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Synthesis, labelling and evaluation of hydantoin-substituted indole carboxylic acids as potential ligands for positron emission tomography imaging of the glycine binding site of the *N*-methyl-D-aspartate receptor

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The *N*-methyl-D-aspartate (NMDA) receptor as a type of ionotropic glutamatergic receptors is essential for physiological processes such as learning, memory and synaptic plasticity. A glutamate-induced overactivation of these receptors, accompanied by increased intracellular calcium concentration, causes cell injury and leads to a large number of acute or chronic neurological disorders, such as stroke, trauma, Parkinson's disease and Alzheimer's disease. In an attempt to visualise the glutamatergic neurotransmission *in vivo* with positron emission tomography, novel fluoroethoxy- and methoxy-substituted reference compounds based on the lead structure of a hydantoin-substituted indole-2-carboxylic acid were synthesised. The affinities towards the glycine binding site of the NMDA receptor showed K_i values between 322 and 11 nM and the lipophilicities ranged from log*D* values of 1.51 to 2.53. On the basis of these results, precursor compounds were synthesised containing a phenolic hydroxy moiety to obtain the radiolabelled ligands through an alkylation reaction. Radiosynthesis was achieved by labelling the precursor ethyl 4,6-dichloro-3-((3-(4-hydroxyphenyl)-2,4-dioxoimidazolidin-1-yl)methyl)-indole-2-carboxylate with 2-[^{18}F]fluoroethyl tosylate or [^{11}C]methyl iodide and subsequent cleavage of the ethyl ester moiety. This gave the final products in overall decay-corrected radiochemical yields of 5–7% and 6–9% and specific activities of 24–67 GBq/μmol and 8–26 GBq/μmol, respectively.

Keywords: glutamate; NMDA receptor; glycine binding site; ^{18}F-fluoroalkylation; ^{11}C-methylation

Introduction

Glutamate, a major excitatory amino acid in the central nervous system of mammals, plays an important role not only in neuronal plasticity and neurotoxicity[1–3] but also in neurological disorders such as Parkinson's and Alzheimer's diseases, schizophrenia and Huntington's chorea.[4,5] Receptors responding to glutamate can be split into two groups: metabotropic glutamate receptors, which are G-protein-coupled receptors that mediate slow modulatory responses, and ionotropic receptors, which are gated ion channels that mediate fast neurotransmission. The ionotropic receptors can be divided into three families, namely (1) *N*-methyl-D-aspartate (NMDA), (2) α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) and (3) kainate,[6] according to their selective agonists. Of these, especially the NMDA receptor is an important target for medicinal chemistry because of its high permeability to calcium, which is an important second messenger in the central nervous system. NMDA receptors have a tetrameric structure and are assembled from seven known subunits (NR1, NR2A–NR2D, NR3A–NR3B).[7] Functional receptors, which are composed of at least one NR1 subunit and one or more of the NR2A–NR2D subunits, can be opened by simultaneous binding of glycine and glutamate to the NR1 and NR2 subunits.[8] The NR3 subunits also can co-assemble with NMDA receptors, although their stoichiometry and function are yet unclear. Besides the binding sites for glutamate and glycine, functional NMDA receptors also possess a binding site in the open ion

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channel and a binding site for polyamines, thought to be located on the NR2B subunit.

Over the past years, a variety of positron emission tomography (PET) and single-photon emission computed tomography studies with ligands for the different binding sites of the receptor have been performed. They mainly represented high-affinity open ion channel blockers based on derivatives of MK801,[9] phenylcyclidine[10] and memantine.[11] These ligands can only bind to their binding site if the receptor is activated and the ion channel is open. In combination with their typically high lipophilicity, which might be accompanied by a high unspecific binding, the obtained data were complicated and difficult to interpret and did not allow clear pharmacological conclusions.

An alternative approach focuses on ligands for the glycine binding site using derivatives of quinoxaline-2,3-diones,[12] 2-quinolones[13] and 2-carboxytetrahydroquinolines.[14] They offer nanomolar affinities, a moderate lipophilicity and fewer side effects compared with ligands of the glutamate and ion channel binding site. Whereas optimization of the 2-carboxytetrahydroquinolines led to ligands that have high affinities in the nanomolar range (**1**), it revealed log*D* values of about 1.3, resulting in a low brain uptake.[14] Therefore, indole 2-carboxylic acid analogues comprising a hydantoin moiety were synthesised,[5,15] in order to improve their lipophilicities and to obtain promising candidates for the *in vivo* visualisation of the NMDA receptor (Figure 1).[14]

Results and discussion

Organic syntheses

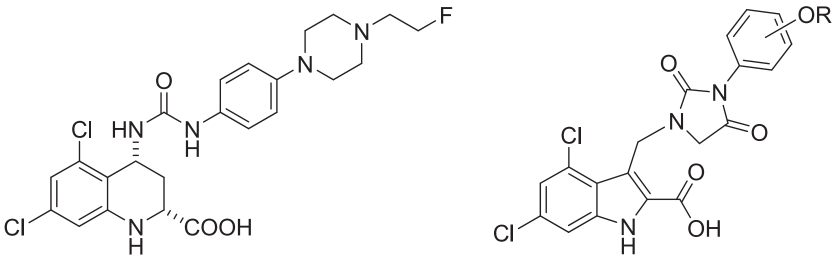
A series of hydantoin-containing compounds based on 4,6-dichloro-3-((2,4-dioxo-3-phenylimidazolidin-1-yl)methyl)-indole-2-carboxylic acid (**15**) were synthesised for their use as labelling precursors and potential imaging agents for the glycine-binding site of NMDA receptors (Figure 2).

To prepare these compounds, ethyl 4,6-dichloro-3-((2-methoxy-2-oxoethylamino)methyl)-indole-2-carboxylate (**7**) was synthesised as a building block and used for coupling with O-fluoroethylated, O-methylated and O-benzylated aminophenols (Scheme 1). The synthesis was started with 4,6-dichlorohydrazine (**3**), which was converted quantitatively into (*E*/*Z*)-ethyl 2-(2-(3,5-dichlorophenyl)hydrazono)propanoate (**4**) by refluxing with ethyl pyruvate. The resulting *E*/*Z* isomers can be separated by column chromatography (hexane/ethyl acetate 10:1 (v/v)), but typically were used as mixture for the next reaction step. The hydrazone was converted into the corresponding ethyl 4,6-dichloro-indole-2-carboxylate (**5**) in a proton-catalysed Fischer indole synthesis, which had to be carefully hydrolyzed after the reaction. The aldehyde derivative ethyl 4,6-dichloro-3-formyl-indole-2-carboxylate (**6**) was obtained under the conditions of a Vilsmeier synthesis using *N*-methyl formanilide as a reagent. Finally, **7** was formed by reductive amination with methyl glycinate, which had to be pestled to obtain high yields, and sodium triacetoxyborohydride.[16]

The synthesis of the fluoroethyl-substituted aminophenols was achieved in two steps from the corresponding nitrophenols **8a–8d** (Scheme 2). The fluoroethyl moiety was introduced with 1-bromo-2-fluoroethane using K_2CO_3 in acetone. The workup of the reaction mixture was realised by extraction with toluene and 10% NaOH solution at pH 12, and afforded sufficient pure compounds **9a–9d** for the conversion of the nitro group into the amino group by catalytic hydrogenation with 10% Pd/C to **10a–10d**. The benzyl-protected aminophenols could not be obtained in the direct way by refluxing aminophenols with benzylchloride and K_2CO_3 in acetone because N-benzylation was the main reaction route. Therefore, a fast and simple way to synthesise these compounds (**12a–12d**) in a microwave-assisted one-step reaction was performed.[17]

Compounds **13a–13e** were synthesised using *bis*-(trichloromethyl) carbonate (triphosgene), which is a less hazardous substitute for phosgene (Scheme 3).[18] Treatment of the amine components **10a–10e** with triphosgene at 0 °C resulted in the formation of the corresponding carbamoyl chlorides, which were then reacted at ambient temperature with **7** to get the unsymmetrical ureas **13a–13e**. To keep the formation of symmetrical 1,3-diarylureas low, the initial step was carried out slowly over a period of 30 min at reduced temperature.[19]

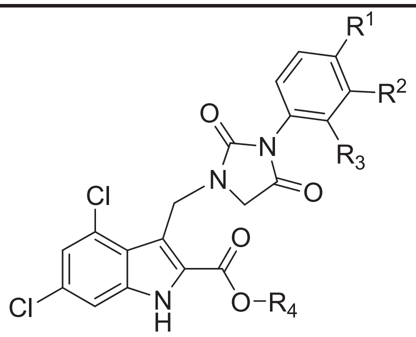
Nuclear magnetic resonance (NMR) and mass spectral analyses revealed the concomitant ring closure of the ureas **13a–13e** to the cyclised hydantoins **14a–14e** for up to 20% during the triphosgene coupling (Scheme 4). As this step was next in the synthesis scheme, the isolation of **13a–13e** was not necessary, and the subsequent cyclisation step was continued by treatment with freshly prepared sodium ethanolate in ethanol. The reaction was stopped after 1 h, and because no satisfying conditions for the purification were found using silica gel as solid phase, the workup was performed by column chromatography using basic alumina oxide and THF/hexane or $CHCl_3$/acetone mixtures as mobile phases. In the last step, **14a–14e** were saponified with 2 M NaOH and purified by column chromatography to yield the reference compounds **15a–15e**.



1
lead structure
K_i = 12 nmol ([^{3}H]MDL 105,519)

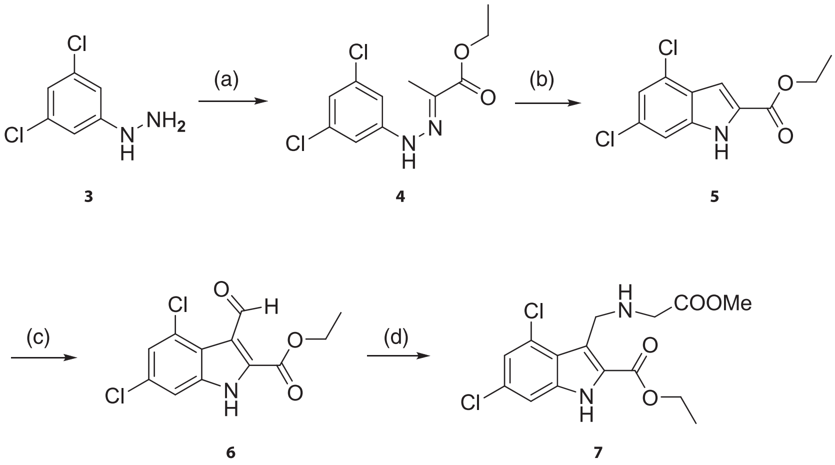
2 (R = C_2H_4F, CH_3)
indole derivatives

Figure 1. Lead structure (**1**) and the methylated and fluoroethylated indole derivatives (**2**).



Compound	R1	R2	R3	R4
15	H	H	H	H
15a	$O(CH_2)_2F$	H	H	H
15b	$O(CH_2)_2F$	Cl	H	H
15c	H	$O(CH_2)_2F$	H	H
15d	H	H	$O(CH_2)_2F$	H
15e	OCH_3	H	H	H
19a	OH	H	H	H
19b	OH	H	H	CH_2CH_3
20	H	OH	H	CH_2CH_3

Figure 2. Lead structure, reference compounds and labelling precursors.



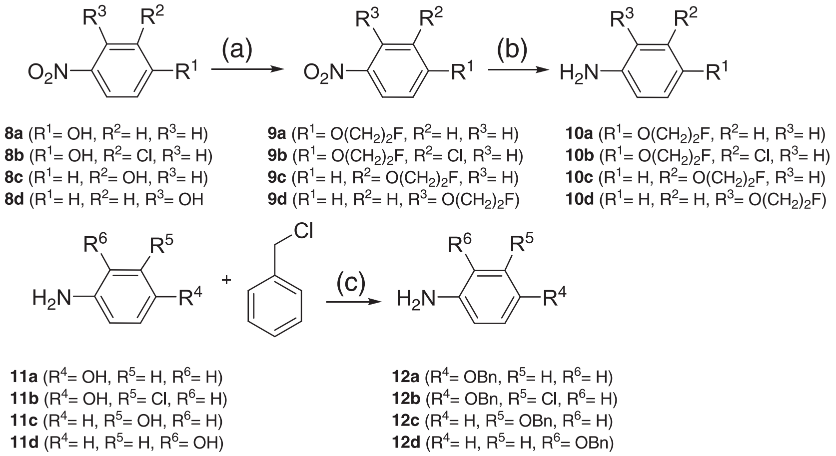
Scheme 1. Synthesis route to **7**. Reagents and conditions: (a) ethyl pyruvate, reflux, 16 h; (b) polyphosphoric acid, 120–130 °C; (c) *N*-methyl formanilide, $POCl_3$; (d) methyl glycinate, $Na(OAc)_3BH$.

The reference compounds were obtainable in nine steps using this reaction procedure in a good overall yield of about 8%.

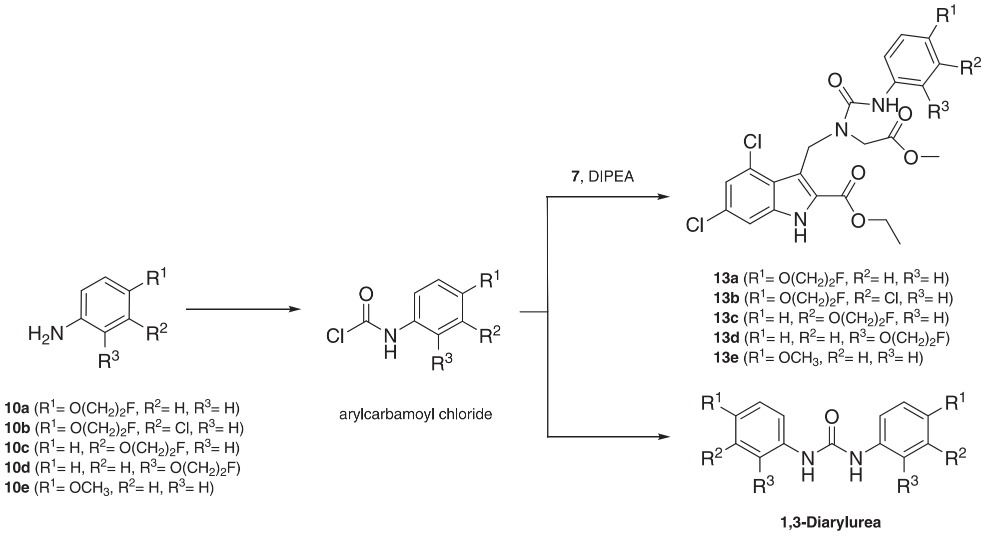
Considering the lipophilicities and *in vitro* affinities of the reference substances, **15a**, **15c** and **15e** seem to be the most promising compounds; thus, only their respective precursors were synthesised. The reaction scheme for the preparation of the labelling precursor compounds **19a**, **19b** and **20** is almost identical to the procedure described above (Scheme 5), except the synthesis of the benzyloxy anilines. In a first attempt, a reaction of the appropriate aminophenols with benzylchloride under basic conditions was examined, resulting in the formation of the N-benzylated compounds as main product. Therefore, an alternative reaction route was used, that is, reacting both educts under microwave-assisted conditions, which gave the O-benzylated compounds in moderate yields of about 45%. Benzyloxy anilines **12a** and **12c** were coupled with **7** to ureas **16a** and **16b**, cyclised with sodium ethanolate to **17a**, **17b** and **17a** saponified with 2 M NaOH to **18**. With these compounds, labelling precursors for a one-step and a two-step labelling reaction were accessible. Therefore, **17a**, **17b** and **18** were converted into **19a**, **19b** and **20** by catalytic hydrogenation with 10% Pd/C. The labelling precursors were obtainable using this reaction procedure in eight steps for **19a** and **19b** and in nine steps for **20** in a moderate overall yield of about 5–7%.

In vitro affinity and lipophilicity

In vitro binding assays for the glycine binding site of the NMDA receptor were performed according to reported methods using



Scheme 2. Synthesis route to **10a–10d** and **12a–12d**. Reagents and conditions: (a) 1-bromo-2-fluoroethane, K_2CO_3, acetone, reflux, 48 h; (b) H_2, Pd (10%)/C; (c) K_2CO_3, KOH, TBABr, microwave (300 W), 40 s.



Scheme 3. Formation of the unsymmetrical ureas using triphosgene as coupling reagent.

rat cortex membranes and [^{3}H]MDL 105,519 as radioligand.[20] Relative affinities of the reference compounds **15a–15e** were determined as IC_{50} values from displacement curves. The K_i values were calculated according to Cheng and Prusoff.[21] Within this series of compounds, the inhibition constants of the 4-fluoroethoxy, 3-fluoroethoxy and 4-methoxy derivatives (**15a**, **15c**, **15e**) were 81 ± 24 nM, 43 ± 13 nM and 47 ± 14 nM, respectively. The electron deficient chloro-derivative **15b** had an inhibition constant of 493 ± 138 nM, the lowest affinity, and the 2-fluoroethoxy derivative **15 d** had 17 ± 5 nM, the highest affinity towards the receptor.

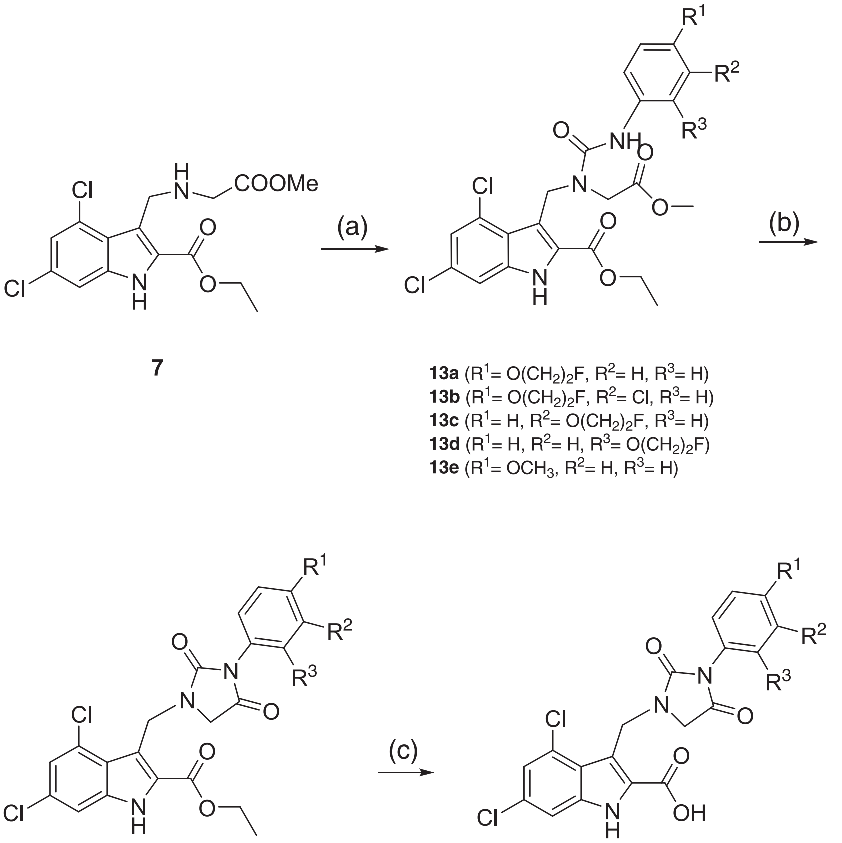
Lipophilicity of the reference compounds **15a–15e** was determined by reversed phase HPLC. Whereas the lipophilicities of compounds **15a**, **15c** and **15e** range very close together, with log*D* values of 2.07 to 2.26, the chloro derivative **15b** shows the highest lipophilicity, with a log*D* value of 2.53, and the *O*-fluoroethoxy derivative **15 d** has the lowest value, with a log*D* of 1.51.

Clearly, the affinity and lipophilicity of the compounds are dependent on the electronic properties of the phenyl ring and the position of the fluoroalkyl moiety. In an earlier study, the influence of lipophilicity was correlated to the blood-brain barrier penetration resulting in a parabolic relationship, with its maximum *in vivo* activity in the middle of the log*D* range of 1.5–2.5.[22] With these considerations, compounds **15a**, **15c** and **15e** were chosen for further investigations because of their reasonable log*D* for good brain uptake and similar affinities for the NMDA receptor (Table 1).

Radiosyntheses

Initial synthesis of 4,6-dichloro-3-((3-(4-(2-[^{18}F]fluorethoxy)phenyl)-2,4-dioxoimidazolidin-1-yl)methyl)-indole-2-carboxylic acid ([^{18}F] **15a**) was performed in a one-step reaction by labelling the precursor compound **20** with 2-[^{18}F]fluoroethyl tosylate ([^{18}F] FETos) in aprotic solvents and 5 M NaOH as a base. This resulted in yields of ≤4% for [^{18}F]**15a** (Scheme 6) owing to the formation of two unknown by-products. Consequently, a two-step synthesis route was developed starting from the ethyl-protected derivatives **19a** and **19b**.

To identify the most suitable reaction conditions, several parameters such as solvent, temperature, base, equivalents of base, precursor amount and reaction time were examined. Optimisation of the used base showed that 5 M NaOH gave the



7

13a (R^1= O(CH$_2$)$_2$F, R^2= H, R^3= H)
13b (R^1= O(CH$_2$)$_2$F, R^2= Cl, R^3= H)
13c (R^1= H, R^2= O(CH$_2$)$_2$F, R^3= H)
13d (R^1= H, R^2= H, R^3= O(CH$_2$)$_2$F)
13e (R^1= OCH$_3$, R^2= H, R^3= H)

14a (R^1= O(CH$_2$)$_2$F, R^2= H, R^3= H)
14b (R^1= O(CH$_2$)$_2$F, R^2= Cl, R^3= H)
14c (R^1= H, R^2= O(CH$_2$)$_2$F, R^3= H)
14d (R^1= H, R^2= H, R^3= O(CH$_2$)$_2$F)
14e (R^1= OCH$_3$, R^2= H, R^3= H)

15a (R^1= O(CH$_2$)$_2$F, R^2= H, R^3= H)
15b (R^1= O(CH$_2$)$_2$F, R^2= Cl, R^3= H)
15c (R^1= H, R^2= O(CH$_2$)$_2$F, R^3= H)
15d (R^1= H, R^2= H, R^3= O(CH$_2$)$_2$F)
15e (R^1= OCH$_3$, R^2= H, R^3= H)

Scheme 4. Synthesis of reference compounds. Reagents and conditions: (a) triphosgene, **10a–10e**, DIPEA, DCM; (b) fresh NaOEt/EtOH; (c) 2 M NaOH, THF.

best results, whereas potassium carbonate and lithium diisopropylamide only resulted in unsatisfactory yields. Under optimised conditions, using 3 mg (6.9 mmol) of precursor (**19a** or **19b**) in DMSO with 1.9 equiv. of 5 M NaOH and a reaction time of 5 min at a temperature of 100 °C, radiochemical yields of 30–40% for [^{18}F]**14a** and 25–30% for [^{18}F]**14c** were achieved. A prolongation of the reaction time led in all cases to a drop in radiochemical yields because of an increasing cleavage of the ester moiety and thermal decomposition.

Because of the similar affinity and lipophilicity of **15a** and **15c** and lower labelling yields for [^{18}F]**14c**, further investigations on ^{11}C labelling chemistry were carried out with **19a** only.

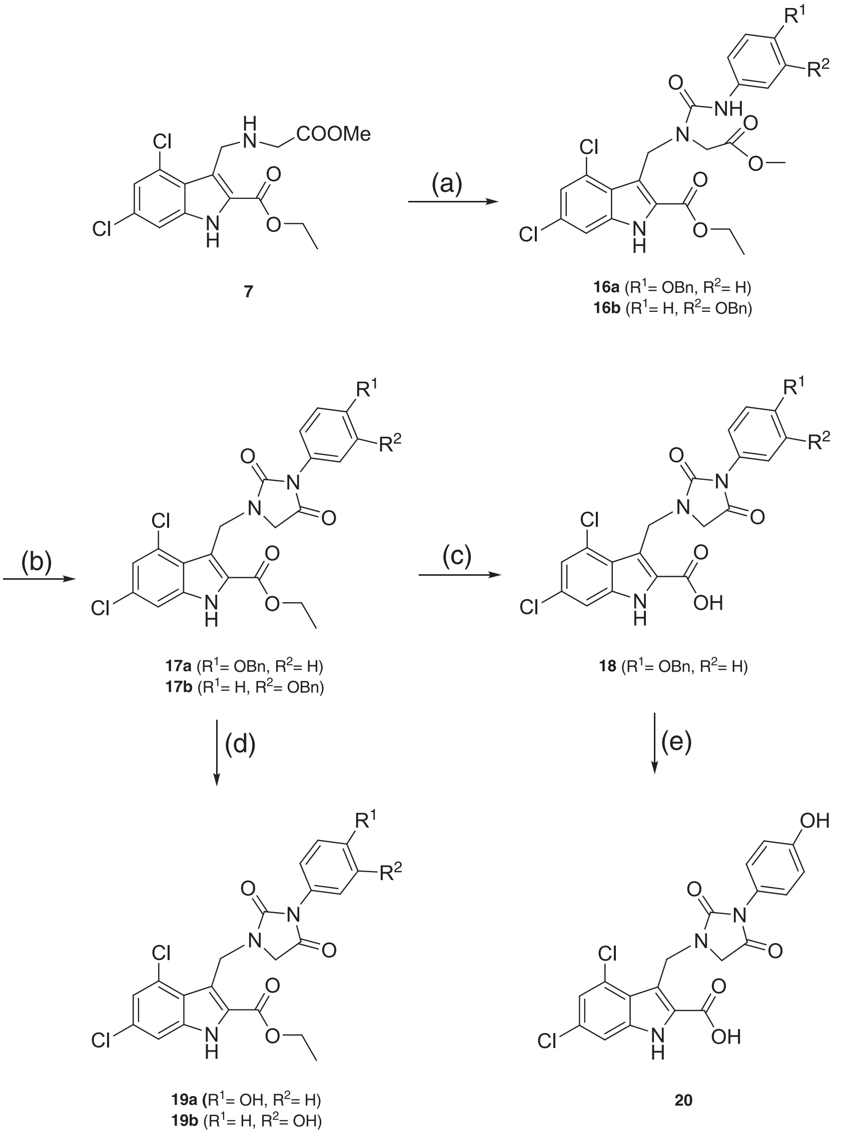
Labelling reactions with [^{11}C]methyl iodide ([^{11}C]CH$_3$I) and **19a** were performed in a similar way. Because of the higher reactivity of [^{11}C]CH$_3$I, the reaction times in the synthesis of [^{11}C]**14e** were decreased to 2.5 min. Reducing the reaction temperature to 50 °C or lower led to no significant product formation. However, the use of a 0.33 M solution of NaOEt in DMSO instead of the 5 M NaOH solution raised the radiochemical yields of [^{11}C]**14e** to 25–35%.

Purification of the crude products [^{18}F]**14a** and [^{11}C]**14e** was accomplished by semipreparative HPLC. The subsequent cleavage of the ethyl protecting group with 1 M NaOH solution at room temperature was incomplete after 30 min, and a decomposition of the final products [^{18}F]**15a** and [^{11}C]**15e** was observed. Trials to improve the ester cleavage by using different base systems as well as by increasing the reaction temperature and shortening the reaction time gave unsatisfactory results. Thus, the reaction was terminated after 30 min, and a second purification by semipreparative HPLC was performed. Attempts to perform this reaction procedure in a one-pot reaction were not successful because no HPLC conditions were found to separate the final product from the by-products formed during ^{18}F-fluoroethylation and saponification. After formulation in physiological saline solution, [^{18}F]**15a** and [^{11}C]**15e** were obtained in decay-corrected yields of 5–7% and 6–9% in a synthesis time of about 130 min and 83 min, respectively.

Experimental

General

Solvents were purchased from Fluka, Acros and Fisher Scientific. (3,5-Dichlorophenyl)hydrazine (**3**; ABCR), benzylchloride (Fluka), 1-bromo-2-fluoroethane (Lancaster), potassium carbonate (Fluka) and 4-methoxyaniline (**10e**; Fluka) were purchased from the companies shown in brackets; all other chemicals were obtained from Merck. All reagents were used without further purification. For solid-phase extraction, Sep-Pak®-QMA, Sep-Pak®-C$_{18}$ (Waters, USA) and Strata X cartridges (1 mL, 30 mg; Phenomenex, USA)



Scheme 5. Synthesis of labelling precursors. Reagents and conditions: (a) triphosgene, **12a**, **12c**, DIPEA, DCM; (b) fresh NaOEt/EtOH; (c) 2 M NaOH, THF; (d and e) H_2, Pd (10%)/C.

were used. Thin layer chromatography (TLC) was performed using plates from Merck (silica gel 60 F_{254} and alumina oxide 60 F_{254}). ^{1}H- and ^{13}C-NMR spectra were recorded using a Bruker AC 300 or Bruker AM 400 spectrometer. Chemical shifts were quoted as δ values (ppm) downfield from tetramethylsilane. Field desorption (FD) mass spectra were recorded using a Finnigan MAT90 spectrometer. Analytical and semipreparative HPLC for nonradioactive syntheses were performed on a system from Dionex consisting of a P680A pump and a UVD 170U UV detector. Analytical HPLC for ^{18}F-compounds was performed with a system from Sykam consisting of a S1121 pump, a Knauer K2501 UV detector and a Berthold LB506 radiodetector. Analytical HPLC for ^{11}C-labelled compounds was performed with a system from Dionex consisting of a P680A pump, a UVD 170U UV detector and a Gabi Star (Raytest) radiodetector. Preparative HPLC was performed for ^{18}F- and ^{11}C-labelled compounds with a system from Sykam, consisting of a S1021 pump, a S3200 UV detector and a NaI(Tl) radioactivity detector, which was integrated in the synthesis module (^{11}C: automated synthesiser system C-11 methylation, GE Medical Systems; ^{18}F: noncommercial synthesis module).

The log*D* values were examined according to the OECD guidelines for testing of chemicals using the HPLC method (stationary phase: Phenomenex Gemini RP18-EC, 5 µm, 250 × 4.6 mm; mobile phase: Sörensen buffer (phosphate buffer/MeOH 25:75 (v/v)) at pH 7.4, flow: 1 mL/min).[23] To obtain the calibration curve, the capacity factors of five compounds with reported lipophilicity were determined by measuring the retention time t_r and dead time t_0, and the resulting log*k* values were plotted versus log*D*. Determination of the *in vitro* binding affinity was performed using a [^{3}H]MDL 105,519 binding assay according to the method of a previous study.[8]

Organic syntheses

*(E/Z)-Ethyl 2-(2-(3,5-dichlorophenyl)hydrazono)propanoate (**4**)*

Five grams of (3,5-dichlorophenyl)hydrazine (28.2 mmol) was dissolved in 45 mL of EtOH. Three grams (3.11 mL, 28.2 mmol) of ethyl pyruvate in 10 mL of EtOH was added dropwise, and the mixture was stirred overnight. The solvent was evaporated and the residue purified by column chromatography (hexane/ethyl

Table 1. IC_{50}, K_i and log *D* values of the reference compounds **15a–15e**

Compound	IC_{50} [nM]	K_i [nM]	Log*D*
15a	81 ± 24*	53 ± 16	2.07
15b	493 ± 148	322 ± 97	2.53
15c	43 ± 13	28 ± 8	2.12
15 d	17 ± 5	11 ± 3	1.51
15e	47 ± 14	31 ± 9	2.26

acetate 10:1 (v/v); R_f (*Z*-isomer): 0.68; R_f (*E*-isomer): 0.22) to yield 7.6 g (27.6 mmol, 98%) of *E*/*Z*-isomers as an orange solid.

MS (FD) *m/z* (% rel. int.): 274.1 (100, $[M]^+$).

Z-isomer: ^{1}H-NMR (300 MHz, DMSO-d_6), δ [ppm]: 11.79 (s, 1H, NH), 7.27 (t, 2H, Ar-CH), 6.98 (t, 1H, Ar-CH), 4.23 (q, 2H, CH_2), 2.08 (s, 3H, CH_3), 1.27 (t, 3H, CH_3).

E-isomer: ^{1}H-NMR (300 MHz, DMSO-d_6), δ [ppm]: 10.08 (s, 1H, NH), 7.21 (t, 2H, Ar-CH), 7.02 (t, 1H, Ar-CH), 4.19 (q, 2H, CH_2), 2.05 (s, 3H, CH_3), 1.25 (t, 3H, CH_3).

*Ethyl 4,6-dichloro-indole-2-carboxylate (**5**)*

To an open 250 mL beaker containing 25 mL of polyphosphoric acid, 7.6 g (28 mmol) of **4** was added slowly at 70 °C. The suspension was heated to 120–130 °C for 10 min and then hydrolyzed by cautious addition of ice. Afterwards, the mixture was treated with ethyl acetate until the precipitate had dissolved. The solution was neutralised with NaOH pellets and extracted with ethyl acetate, and the combined organic phases were dried with $MgSO_4$. After the solvent was evaporated *in vacuo*, the crude product was purified by column chromatography (hexane/ethyl acetate 3:1 (v/v); R_f: 0.77) to yield 6.6 g (26 mmol, 93%) of a light brown solid.

MS (FD) *m/z* (% rel. int.): 257.0 (100, $[M]^+$).

^{1}H-NMR (300 MHz, DMSO-d_6), δ [ppm]: 12.41 (s, 1H, In-NH), 7.43 (t, 1H, Ar-CH), 7.26 (d, 1H, Ar-CH), 7.10 (d, 1H, CH), 4.34 (q, 2H, CH_2), 1.36 (t, 3H, CH_3).

*Ethyl 4,6-dichloro-3-formyl-indole-2-carboxylate (**6**)*

To 6.1 g (45 mmol) of *N*-methyl formanilide was added 3.7 g (43.2 mmol) of phosphoryl chloride, and the resulting solution was stirred at ambient temperature under argon atmosphere until an orange solid precipitated. A solution of 7 g (27 mmol) of **5** in 30 mL of 1,2-dichloroethane was then added, and the resulting mixture was stirred under reflux for 8 h. Afterwards, it was poured on a mixture of 29.7 g of NaOAc·3H_2O and 60 g of ice and stored overnight in a refrigerator. The precipitate was filtered off, dried *in vacuo* and recrystallised using ethyl acetate to yield 5.9 g (20.6 mmol, 76%) of a yellow solid.

MS (FD) *m/z* (% rel. int.): 285.3 (100, $[M]^+$).

^{1}H-NMR (300 MHz, DMSO-d_6), δ [ppm] = 13.16 (s, 1H, In-NH), 10.64 (s, 1H, CHO), 7.53 (d, 1H, Ar-CH), 7.38 (d, 1H, Ar-CH), 4.43 (q, 2H, CH_2), 1.34 (t, 3H, CH_3).

*Ethyl 4,6-dichloro-3-((2-methoxy-2-oxoethylamino)methyl)-indole-2-carboxylate (**7**)*

To a mixture of 880 mg (7 mmol) of methyl glycinate in 20 mL of CH_2Cl_2, 1.06 g (1460 µL, 10.5 mmol) of triethylamine was added under argon atmosphere. After 10 min, a solution of 2 g (7 mmol) of **6** was added, and the mixture was stirred for 15 min; 2.4 g (11 mmol) of $Na(OAc)_3BH$ was then added, and the mixture was stirred for 1.5 h. The reaction was quenched with 20 mL of saturated $NaHCO_3$ solution and diluted with ethyl acetate. The layers were separated, and the organic phase was dried with $MgSO_4$. After purification of the residue by column chromatography (ethyl acetate/hexane 5:1 (v/v); R_f: 0.44), 1.9 g (5.2 mmol, 75%) of a light brown solid was obtained.

MS (FD) *m/z* (% rel. int.): 358.5 (100, $[M]^+$).

^{1}H-NMR (300 MHz, DMSO-d6), δ [ppm] = 12.14 (s, 1H, In-NH), 7.42 (d, 1H, Ar-CH), 7.20 (d, 1H, Ar-CH), 4.35 (q, 2H, CH_2), 3.48 (s, 3H, CH_3O), 3.33 (s, 2H, CH_2), 3.33 (s, 2H, CH_2), 1.98 (s, 1H, NH), 1.35 (t, 3H, CH_3).

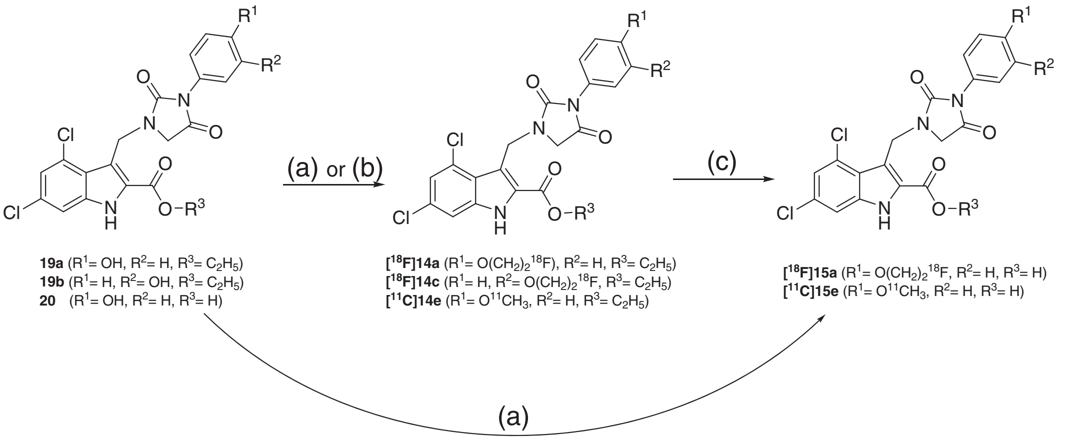
General procedure I: fluoroethylation of nitrophenols

A mixture of 44 mmol 1-bromo-2-fluoroethane, 40 mmol of the appropriate nitrophenol and 44 mmol K_2CO_3 in 40 mL of acetone was refluxed for 48 h. The solvent was evaporated, and the residue was taken up in water and extracted twice with 75 mL of toluene. The combined organic layers were washed three times with 50 mL of a 10% NaOH solution and dried with Na_2SO_4, and the solvent was evaporated *in vacuo*.

*1-(2-Fluoroethoxy)-4-nitrobenzene) (**9a**)*

Yield: 45%, TLC: R_f (ethyl acetate/hexane 1:1 (v/v)): 0.87.

MS (FD) *m/z* (% rel. int.): 184.5 (100, $[M]^+$).



Scheme 6. Radioactive labelling of **20** with [^{18}F]FETos yielding [^{18}F]**15a** in a direct approach and of **19a** with [^{18}F]FETos and [^{11}C]CH_3I yielding [^{18}F]**15a** and [^{11}C]**15e** via [^{18}F]**14a** and [^{11}C]**14e**. Reagents and conditions: (a) [^{18}F]FETos, 1.9 equiv. 5 M NaOH, DMSO 100 °C, 5 min; (b) [^{11}C]CH_3I, 1.9 equiv. 0.33 M NaOEt, DMSO, 50 °C, 2.5 min; (c) EtOH, aq. 1 M NaOH, 30 min.

^{1}H-NMR (300 MHz, $CDCl_3$), δ [ppm] = 8.23 (d, 2H, Ar-CH), 7.01 (d, 2H, Ar-CH), 4.77 (dt, 2H, CH_2), 4.28 (dt, 2H, CH_2).

*2-Chloro-1-(2-fluoroethoxy)-4-nitrobenzene (**9b**)*

Yield: 46%, TLC: R_f (ethyl acetate/hexane 3:2 (v/v)): 0.85.

MS (FD) *m/z* (% rel. int.): 219.2 (100, $[M]^+$).

^{1}H-NMR (300 MHz, $CDCl_3$), δ [ppm] = 8.29 (d, 1H, Ar-CH), 8.13 (dd, 1H, Ar-CH), 6.99 (d, 1H, Ar-CH), 4.92–4.90 (m, 1H CH_2), 4.77–4.74 (m, 1H CH_2), 4.45–4.42 (m, 1H CH_2), 4.35–4.32 (m, 1H, CH_2).

*1-(2-Fluoroethoxy)-3-nitrobenzene (**9c**)*

Yield: 81%, TLC: R_f (ethyl acetate/hexane 1:1 (v/v)): 0.93.

MS (FD) *m/z* (% rel. int.): 184.5 (100, $[M]^+$).

^{1}H-NMR (300 MHz, $CDCl_3$), δ [ppm] = 7.84 (dd, 1H, Ar-CH), 7.73 (t, 1H, Ar-CH), 7.43 (t, 1H, Ar-CH), 7.27 (d, 1H, Ar-CH), 4.86 (m, 1H CH_2), 4.70 (m, 1H CH_2), 4.33 (m, 1H CH_2), 4.24 (m, 1H CH_2).

*1-(2-Fluoroethoxy)-2-nitrobenzene (**9d**)*

Yield: 53%, TLC: R_f (ethyl acetate/$CHCl_3$ 1:10 (v/v)): 0.91.

MS (FD) *m/z* (% rel. int.): 184.5 (100, $[M]^+$).

^{1}H-NMR (300 MHz, $CDCl_3$), δ [ppm] = 7.82 (dd, 1H, Ar-CH), 7.51 (dt, 1H, Ar-CH), 7.10–7.03 (m, 2H, Ar-CH), 4.87–4.84 (m, 1H, CH_2), 4.71–4.68 (m, 1H, CH_2), 4.42–4.38 (m, 1H CH_2), 4.32–4.29 (m, 1H CH_2).

General procedure II: hydrogenation

A mixture of 16 mmol of the appropriate fluoroethylated nitrophenol and 400 mg of Pd (10%)/C in 80 mL of MeOH was hydrogenated for 4 h at 0 °C and 1 bar pressure. The suspension was filtered, the solvent evaporated and the residue purified by column chromatography.

*4-(2-Fluoroethoxy)aniline (**10a**)*

Yield: 54%, TLC: R_f (ethyl acetate/hexane 1:1 (v/v)): 0.44.

MS (FD) *m/z* (% rel. int.): 155.1 (100, $[M]^+$).

^{1}H-NMR (200 MHz, $CDCl_3$), δ [ppm] = 6.61 (d, 2H, Ar-CH_a), 6.49 (d, 2H, Ar-CH_b), 4.56 (dt, 2H, CH_2), 3.98 (dt, 2H, CH_2), 3.43 (br s, 2H, NH_2).

*3-Chloro-4-(2-fluoroethoxy)aniline (**10b**)*

Yield: (54%), TLC: R_f (ethyl acetate/hexane/3/2): 0.67.

MS (FD) *m/z* (% rel. int.): 189.2 (100, $[M]^+$).

^{1}H-NMR (300 MHz, DMSO-d_6), δ [ppm] = 6.85 (d, 1H, Ar-CH), 6.62 (d, 1H, Ar-CH), 6.46 (dd, 1H, Ar-CH), 4.94 (s, 2H, NH_2), 4.75–4.72 (m, 1H CH_2), 4.59–4.56 (m, 1H CH_2), 4.16–4.13 (m, 1H CH_2), 4.06–4.03 (m, 1H, CH_2).

*3-(2-Fluoroethoxy)aniline (**10c**)*

Yield: 84%, TLC: R_f (ethyl acetate/hexane 1:1 (v/v)): 0.65.

MS (FD) *m/z* (% rel. int.): 155.1 (100, $[M]^+$).

^{1}H-NMR (300 MHz, DMSO-d_6), δ [ppm] = 6.88 (t, 1H, Ar-CH), 6.17–6.06 (m, 3H, Ar-CH), 5.05 (s, 2H, NH_2), 4.76 (t, 1H, CH_2), 4.60 (t, 1H, CH_2), 4.13 (t, 1H, CH_2), 4.03 (t, 1H, CH_2).

*2-(2-Fluoroethoxy)aniline (**10d**)*

Yield: 68%, TLC: R_f (ethyl acetate/$CHCl_3$ 1:10 (v/v)): 0.69.

MS (FD) *m/z* (% rel. int.): 155.1 (100, $[M]^+$).

^{1}H-NMR (300 MHz, DMSO-d_6), δ [ppm] = 6.79 (d, 1H, Ar-CH), 6.74–6.62 (m, 2H, Ar-CH), 6.52–6.43 (m, 1H, CH_2), 4.68 (s, 2H, NH_2), 4.65 (t, 1H, CH_2), 4.21 (t, 1H, CH_2), 4.11 (t, 1H, CH_2).

General procedure III: synthesis of benzyloxy anilines

A mixture of 5 mmol of the appropriate aminophenol, 20 mmol K_2CO_3, 20 mmol fine powdered KOH and 0.5 mmol tributylammonium bromide was triturated in a mortar, divided into four equal portions and transferred to four Erlenmeyer flasks (100 mL). To each flask, 760 mg (690 μL, 6 mmol) of benzylchloride was added dropwise. The flasks were covered with a beaker and carefully heated in a household microwave (360 W) for 40 s. To prevent the appearance of vapours in the flasks, heating was interrupted a few times. After cooling the mixtures, they were combined and extracted twice with 100 mL of ethyl acetate. The organic layer was washed with saturated Na_2CO_3 solution, saline solution, the organic phase dried with $MgSO_4$ and the solvent evaporated *in vacuo*. The obtained black residue was purified by column chromatography.

*4-(Benzyloxy)aniline (**12a**)*

Yield: 61%, TLC: R_f (ethyl acetate): 0.75.

MS (FD) *m/z* (% rel. int.): 199.5 (100, $[M]^+$).

^{1}H-NMR (300 MHz, DMSO-d_6), δ [ppm] = 7.41–7.25 (m, 5H, Ar-CH), 6.70 (dt, 2H, Ar-CH), 6.48 (dt, 2H, Ar-CH), 4.92 (s, 2H, CH_2), 4.62 (s, 2H, NH_2).

*4-(Benzyloxy)-3-chloroaniline (**12b**)*

Yield: 24%, TLC: R_f (ethyl acetate/hexane 1:4 (v/v)): 0.5.

MS (FD) *m/z* (% rel. int.): 233.5 (100, $[M]^+$).

^{1}H-NMR (300 MHz, DMSO-d_6), δ [ppm] = 7.45 (d, 2H, Ar-CH), 7.40–7.28 (m, 3H, Ar-CH), 6.81 (d, 1H, Ar-CH), 6.65 (d, 1H, Ar-CH), 6.45 (dd, 1H, Ar-CH), 5.06 (s, 2H, CH_2), 5.04 (s, 2H, NH_2).

*3-(Benzyloxy)aniline (**12c**)*

Yield: 35%, TLC: R_f (ethyl acetate/hexane 1:3 (v/v)): 0.48.

MS (FD) *m/z* (% rel. int.): 199.5 (100, $[M]^+$).

^{1}H-NMR (300 MHz, DMSO-d_6), δ [ppm] = 7.41–7.27 (m, 5H, Ar-CH), 6.88 (t, 1H, Ar-CH), 6.19 (t, 1H, Ar-CH), 6.14 (dd, 2H, Ar-CH), 5.04 (s, 2H, CH_2), 4.97 (s, 2H, NH_2).

*2-(Benzyloxy)aniline (**12d**)*

Yield: 53%, TLC: R_f (ethyl acetate/hexane 1:2 (v/v)): 0.64.

MS (FD) *m/z* (% rel. int.): 199.5 (100, $[M]^+$).

^{1}H-NMR (300 MHz, $CDCl_3$), δ [ppm] = 7.45–7.30 (m, 5H, Ar-CH), 6.86–6.70 (m, 4H, Ar-CH), 5.07 (s, 2H, CH_2), 4.40–3.00 (br s, 2H, NH_2).

General procedure IV: coupling with bis-(trichloromethyl) carbonate (triphosgene)

To a stirred solution of 0.37 mmol triphosgene in 4 mL of dry CH_2Cl_2 at 0 °C under argon atmosphere was slowly added, over a period of 30 min, a solution of 1 mmol of the appropriate amino compound and 1.1 mmol *N*-ethyldiisopropylamine in 4 mL of dry CH_2Cl_2. After stirring for 30 min, a solution of 1 mmol **7** and 1.1 mmol *N*-ethyldiisopropylamine in 5 mL of dry CH_2Cl_2 was quickly added. The mixture was warmed to ambient temperature and stirred for another 60 min. The solvent was evaporated, the residue taken up in 30 mL of ethyl acetate and then washed with 5% HCl solution, saturated $NaHCO_3$ solution and water. The organic layer was dried, the solvent removed *in vacuo* and the residue taken up in 15 mL of diethyl ether. After treating the suspension for 15 min in an ultrasonic bath, the remaining solid was filtered off and dried *in vacuo*.

*Ethyl 4,6-dichloro-3-((3-(4-(2-fluoroethoxy)phenyl)-1-(2-methoxy-2-oxoethyl)ureido)methyl)-indole-2-carboxylate (**13a**)*

Yield: 61%, TLC: R_f (Al_2O_3, $CHCl_3$/acetone 4:1 (v/v)): 0.63.

MS (FD) *m/z* (% rel. int.): 539.4 (100, $[M]^+$).

^{1}H-NMR (300 MHz, DMSO-d6), δ [ppm] = 12.44 (s, 1H, In-NH), 8.55 (s, 1H, NH), 7.46 (d, 1H, Ar-CH), 7.26 (m, 2H, Ar-CH), 7.22 (d, 1H, Ar-CH), 6.87 (dd, 2H, Ar-CH), 5.19 (s, 2H, CH_2), 4.79 (m, 1H, CH_2), 4.62 (m, 1H, CH_2), 4.33 (q, 2H, CH_2), 4.23 (m, 1H, CH_2), 4.12 (m, 1H, CH_2), 3.49 (s, 2H, CH_2), 3.46 (s, 3H, OCH_3) 1.32 (t, 3H, CH_3).

*Ethyl 4,6-dichloro-3-((3-(3-chloro-4-(2-fluoroethoxy)phenyl)-1-(2-methoxy-2-oxoethyl)ureido)methyl)-indole-2-carboxylate (**13b**)*

Yield: 70%, TLC: R_f (Al_2O_3, THF/hexane 1:3 (v/v)): 0.22.
MS (FD) *m/z* (% rel. int.): 543.1 (100, $[M\text{-}MeOH]^+$).
^{1}H-NMR (300 MHz, DMSO-d_6), δ [ppm] = 12.39 (s, 1H, In-NH), 8.70 (s, 1H, NH),7.65–7.61 (m, 1H, Ar-CH), 7.47–7.44 (m, 1H, Ar-CH) 7.27–7.22 (m, 2H, Ar-CH), 7.08 (d, 1H, Ar-CH), 5.18 (s, 2H, CH_2), 4.84–4.80 (m, 1H, CH_2), 4.68–4.64 (m, 1H, CH_2), 4.41–4.29 (m, 3H), 4.21–4.19 (m, 1H, CH_2), 3.82 (s, 2H, CH_2), 3.48 (s, 3H, OCH_3), 1.31 (t, 3H, CH_3).

*Ethyl 4,6-dichloro-3-((3-(3-(2-fluoroethoxy)phenyl)-1-(2-methoxy-2-oxoethyl)ureido)methyl)-indole-2-carboxylate (**13c**)*

Yield: 48%, TLC: R_f (Al_2O_3, $CHCl_3$/acetone 10:1 (v/v)): 0.63.
MS (FD) *m/z* (% rel. int.): 539.3 (100, $[M]^+$).
^{1}H-NMR (300 MHz, DMSO-d_6), δ [ppm] = 12.40 (s, 1H, In-NH), 8.69 (s, 1H, NH), 7.46 (d, 1H, Ar-CH), 7.22 (d, 1H, Ar-CH), 7.20–7.05 (m, 3H, Ar-CH), 6.55 (dd, 1H, Ar-CH), 5.20 (s, 2H, CH_2), 4.80 (t, 1H, CH_2), 4.64 (t, 1H, CH_2), 4.33 (q, 2H, CH_2), 4.22 (t, 1H, CH_2) 4.11 (t, 1H, CH_2), 3.85 (s, 2H, CH_2), 3.50 (s, 3H, OCH_3), 1.31 (t, 3H, CH_3).

*Ethyl 4,6-dichloro-3-((3-(2-(2-fluoroethoxy)phenyl)-1-(2-methoxy-2-oxoethyl)ureido)methyl)-indole-2-carboxylate (**13d**)*

Yield: 63%. TLC: R_f (Al_2O_3, $CHCl_3$/acetone 10:1 (v/v)): 0.47.
MS (FD) *m/z* (% rel. int.): 538.9 (100, $[M]^+$).
^{1}H-NMR (300 MHz, DMSO-d_6), δ [ppm] = 12.42 (s, 1H, In-NH), 7.88 (s, 1H, NH), 7.79 (dd, 1H, Ar-CH) 7.46 (dd, 1H, Ar-CH), 7.23 (dd, 1H, Ar-CH), 7.05–6.88 (m, 3H, Ar-CH), 5.19 (s, 2H, CH_2), 4.80 (t, 1H, CH_2), 4.64 (t, 1H, CH_2), 4.35 (q, 2H, CH_2), 4.21 (t, 1H, CH_2) 4.31 (t, 1H, CH_2), 3.87 (s, 2H, CH_2), 3.41 (s, 3H, OCH_3), 1.32 (t, 3H, CH_3).

*Ethyl 4,6-dichloro-3-((1-(2-methoxy-2-oxoethyl)-3-(4-methoxyphenyl)ureido)methyl)-indole-2-carboxylate (**13e**)*

Yield: 67%, TLC: R_f (Al_2O_3, $CHCl_3$/acetone 10:1 (v/v)): 0.42.
MS (FD) *m/z* (% rel. int.): 506.9 (100, $[M]^+$).
^{1}H-NMR (300 MHz, DMSO-d6), δ [ppm] = 12.38 (s, 1H, In-NH), 8.52 (s, 1H, NH), 7.46 (d, 1H, Ar-CH) 7.35–7.30 (m, 2H, Ar-CH), 7.22 (d, 1H, Ar-CH), 6.82 (d, 2H, Ar-CH), 5.18 (s, 2H, CH_2), 4.33 (q, 2H, CH_2), 3.82 (s, 2H, CH_2), 3.69 (s, 3H, OCH_3), 3.48 (s, 3H, OCH_3), 1.31 (t, 3H, CH_3).

*Ethyl 3-((3-(4-(benzyloxy)phenyl)-1-(2-methoxy-2-oxoethyl)ureido)methyl)-4,6-dichloro-indole-2-carboxylate (**16a**)*

Yield: 64%, TLC: R_f (ethyl acetate/$CHCl_3$ 4:1 (v/v)): 0.75.
MS (FD) *m/z* (% rel. int.): 551.8 (100, $[M]^+$).
^{1}H-NMR (300 MHz, DMSO-d_6), δ [ppm] = 12.37 (s, 1H, In-NH), 7.51–7.31 (m, 9H, Ar-CH), 6.91 (d, 2H, Ar-CH), 5.91 (s, 2H, CH_2), 5.05 (s, 2H, CH_2), 4.33 (q, 2H, CH_2), 3.82 (s, 2H, CH_2), 3.49 (s, 3H, OCH_3), 1.32 (t, 3H, CH_3).

*Ethyl 3-((3-(3-(benzyloxy)phenyl)-1-(2-methoxy-2-oxoethyl)ureido)methyl)-4,6-dichloro-indole-2-carboxylate (**16b**)*

Yield: 46%, TLC: R_f (Al_2O_3, $CHCl_3$/acetone 8:1 (v/v)): 0.38.
MS (FD) *m/z* (% rel. int.): 583.4 (100, $[M]^+$).
^{1}H-NMR (300 MHz, DMSO-d_6), δ [ppm] = 12.41 (s, 1H, In-NH), 7.46 (d, 1H, Ar-CH), 7.43–7.31 (m, 9H, Ar-CH), 7.23 (d, 1H, Ar-CH), 5.20 (s, 2H, CH_2), 5.06 (s, 2H, CH_2), 4.33 (q, 2H, CH_2), 3.85 (s, 2H, CH_2), 3.49 (s, 3H, OCH_3), 1.32 (t, 3H, CH_3).

General procedure V: cyclisation

To a stirred suspension of 1.0 mmol of the appropriate ureido compound in 5 mL of EtOH under argon atmosphere, a solution of 0.5 mmol NaOEt in 1 mL of EtOH was added. The mixture was stirred for 60 min at room temperature, then acidified with 10% HCl solution and diluted with CH_2Cl_2. The organic layer was separated and dried with $MgSO_4$. The solvent was removed *in vacuo*, and the crude product purified by column chromatography.

*Ethyl 4,6-dichloro-3-((3-(4-(2-fluoroethoxy)phenyl)-2,4-dioxoimidazolidin-1-yl)methyl)-indole-2-carboxylate (**14a**)*

Yield: 70%, TLC: R_f (Al_2O_3, $CHCl_3$/acetone 4:1 (v/v)): 0.87.
MS (FD) *m/z* (% rel. int.): 507.4 (100, $[M]^+$).
^{1}H-NMR (300 MHz, DMSO-d_6), δ [ppm] = 12.43 (s, 1H, In-NH), 7.48 (d, 1H, Ar-CH), 7.26 (s, 1H, Ar-CH), 7.23 (d, 2H, Ar-CH), 7.04 (d, 2H, Ar-CH), 5.24 (s, 2H, CH_2), 4.82 (t, 1H, CH_2), 4.66 (t, 1H, CH_2), 4.40 (q, 2H, CH_2), 4.31 (t, 1H, CH_2), 4.21 (t, 1H, CH_2), 3.79 (s, 2H, CH_2), 1.37 (t, 3H, CH_3).

*Ethyl 4,6-dichloro-3-((3-(3-chloro-4-(2-fluoroethoxy)phenyl)-2,4-dioxoimidazolidin-1-yl)methyl)-indole-2-carboxylate (**14b**)*

Yield: 86%, TLC: R_f (Al_2O_3, THF/hexane 1:3 (v/v)): 0.82.
MS (FD) *m/z* (% rel. int.): 543.1 (100, $[M]^+$).
^{1}H-NMR (300 MHz, DMSO-d_6), δ [ppm] = 12.43 (s, 1H, In-NH), 7.45 (dd, 2H, Ar-CH), 7.26 (dd, 3H, Ar-CH), 5.24 (s, 2H, CH_2), 4.86–4.84 (m, 1H, CH_2), 4.70–4.68 (m, 1H, CH_2), 4.43–4.36 (m, 3H), 4.33–4.30 (m, 1H, CH_2), 3.79 (s, 2H, CH_2), 1.36 (t, 3H, CH_3).

*Ethyl 4,6-dichloro-3-((3-(3-(2-fluoroethoxy)phenyl)-2,4-dioxoimidazolidin-1-yl)methyl)-indole-2-carboxylate (**14c**)*

Yield: 88%, TLC: R_f (Al_2O_3, $CHCl_3$/acetone 10:1 (v/v)): 0.88.
MS (FD) *m/z* (% rel. int.): 507.2 (100, $[M]^+$).
^{1}H-NMR (300 MHz, DMSO-d_6), δ [ppm] = 12.44 (s, 1H, In-NH), 7.47 (d, 1H, Ar-CH), 7.38 (t, 1H, Ar-CH), 7.25 (d, 1H, Ar-CH), 7.00–6.94 (m, 3H, Ar-CH), 5.25 (s, 2H, CH_2), 4.81 (t, 1H, CH_2), 4.66 (t, 1H, CH_2), 4.39 (q, 2H, CH_2), 4.28 (t, 1H, CH_2) 4.17 (t, 1H, CH_2), 3.80 (s, 2H, CH_2), 1.37 (t, 3H, CH_3).

*Ethyl 4,6-dichloro-3-((3-(2-(2-fluoroethoxy)phenyl)-2,4-dioxoimidazolidin-1-yl)methyl)-indole-2-carboxylate (**14d**)*

Yield: 69%, TLC: R_f (Al_2O_3, $CHCl_3$/acetone 10:1 (v/v)): 0.54.
MS (FD) *m/z* (% rel. int.): 507.2 (100, $[M]^+$).
^{1}H-NMR (300 MHz, DMSO-d_6), δ [ppm] = 12.44 (s, 1H, In-NH), 7.48 (d, 1H, Ar-CH) 7.42 (dt, 1H, Ar-CH), 7.25 (d, 1H, Ar-CH), 7.21 (dt, 2H, Ar-CH), 7.06 (t, 1H, Ar-CH), 5.29 (d, 1H, CH_2), 5.15 (d, 1H, CH_2) 4.69 (t, 1H, CH_2), 4.53 (t, 1H, CH_2), 4.40 (q, 2H, CH_2), 4.28 (m, 1H, CH_2) 4.18 (t, 1H, CH_2), 3.88 (d, 1H, CH_2), 3.72 (d, 1H, CH_2), 1.35 (t, 3H, CH_3).

*Ethyl 4,6-dichloro-3-((3-(4-methoxyphenyl)-2,4-dioxoimidazolidin-1-yl)methyl)-indole-2-carboxylate (**14e**)*

Yield: 77%, TLC: R_f (Al_2O_3, $CHCl_3$/acetone 10:1 (v/v)): 0.85.
MS (FD) *m/z* (% rel. int.): 475.9 (100, $[M]^+$).
^{1}H-NMR (300 MHz, DMSO-d_6), δ [ppm] = 12.43 (s, 1H, In-NH), 7.47 (d, 1H, Ar-CH) 7.25 (d, 2H, Ar-CH), 7.21 (m, 1H, Ar-CH), 7.00 (d, 2H, Ar-CH), 5.24 (s, 2H, CH_2), 4.53 (t, 1H, CH_2), 4.40 (q, 2H, CH_2), 3.78 (s, 2H, CH_2), 3.76 (s, 2H, CH_2), 1.37 (t, 3H, CH_3).

*Ethyl 3-((3-(4-(benzyloxy)phenyl)-2,4-dioxoimidazolidin-1-yl)methyl)-4,6-dichloro-indole-2-carboxylate (**17a**)*

Yield: 81%, TLC: R_f (ethyl acetate/hexane 2:1 (v/v)): 0.92.
MS (FD) *m/z* (% rel. int.): 551.8 (100, $[M]^+$).

^{1}H-NMR (300 MHz, DMSO-d6), δ [ppm] = 12.43 (s, 1H, In-NH), 7.48–7.31 (m, 9H, Ar-CH), 7.23 (d, 1H, Ar-CH), 7.08 (d, 1H, Ar-CH), 5.24 (s, 2H, CH$_2$), 5.13 (s, 2H, CH$_2$), 4.40 (q, 2H, CH$_2$), 3.78 (s, 2H, CH$_2$), 1.36 (t, 3H, CH$_3$).

*Ethyl 3-((3-(3-(benzyloxy)phenyl)-2,4-dioxoimidazolidin-1-yl)methyl)-4,6-dichloro-indole-2-carboxylate (**17b**)*

Yield: 61%, TLC: R_f (Al$_2$O$_3$, CHCl$_3$/acetone 8:1 (v/v)): 0.52.
MS (FD) *m/z* (% rel. int.): 550.8 (100, [M]$^+$).
^{1}H-NMR (300 MHz, DMSO-d$_6$), δ [ppm] = 12.44 (s, 1H, In-NH), 7.47–7.34 (m, 7H, Ar-CH), 7.25 (d, 1H, Ar-CH), 7.05–6.99 (m, 2H, Ar-CH), 6.93 (d, 1H, Ar-CH), 5.24 (s, 2H, CH$_2$), 5.09 (s, 2H, CH$_2$), 4.39 (q, 2H, CH$_2$), 3.80 (s, 2H, CH$_2$), 1.36 (t, 3H, CH$_3$).

General procedure VI: saponification

To a solution of 0.5 mmol of the appropriate ester compound in 9 mL of THF, 1 mL of H$_2$O and 400 μL of 2 M NaOH solution were added. The mixture was stirred at room temperature till completion (TLC-control) and then diluted with 10 mL of ethyl acetate and 10 mL of H$_2$O. The layers were separated, and the aqueous phase was acidified with 2 M HCl. The suspension was stored in the refrigerator overnight, and the precipitate filtered off and dried *in vacuo*.

*4,6-Dichloro-3-((3-(4-(2-fluoroethoxy)phenyl)-2,4-dioxoimidazolidin-1-yl)methyl)-indole-2-carboxylic acid (**15a**)*

Yield: 79%, TLC: R_f (CHCl$_3$/acetone 4:1 (v/v) + 3% HCOOH): 0.49.
MS (FD) *m/z* (% rel. int.): 480.0 (100, [M]$^+$).
^{1}H-NMR (300 MHz, DMSO-d$_6$), δ [ppm] = 11.91 (s, 1H, In-NH), 7.42 (d, 2H, Ar-CH), 7.40 (s, 1H, Ar-CH), 7.12 (s, 1H, Ar-CH), 6.82 (d, 2H, Ar-CH), 5.22 (s, 2H, CH$_2$), 4.78 (t, 1H, CH$_2$), 4.62 (t, 1H, CH$_2$), 4.20 (t, 1H, CH$_2$), 4.10 (t, 1H, CH$_2$), 3.79 (s, 2H, CH$_2$).

*4,6-Dichloro-3-((3-(3-chloro-4-(2-fluoroethoxy)phenyl)-2,4-dioxoimidazolidin-1-yl)methyl)-indole-2-carboxylic acid (**15b**)*

Yield: 46%, TLC: R_f (CHCl$_3$/acetone 4:1 (v/v) + 3% HCOOH): 0.36.
MS (FD) *m/z* (% rel. int.): 514.7 (100, [M]$^+$).
^{1}H-NMR (300 MHz, DMSO-d$_6$), δ [ppm] = 12.11 (s, 1H, In-NH), 7.44 (s, 2H, Ar-CH), 7.31–7.23 (m, 2H, Ar-CH), 7.15 (d, 1H, Ar-CH), 5.31 (s, 2H, CH$_2$), 4.87–4.82 (m, 1H, CH$_2$), 4.70–4.68 (m, 1H, CH$_2$), 4.44–4.38 (m, 1H, CH$_2$), 4.33–4.29 (m, 1H, CH$_2$), 3.82 (s, 2H, CH$_2$).

*4,6-Dichloro-3-((3-(3-(2-fluoroethoxy)phenyl)-2,4-dioxoimidazolidin-1-yl)methyl)-indole-2-carboxylic acid (**15c**)*

Yield: 77%, TLC: R_f (CHCl$_3$/acetone 4:1 (v/v) + 3% HCOOH): 0.41.
MS (FD) *m/z* (% rel. int.): 479.3 (100, [M]$^+$).
^{1}H-NMR (300 MHz, DMSO-d$_6$), δ [ppm] = 12.32 (s, 1H, In-NH), 7.44 (d, 1H, Ar-CH), 7.38 (t, 1H, Ar-CH), 7.21 (d, 1H, Ar-CH), 7.00–6.94 (m, 3H, Ar-CH), 5.27 (s, 2H, CH$_2$), 4.81 (t, 1H, CH$_2$), 4.65 (t, 1H, CH$_2$), 4.28 (t, 1H, CH$_2$) 4.17 (t, 1H, CH$_2$), 3.81 (s, 2H, CH$_2$).

*4,6-Dichloro-3-((3-(2-(2-fluoroethoxy)phenyl)-2,4-dioxoimidazolidin-1-yl)methyl)-indole-2-carboxylic acid (**15d**)*

Yield: 53%, TLC: R_f (CHCl$_3$/acetone 4:1 (v/v) + 3% HCOOH): 0.36.
MS (FD) *m/z* (% rel. int.): 479.2 (100, [M]$^+$).
^{1}H-NMR (300 MHz, DMSO-d$_6$), δ [ppm] = 12.32 (s, 1H, In-NH), 7.44 (d, 1H, Ar-CH) 7.41–7.38 (m, 1H, Ar-CH), 7.26–7.17 (m, 3H, Ar-CH), 7.05 (dt, 1H, Ar-CH), 5.24 (q, 2H, CH$_2$), 4.69 (t, 1H, CH$_2$), 4.53 (t, 1H, CH$_2$), 4.28 (m, 1H, CH$_2$), 4.17 (m, 1H, CH$_2$), 3.80 (q, 2H, CH$_2$).

*4,6-Dichloro-3-((3-(4-methoxyphenyl)-2,4-dioxoimidazolidin-1-yl)methyl)-indole-2-carboxylic acid (**15e**)*

Yield: 54%, TLC: R_f (CHCl$_3$/acetone 4:1 (v/v) + 3% HCOOH): 0.42.
MS (FD) *m/z* (% rel. int.): 447.7 (100, [M]$^+$).
^{1}H-NMR (300 MHz, DMSO-d$_6$), δ [ppm] = 12.23 (s, 1H, In-NH), 7.44 (d, 1H, Ar-CH), 7.24–7.19 (m, 3H, Ar-CH), 7.00 (d, 2H, Ar-CH), 5.27 (s, 2H, CH$_2$), 3.78 (s, 2H, CH$_2$), 3.76 (s, 3H, OCH$_3$).

*3-((3-(4-(Benzyloxy)phenyl)-2,4-dioxoimidazolidin-1-yl)methyl)-4,6-dichloro-indole-2-carboxylic acid (**18**)*

Yield: 87%, TLC: R_f (CHCl$_3$/acetone 1:1 (v/v) +3% HCOOH): 0.60.
MS (FD) *m/z* (% rel. int.): 523.8 (100, [M]$^+$).
^{1}H-NMR (300 MHz, DMSO-d$_6$), δ [ppm] = 12.27 (s, 1H, In-NH), 7.48–7.31 (m, 8H, Ar-CH), 7.18 (d, 1H, Ar-CH), 6.90 (d, 2H, Ar-CH), 5.18 (s, 2H, CH$_2$), 5.04 (s, 2H, CH$_2$), 3.74 (s, 2H, CH$_2$).

General procedure VII: deprotection of benzyl groups

The benzyl-protected compound (0.31 mmol) was dissolved in 200 mL of MeOH at 60 °C. Pd (10%)/C (30 mg) was added and hydrogenated at 1 bar pressure until the reduction was complete (TLC-control). The catalyst was removed by filtration, the solvent removed *in vacuo* and the crude product purified by column chromatography.

*4,6-Dichloro-3-((3-(4-hydroxyphenyl)-2,4-dioxoimidazolidin-1-yl)methyl)-indole-2-carboxylic acid (**20**)*

Yield: 52%, TLC: R_f (CHCl$_3$/acetone 4:1 (v/v) + 3% HCOOH): 0.31.
MS (FD) *m/z* (% rel. int.): 433.7 (100, [M]$^+$).
^{1}H-NMR (300 MHz, DMSO-d$_6$), δ [ppm] = 12.22 (s, 1H, In-NH), 9.62 (s, 1H, OH), 7.40 (d, 1H, Ar-CH), 7.17 (d, 1H, Ar-CH), 7.04 (d, 2H, Ar-CH), 6.76 (d, 2H, Ar-CH), 5.21 (s, 2H, CH$_2$), 3.73 (s, 2H, CH$_2$). ^{13}C-NMR (100.6 MHz, DMSO-d$_6$), δ [ppm] = 169.7 (C = O), 162.8 (C = O), 157.2 (C-OH), 155.2 (C = O), 137.4 (C-quart.), 130.3 (C-quart.), 128.9 (C-quart.), 128.4 (2 CH.), 127.3 (C-quart.), 123.6 (C-quart.), 122.9 (C-quart.), 121.2 (CH), 115.5 (2 CH), 113.2 (C-quart.), 111.6 (CH), 48.8 (CH$_2$), 36.3 (CH$_2$).

*Ethyl 4,6-dichloro-3-((3-(4-hydroxyphenyl)-2,4-dioxoimidazolidin-1-yl)methyl)-indole-2-carboxylate (**19a**)*

Yield: 63%, TLC: R_f (ethyl acetate/hexane 2:1 (v/v)): 0.75.
MS (FD) *m/z* (% rel. int.): 461.0 (100, [M]$^+$).
^{1}H-NMR (300 MHz, DMSO-d$_6$), δ [ppm] = 12.46 (s, 1H, In-NH), 9.73 (s, 1H, OH), 7.48 (d, 1H, Ar-CH), 7.26 (d, 1H, Ar-CH), 7.08 (d, 2H, Ar-CH), 6.81 (d, 2H, Ar-CH) 5.23 (s, 2H, CH$_2$), 4.39 (q, 2H, CH$_2$), 3.77 (s, 2H, CH$_2$), 1.37 (t, 3H, CH$_3$). ^{13}C-NMR (100.6 MHz, DMSO-d$_6$), δ [ppm] = 169.7 (C = O), 159.7 (C = O), 157.2 (C-OH) 155.2 (C = O), 137.7 (C-quart.), 129.4 (C-quart.), 128.4 (C-quart.), 128.3 (2 CH), 127.5 (C-quart.), 123.6 (C-quart.), 122.8 (C-quart.), 121.7 (CH), 115.6 (2 CH), 114.2 (C-quart.), 111.8 (CH), 61.5 (CH$_2$), 48.8 (CH$_2$), 36.3 (CH$_2$), 14.5 (CH$_3$).

*Ethyl 4,6-dichloro-3-((3-(3-hydroxyphenyl)-2,4-dioxoimidazolidin-1-yl)methyl)-indole-2-carboxylate (**19b**)*

Yield: 53%, TLC: R_f (ethyl acetate/hexane 1:1 (v/v)): 0.63.
MS (FD) *m/z* (% rel. int.): 461.4 (100, [M]$^+$).
^{1}H-NMR (300 MHz, DMSO-d$_6$), δ [ppm] = 12.42 (s, 1H, In-NH), 9.69 (s, 1H, OH), 7.46 (d, 1H, Ar-CH), 7.23 (d, 2H, Ar-CH), 6.77–6.73 (m, 3H, Ar-CH), 5.23 (s, 2H, CH$_2$), 4.39 (q, 2H, CH$_2$), 3.78 (s, 2H, CH$_2$), 1.37 (t, 3H, CH$_3$). ^{13}C-NMR (100.6 MHz, DMSO-d$_6$), δ [ppm] = 169.3 (C = O), 161.1 (C = O), 157.8 (C-OH) 154.7 (C = O), 137.8 (C-quart.), 133.4 (C-quart.), 129.6 (C-quart.), 129.3 (CH), 128.6 (C-quart.), 127.4 (C-quart.), 122.8 (C-quart.), 121.6 (CH), 117.3 (CH), 115.0 (CH), 114.0 (CH), 113.9 (C-quart.), 111.8 (CH), 61.5 (CH$_2$), 48.8 (CH$_2$), 36.3 (CH$_2$), 14.5 (CH$_3$).

Radiochemistry

2-[^{18}F]Fluoroethyl tosylate ([^{18}F]FETos)

A solution of aqueous [^{18}F]F$^-$ (500–8000 MBq) produced by the ^{18}O(p,n)^{18}F nuclear reaction on an isotopically enriched [^{18}O]H$_2$O target was added to a solution of 15 μL of 1 M K_2CO_3 and 10–15 mg of Kryptofix® 222 in 0.8 mL of MeCN. The water was removed using a stream of N_2 at 80 °C and co-evaporated to dryness with MeCN (2 × 1 mL). To the dried K/[^{18}F]F$^-$/Kryptofix® 222/carbonate complex, 4 mg (15 mmol) of 1,2-ditosyloxyethane in 1 mL of MeCN was added, and the mixture was heated at 85 °C for 3 min. Purification of the crude product was accomplished using semipreparative HPLC under isocratic conditions (column: Lichrospher RP18-EC5, 250 × 10 mm, eluent: MeCN/H_2O 50:50 (v/v), flow rate: 5 mL/min, R_t: 8 min). After diluting the HPLC fraction containing [^{18}F]FETos with 40 mL of H_2O, the product was loaded on a Sep-Pak C18-column, dried in a stream of N_2 and eluted with 1 mL of DMSO to yield [^{18}F]FETos after a total synthesis time of 50 min, with a radiochemical yield of about 70%.

[^{11}C]Methyl iodide ([^{11}C]CH_3I)

Carbon-11 was produced at the PET Center Tübingen as [^{11}C]CO_2 with a PETtrace cyclotron (GE Medical Systems, Uppsala, Sweden) by irradiation of N_2 (0.5% O_2) using the ^{14}N(p,α)^{11}C nuclear reaction with 16.5 MeV protons. [^{11}C]CH_3I was obtained by an automated synthesis module (MeI MicroLab, GE Medical Systems). First, [^{11}C]CO_2 was trapped on a molecular sieve (4 Å) and converted to [^{11}C]CH_4 in the presence of a Ni catalyst and H_2 at 360 °C, and then [^{11}C]CH_4 was reacted with iodine at 760 °C to obtain [^{11}C]CH_3I.

*Automated synthesis of 4,6-dichloro-3-((3-(4-(2-[^{18}F]fluorethoxy)phenyl)-2,4-dioxoimidazolidin-1-yl)methyl)-indole-2-carboxylic acid ([^{18}F]**15a**)*

A solution of 3 mg (6.9 μmol) of **19a** and 2.55 μL of 5 M NaOH in 1 mL of DMSO was transferred to a reaction vessel of an automated synthesis module and tempered for 5 min at 100 °C. After addition of 2.5 to 3 GBq of [^{18}F]FETos in 1 mL of DMSO, the reaction was stirred for 5 min at 100 °C, then diluted with 4 mL of eluent and purified with HPLC (MeCN/0.25 M NH_4OAc (pH 5) 60:40 (v/v), flow rate: 6 mL/min, t_r: 14.6 min). After diluting the HPLC fraction containing the product with 50 mL of H_2O, it was loaded on a solid-phase column (Strata X, Phenomenex), washed with 10 mL of H_2O, dried with N_2 and eluted in a second reaction vessel with 1 mL of EtOH. After addition of 100 μL of a 1 M NaOH solution, the reaction was stirred at ambient temperature for 30 min, diluted with 4 mL of H_2O and again purified by HPLC (MeCN/0.25 M NH_4OAc (pH 5) 40:60 (v/v), flow rate: 6 mL/min, t_r: 7.3 min). The isolated product fraction was transferred into a vessel containing 50 mL of H_2O, passed on a Strata X solid-phase extraction column, washed with 10 mL of H_2O, dried with N_2 and eluted with 1.5 mL of EtOH. The solvent was removed *in vacuo*, and the residue taken up in physiological saline solution to yield 55–90 MBq of [^{18}F]**15a** in a decay-corrected radiochemical yield of 5–7% and specific activities between 24 and 67 GBq/μmol after a synthesis time of about 130 min.

*Automated synthesis of 4,6-dichloro-3-((3-(4-(2-[^{11}C]methoxy)phenyl)-2,4-dioxoimidazolidin-1-yl)methyl)-indole-2-carboxylic acid ([^{11}C]**15e**)*

To the reaction vessel of the automated synthesis module, 1.5 mg (3.5 μmol) of **19a**, 500 μL of DMSO and 20 μL of a 0.33 M NaOEt solution were added. The mixture was tempered for 3 min at 100 °C. After cooling to room temperature, 40 to 45 GBq of [^{11}C]CH_3I, carried by a flow of He-gas, was trapped in the reaction solution. The mixture was heated to 100 °C for 2.5 min, then diluted with 4 mL of HPLC eluent and purified by semipreparative radio-HPLC (stationary phase: Luna 5 μm C18(2), 250 × 10 mm; mobile phase: MeCN/0.25 M NH_4OAc (pH 5) 70:30 (v/v), flow rate: 6 mL/min, t_r: 7.1 min). After diluting the product-containing HPLC fraction with 50 mL of H_2O, it was loaded on a solid-phase extraction column (Strata X, Phenomenex; conditioned with 10 mL EtOH and 10 mL water), washed with 10 mL of H_2O, dried with He and eluted in a second reaction vessel with 1.5 mL of EtOH. After addition of 100 μL of a 1 M NaOH solution, the reaction was stirred at ambient temperature for 30 min, diluted with 4 mL of H_2O and again purified by semipreparative radio-HPLC (stationary phase: Luna 5 μm C18(2), 250 × 10 mm; mobile phase: MeCN/0.25 M NH_4OAc (pH 5) 40:60 (v/v), flow rate: 6 mL/min, t_r: 7.3 min). The isolated product fraction was transferred into a vessel containing 50 mL of H_2O, passed on a Strata X solid-phase extraction column, washed with 10 mL of H_2O, dried with He and eluted with 1.5 mL of EtOH. The solvent was removed *in vacuo* and the residue taken up in physiological saline solution to yield 130–220 MBq of [^{11}C]**15e** in a radiochemical yield of 6–9% and specific activities between 8 and 26 GBq/μmol after a synthesis time of about 85 min.

Conclusion

On the basis of a hydantoin-substituted indole 2-carboxylic acid lead structure, five compounds for the glycine binding site of the NMDA receptor were synthesised. From these reference compounds, the *in vitro* affinity and lipophilicity were determined. They exhibit IC_{50} values ranging from 17 to 453 nM and have log*D* values from 1.51 to 2.53, depending on the substitution pattern and the electronic properties. With these results, labelling precursors for ^{18}F- and ^{11}C-derivatives were synthesised for a two-step synthesis route using the secondary labelling precursors [^{18}F]FETos and [^{11}C]CH_3I. The radiosynthesis of [^{18}F]**15a** and [^{11}C]**15e** was hampered to a high degree by side reactions and partial decomposition of the final product during the ester cleavage. Decay-corrected radiochemical yields of 5–7% and 6–9% were obtained for the final compounds.

Therefore, future efforts will focus on improvement of the radiochemical yields of the compounds, for example by examination of different protecting groups for carboxylic acids and their biological evaluation.

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