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# Synthesis and Evaluation of $^{18}$F- and $^{11}$C-Labelled 9,10-Ethanobenzo[*b*]quinolizinium Derivatives for Imaging of the NMDA Receptor at the TCP-Binding Site

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## Summary

Derivatives of 9,10-ethanobenzo[*b*]quinolizinium are potent antagonists for the TCP-site of the NMDA receptor. Two fluoroethyl-substituted analogues were labelled with fluorine-18 by displacement of the tosylate with [$^{18}$F]fluoride, followed by a Diels-Alder reaction. A methoxy-substituted analogue labelled with carbon-11 was obtained by O-methylation of the corresponding hydroxy precursor with [$^{11}$C]iodomethane. In biodistribution studies in mice with these three radioligands, it was found that they have little ability to penetrate the blood-brain barrier.

**Key Words:** NMDA receptor, TCP-binding site, fluorine-18, carbon-11, 9,10-ethanobenzo[*b*]quinolizinium derivatives, biodistribution

## Introduction

The N-methyl-D-aspartate (NMDA) receptors belong to the group of ionotropic glutamate receptors in the mammalian central nervous system (CNS), and have been shown to be essential for neuronal and behavioral plasticity, and hence have influence on learning and memory processes in the development of the brain.[1] Excessive activation of the receptors, in response to brain injury, lead to cell death, probably caused by an excess accumulation of intracellular $Ca^{2+}$.[2] It seems likely that the NMDA receptor has been implicated as the major factor for a number of neurodegenerative disorders such as ischemia, hypoxic, hypoglycaemic, traumatic CNS injury, AIDS dementia, Alzheimer's disease, and Huntington's chorea.[2,3] Thus, there has been great interest in the development of useful radioligands for imaging the NMDA receptor in living human brain by non-invasive tomographic techiques.


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A site within the NMDA ion channel is specifically identified by [$^3$H]TCP ([1-(2-thienyl)-cyclohexyl]piperidine) binding. The flow of $Ca^{2+}$ through the NMDA receptor channel is inhibited by the binding of TCP-site ligands such as phencyclidine (PCP), ketamin, and MK-801, which are expected to be useful for therapeutic treatment. Several radio-labelled ligands have been developed for *in vivo* studies with positron emission tomography (PET) or single photon emission tomography (SPECT),[4] but none of these ligands has proved to be useful for imaging the NMDA receptor because of their undesirable non-specific interactions with the brain components. The highly lipophilic nature of these ligands was thought to be attributable to non-specific binding *in vivo*.

Recently, a new type of highly hydrophilic TCP-site ligands, 9.10-ethanobenzo [*b*]quinolizinium derivatives (**1**), was reported to be specific to the open state of the NMDA ion channel.[5] In order to develop a tracer for studies of the NMDA TCP-site using PET, we chose this skeleton as a new lead structure. We designed new fluorine-18 ((±)-**2** and (±)-**3**) and carbon-11 ((±)-**4**) labelled ligands based on the 9,10-ethanobenzo[*b*]quinolizinium skeleton, whose non-radiolabelled correspondences showed relatively high *in vitro* affinity to the NMDA receptors ((±)-**2**: $IC_{50}$=47 nM, (±)-**3**: $IC_{50}$=89 nM, (±)-**4**: $IC_{50}$=19 nM) in displacement of the binding of [$^3$H]TCP to rat nervous membranes.[6] We report here in detail the radiosyntheses of two $^{18}$F-labelled compounds ((±)-[$^{18}$F]**2** and (±)-[$^{18}$F]**3**) and a $^{11}$C-labelled compound ((±)-[$^{11}$C]**4**). In addition, *in vivo* study for investigation of biodistribution in mice is also described.

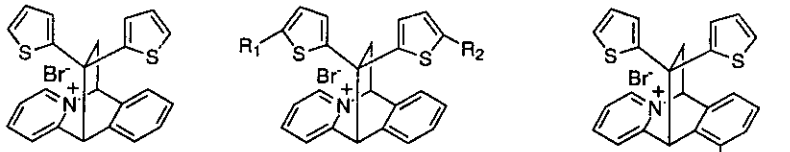
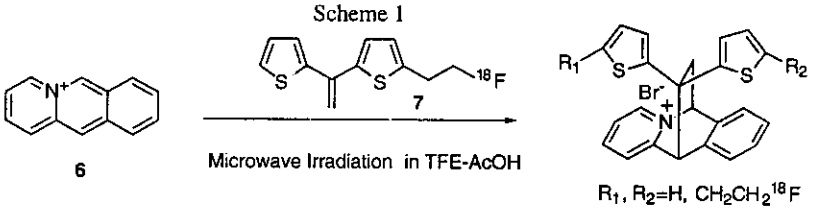



**Fig. 1.** Design of New $^{18}$F- and $^{11}$C-labelled NMDA Antagonist Analogs

## Results and Discussion

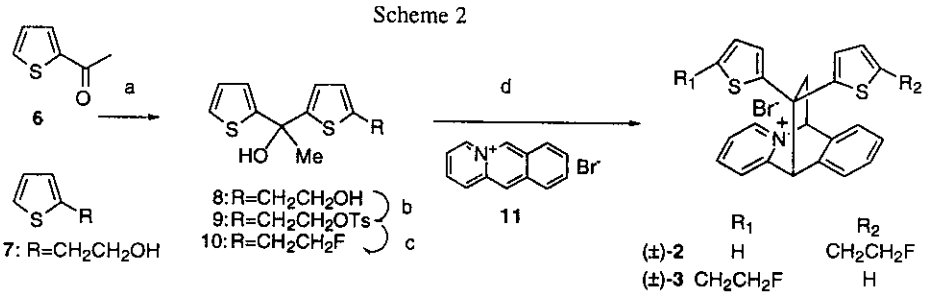
In the investigation of new fluorinated ligands, radio-fluorination *via* $S_N2$ displacement of the mesylate ((±)-**5** having the same skeleton as (±)-**2** or (±)-**3** with $R_1$, $R_2$=H, $CH_2CH_2OMs$) using the complex of potassium [$^{18}$F]fluoride with Kryptofix[2.2.2] was unsuccessful, probably because the $^{18}$F-anion was trapped by the ammonium cation. Then, an alternative synthetic route was devised to include early fluorination of dienophile prior to the Diels-Alder reaction (Scheme 1). We have already established a very efficient D-A reaction by microwave irradiation in a trifluoroethanol (TFE) solution containing acetic acid for several minutes for completion.[6] This method was applied to the synthesis of radio-labelled ligands with $^{18}$F in this study.

Scheme 1



The racemic non-radioactive fluoroethyl-substituted analogues corresponding to (±)-**2** and (±)-**3** were synthesized starting from commercially available thiophene derivatives (**6** and **7**) (Scheme 2).[5] The hydroxyethyl-substituted thiophene **7** was lithiated with *n*-BuLi and reacted with acetylthiophene **6** to give the corresponding alchohol **8**. The bisthienyl dienophile precursor (**8**) was tosylated, followed by fluoride anion substitution with $n$Bu$_4$NF. An attempt to isolate the eliminated olefin from **10** was unsuccessful, because the bisthiophene-substituted ethylene was unstable and easily polymerized. Then, thermal elimination of **10** and *in-situ* trapping by the D-A reaction with **11** was examined. Thus, a mixture of **10** and **11** was heated in TFE to give the corresponding D-A adducts.

Scheme 2




a) **7**, *n*BuLi, THF, then **6**, 69% for **8**, b) TsCl, TEA, $CH_2Cl_2$, 76%,
c) $n$Bu$_4$NF, THF, 52%, d) TFE, reflux, 54% (2:3=1:1).

Their structures were unambiguously determined by $^1$H-$^1$H COSY as well as NOESY spectra. All proton signals were assigned by H-H COSY and a typical NOE correlation indicative of the stereochemistry of (±)-**3** is shown by the arrow "a" in Figure 2. These non-radiolabelled fluorinated derivatives ((±)-**2** and (±)-**3**) were used for the standard in HPLC comparison in radiosynthesis.

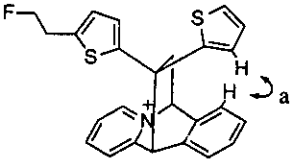


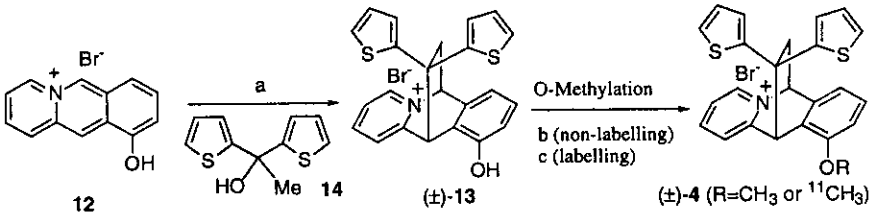

**Fig. 2.** Selected NOE correlation of (±)-**3** indicating its stereochemistry

The radiosynthesis was carried out with the tosylate precursor (**9**) in dry acetonitrile using the complex of potassium [$^{18}$F]fluoride with Kryptofix[2.2.2] as the radiofluorinating agent. The closed TPX vessel was heated in an oil bath at 80°C for 20 min. The mixture was briefly cooled and the product was purified through a silica gel Sep-Pak cartridge. $^{18}$F-Labelled thiophene derivative ([$^{18}$F]**10**) was obtained in 70 % radiochemical yield (not corrected for decay). The following D-A reaction under microwave irridiation in AcOH/TFE for 6 min gave (±)-[$^{18}$F]**2** and (±)-[$^{18}$F]**3** in 20 % radiochemical yield (not corrected for decay) in a 1:1 ratio at the total synthesis

time of 96-98 min including HPLC purification with a reverse-phase ODS column. The specific activity of the product was estimated by UV spectroscopy to be more than 263 MBq/μmol. These $^{18}$F-fluorinated ligands ((±)-[$^{18}$F]**2** and (±)-[$^{18}$F]**3**) were used for the *in vivo* study without further optical resolution.

$^{11}$C-Labelled ((±)-[$^{11}$C]**4**) and its nonradioactive compound were synthesized by *O*-methylation of the corresponding hydroxyl precursor (Scheme 3). The Diels-Alder reaction between the benzo[*b*]quinolizinium (**12**)[8] and **14** in TFE as described above produced the hydroxyl precursor (±)- (**13**), which was used for the methylation This precursor was carefully purified by HPLC to prevent lowering of the specific activity of the labelled compounds. The non-radiolabelled (±)-**4** was obtained by *O*-methylation of the corresponding hydroxy precursor ((±)-**13**) with iodomethane.

Scheme 3




a) TFE, reflux, 87 %, b) $CH_3I$, $K_2CO_3$, DMF, 91 %, c) [$^{11}$C]$CH_3I$, NaH, DMF, 27 %.

Radiosynthesis was carried out in an automated synthesis apparatus for $^{11}$C-labelled compound developed by Suzuki *et al.*[7] Reaction of (±)-**13** with [$^{11}$C]iodomethane in dimethylformamide in the presence of about 2 equivalents of NaH at 50 °C for 5 min gave (±)-[$^{11}$C]**4** with 27 % incorporation of the radioactivity determined by HPLC of the $^{11}$C-reaction mixture. The total synthesis time, including reverse-phase HPLC purification and formulation for injection, was 30 min from the end of bombardment (EOB). The specific activity was about 63 MBq/μmol at the end of synthesis. Radiochemical purity was greater than 98 %. (±)-[$^{11}$C]**4** was used for the *in vivo* study without further optical resolution.

The biodistribution of $^{18}$F activity in the various tissues in ddy mice following intravenous administration of (±)-[$^{18}$F]**2** and (±)-[$^{18}$F]**3** is shown in Table 1 and 2. The result of the biodistribution study with (±)-[$^{11}$C]**4** is shown in Table 3. All three radioligands exhibited rapid clearance from blood and gave very similar distribution patterns. The highest accumulation of radioactivity was in the kidney, which was expected since most compounds containing quaternary ammonium moieties are known to be excreted through the kidney. The radioactivity in the liver and heart was also high and remained high over 30 min. Low uptake of (±)-[$^{18}$F]**2** and (±)-[$^{18}$F]**3** in the bone indicated no extensive defluorination of the tracers *in vivo*. Brain uptake of these three radioligands was initially low and the regional brain distribution did not show selective accumulation in any tissues. These results suggest that these ligands (±)-[$^{18}$F]**2**, (±)-[$^{18}$F]**3**, and

(±)-[$^{11}$C]**4** have a charged cation and have low lipophilicity, so they can not penetrate the intact blood-brain barrier (BBB). Design based on prodrug strategy will be necessary for developing useful *in vivo* radioligands for PET studies of the NMDA receptor. Further studies are now ongoing along this line.

Table 1. Biodistribution of Radioactivity in Tissues of Mice Following Intravenous of (±)-[$^{18}$F]**2**.[a]

| Tissue | 5min | 15min | 30min |
|---|---|---|---|
| Brain | 0.04±0.01 | 0.03±0.03 | 0.05±0.03 |
| Hippocampus | 0.03±0.02 | 0.04±0.06 | 0.06±0.03 |
| Striatum | 0.05±0.01 | 0.03±0.02 | 0.04±0.03 |
| Cortex | 0.05±0.03 | 0.02±0.02 | 0.03±0.01 |
| Cerebellum | 0.07±0.08 | 0.04±0.06 | 0.07±0.03 |
| Lung | 5.99±8.35 | 0.79±1.06 | 1.06±0.43 |
| Liver | 6.22±2.31 | 2.83±2.91 | 5.46±0.88 |
| Kidney | 23.5±9.35 | 17.4±17.4 | 27.3±8.30 |
| Heart | 2.32±0.76 | 2.16±3.17 | 3.37±1.91 |
| Bone | 0.41±0.23 | 1.04±1.88 | 2.40±1.66 |
| Blood | 0.76±0.22 | 0.56±0.46 | 0.76±0.26 |

a) Tissue radioactivity was expressed as %dose/g with means±S.D. from four mice.

Table 2. Biodistribution of Radioactivity in Tissues of Mice Following Intravenous of (±)-[$^{18}$F]**3**.[a]

| Tissue | 5min | 15min | 30min |
|---|---|---|---|
| Brain | 0.02±0.01 | 0.04±0.01 | 0.02±0.01 |
| Hippocampus | 0.02±0.03 | 0.05±0.03 | 0.05±0.01 |
| Striatum | 0.02±0.01 | 0.05±0.02 | 0.04±0.03 |
| Cortex | 0.02±0.01 | 0.03±0.01 | 0.02±0.00 |
| Cerebellum | 0.03±0.03 | 0.04±0.02 | 0.03±0.01 |
| Lung | 0.72±0.71 | 1.40±0.64 | 0.92±0.41 |
| Liver | 4.66±7.24 | 12.4±6.53 | 7.01±1.48 |
| Kidney | 14.0±21.8 | 35.8±16.4 | 22.5±9.19 |
| Heart | 1.41±1.67 | 4.15±2.43 | 3.12±1.80 |
| Bone | 0.30±0.34 | 1.10±0.96 | 0.62±0.38 |
| Blood | 0.44±0.23 | 0.49±0.13 | 0.37±0.01 |

a) Tissue radioactivity was expressed as %dose/g with means±S.D. from four mice.

Table 3. Biodistribution of Radioactivity in Tissues of Mice Following Intravenous of (±)-[$^{11}$C]**4**.[a]

| Tissue | 1min | 5min | 20min |
|---|---|---|---|
| Hippocampus | 0.04±0.01 | 0.02±0.01 | 0.02±0.00 |
| Striatum | 0.03±0.01 | 0.02±0.01 | 0.05±0.01 |
| Cortex | 0.04±0.01 | 0.02±0.02 | 0.07±0.02 |
| Cerebellum | 0.07±0.03 | 0.03±0.01 | 0.07±0.02 |
| Lung | 2.38±0.11 | 2.31±1.16 | 1.32±0.22 |
| Liver | 9.30±1.08 | 10.6±1.75 | 10.5±0.93 |
| Kidney | 17.9±4.70 | 20.8±0.13 | 22.8±4.20 |
| Heart | 4.55±0.68 | 5.97±1.59 | 6.12±1.41 |
| Blood | 1.02±0.27 | 0.57±0.01 | 0.39±0.09 |

a) Tissue radioactivity was expressed as %dose/g with means±S.D. from three mice.

## Experimental

### General

$^1$H-NMR spectra were obtained on a JNM GSX-500 or JNM GX-270 spectrometer with $SiMe_4$ as an internal standard, and IR spectra were recorded with JASCO IR Report 100 spectrometer. Low-resolution FAB mass spectra were obtained on a JEOL JMS-D300 spectrometer, and high-resolution FAB mass spectra (HR-FABMS) were obtained on a JEOL NMS-SX 102-SX spectrometer. Column chromatography was done on Merck kieselgel 60 (70-230 mesh) or Fuji gel FL-60D. Thin-layer chromatography (TLC) was carried out on Merck Kieselgel 60 $F_{254}$ plates. Fluorine-18 was produced from 10% enriched [$^{18}$O]$H_2O$ by the $^{18}O(p,n)^{18}F$ reaction as described previously.[9] Aminopolyether (Kryptofix2,2,2) supported potassium[$^{18}$F]fluoride ([K/2,2,2]$^+$$^{18}F^-$) was prepared by the addition of $K_2CO_3$(1.5mg) and Kryptofix2,2,2 (4.5mg) to the irradiated water in a TPX (polymetylpentene) vessel and subsequent removal of the water by co-evaporation with dry acetonitrile as reported previously.[9] Radiochemical yields were expressed as the percentage of the initial activity of the [$^{18}$F]fluorinating agent. Carbon-11 was generated by the $^{14}N(p, \alpha)^{11}C$ nuclear reaction using a CYPRIS HM-18 cyclotron (Sumitomo Heavy Industries, Ltd.). Preparation of [$^{11}$C]$CH_3I$ and $^{11}$C-metylations was carried out automatically by using a synthetic apparatus for $^{11}$C-labelled compounds developed by Suzuki *et al.*[6]

**1-(5'-Hydroxyethyl-2'-thienyl)-1-(2'-thienyl)ethanol (8).** A solution of n-BuLi (1.56 M in hexane, 30 mL, 46.8 mmol) was slowly added over 20 min into a solution of 2-(2'-thienyl)ethanol (**7**, 2.6ml, 23.4 mmol) in THF (50 mL) at -78°C, and the reaction mixture was stirred at -78°C for 2 hours. A solution of 2-acetylthiophene **6** (2 mL, 18 mmol) in THF (10mL) was added into the above reaction mixture at -78°C and the mixture was allowed to warm up to room temperture, and stirred for 16 hours. The reaction was quenched with saturated $NaHCO_3$ solution and extracted with ethyl acetate. Then the organic layer was dried over $Na_2SO_4$. Filtration and evaporation gave a crude oil which was chromatographed on a silica gel column (hexane:AcOEt=10:1) to provide **8** as a brown gum (3.15 g, 69%). $^1$H-NMR ($CD_3OD$) δ: 1.97 (3H, s), 2.95 (2H, dt, $J$ = 0.1, 6.9), 3.72 (2H, t, $J$ = 6.9), 6.65 (1H, dt, $J$ = 0.1, 3.3), 6.74 (1H, d, $J$ = 3.6), 6.90 (1H, dd, $J$ = 3.6, 5.3), 6.94 (1H, dd, $J$ = 1.3, 3.6), 7.24 (1H, dd, $J$ = 1.7, 5.3), IR (neat) cm$^{-1}$: 3400.

**1-(5'-*p*-Toluenesulfonylethyl-2'-thienyl)-1-(2'-thienyl)ethanol (9).** *p*-Toluenesulfonyl chloride (2.52 g, 13.2 mmol) was added into a solution of **8** (3.15 g, 12.4 mmol) in anhydrous pyridine (20 mL) at 0°C and the reaction mixture was cooled at -4°C for 40 hours, and then diluted with ether. The organic layer was washed with brine and dried over $Na_2SO_4$. Filtration and evaporation gave a crude oil which was chromatographed on a silica gel column (hexane:AcOEt=10:1) to provide **9** as a colorless oil (3.87 g, 76%). $^1$H-NMR ($CD_3OD$) δ: 1.95 (3H, s), 2.41 (3H, s), 3.06 (2H, dt, $J$ = 0.1, 6.3), 4.14 (2H, t, $J$ = 6.6), 6.58 (1H, dt, $J$ = 0.1, 3.6), 6.71 (1H, d, $J$ = 3.6), 6.89-6.94 (2H, m), 7.26 (1H, dd, $J$ = 1.7, 4.6), 7.35 (2H, d, $J$ = 8.3), 7.68 (2H, d, J=8.3), IR (neat) cm$^{-1}$: 1350, FAB Mass (*m/z*): 410 ($M^+$).

**1-(5'-Fluoroethyl-2'-thienyl)-1-(2'-thienyl)ethanol (10).** Tetrabuthylammonium fluoride (1.0 M in THF, 1mL, 1 mmol) was added into a solution of **9** (97 mg, 0.23 mmol) in THF (5 mL) at 50°C and stirred for 20 min. The solvent was evaporated, and the residue was chromatographed on a silica gel column (hexane:AcOEt=10:1) to provide **10** as a pale yellow oil

(32.5 mg, 52%). $^1$H-NMR ($CD_3OD$) δ: 1.98 (3H, s), 3.13 (2H, dt, $J$ = 6.3, 24.1), 4.56 (2H, t, $J$ = 6.3, 47.2), 4.60 (1H, bs), 6.70 (1H, d, $J$ = 3.3), 6.75 (1H, d, $J$ = 3.3), 6.91 (1H, dd, $J$ = 3.6, 4.9), 6.94 (1H, dd, $J$ = 1.3, 3.6), 7.26 (1H, dd, $J$ = 1.3, 4.9), IR (neat) cm$^{-1}$: 3400.

**(±)-12-(5-Fluoroethyl-2-thienyl)-12-(2-thienyl)-9,10-ethano-4a-azonia-anthracene bromide ((±)-2 and (±)-3).** Benzo[*b*]quinolizinium bromide (**11**, 18 mg, 67 μmol)[5] was added into a solution of **10** (18 mg, 67 μmol) in 2,2,2-trifluoroethanol at 50°C and stirred for 24 hours. The solvent was evaporated, and the residue was purified by HPLC (Column: Nakarai 5C-18 10.0x250 mm, Solvent: $H_2O$ containing 0.1% TFA: MeOH=9:11, Flow rate: 7 mL/min) to provide **2** (10.1 mg, 29%) and (±)-**3** (8.7 mg, 25%) as a brown gum. (±)-**2**: $^1$H-NMR ($CD_3OD$) δ: 3.00 (2H, dt, $J$ = 6.2, 18.8), 3.08 (1H, dd, $J$ = 1.8, 14.4), 3.39 (1H, dd, $J$ = 3.4, 14.4), 4.46 (2H, dt, $J$ = 6.2, 41.2), 5.89 (1H, s), 6.46 (1H, d, $J$ = 3.7), 6.54 (1H, d, $J$ = 3.7), 6.61 (1H, d, $J$ = 2.3), 6.84 (1H, dd, $J$ = 3.7, 5.3), 6.99 (1H, dd, $J$ = 0.9, 3.7), 7.25 (1H, dd, $J$ = 0.9, 5.3), 7.33 (1H, dd, $J$ = 1.1, 7.6), 7.37 (1H, dd, $J$ = 1.1, 7.6), 7.46 (1H, d, $J$ = 7.3), 7.65 (1H, d, $J$ = 6.9), 7.86 (1H, t, $J$ = 6.4), 8.01 (1H, d, $J$ = 7.8), 8.34 (1H, dt, $J$ = 1.1, 7.8), 9.16 (1H, d, $J$ = 6.0), IR (neat) cm$^{-1}$: 1680, HR-FAB Mass (*m/z*): calcd for $C_{25}H_{22}ONS_2$: 418.1099, found: 418.1109. **3**: $^1$H-NMR ($CD_3OD$) δ: 3.03 (2H, dt, $J$ = 6.0, 25.2), 3.08 (1H, dd, $J$ = 1.8, 3.6), 3.39 (1H, dd, $J$ = 3.6, 14.6), 4.49 (2H, dt, $J$ = 6.1, 47.2), 5.89 (1H, s), 6.48 (1H, bs), 6.63 (1H, d, $J$ = 3.7), 6.66 (1H, dd, $J$ = 1.1, 3.7), 6.75 (1H, dd, $J$ = 3.6, 5.3), 6.81 (1H, d, $J$ = 3.6), 7.18 (1H, dd, $J$ = 1.2, 5.1), 7.31 (1H, dt, $J$ = 1.1, 7.5), 7.36 (1H, dd, $J$ = 1.1, 7.5), 7.43 (1H, d, $J$ = 7.3), 7.65 (1H, d, $J$ = 7.1), 7.87 (1H, t, $J$ = 6.4), 8.09 (1H, d, $J$ = 7.9), 8.36 (1H, dt, $J$ = 1.4, 8.0), 9.16 (1H, d, $J$ = 6.0), IR (neat) cm$^{-1}$: 1680, HR-FAB Mass (*m/z*): calcd for $C_{25}H_{22}ONS_2$: 418.1099, found: 418.1096.

**(±)-12,12-Bis(2-thienyl)-9,10-ethano-8-hydroxy-4a-azoniaanthracene bromide (13).** 8-Hydroxybenzo[b]quinolizinium bromide (**12**, 87 mg, 0.32 mmol)[8] was added into a solution of 1,1-di(2,-thienyl)ethanol (**14**, 121 mg, 0.58 mmol) in 2,2,2-trifluoroethanol/acetic acid (9:1, 3 mL) and the mixture was heated to reflux with stirring for 48 hours. The solvent was evaporated, and the residue was chromatographed on a silica gel column ($CHCl_3$:MeOH=98:2) to provide (±)-**13** as a colorless oil (32.5 mg, 52%). $^1$H-NMR ($CD_3OD$) δ: 3.03 (1H, dd, $J$ = 2.2, 14.5), 3.51 (1H, dd, $J$ = 3.3, 14.5), 6.01 (1H, s), 6.60 (1H, dd, $J$ = 1.2, 3.3), 6.76-6.93 (4H, m), 7.05 (1H, d, $J$ = 2.6), 7.09-7.24 (4H, m), 7.74 (1H, dt, $J$ = 1.3, 6.3), 8.01 (1H, d, $J$ = 7.6), 8.34 (1H, t, $J$ = 7.6), 9.16 (1H, d, $J$ = 6.3), IR (neat) cm$^{-1}$: 3450, 3000, HR-FAB Mass (*m/z*): calad for $C_{23}H_{18}ONS_2$: 388.0830, found: 388.0826.

**(±)-12,12-Bis(2-thienyl)-9,10-ethano-8-methoxy-4a-azoniaanthracene bromide (4).** Iodomethane (1.1 μL, 18 μmol) was added into a solution of (±)-**13** (2.8 mg, 5.9 μmol) in dimethylformamide in the presence of potassium carbonate (0.9 mg, 6.5 μmol) and stirred for 24 hours at room temperature. The solvent was evaporated, and the residue was chromatographed on a silica gel column ($CHCl_3$:MeOH=10:1) to provide (±)-**4** as a pale yellow oil (2.6 mg, 91%). $^1$H-NMR ($CD_3OD$) δ: 3.05 (1H, dd, $J$ = 2.6, 14) 3.43 (1H, dd, $J$ = 3.3, 14.2), 3.82 (3H, s), 6.07 (1H, s), 6.59 (1H, bs), 6.60-6.96 (4H, m), 7.00 (1H, d, $J$ = 1.3), 7.18-7.37 (4H, m), 7.87 (1H, dt, $J$ = 1.7, 6.3), 8.06 (1H, d, $J$ = 7.3), 8.34 (1H, dt, $J$ = 1.7, 7.9), 9.17 (1H, d, $J$ = 6.3), IR (neat) cm$^{-1}$: 3050, 2950, 1630, 1600, HR-FAB Mass (*m/z*): calad for $C_{24}H_{20}ONS_2$: 402.0986, found: 402.0990.

**Radiosynthesis of (±)-[$^{18}$F]2 and (±)-[$^{18}$F]3.** A solution of the tosylate **9** (6 mg, 14.7 μmol) in acetonitrile (250 μL) was added to a TPX vessel containing the [K/2.2.2]$^{18}$F$^-$ (37-74 MBq). The closed vessel was heated in an oil bath at 80 °C for 20 min. The mixture was briefly cooled and passed through a silica gel Sep-Pak cartridge. The column was eluted with hexane/AcOEt (1/1, 3 mL), and the solvent was evaporated to give $^{18}$F-labelled thiophene derivative ([$^{18}$F]**10**) in 70% radiochemical yield (not corrected for decay). The residue diluted with 2,2,2-trifluoroethanol (100 μL) was added into a solution of benzo[*b*]quinolizinium bromide (**12**, 2.6 mg, 10 μmol) in 2,2,2-trifluoroethanol/acetic acid (99:1, 100 μL) and the solvent was irradiated in a microwave (a kitchen-type microwave oven, 500 W) for 6 min. The mixture was evaporated, and the residue was purified by HPLC (Column: SHISEIDO CAPCELL PAK 10.0x250 mm, Solvent: $H_2O$ containing 0.1% TFA: MeOH=1:1, Flow rate: 3 mL/min) to provide (±)-[$^{18}$F]**2** and (±)-[$^{18}$F]**3** in 20% radiochemical yield (not corrected for decay) in 1:1 ratio following an overall preparation time of 96-98 min. Radioactive peaks ($t_R$=18 and 22 min) corresponding to the retention time of authentic non-radiolabelled ligands corresponding to (±)-**2**, (±)-**3** were collected. The specific activity of the products was estimated by UV spectroscopy to be >263 MBq/μmol. The solvent was evaporated and the residual radioactive material was dissolved in saline which was used for animal experiments.

**Radiosynthesis of (±)-[$^{11}$C]4.** [$^{11}$C]Iodomethane was preparated from [$^{11}$C]$CO_2$ by the conventional method, and introduced into a reaction vessel containing (±)-**13** (2.0 mg) and NaH (7.5 μL, 0.5 g/20 mL DMF in DMF (250 μL) at -50°C. The temperature of the reaction vessel was raised to 50°C and the mixture was stirred for 5 min. After addition of a HPLC mobile phase (500 μL), the reaction mixture was transferred onto a HPLC column (Column: SHISEIDO CAPCELL PAK 10.0x250 mm, Solvent: $H_2O$ containing 0.1% TFA: MeOH=9:11, Flow rate: 3.5 mL/min) to provide (±)-[$^{11}$C]**4** in a synthesis time of 30 min from EOB. A radioactive peak ($t_R$=16 min) corresponding to the retention time of authentic non-radiolabelled ligands corresponding to (±)-**4** was collected. The specific activity of the product was estimated by UV spectroscopy to be >63 MBq/μmol. The solvent was evaporated and the residual radioactive material was dissolved in saline which was used for animal experiments.

***In vivo* studies.** The radiolabelled ligands were injected into ddy mouse (8-9 W.O., three or four mise as indicated in the footnote of Talbe 1-3) through the tail vein in a volume of 200 μL with activities ranging from 270-555KBq ([$^{18}$F]-labelled ligands), 5.2-7.2MBq ([$^{11}$C]-labelled ligands). The animals were killed under ether anesthesia at various time points. Samples of blood and the organs of interest were taken, weighed and assayed for radioactivity in a Packard autogamma scintillation counter and corrected for decay. Samples from different brain regions (cerebellum, cortex, striatum and hippocampus) were dissected, weighted, counted. The results are expressed as the percent administered dose per gram of tissue (% dose/g).

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