleaving of the methyl ester moiety, resulting in an overall decay corrected yield of 35% of the final product *trans*-5,7-dichloro-4-(3-{4-[4-(2-[^{18}F]fluoroethyl)-piperazin-1-yl]-phenyl}-ureido)-1,2,3,4-tetrahydroquinoline-2-carboxylic acid. The biodistribution kinetics of this compound were determined with Sprague Dawley rats *ex vivo* for brain, liver, kidney, and bone. The ligand showed a maximum brain uptake 30 min.p.i. of about 0.1% ID/g.

Key Words: Glutamate; NMDA receptor; Glycine binding site; ^{18}F-fluoroalkylation

Introduction

Glutamate is the most abundant excitatory amino acid (EAA) in the central nervous system of mammals and plays an important role in neurodevelopment, synaptic plasticity and neurotoxicity.[1,2] Glutamate is recognized by metabotropic and ionotropic receptors, the latter ones subdivided into the three subtypes named after their selective agonists: *N*-methyl-*D*-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) and kainate.

NMDA receptors have been extensively characterised since their discovery in 1977.[3] They represent an important target for medicinal chemistry due to their involvement in Morbus Parkinson, Alzheimer's disease and schizophrenia.[4] Like other ligand-gated ion channels they are proposed to have a heteropentameric structure, though their transmembrane regions are at variance with e.g. $GABA_A$ and nicotinic acetylcholine receptors. They posses a variety of binding sites both in the extracellular domains of the receptor for glycine, glutamate and polyamines, and in the ion channel itself for Zn^{2+}, Mg^{2+} and the use-dependent psychotropic drug MK 801.[5]

Glycine has been defined as a co-agonist of NMDA receptors. Thus for the synthesis of new ligands the glycine binding site is an important target, especially as these ligands have less side effects compared to ligands of the glutamate and ion channel binding site. Therefore several studies have dealt with positron emitter labelled ligands for the *in vivo* visualisation of the NMDA receptor using PET.[6–8]

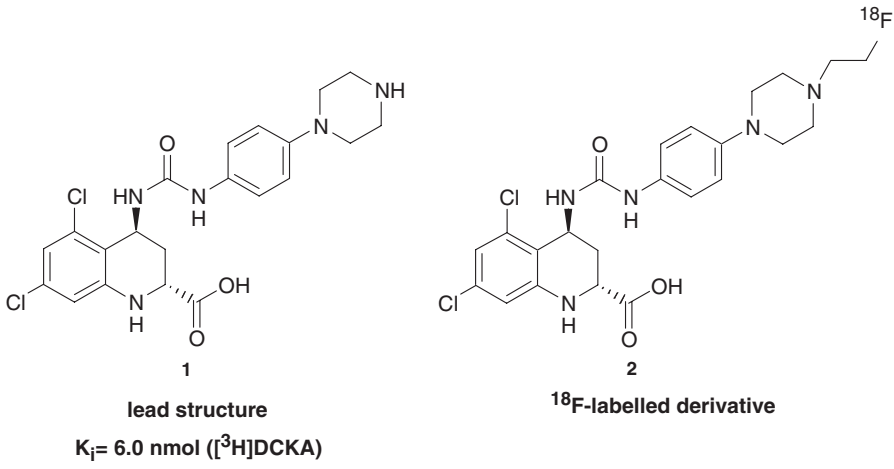


Figure 1. Lead structure 1 and the fluoroethylated derivative *trans*-5,7-dichloro-4-(3-{4-[4-(2-[^{18}F]fluoroethyl)-piperazin-1-yl]-phenyl}-ureido)-1,2,3,4-tetrahydroquinoline-2-carboxylic acid (2)

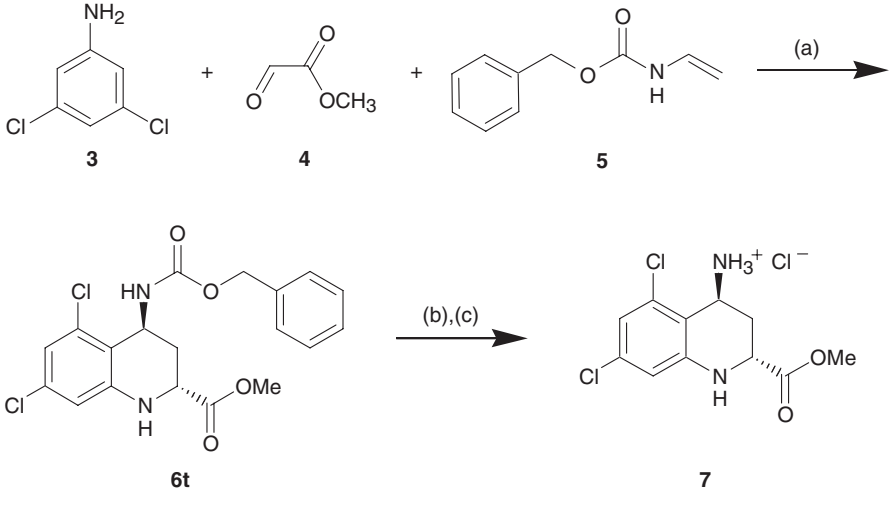
Since the discovery of kynurenic acid as an antagonist of the glycine binding site its derivatives were optimised for improved affinity and selectivity resulting in the development of the *trans*-5,7-dichloro 4-substituted 2-carboxytetrahydroquinolines, providing affinities in the nanomolar range.[9] The [^{18}F]fluorine labelled derivative *trans*-5,7-dichloro-4-(3-{4-[4-(2-[^{18}F]fluoroethyl)-piperazin-1-yl]-phenyl}-ureido)-1,2,3,4-tetrahydroquinoline-2-carboxylic acid (**2**) of the lead structure **1** was considered for *in vivo* visualisation of the NMDA receptor. In the following figures and schemes the 2R,4S-configuration represents the *trans*-isomers, whilst the 2S,4S-configuration represents the *cis*-isomers (Figure 1).

Results and discussion

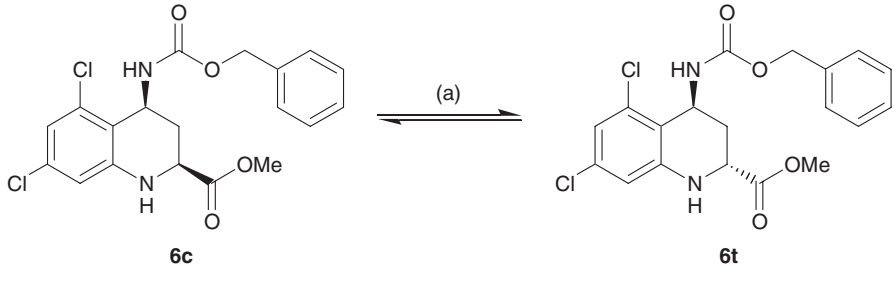
Chemistry

The aim of this study was the labelling of *trans*-5,7-dichloro-4-[3-(4-piperazin-1-yl-phenyl)-ureido]-1,2,3,4-tetrahydroquinoline-2-carboxylic acid with 2-[^{18}F]fluoroethyltosylate. In a first step the ^{19}F-analogue of this molecule was synthesised to evaluate the *in vitro* affinity and lipophilicity of the ligand.

The treatment of acryloyl acid chloride with sodium azide afforded the appropriate acid azide which was rearranged according to Curtius at 120°C. The forming vinyl isocyanate was converted *in situ* into the appropriate benzyl vinylcarbamate **5** by addition of benzyl alcohol and purified by distillation. In a further step 3,5-dichloro aniline (**3**) was converted with methyl glyoxylate to the Schiff base and afterwards *in situ* with the benzyl vinylcarbamate to form compounds **6c** and **6t**. Because this cyclization did not favour one stereochemical route, the synthesised diastereomers were separated by means of column chromatography. The *cis*-isomers however, can be converted into the *trans*-isomers using catalytic quantities of sodium methylate (Scheme 2). After removing the benzyloxycarbonyl protecting group using iodotrimethyl silane, compound **7** was separated as hydrochloride (Scheme 1 and 2).

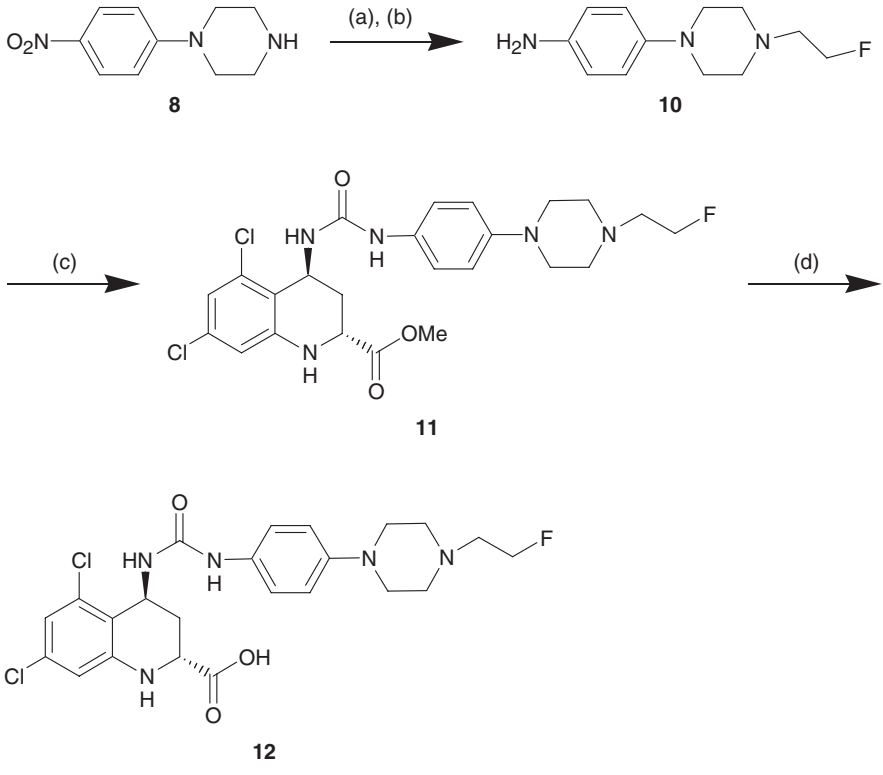


Scheme 1. (a) sodium sulfate, boron trifluoride diethyl ether complex (b) iodotrimethyl silane, methanol (c) HCl, ethyl acetate



Scheme 2. (a) potassium methylate, methanol

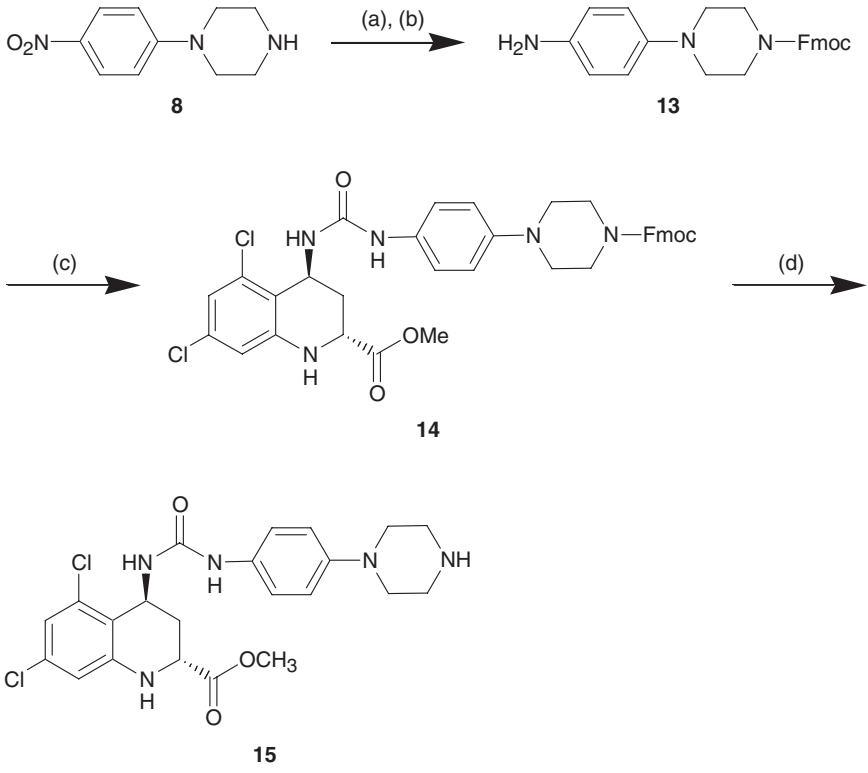
The synthesis of **12** could be achieved by carrying out the following reaction steps: 1-(4-nitrophenyl)-piperazine (**8**) was first reacted with 1-bromo-2-fluoroethane and subsequently reduced with H_2/Pd to obtain compound **10**. This compound was then coupled with **7** using triphosgene to yield compound **11**. After cleavage of the ester moiety with 1 N LiOH compound **12** was obtained (Scheme 3).



Scheme 3. (a) 1-bromo-2-fluoroethane, [1,4]dioxane (b) H_2/palladium, methanol (c) triphosgene, dichloro methane, DIEA, 7 (d) THF, LiOH (1 N)

The precursor **15** was synthesised by reacting FmocCl and **8**, followed by the reduction of the nitro moiety using H_2/Pd to obtain **13**. After coupling this compound with **7** using triphosgene, the Fmoc moiety was removed with piperidine to yield the desired precursor **15** (Scheme 4).

The radiolabelling with 2-[^{18}F]fluoroethyltosylate was performed in DMSO at a temperature of 140°C within 25 min yielding the desired product **16** in 55% radiochemical yield (referring to the starting activity

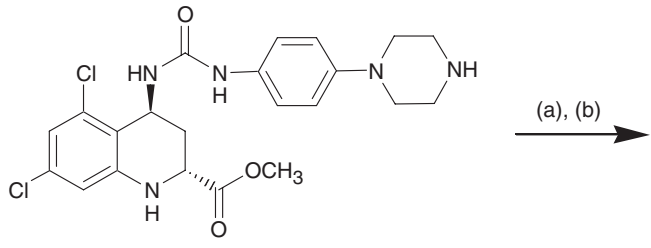


Scheme 4. (a) 9-fluorenylmethyl chloroformate, [1,4]dioxane, $NaHCO_3$ (10%) (b) H_2/palladium, methanol (c) triphosgene, dichloro methane, DIEA, 7 (d) piperidine, THF

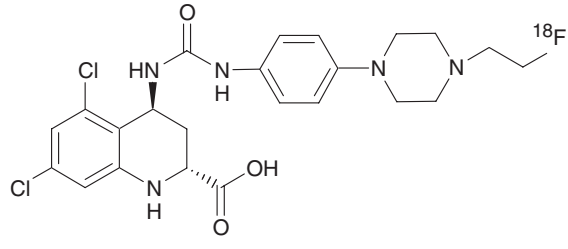
of 2-[^{18}F]fluoroethyltosylate). Purification of the crude product was accomplished by HPLC. Subsequent quantitative cleavage of the remaining methyl ester moiety within 5 min gave the final product **2** in an overall radiochemical yield of 35%. The total preparation time until a solution for the biodistribution studies was obtained was approximately 110 min (Scheme 5).

Pharmacology

Preliminary pharmacological experiments were performed with compound **12**. The lipophilicity of the molecule was determined using the HPLC method[10] and Sörensen buffer as eluent, resulting in a logD of 1.3. In [H^3]MDL-105,519 binding assays[11] the *in vitro* affinity was examined, which showed a high affinity of the ligand to the glycine



15



2

Scheme 5. Radioactive labelling of 13 with a) 2-[^{18}F]fluoroethyltosylate, DMSO and subsequent ester hydrolysis to 1 with b) LiOH (1.25 N)

binding site with a K_i of 12 nM, favourably comparing with the K_i of 6 nM of the parental compound.

The *in vitro* stability of **2** was measured 8 h after the radiosynthesis with radio TLC and showed 97% intact radioligand. The maximum brain uptake of *trans*-5,7-dichloro-4-(3-{4-[4-(2-[^{18}F]fluoroethyl)-piperazin-1-yl]-phenyl}-ureido)-1,2,3,4-tetrahydroquinoline-2-carboxylic acid of about 0.1% ID/g was reached after 30 min p.i. (Table 1). Excretion was primarily via the urine, while kidney and liver showed an uptake of up to 8% ID/g and 3% ID/g within 60 min.The low ^{18}F-activity in the bone, with a maximum of 2% ID/g, indicates a small metabolic hydrolysis of the tracer under release of ^{18}F-fluoride.

In a preliminary autoradiographic study the biodistribution of the radioligand, using sagittal brain sections (including cerebellum and hippocampus) after 30 min past application, was evaluated. According to previous studies,[12] the NMDA receptor density in cortex and hippocampus is very high, whilst it is very low in the cerebellum. Due to an ubiquitous low amount of accumulation of the ligand in all brain structures this distribution could not be detected and therefore no blocking experiments were undertaken.

Table 1. Organ uptake of 2 measured *ex vivo* in Sprague Dawley rats ($n = 3$) at 5, 15, 30 and 60 minutes p.i

Tissue	^{18}F-activity [%ID/g]			
	5 min p.i.	15 min p.i.	30 min p.i.	60 min p.i.
Brain	0.07 ± 0.01	0.07 ± 0.01	0.09 ± 0.02	0.03 ± 0.01
Liver	2.69 ± 0.99	0.92 ± 0.09	0.83 ± 0.65	1.12 ± 0.99
Kidney	7.99 ± 2.01	1.02 ± 0.04	0.79 ± 0.63	0.73 ± 0.71
Bone	2.04 ± 1.43	0.74 ± 0.01	0.80 ± 0.74	0.45 ± 0.38
Blood	0.37 ± 0.10	0.08 ± 0.01	0.07 ± 0.03	0.08 ± 0.04

Materials and methods

General

All reagents were purchased from commercial sources and were used without further purification. Methyl glyoxylate (**4**) and benzyl vinylcarbamate (**5**) were prepared according to the literature.[13,14] Solid phase columns were purchased from Merck (Lichrolut EN). Analytical thin layer chromatography (TLC) was performed using plates from Merck (Silicagel 60 F_{254}). High performance liquid chromatography (HPLC) was performed with an HPLC system from Sycam S1100, UV detection was obtained using a UV detector from Sycam S3200. NMR spectra were recorded using a Bruker 200-MHz-FT-NMR spectrometer AC 200. Chemical shifts are quoted in δ (ppm) downfield from tetramethylsilane (TMS) as an internal standard. MS spectra were obtained on a Finnigan MAT90 spectrometer. Detection of radioactivity was performed using an instant imager (Packard Canberra) for radio-TLC and detection of radio-HPLC was performed using a NaI-radiodetector (Canberra Packard). [^{18}F]Fluoride, produced via the ^{18}O(p,n)^{18}F reaction, was purchased. Purification of the labelling precursor 2-[^{18}F]fluoroethyltosylate and of the labelled compound **16** was accomplished using a HPLC column (Lichrospher RP18-EC5, 250 × 10 mm), while for the HPLC analysis of **2** a Lichrospher RP18-EC5, 250 × 4 mm HPLC column was used.

Pharmacological procedures

The logD value of **12** was examined according to the OECD-guideline for testing chemicals using the high performance liquid chromatography (HPLC) method.[10] Determination of the *in vitro* binding affinity was

performed using [^{3}H]MDL-105,519 binding assay according to the method of a previous study.[11]

The animal experiments were carried out in compliance with the German legal regulation of animal experimentation. About 12 MBq of the labelled compound **2** were injected intravenously in the tail vein of 12 Sprague-Dawley rats (220-250 g). After 5, 15, 30 and 60 min in each case 3 rats were anaesthetised using fluothane (Zeneca), and sacrificed. Brain, liver, kidney and bones were removed and the biodistribution was measured. Detection of the radioactivity in organ tissue was carried out using a NaI(TI)-detector. One of the brains removed after 30 min was rapidly frozen on dry ice and 30 μm sagittal sections cut on a Jung microtom. The sections were exposed over night to a phospho image screen and the screens analysed in a Storm 860 (Molecular Dynamics).

Chemistry

Trans-4-benzyloxycarbonylamino-5,7-dichloro-1,2,3,4-tetrahydroquinoline-2-carboxylic acid methyl ester (6t)[15]. 44.1 g (0.27 mol) 3,5-Dichloro-aniline (**3**) and 44 g (0.31 mol) sodium sulfate were added to 1 l dry dichloro methane and the resulting suspension stirred at ambient temperature. After adding rapidly 27.3 g (0.31 mol) methyl glyoxylate (**4**) the mixture was stirred for another 30 min at ambient temperature and then filtrated to separate the sodium sulfate. To this solution 55 g (0.31 mol) benzyl vinylcarbamate (**5**) were added under argon atmosphere and the resulting mixture cooled to 10°C. Under strong stirring 4.5 ml (0.042 mol) boron trifluoride diethyl ether complex were then added dropwise to the solution, whereby the mixture turned orange slowly. The reaction stirred for another hour at ambient temperature after which the solvent was removed in vacuum and the resulting oil was purified via column chromatography (dichloro methane/ethyl acetate: 9/1) to yield 20.3 g (0.05 mol, 18%) of the *trans*- (**6t**) and 34.1 g (0.08 mol, 31%) of the *cis*-4-benzyloxycarbonylamino-5,7-dichloro-1,2,3,4-tetrahydroquinoline-2-carboxylic acid methyl ester (**6c**).

trans-isomers. ^{1}H-NMR (200 MHz, $CDCl_3$) δ [ppm]: 7.33 (s, 5 H), 6.73 (d, 1 H), 6.53 (d, 1 H), 5.15 (s, 2 H), 5.05 (m, 1 H), 4.84 (m, 1 H), 4.78 (m, 1 H), 3.98 (dd, 1 H), 3.80 (s, 3 H), 2.65 (dm, 1 H), 1.67 (ddd, 1 H), MS (FD): m/z (% rel. int.) 407.9 (100.0, $[M]^+$), mp 155–157°C.

cis-isomers: ^{1}H-NMR (200 MHz, $CDCl_3$) δ [ppm]: 7.31 (s, 5 H), 6.72 (d, 1 H), 6.53 (d, 1 H), 5.12 (s, 2 H), 4.95 (m, 1 H), 4.69 (m, 1 H), 4.60 (m,

1 H), 4.04 (dd, 1 H), 3.47 (s, 3 H), 2.88 (dm, 1 H), 2.03 (ddd, 1 H), MS (FD): *m*/*z* (% rel. int.) 407.9 (100.0, $[M]^+$), mp 152–154°C.

Isomerization of the diastereomers:. To a stirred solution of 4 g (9.8 mmol) of the *cis*-isomers (**6c**) in 300 ml dry methanol, catalytic quantities of sodium methylate (3-5 mg) were added and the resulting mixture was refluxed for 36 h at 85°C. After adding a small amount of sodium hydrogen carbonate the solvent was evaporated and the residue purified with column chromatography (dichloro methane/ethyl acetate: 9/1) to yield 2.39 g (5,9 mmol, 60%) *trans*- and 1.39 g (3,4 mmol, 35%) *cis*-isomers.

Trans-4-amino-5,7-dichloro-1,2,3,4-tetrahydroquinoline-2-carboxylic acid methyl ester hydrochloride (7). Following reported methods,[16] to a stirred solution of 4.23 g (10 mmol) **6t** in 150 ml dry dichloro methane at 0°C under an argon atmosphere were slowly added 2.2 g (11 mmol) iodotrimethyl silane, whereby the solution clouded. The cooling was removed and the mixture stirred for 4 h at ambient temperature. After adding 10 ml methanol and stirring the resulting clear solution for another 15 min the solvent was removed in vacuum. The product was purified by precipitation of the corresponding hydrochloride using 150 ml ethyl acetate, saturated with gaseous HCl, to yield 2.4 g (7.4 mmol, 74%) of **7** as a white solid.^{1}H-NMR (200 MHz, DMSO-d_6) δ [ppm]: 8.43 (s, 3 H), 7.22 (s, 1 H), 6.91 (s, 1 H), 6.78 (s, 1 H), 4.61 (s, 1 H), 4.34 (d, 1 H), 3.77 (s, 3 H), 2.53 (d, 1 H), 1.93 (ddd, 1 H), mp 192–194°C (decomposition).

1-(2-Fluoroethyl)-4-(4-nitrophenyl)-piperazine (9). A mixture of 3.1 g (24 mmol) 1-bromo-2-fluoroethane, 5 g (24 mmol) 1-(4-nitrophenyl)-piperazine (**8**), 9.95 g (72 mmol) potassium carbonate and 45 mg sodium iodide in 30 ml dioxane was heated overnight under reflux. Afterwards the solvent was evaporated, the residue taken up in ethyl acetate and washed with water. The organic layer was dried with sodium sulfate, evaporated and the residue purified via column chromatography (methanol/chloroform: 1/1) to obtain 4.0 g (16 mmol, 65%) of **9** as a yellow solid. ^{1}H-NMR (200 MHz, $CDCl_3$) δ [ppm]: 8.10 (dd, 2 H), 6.80 (dd, 2 H), 4.60 (dt, 2 H, 48 Hz), 3.43(t, 4 H), 2.74 (dt, 2 H, 29 Hz), 2.68 (t, 4 H), MS (FD): *m*/*z* (% rel. int.) 253.5 (100.0, $[M]^+$), mp 101°C.

4 -[4-(2-Fluoroethyl)-piperazin-1-yl]-phenylamine (10). 4.0 g (15.7 mmol) **9** were dissolved in 80 ml methanol, 400 mg palladium (10% on activated charcoal) were added and the mixture was hydrogenated for 4 h at 0°C and 1 bar hydrogen pressure. The resulting suspension was filtered, the solvent evaporated and the residue separated through column chromatography (ethyl acetate) to yield 2.7 g (12 mmol, 77%) of **10**. ^{1}H-NMR (200 MHz, $CDCl_3$) δ [ppm]: 6.80 (dd, 2 H), 6.63 (dd, 2 H), 4.60 (dt, 2 H, 48 Hz), 3.07 (t, 4 H), 2.75 (dt, 2 H, 29 Hz), 2.69 (t, 4 H), MS (FD): *m*/*z* (% rel. int.) 222.8 (100.0, $[M]^+$), mp 52°C.

Trans-5,7-dichloro-4-(3-{4-[4-(2-fluoroethyl)-piperazin-1-yl]-phenyl}-ureido)-1,2,3,4-tetrahydroquinoline-2-carboxylic acid methyl ester (11). To a stirred solution of 110 mg (0.37 mmol) triphosgene in 4 ml dry dichloro methane at 0°C under argon atmosphere was slowly added, over a period of 30 minutes, a solution of 223 mg (1 mmol) **10** and 189 µl (1.1 mmol) *N*-ethyldiisopropylamine in 3.5 ml dry dichloro methane.[17] After further 15 min stirring a solution of 312 mg (1 mmol) **7** and 378 µl (2.2 mmol) *N*-ethyldiisopropylamine in 2 ml dry dichloro methane were quickly added, the mixture was warmed to ambient temperature and stirred for another 20 min.The solvent was evaporated and the residue was taken up in 30 ml ethyl acetate and washed with water. The organic solvent was removed in vacuum and the remaining crude product separated by column chromatography (methanol/chloroform: 1/9) to yield 460 mg (0.88 mmol, 88%) of **11**. ^{1}H-NMR (200 MHz, DMSO-d6) δ [ppm]: 7.91 (s, 1 H), 7.23 (d, 2 H), 6.91 (s, 1 H), 6.87 (d,), 6.83 (d, 2 H), 6.72 (d, 1 H), 6.42 (d, 1 H), 4.89(m, 1 H), 4.56 (dt, 2 H, 48 Hz), 3.99 (dm, 1 H), 3.72 (s, 3 H), 3,02 (t, 4 H), 2.65 (dt, 2 H, 28 Hz), 2.58 (t, 4 H), 2.32 (dm, 1 H), 1.65 (ddd, 1 H), MS (FD): *m*/*z* (% rel. int.) 523.2 (100.0, $[M]^+$).

Trans-5,7-dichloro-4-(3-{4-[4-(2-fluoroethyl)-piperazin-1-yl]-phenyl}-ureido)-1,2,3,4-tetrahydroquinoline-2-carboxylic acid (12). 200 mg (0.38 mmol) **11** were dissolved in 4 ml tetrahydrofurane, diluted with 1 ml water and 400 µl of an aqueous 1 N lithium hydroxide solution. The mixture was stirred for 6 h at ambient temperature (TLC control), diluted with 10 ml ethyl acetate and 10 ml water and the two phases allowed to separate. The aqueous phase was filtered and acidified with 2 N hydrochloric acid until the solution clouded and a precipitate formed. The precipitate was filtered off to obtain 140 mg (0.27 mmol, 73%) of **12**. ^{1}H-NMR (200 MHz, DMSO-d6) *δ* [ppm]: 7.93 (s, 1 H), 7.23 (d, 2 H)

6.87 (d, 1 H), 6.83 (d, 2 H), 6.67 (d, 1 H), 6.41 (s, 1 H), 4.92 (m, 1 H), 4.56 (dt, 2 H, 48 Hz), 3.85 (dm, 1 H), 3,03 (t, 4 H), 2.64 (dt, 2 H, 28 Hz), 2.58 (t, 4 H), 2.30 (dm, 1 H), 1.59 (ddd, 1 H), MS (FD): *m/z* (% rel. int.) 509.9 (28.8, $[M]^+$), 465.9 (18.0, $[M\text{-}CO_2]^+$), 266.7 (100.0 $[M\text{-}C_{10}H_7NO_2Cl_2]^+$), elemental analysis ($C_{23}H_{26}Cl_2FN_5O_3$) C, H, N, mp 227-229°C (decomposition).

4-(4-Aminophenyl)-piperazine-1-carboxylic acid 9h-fluoren-9-ylmethyl ester (13). To a solution of 4.0 g (21.7 mmol) 1-(4-nitrophenyl)-piperazine (**8**) in 80 ml dioxane was added a sodium hydrogen carbonate solution (10%, 40 ml). The mixture was rigorously stirred, cooled to 0°C and a solution of 5.0 g (19.3 mmol) 9-fluorenylmethyl chloroformate in 40 ml dry dioxane was slowly added. Afterwards the cooling was removed and the mixture was stirred for another 10 h at ambient temperature. The reaction mixture was diluted with 80 ml brine and extracted several times with 200 ml ethyl acetate. The organic phases were dried, the solvent evaporated and the residue was taken up in 160 ml methanol. After adding 800 mg palladium (10% on activated charcoal) the mixture was hydrogenated for 4 h (0°C, 1 bar hydrogen pressure), the catalyst was filtered off and the solvent evaporated. The crude product was separated by column chromatography (ethyl acetate) to yield 5.9 g (15 mmol, 76%) of **13**. ^{1}H-NMR (200 MHz, $CDCl_3$) δ [ppm]: 7.76 (d, 2 H), 7.58 (d, 2 H), 7.34 (m, 4 H), 6.80 (d, 2 H), 6.65 (d, 2 H), 4.45 (d, 2 H), 4.25 (t, 1 H), 3.60(s, 4 H), 3.47 (s, 2 H), 2.94 (s, 4 H), MS (FD): m/z (% rel. int.): 399.0 (100.0, $[M]^+$), mp 126°C.

Trans-5,7-dichloro-4-(3-{4-[4-(9H-fluoren-9-ylmethoxycarbonyl)-piperazin-1-yl]-phenyl}-ureido)-1,2,3,4-tetrahydroquinoline-2-carboxylic acid methyl ester (14). To a stirred solution of 110 mg (0.37 mmol) triphosgene in 4 ml dry dichloro methane at 0°C under argon atmosphere were slowly added, over a period of 30 min, a solution of 400 mg (1 mmol) **13** and 189 µl (1.1 mmol) *N*-ethyldiisopropylamine in 3.5 ml dry dichloro methane. After further 15 min stirring a solution of 312 mg (1 mmol) **7** and 378 µl (2.2 mmol) *N*-ethyldiisopropylamine in 2 ml dry dichloro methane were quickly added, the mixture was warmed to ambient temperature and stirred for another 20 min.The solvent was evaporated, the residue taken up in 30 ml ethyl acetate and washed 2 times with hydrochloric acid (3%), saturated sodium hydrogen carbonate solution and saturated sodium chloride solution. The organic layer was removed in vacuo and the remaining crude product separated

by column chromatography (ethyl acetate/chloroform: 1/1) to yield 530 mg (0.76 mmol, 76%) of **14**. ^{1}H-NMR (200 MHz, DMSO-d6) δ [ppm]: 7.92 (s, 1H), 7.88 (d, 2H), 7.63 (d, 2H), 7.36 (m, 4H), 7.25 (d, 2H), 6.91 (m, 1H), 6.86 (d, 1H), 6.83 (d, 2H), 6.71 (d, 1H), 6.43 (d, 1H), 4.91 (m, 1H), 4.40 (s, 2H), 4.27 (t, 1H), 3.98 (dm, 1H), 3.70 (s, 3H), 3,41 (s, 4H), 2,89 (t, 4H), 2.31 (dm, 1H), 1.64 (ddd, 1H), MS (FD): *m/z* (% rel. int.) 477.0 (100.0, $[M\text{-Fmoc}]^+$), 699.3 (31.4, $[M]^+$).

Trans-5,7-dichloro-4-[3-(4-piperazin-1-yl-phenyl)-ureido]-1,2,3,4-tetrahydroquinoline-2-carboxylic acid methyl ester (15). To a solution of 350 mg (0.5 mmol) **14** in 2.7 ml in dry tetrahydrofurane were added 300 µl piperidine, which led to a rapid formation of carbon dioxide. The solution was stirred for $\frac{1}{2}$ h, the solvent was evaporated, and the residue was taken up in 10 ml dry diethyl ether and treated for 10 min in the ultrasonic bath. The mixture was filtered and the solid washed with 5 ml diethyl ether. This crude product was purified by column chromatography (methanol + 3% *N*-ethyldiisopropylamine) to yield 210 mg (0.44 mmol, 88%) of **15** as a white solid. ^{1}H-NMR (200 MHz, DMSO-d6) δ [ppm]: 8.13 (s, 1H), 7.18 (d, 2H), 7.10 (d, 1H), 6.80 (d, 2H), 6.74 (d, 1H), 6.71 (d, 1H), 5.57 (d, 1H), 4.76 (m, 1H), 4.17 (m, 1H), 3.53 (s, 3H,), 2,95 (t, 4H), 2,86 (s, 4H), 2.75 (dm, 1H), 1.84 (ddd, 1H), MS (FD): *m/z* (% rel. Int.) 477.0 (100.0, $[M]^+$).

2-[^{18}F]Fluoroethyltosylate. To an aqueous [^{18}F]fluoride solution (1400-1600 MBq) were added Kryptofix 2.2.2. (10 mg, 25 µmol), 12.5 µl potassium carbonate (1N) and 1 ml acetonitrile.[18] The mixture was dried in a stream of nitrogen at 80°C. The drying procedure was repeated three times until the reaction mixture was absolutely dry. The dried Kryptofix 2.2.2./[^{18}F]fluoride-complex was then dissolved in 1 ml acetonitrile and 4 mg (10 µmol) ethylenglycol-1,2-ditosylate were added and heated under stirring in a sealed vial for 3 min. Purification of the crude product was accomplished using HPLC (acetonitrile/water 50:50, flow rate: 5 ml/min, r_t: 8 min). After diluting the HPLC fraction containing the 2-[^{18}F]fluoroethyltosylate with water, the product was loaded on a Sep-Pak C_{18} column, dried with nitrogen and eluated with 1 ml of tempered (40-50°C) DMSO (760-890 MBq)

Trans-5,7-dichloro-4-(3-{4-[4-(2-[^{18}F]fluoroethyl)-piperazin-1-yl]-phenyl}-ureido)-1,2,3,4-tetrahydroquinoline-2-carboxylic acid methyl ester (16). To 3 mg (6.3 µmol) **15** were added 0.5 ml DMSO and the

resulting solution tempered for 5 min at 140°C. A solution of 2-[^{18}F]fluoroethyltosylate (480-560 MBq) in DMSO (500 µl) were added and stirred in a sealed reaction vessel at 140°C for 25 min.The product was diluted with 1 ml 0.1% trifluoro acetic acid in water and purified with HPLC (acetonitrile/water 35:65 + 0.05% trifluoro acetic acid, flow rate: 5 ml/min, t_r: 22.9 min). After diluting the HPLC fraction containing the product with water, it was loaded on a solid phase column (LiChrolut EN, Merck), dried with nitrogen and eluated with 2 ml methanol to yield 180-250 MBq of **16**.

Trans-5,7-dichloro-4-(3-{4-[4-(2-[^{18}F]fluoroethyl)-piperazin-1-yl]-phenyl}-ureido)-1,2,3,4-tetrahydroquinoline-2-carboxylic acid (2). To the methanolic solution containing **16** were added a LiOH solution (1.25N, 200 µl) and stirred at ambient temperature for 5 min. The solution was then diluted with a ten-fold excess of water and passed on a solid phase column (LiChrolut EN, Merck), dried with nitrogen, eluated with 1 ml ethanol and diluted with physiological saline solution (160-200 MBq). HPLC analysis (acetonitrile/0.05 M Na_2HPO_4 buffer 30:70, flow rate: 1 ml/min, r_t: 6.1 min) showed that the radiochemical purity was > 99%, while the specific activity (determined via UV-calibration curve) was between 50 and 75 GBq/µmol.

Conclusion

The synthesis of the radioligand *trans*-5,7-dichloro-4-(3-{4-[4-(2-[^{18}F]fluoroethyl)-piperazin-1-yl]-phenyl}-ureido)-1,2,3,4-tetrahydroquinoline-2-carboxylic acid (**2**) for the glycine binding site of the NMDA receptor was carried out in an overall radiochemical yield of 35% by labelling the precursor with 2-[^{18}F]fluoroethyltosylate.

The lipophilicity and the *in vitro* affinity of compound **12** were determined, showing that the ligand has a moderate lipophilicity (logD = 1.3) and a high affinity (K_i = 12 nM) for the glycine binding site.

Biodistribution studies resulted in a maximum uptake of 0.1% ID/g in rat brain, while urinary excretion was predominating liver uptake. The relatively low brain uptake may be due to the deprotonated acid function, which might indicate that an increased brain uptake of this high-affinity tracer could be achieved by further heightening the lipophilicity.

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