Synthesis, radiosynthesis and biological evaluation of 1,4-dihydroquinoline derivatives as new carriers for specific brain delivery†

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In spite of numerous reports dealing with the use of 1,4-dihydropyridines as carriers to deliver biological active compounds to the brain, this chemical delivery system (CDS) suffers from poor stability of the 1,4-dihydropyridine derivatives towards oxidation and hydration reactions seriously limiting further investigations *in vivo*. In an attempt to overcome these limitations, we report herein the first biological evaluation of more stable annellated NADH models in the quinoline series as relevant neuroactive drug-carrier candidates. The radiolabeled 1,4-dihydroquinoline [^{11}C]**1a** was prepared to be subsequently peripherally injected in rats. The injected animals were sacrificed and brains were collected. The radioactivity measured in rat brain indicated a rapid penetration of the carrier [^{11}C]**1a** into the CNS. HPLC analysis of brain homogenates showed that oxidation of [^{11}C]**1a** into the corresponding quinolinium salt [^{11}C]**4a** was completed in less than 5 min. An *in vivo* evaluation in mice is also reported to illustrate the potential of such 1,4-dihydroquinoline derivatives to transport a neuroactive drug in the CNS. For this purpose, γ-aminobutyric acid (GABA), well known to poorly cross the brain blood barrier (BBB) was connected to this 1,4-dihydroquinoline-type carrier. After i.p. injection of 1,4-dihydroquinoline-GABA derivative **1b** in mice, a significant alteration of locomotor activity (LMA) was observed presumably resulting from an enhancement of central GABAergic activity. These encouraging results give strong evidence for the capacity of carrier-GABA derivative **1b** to cross the BBB and exert a pharmacological effect on the CNS. This study paves the way for further progress in designing new redox chemical delivery systems.

Introduction

The delivery of drugs to the brain remains a major challenge in the treatment of central nervous system (CNS) disorders. The blood-brain barrier (BBB), a physiological barrier between blood and brain, constitutes the main obstacle for CNS therapy and a serious bottleneck in drug development for CNS diseases. The BBB resides mainly within the endothelial cells of cerebral capillaries that are connected with tight continuous circumferential junctions hampering the passage of hydrophilic drugs to the brain. As a result, more than 98% of small molecules do not cross the BBB while transport of nutriments and hormones that are essential for brain functions are regulated by specific BBB transporters. The BBB being essentially impermeable to hydrophilic and/or charged compounds, one of the most straightforward strategies to surmount this transport problem is to administrate prodrugs sufficiently lipophilic to facilitate their passive diffusion. Among the various pharmacological strategies to target drugs to the brain, one can mention Bodor's chemical delivery systems (CDS). In this approach, the drug is covalently coupled to a lipophilic redox carrier; *i.e.* a 1,4-dihydropyridine derived from nicotinic acid. The first task of this 1,4-dihydropyridine is to increase sufficiently the lipophilicity of the drug to facilitate crossing of the BBB *via* passive diffusion. Once in the brain, the 1,4-dihydropyridine moiety is converted into the corresponding pyridinium salt by oxido-reductases. The so-obtained permanently charged carrier-drug can no longer cross back the BBB by passive diffusion and remains locked in the CNS. Subsequently, the parent drug is released from the carrier by enzymatic cleavage providing brain-specific release of the active drug while reducing putative peripheral effects. This redox CDS has been explored for brain targeting of a wide variety of drug classes including steroids, antiviral agents, anti-tumor agents, antibiotics, anticonvulsants and neuropeptides.[1] A serious limitation observed with the 1,4-dihydropyridine carrier arose from its too high sensitivity to oxidation and/or hydration on the enamine 5,6-double bond of the dihydropyridine ring.[2] Although some attempts have been made to address this problem through chemical modifications of the 1,4-dihydopyridine ring,[3] the modest stability of this class of carriers remains an obstacle to further development of this CDS approach.

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In this context, our expertise in the preparation of more stable annellated NADH models in the quinoline series and their use in biomimetic reductions[4] prompted us to evaluate their potential as carriers to target drugs in the CNS.[5] A survey of the literature reveals that little attention has been paid to the evaluation of 1,4-dihydroquinoline derivatives as potential carriers.[6] However, the use of 1,4-dihydroquinolines may display attractive advantages over the more conventional 1,4-dihydropyridines: (1) annelation of the 1,4-dihydopyridine ring protects the enamine 5,6-double bond from hydration, (2) additional electron withdrawing or donating functional groups (FG) may be easily installed on the phenyl ring, offering the possibility to tune the redox potential of the carrier to find a good balance between stabilization of the 1,4-dihydroquinoline at the periphery and its oxidation in the CNS (Fig. 1).

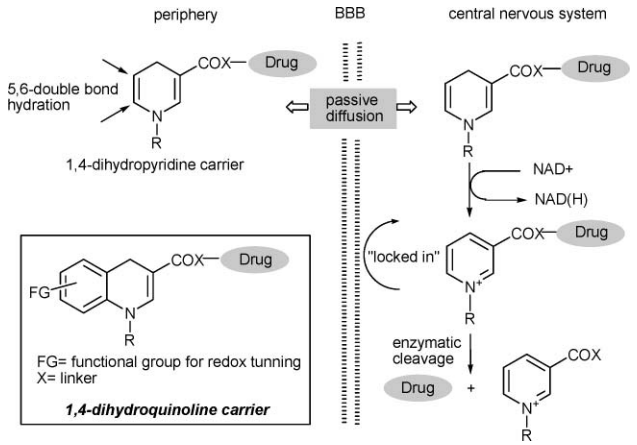


Fig. 1 Bodor's chemical delivery system (CDS) by means of 1,4-dihydropyridines and 1,4-dihydroquinolines as new candidates.

To further explore the potential of such 1,4-dihydroquinolines as carriers, we report herein the labelling of **1a** with carbon-11, a radionuclide used in Positron Emission Tomography (PET). PET is an imaging technique that has become a powerful scientific tool probing biochemical processes *in vivo*. For this purpose, we achieved the radiosynthesis of *N*-[^{11}C]methyl-1,4-dihydroquinoline **1a** and studied its biological behavior in rats (Fig. 2a). This approach has consisted in evaluating the ability of [^{11}C]**1a** to cross the BBB and to be subsequently enzymatically oxidized and "locked-in" in the brain as its corresponding quinolinium salt. As an illustrative application of this new 1,4-dihydroquinoline-type redox carrier system, we also reported its coupling with γ-aminobutyric acid (GABA) derivatives, an inhibitory neurotransmitter well-known for not crossing the BBB owing to its hydrophilic nature and the absence of active transport system.[7] *In vivo* studies in mice are reported herein to give evidence

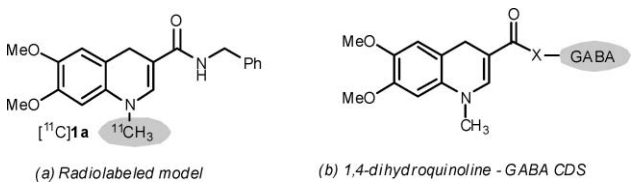


Fig. 2 Design of a new 1,4-dihydroquinoline carrier: (a) radiolabeled model [^{11}C]**1a**, (b) illustrative application to brain delivery of GABA.

of BBB penetration and central activities of administrated 1,4-dihydroquinoline-GABA derivatives by investigating a putative diminution of the locomotor activity (LMA) (Fig. 2b).[8]

Results and discussion

Design of the redox chemical delivery system (lipophilicity, redox potential, linker)

The redox CDS was designed to satisfy several physicochemical parameters regarding the lipophilicity and redox properties of the system and implementation of an appropriate covalent connection between the carrier and GABA compatible with enzymatic cleavage in the brain (Fig. 3). For a given neuropharmacological agent, its ability to gain access to the brain through the BBB by passive diffusion is roughly related to its overall lipophilicity and is reflected by its *n*-octanol-water partition coefficient (*log P* value). Log *P* values around 2 are usually considered as satisfactory to cross the BBB. Consequently, the *log P* values of compounds **1a**, **1b** and **1e** were calculated.[9] As depicted in Fig 3, *log P* values ranged from 1.88 to 2.2 indicating that compounds **1a**, **1b** and **1e** are lipophilic enough to cross the BBB. One can anticipate that the presence of the stabilizing phenyl ring, installed to protect the 5,6-double bond from hydration, previously observed with 1,4-dihydropyridines, may also hamper the required bio-oxidation step of the carrier once in the brain. To overcome this problem, two electron-donating methoxy groups were installed to restore adequate reducing properties of the carrier. Two strategies were investigated to connect GABA to the quinoline moiety leading either to an amide or ester linkage between the drug and the carrier. In such systems, release of GABA derivatives from **1b** or **1d** would be ensured *via in vivo* hydrolysis in the brain by amidase or esterase respectively (Fig 3).

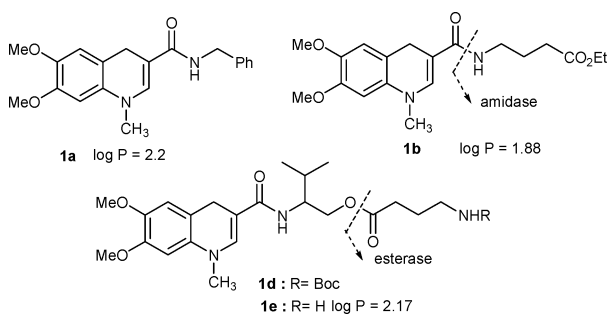
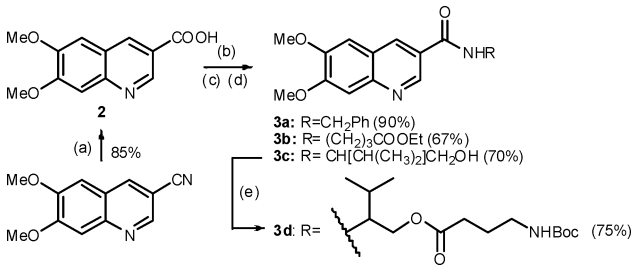


Fig. 3 Design of the redox CDS (lipophilicity, redox potential, linker).

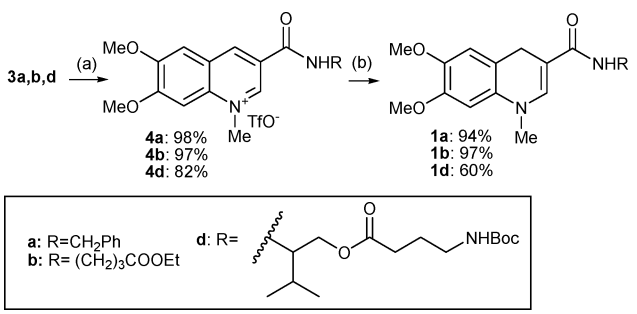
Preparation of 1,4-dihydroquinoline carrier 1a and 1,4-dihydroquinoline-GABA derivatives 1b and 1d

Hydrolysis of the available 6,7-dimethoxyquinoline-3-carbonitrile[10] furnished the corresponding carboxylic acid **2** in 85% yield. The latter was subsequently involved in the coupling with benzylamine, GABA ethyl ester and valinol by means of oxalyl chloride to yield respectively amides **3a–c** in fair to good yields. The amidoalcohol intermediate **3c** was further coupled with N-Boc GABA in the presence of DCC/DMAP to afford **3d** in 72% yield (Scheme 1).



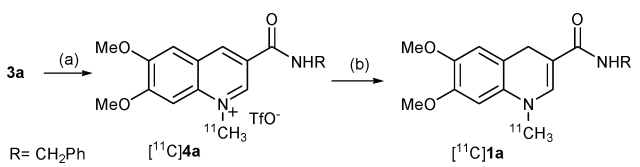
Scheme 1 Preparation of quinoline amides **3a–d**. *Reagents and conditions:* (a) 25% aqueous NaOH/EtOH/reflux/20h (85%); (b) $(COCl)_2$/CH_2Cl_2/few drops of DMF/8h/rt/then benzylamine/NEt_3/−10°C to rt/5h (90%); (c) $(COCl)_2$/CH_2Cl_2/few drops of DMF/2h/rt/then GABA ethyl ester HCl/NEt_3/CH_2Cl_2/rt (67%); (d) $(COCl)_2$/CH_2Cl_2/few drops of DMF/2h/rt/then valinol/NEt_3/−10°C to rt/16h (70%); (e) DCC/DMAP/CH_2Cl_2/rt/16h (75%).

The synthesis was completed by quaternization of quinoline amides **3a**,**b** and **3d** followed by regioselective 1,4-reduction of the corresponding quinolinium salts **4a**,**b** and **4d** (Scheme 2). The quaternization step occurred smoothly at room temperature in the presence of methyltrifluoromethanesulfonate as a methylating agent to afford quinolinium salts **4a**,**b** and **4d** in almost quantitative yields. The reduction took place regioselectively by using sodium dithionite under basic conditions affording the desired 1,4-dihydroquinolines **1a**,**b** in excellent yields and **1d** in somewhat lower yield (60%). It is worth noting that both 1,4-dihydroquinolines **1a**,**b** could be stored several days either at the solid state or in chloroform without any degradation being observed. The higher chemical stability of 1,4-dihydroquinoline derivatives compared to their 1,4-dihydropyridine analogues has already been reported[11] and may constitute a real advantage for further *in vivo* development of these potential drug carriers.



Scheme 2 Access to 1,4-dihydroquinolines **1a–b** and **1d**. *Reagents and conditions:* (a) MeOTf/CH_2Cl_2/rt/2h. (b) $Na_2S_2O_4$/$NaHCO_3$/MeOH/H_2O/rt/2h.

Radiosynthesis of [^{11}C]1a. The radiosynthesis of [^{11}C]**1a** was carried out as illustrated in Scheme 3. The quaternization reaction



Scheme 3 Radiosynthesis of [^{11}C]**1a**. *Reagents and conditions:* (a) [^{11}C]MeOTf/CH_3CN/rt/7min (b) $Na_2S_2O_4$/H_2O/rt/5min then Na_2CO_3/H_2O/rt/3min.

of the quinoline **3a** with [^{11}C]methyl triflate[12] afforded the quaternary salt [^{11}C]**4a** with an incorporation of [^{11}C]CH_3OTf up to 90%. Reduction of [^{11}C]**4a** by using sodium dithionite and sodium carbonate led to the corresponding 1,4-dihydroquinoline [^{11}C]**1a** with a radiochemical yield ranging from 35 to 66% based on initial [^{11}C]methyl triflate (Fig. 4). After HPLC purification and formulation, [^{11}C]**1a** was obtained with a radiochemical purity higher than 99%.

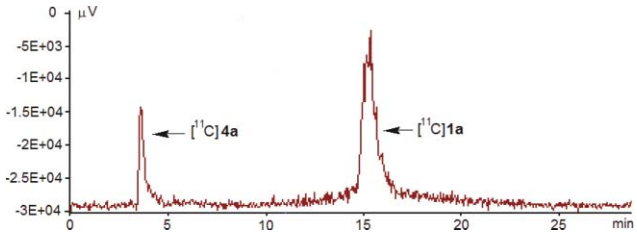


Fig. 4 Radiochromatogram from HPLC analysis after [^{11}C]**1a** radiosynthesis.

Biological investigations of [^{11}C]1a in rats

***In vitro* study.** The chemical stability of [^{11}C]**1a** towards oxidation was firstly investigated *in vitro* on blood samples to establish whether it is stable enough to start developing an *ex vivo* evaluation. To carry out this investigation, [^{11}C]**1a** was added to rat blood and after treatment, the plasma samples were analyzed using reversed phase HPLC analysis. The chromatograms obtained at various time points (5, 10 and 20 min) did not show any trace of the quaternary form [^{11}C]**4a**. Additionally, no by-products which could have arisen from hydration reactions were detected indicating that [^{11}C]**1a** exhibits good stability in rat blood.

***Ex vivo* evaluation.** To evaluate the potential of 1,4-dihydroquinoline **1a** as a carrier *in vivo*, rats were injected into the tail vein with [^{11}C]**1a** and sacrificed at different time intervals (1, 2.5, 5, 10 and 20 minutes) post injection. Then, the brain penetration of [^{11}C]**1a** through the BBB and its oxidation to the corresponding "quaternary form" [^{11}C]**4a** expected *in vivo* were examined. The results indicated a moderate but appreciable and rapid penetration of [^{11}C]**1a** into the CNS wherein the radioactivity brain uptake ranged from 0.78% ± 0.19 ID/g at 1 min to 0.24% ± 0.04 ID/g at 20 min (Fig. 5). The oxidation rate of [^{11}C]**1a** to the

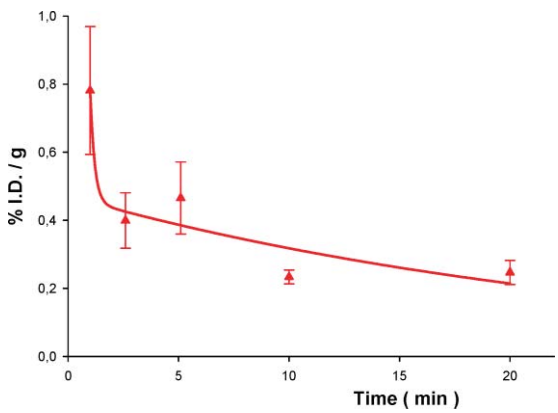


Fig. 5 Time course for radioactivity expressed as %ID/g in brain after i.v. injection of [^{11}C]**1a** in rat.

corresponding quinolinium salt [^{11}C]**4a** was monitored by HPLC analysis from brain homogenates over a period of 20 min. Our findings showed the disappearance of [^{11}C]**1a** beyond 5 min post injection in favour of the quaternary form [^{11}C]**4a** present at 100% which revealed a rapid *in vivo* oxidation of the carrier in the brain (Fig. 6).

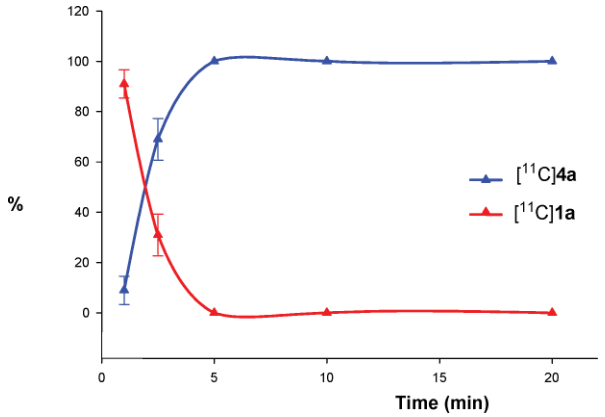


Fig. 6 Percentage of [^{11}C]**1a** and [^{11}C]**4a** in rat brain.

A control experiment was deemed necessary to confirm that the oxidized form [^{11}C]**4a** does not cross the BBB. As expected, after peripheral administration of [^{11}C]**4a** in rats, no radioactivity could be detected in the brain confirming that [^{11}C]**4a** is unable to cross the BBB by passive diffusion or by means of specific transporters.[13]

Biological investigations of 1b in mice

Locomotor activity. The next aspect of this study was to evaluate the CNS activity of the 1,4-dihydroquinoline-GABA derivative **1b** by examining diminution of locomotor activity (LMA) in mice associated with enhancement of central GABAergic activity (Fig. 7). Compound **1d** was revealed to be insoluble in water and was unfortunately excluded from this *in vivo* assay. Compound **1b** was injected intraperitoneally (i.p.) into mice at doses from 50–150 mg/kg and after 30 min the vertical and horizontal LMA was recorded during 1 hour. While a slight but significant decrease in LMA was noted at 50 and 100 mg/kg, LMA was severely affected at 150 mg/kg. At this dose, although not substantial, the diminution in LMA was still evident after 90 min, while LMA was completely restored after 120 min. As expected, no significant

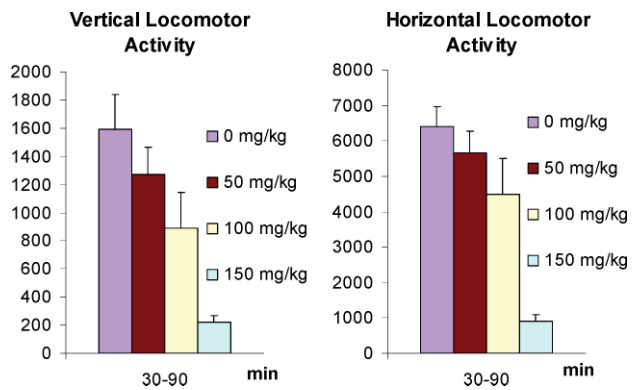


Fig. 7 Effect of 1,4-dihydroquinoline-GABA **1b** on locomotor activity (LMA) in mice.

inhibition of LMA could be observed after administration of GABA at a dose of 150 mg/kg. These findings provide further evidences that 1,4-dihydroquinoline-GABA derivative **1b**, after i.p. administration, is able to reach the brain and exert a central activity after having passed the BBB.

***In vitro* study.** Two hypotheses may be put forward to account for the observed enhancement of central GABAergic activity; (i) 1,4-dihydroquinoline-GABA derivative **1b** or the corresponding quinolinium salt **4b** act as true ligands for GABA receptors; (ii) a delivery mechanism whereby, once in the brain, GABA is released from the quinoline carrier through enzymatic hydrolysis at the amide function. To partly address this question, *in vitro* assays to assess the interaction of 1,4-dihydroquinoline-GABA derivative **1b** and the corresponding quinolinium salt **4b** with $GABA_A$ receptors were performed by measuring their ability to displace [^{3}H] muscimol from $GABA_A$ receptors of rat cortical membrane preparations. It was found that both compounds **1b** and **4b** failed to displace [^{3}H] muscimol at the two concentrations tested (*i.e.* 1 μM and 10 μM). At this stage, although these preliminary *in vitro* assays do not rule out binding of **1b** and/or **4b** to other GABA receptors which could account for the observed enhancement of GABAergic activity, the failure of **1b** and **4b** to displace [^{3}H] muscimol binding argue for a release of GABA from the redox CDS in the CNS.

Conclusion

To conclude, we reported the first biological evaluation of 1,4-dihydroquinoline derivatives as new carriers for specific brain delivery. The radiosynthesis of [^{11}C]**1a** and an *ex vivo* evaluation in rats showed not only that a moderate but significant penetration of the carrier in the CNS was observed, but also that once in the brain, the 1,4-dihydroquinoline [^{11}C]**1a** was rapidly oxidized to the quaternary form [^{11}C]**4a**. An *in vivo* evaluation of 1,4-dihydroquinoline-GABA derivative **1b**, based on alterations of locomotor activity (LMA) in mice, demonstrated the potential of such 1,4-dihydroquinolines to transport neuroactive drugs in the CNS at a sufficiently high concentration and duration to exert a pharmacological effect. These promising findings designate 1,4-dihydroquinoline derivatives as appealing chemical tools for targeting neuroactive drugs to the brain. The potential of such 1,4-dihydroquinolines is currently exploited in the design of original "bio-oxidative" prodrugs for specific delivery of acetylcholinesterase inhibitors to the CNS[14] and in the targeting of PET radioligands for brain imaging.[15]

Experimental

General methods

Melting points (°C) were determined with a WME Köfler apparatus. IR spectra were recorded on a PERKIN ELMER IRFT 1650 spectrometer. Liquids were applied as a film between KBr windows and solids were dispersed in KBr tablets. Absorption bands are given in cm^{-1}. NMR spectra were recorded on BRUKER AVANCE-300 (300 MHz) spectrometer. ^{1}H at 300 MHz, ^{13}C at 75 MHz at 282.5 MHz using $CDCl_3$ or DMSO-d_6 as solvents and with the residual solvent signals as internal standards unless otherwise indicated. The following abbreviations are used to

describe peak pattern: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad). Elemental analyses were carried out at the University of Rouen (Microanalytical Service Laboratory) on a CARLO ERBA 1160. Measurement accuracy is around ±0.4% on carbon. Mass spectra (EI, CI, FAB) were recorded on JEOL JMS AX-500. Analytical thin layer chromatography (TLC) was performed on silica gel plates (Kieselgel $60F_{254}$) from Merck laboratory. Flash chromatography on silica gel was carried out using silica (70–230 mesh ASTM) from Merck laboratory. In the radiochemistry, the labelled compounds were isolated on a HPLC system equipped with a Waters 501 pump, a spectrophotometer ($\lambda = 254$ nm) coupled with a Geiger-Müller probe (SDS). Radiochemical purity analyses were performed on a Merck HPLC system coupled with a NaI radioactive detector (Novelec). For each labeled compound, the characteristics of the column and solvent used will be described. The identities of the labeled compounds were confirmed by their co-elution with the unlabeled reference compound in the HPLC system.

6,7-Dimethoxyquinoline-3-carboxylic acid (2). A mixture of 6,7-dimethoxyquinoline-3-carbonitrile[10] (2.55 g, 11.9 mmol) and 25% aqueous sodium hydroxide (1.47 mL) in ethanol (50 mL) was refluxed with stirring for 20h. After cooling, the reaction mixture was acidified with diluted hydrochloric acid. The precipitate was filtered, dried and purified by recrystallization from $EtOH/H_2O$ (1:1) to yield **2** as a white solid (2.4 g, 85%); mp 262 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.96 (1H, s), 8.61 (1H, s), 7.42 (1H, s), 7.31 (1H, s), 3.83 (3H, s), 3.79 (3H, s); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 167.0, 154.4, 150.3, 147.8, 147.1, 136.5, 122.5, 121.9, 107.7, 107.0, 56.2, 56.1. IR (KBr) ν = 3378, 3062, 1727, 1657, 1614, 1509, 1439, 1381, 1259, 1150, 993 cm^{-1}.

N-Benzyl-6,7-dimethoxyquinoline-3-carboxamide (procedure A) (3a). Oxalyl chloride (0.54 mL, 6.44 mmol) was added to a solution of carboxylic acid **2** (0.5 g, 2.15 mmol) in dichloromethane (30 mL) under nitrogen followed by one drop of freshly distilled DMF. After stirring at room temperature for 2h, dichloromethane was removed under vacuum to afford the acyl chloride intermediate. The residue was dissolved in dichloromethane (30 mL) and was added to a solution of benzylamine (0.3 mL, 2.15 mmol) in dichloromethane pre-cooled to −10 °C. Triethylamine (0.3 mL, 2.15 mmol) was then added and the solution was stirred for 5 hours at room temperature after which solvent was removed. Water was added and the pH of the aqueous layer was adjusted to 5. The organic layer was then washed with water (3 × 100 mL) before being dried over $MgSO_4$ and concentrated. Flash chromatography (eluent: dichloromethane/ethanol 9:1) provided the pure product **3a** (0.62 g, 90%) as a white solid; mp: 168 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.06 (1H, d, J= 1.9 Hz), 8.47 (1H, d, J= 1.9 Hz), 7.43 (1H, s), 7.37 (5H, m), 7.08 (1H, s), 6.60 (1H, brs), 4.71 (2H, d, J= 5.6 Hz), 4.04 (3H, s), 4.01 (3H, s). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.3, 154.3, 150.8, 147.1, 146.1, 138.4, 134.3, 129.2, 128.4, 128.1, 125.7, 123.0, 108.1, 106.0, 56.6, 56.5, 44.6; IR (KBr) ν = 3265, 1657, 1501, 1246, 1146, 1012, 701 cm^{-1}; Anal. Calcd for $C_{19}H_{18}N_2O_3$: C, 70.79; H, 5.63; N, 8.69. Found: C, 70.72; H, 5.87; N, 8.62.

Ethyl 4-(6,7-dimethoxyquinoline-3- carbonylamino)butanoate (3b). Prepared according to procedure A from oxalyl chloride (1.15 mL, 13 mmol), carboxylic acid 2 (0.79 g, 3.4 mmol), ethyl 4-aminobutyrate hydrochloride (0.62 g, 3.7 mmol) and triethylamine (1 mL, 7.1 mmol). Purification of the crude product by flash chromatography on silica gel (eluent: dichloromethane/ethanol 9:1) yielded **3b** as a white solid (0.79 g, 67%); mp 129 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.07 (1H, s), 8.47 (1H, s), 7.44 (1H, s), 7.09 (1H, s), 6.91 (1H, brs), 4.16 (2H, q, J = 7.1 Hz), 4.05 (3H, s), 4.02 (3H, s), 3.60 (2H, q, J = 7.1 Hz), 2.50 (2H, t, J = 7.1 Hz), 2.02 (2H, quint, J = 7.1 Hz), 1.25 (3H, t, J = 7,1 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.3, 159, 9, 154.2, 150.8, 147.1, 146.2, 134.2, 125.8, 123.1, 108.2, 106.0, 61.2, 56.6, 56.5, 40.4, 32.6, 24.6, 14.6. IR (KBr) ν = 3282, 2915, 1730, 1664, 1547, 1505, 1430, 1250, 1186, 1008 cm^{-1}; MS(ESI$^+$) m/z: 715 [2M + Na$^+$], 692.93 [2M + H$^+$], 347.33 [M + H$^+$]; Anal. Calcd for $C_{18}H_{22}N_2O_5$: C, 62.42; H, 6.40; N, 8.09. Found: C, 62.39; H, 6.54; N, 8.08.

N-(1-Hydroxy-3-methylbutan-2-yl)-6,7-dimethoxyquinoline-3-carboxamide (3c). Prepared according to procedure A from oxalyl chloride (2.92 mL, 34 mmol), carboxylic acid **2** (2.0 g, 8.58 mmol), rac-valinol (1.32 g, 12.9 mmol) and triethylamine (1.32 mL, 9.44 mmol). Purification of the crude product by flash chromatography on silica gel (dichloromethane/ethanol 9:1) afforded **3c** (1.89 g, 70%) as a white solid; mp: 191 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.07 (1H, d, J= 1.9Hz), 9.64 (1H, d, J= 1.9 Hz), 8.21 (1H, d, J= 9.0 Hz), 7.43 (2H, s), 4.63 (1H, t, J= 5.6 Hz), 3.96 (3H, s), 3.92 (3H, s), 3.90 (1H, m), 3.53 (2H, m), 1.90 (1H, m), 0.92 (6H, t); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 165.7, 153.6, 150.2, 146.9, 146.1, 133.9, 126.2, 122.4, 107.7, 106.6, 61.6, 57.0, 56.2, 56.1, 28.9, 20.0, 19.0; Anal. Calcd for $C_{17}H_{22}N_2O_4$: C, 64.13; H, 6.97; N, 8.80; Found: C, 64.34; H, 6.56; N, 8.67.

4-*tert*-Butyloxycarbonylaminobutyric acid 2-(6,7-dimethoxy-3-carbonylaminoquinoline)-3-methylbutyl ester (3d). BocGABA (13.0 mg, 0.06 mmol), DMAP (7.7 mg, 0.06 mmol) and DCC (13.0 mg, 0.06 mmol) were successively added to a solution of compound **3c** (16 mg, 0.05 mmol) in dichloromethane and the resulting mixture was stirred overnight at room temperature. The reaction mixture was subsequently filtered. Flash chromatography using ethyl acetate as eluent provided the desired compound **3d** (19 mg, 75%) as a colorless oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.09 (1H, d, J= 1.3 Hz), 8.46 (1H, d, J= 1.3 Hz), 7.39 (1H, s), 7.33 (1H, d, J= 8.5 Hz), 7.04 (1H, s), 4.78 (1H, brs), 4.49 (1H, d, J= 9.6 Hz), 4.23 (2H, m), 4.01 (3H, s), 3.98 (3H, s), 3.22 (1H, m), 2.97 (1H, m), 2.31 (2H, m), 2.04 (1H, m), 1.70 (2H, m), 1.36 (9H, s), 1.04 (6H, d, J= 6.0 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.6, 166.5, 156.7, 154.1, 150.6, 147.0, 146.8, 134.3, 126.0, 123.0, 108.1, 106.1, 79.8, 64.6, 56.6, 56.5, 55.2, 39.7, 31.3, 29.4, 28.7, 26.0, 19.9; IR (KBr) ν = 3321, 2968, 1743, 1692, 1641, 1502, 1245, 1171, 1007, 755 cm^{-1}; MS(ESI$^+$) m/z: 1045.00 [2MK$^+$], 1029.13 [2MNa$^+$], 1007.13 [2MH$^+$], 526.33 [MNa$^+$], 504.27 [MH$^+$]; HRMS (IC$^+$) m/z [M + H$^+$]: calcd for $C_{26}H_{38}N_30_7$, 504.2710; found, 504.2698.

3-(Benzylaminocarbonyl)-6,7-dimethoxy-1-methylquinolinium triflate (procedure B) (4a). To a solution of quinoline **3a** (0.50 g, 1.5 mmol) in dichloromethane was added methyl trifluoromethanesulfonate (0.19 mL, 1.7 mmol) previously treated with K_2CO_3 to avoid the presence of trifluoromethane sulfonic acid. The mixture was stirred at room temperature for 2 hours. The solvent was then removed under vacuum. Ether or heptane was added to a solution of the quinolinium salt in dichloromethane. The precipitate was filtered and dried to yield the pure quinolinium

salt **4a** (0.70 g, 98%) as a white solid; mp: 191 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.32 (1H, s), 9.31 (1H, s), 8.70 (1H, brs, J= 5.6 Hz), 7.52 (1H, s), 7.31 (6H, m), 4.62 (2H, d, J= 5.6 Hz), 4.51 (3H, s), 4.23 (3H, s), 4.06 (3H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 161.7, 159.6, 152.9, 142.9, 138.5, 137.7, 128.9, 128.6, 127.7, 126.5, 126.2, 108.4, 98.0, 90.0, 58.2, 57.3, 46.3, 44.5; IR (KBr) ν = 3344, 3064, 1660, 1511, 1274, 1167, 1032, 640 cm^{-1}; Anal. Calcd for $C_{21}H_{21}F_3N_2O_6S$: C, 51.85; H, 4.35; N, 5.76; S, 6.59. Found: C, 51.76; H, 4.36; N, 5.72; S, 6.38.

3-(Ethoxycarbonylpropylaminocarbonyl)-6,7-dimethoxy-1-methylquinolinium triflate (4b). Quinolinium salt **4b** (0.56 g, 97%) was obtained from quinoline **3b** (0.40 g, 1.15 mmol) and methyl trifluoromethanesulfonate (143 µL, 1.26 mmol) according to procedure B. mp 152 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.43 (1H, s), 9.30 (1H, s), 8.31 (1H, brt, J = 5.3 Hz), 7.55 (1H, s), 7.51 (1H, s), 4.69 (3H, s), 4.25 (3H, s), 4.12 (2H, m), 4.06 (3H, s), 3.48 (2H, m), 2.41 (2H, t, J = 7.5 Hz), 1.98 (2H, quint, J = 7.2 Hz), 1.24 (3H, t, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.6, 161.8, 159.6, 153.0, 144.3, 142.9, 137.9, 126.6, 126.3, 108.5, 98.1, 60.8, 58.2, 57.3, 45.5, 40.2, 32.0, 24.7, 14.5. IR (KBr) ν = 3408, 2940, 1722, 1678, 1511, 1266, 1028 cm^{-1}; Anal. Calcd for $C_{20}H_{25}F_3N_2O_8S$: C, 47.06; H, 4.94; N, 5.49; S, 6.28. Found: C, 47.03; H, 4.96; N, 5.54; S; 6.30; UV/vis (methanol) 262, 362 nm; MS(ESI$^+$) m/z: 361.33 [M-TfO$^-$]$^+$.

N-(1-tert-Butyloxycarbonylaminopropylcarbonyloxy-3-methylbutan-2-yl)-3-carbonylamino-6,7-dimethoxy-1-methyl quinolinium triflate (4d). Quinolinium salt **4d** (0.14 g, 82%) was obtained from quinoline **3d** (0.12 g, 0.25 mmol) and methyl trifluoromethanesulfonate (28 µL, 0.25 mmol) as a colourless translucent solid, according to procedure B. mp: 87 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.52 (1H, s), 9.32 (1H, s), 8.82 (1H, brd, J= 7.9 Hz), 7.87 (1H, s), 7.69 (1H, s), 6.80 (1H, brt, J= 5.0 Hz), 4.80 (3H, s), 4.30 (1H, m), 4.17 (3H, s), 4.14 (2H, m), 4.02 (3H, s), 2.86 (2H, m), 2.26 (2H, t, J= 7.3 Hz), 1.94 (1H, m), 1.56 (2H, m), 1.30 (9H, s), 0.99 (6H, d). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 173.0, 162.8, 158.0, 155.9, 151.9, 145.7, 141.8, 137.3, 126.0, 125.2, 108.6, 99.2, 77.8, 64.2, 57.8, 56.9, 55.2, 54.4, 45.8, 31.2, 29.4, 28.5, 25.2, 19.7, 19.0; IR (KBr) ν = 3355, 2974, 1737, 1705, 1667, 1513, 1281, 1165, 1030 cm^{-1}; Anal. Calcd for $C_{28}H_{40}F_3N_3O_{10}S$: C, 50.37; H, 6.04; N, 6.29; S, 4.80. Found: C, 50.39; H, 6.02; N, 6.36; S, 4.74; MS(ESI$^+$) m/z: 518.33 [M-TfO$^-$]$^+$.

N-Benzyl-1,4-dihydro-6,7-dimethoxy-1-methylquinoline-3-carboxamide (procedure C) (1a). A mixture of $NaHCO_3$ (50 mg, 0.62 mmol) and $Na_2S_2O_4$ (77 mg, 0.51 mmol) was added to a solution of quinolinium salt **4a** (50 mg, 0.10 mmol) dissolved in a mixture of water (4 mL) and methanol (4 mL) deaerated with nitrogen. The mixture was stirred in darkness, at room temperature for 2h. The same amounts of $NaHCO_3$ and $Na_2S_2O_4$ were added and the mixture was stirred for an additional time of 30 min. After addition of ethyl acetate (10 mL) and phase separation, the aqueous layer was extracted with ethyl acetate (3 × 10 mL) and the combined organic phases were washed with water (3 × 10 mL). After drying over $MgSO_4$, solvent was removed under vacuum, giving **1a** (32.7 mg, 94%) as a yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.29 (6H, m), 6.55 (1H, s), 6.34 (1H, s), 5.67 (1H, brt, J = 5.7 Hz), 4.56 (2H, d, J = 5.7 Hz), 3.88 (3H, s), 3.81 (3H, s), 3.75 (2H, s), 3.22 (3H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.7, 148.2, 144.7, 140.6, 139.2, 132.9, 128.8, 128.0, 127.5, 113.1, 98.2, 97.6, 56.4, 56.3, 43.8, 39.0, 26.6; IR ($CHCl_3$) ν = 3392, 1665, 1602, 1578, 1519, 1237, 1101, 1032 cm^{-1}; HRMS (IC$^+$) m/z [M + H$^+$]: calcd for $C_{20}H_{23}N_20_3$, 339.1709; found, 339.1715

Ethyl 4-(1,4-dihydro-6,7-dimethoxy-1-methylquinoline-3-carbonylamino)butanoate (1b). Prepared according to procedure C from $NaHCO_3$ (0.29 g, 3.44 mmol), $Na_2S_2O_4$ (0.43 g, 2.86 mmol) and quinolinium salt **4b** (0.29 g, 0.57 mmol). Compound **1b** (0.20 g, 97%) was obtained as a yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.20 (1H, s), 6.58 (1H, s), 6.32 (1H, s), 5.55 (1H, brs), 4.11 (2H, q, J = 7.2 Hz), 3.87 (3H, s), 3.82 (3H, s), 3.72 (2H, s), 3.39 (2H, q, J = 7.2 Hz) 3.21 (3H, s), 2.39 (2H, t, J = 7.2 Hz), 1.89 (2H, quint, J = 7.2 Hz), 1.25 (3H, t, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.1, 167.2, 148.0, 144.3, 139.1, 133.4, 113.7, 113.3, 99.2, 99.0, 60.1, 56.2, 56.1, 51.6, 41.4, 31.1, 26.4, 25.2, 14.5. MS (ESI$^+$) m/z 363 [M + H$^+$], 333, 247, 203, 188, 160, 115; UV/vis (methanol) 348 nm; HRMS (IC$^+$) m/z [M + H$^+$]: calcd for $C_{19}H_{27}N_20_5$, 363.1920; found, 363.1927

4-tert-Butyloxycarbonylaminobutyric acid 2-(1,4-dihydro-6,7-dimethoxy-1-methyl-3-carbonylaminoquinoline)-3-methylbutyl ester (1d). Prepared according to procedure C from $NaHCO_3$ (206 mg, 2.4 mmol), $Na_2S_2O_4$ (308 mg, 2.0 mmol) and quinolinium salt **4d** (273 mg, 0.4 mmol). Compound **1d** (124 mg, 60%) was obtained as a yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.30 (1H, s), 6.64 (1H, s), 6.37 (1H, s), 5.53 (1H, d, J= 8.3 Hz), 4.84 (1H, brs), 4.23 (3H, m), 3.92 (3H, s), 3.88 (3H, s), 3.73 (2H, s), 3.27 (3H, s), 3.16 (2H, m), 2.40 (2H, m), 1.82 (3H, m), 1.47 (9H, s), 1.01 (6H, m). HRMS (IC$^+$) m/z [M + H$^+$]: calcd for $C_{27}H_{42}N_30_7$, 520.3023; found, 520.3031

[^{11}C]Methyl triflate[12]. [^{11}C]Carbon dioxide was produced by the ^{14}N(p,α)^{11}C nuclear reaction of a gas target filled with nitrogen containing 0.5% oxygen at pressure of 20 bars irradiated with 16 MeV protons (IBA Cyclone 18/9 cyclotron). The [^{11}C]carbon dioxide formed was carried by a nitrogen flow through P_2O_5 and collected in a stainless loop immersed in liquid argon. Then the loop was removed from the liquid nitrogen and brought back to room temperature. The [^{11}C]carbon dioxide was carried by nitrogen flow into a reactor maintained at 0 °C and containing $LiAlH_4$ in tetrahydrofuran (1M, 200 µL) then tetrahydrofuran was removed at 140 °C and after cooling at 0 °C, hydriodic acid (1.0 mL, 54%) was added. The [^{11}C]methyl iodide was distilled under stream of nitrogen at 140 °C and passed at 200 °C through an oven containing silver triflate-impregnated graphitized carbon and the [^{11}C]methyl triflate was obtained.

***N*-Benzyl-1,4-dihydro-6,7-dimethoxy-1-[^{11}C]methylquinoline-3-carboxamide ([^{11}C]1a).** [^{11}C]Methyl triflate was trapped at room temperature into a reactor containing a solution of quinoline **3a** (1 mg, 3.1 µmol) in acetonitrile (150 µL). When all the radioactivity was collected, a solution of $Na_2S_2O_4$ (4 mg, 23 µmol) in water (150 µL) was added in the reactor. After a time of 5 min, a solution of Na_2CO_3 (2 mg, 18.9 µmol) in water (150 µL) was added in the reactor. The resulting mixture was kept for 3 min at room temperature. The purification was performed by reverse-phase semi-preparative HPLC (Macherey Nagel nucleosil 100–5 protect1 column 10 × 250 mm, 4 mL/min, λ = 254 nm, $CH_3CN/H_2O/Et_3N$ 40:60:0.01). The fraction containing the labelled product [^{11}C]**1a** (t_R = 16.5 min) was collected into a flask containing water (25 mL).

The radioactive solution was transferred onto a Macherey Nagel chromabond C18ec previously conditioned with methanol (2 mL) and water (5 mL). The 1,4-dihydroquinoline [^{11}C]**1a** was eluted with ethanol (200 μL). To the ethanolic solution was added 1 mL of an isotonic saline solution containing 10% phosphate buffer (25 mM) pH = 7.4. The final solution was filtered through a 0.22 μm filter into a sterile vial. The radiochemical and chemical purities were determined by reverse-phase analytical HPLC (Macherey Nagel nucleosil 100–5 protect1 column 4.6 × 250 mm, 1 mL/min, λ = 254 nm, $CH_3CN/H_2O/Et_3N$ 40:60:0.01, t_R = 12.5 min). Radiochemical purity exceeded 99% and [^{11}C]**1a** was ready for injection in about 50 min in batches of 185–740 MBq. The specific activity ranges from 14 to 30GBq/μmol at EOS.

Biological investigations of [^{11}C]1a in rats

Animals

Animal experimental procedures were in accordance with the recommendations of the EEC (86/609/EEC) and the French National Committee (decret 87/848) for the care and use of laboratory animals and were approved by the Normandy Regional Animal Ethics Committee (saisine N/05–04-05–05). Sprague-Dawley male rats weighing 250 to 300 g were used in all experiments. The animals were kept at constant temperature (22 °C) and humidity (50%) with 12 h light/dark cycles and were allowed free access to food and water until experiment time. Anesthesia was induced with 5% isoflurane in a gas mixture of nitrous oxide/oxygen (70/30%) and maintained with 1.5–2.5% isoflurane during the entire surgical procedure. Body temperature was monitored rectally and kept close to 37.5 °C. A catheter was inserted into the tail vein.

***In vitro* stability in rat blood.** Compound [^{11}C]**1a** (14 MBq) formulated in a solution containing NaCl 0.9%/phosphate buffer 25 mM pH= 7.4/ethanol (v/v/v 80/10/10) was added to rat blood. After incubation for 5, 10 and 20 min at 37 °C, blood samples (1 mL) were centrifuged (2000 g, 4 min) and plasma was mixed with an equivalent volume of acetonitrile containing 0.01% Et_3N and centrifuged (2000 g, 10 min). Supernatants were filtered and injected onto HPLC (Macherey Nagel nucleosil 100–5 protect1 column 4.6 × 250 mm, 1 mL/min, λ = 254 nm, $CH_3CN/H_2O/Et_3N$ 40:60:0.01).

Brain uptake. Rats were injected with [^{11}C]**1a** (18.5–70.3 MBq) formulated in a solution containing NaCl 0.9%/phosphate buffer 25 mM pH = 7.4/ethanol (v/v/v 80/10/10) and were sacrificed at 1, 2.5, 5, 10 and 20 min post injection (n = 3 per time). The whole brain was quickly dissected. The brain sample was weighed and the radioactivity was measured in a γ-counter (Cobra 2 gamma counter, Packard). Data were expressed as the percentage of injected dose (decay-corrected) per gram of tissue (% ID/g).

Oxidation rate in brain homogenate. Rats were injected with [^{11}C]**1a** (18.5–70.3 MBq) formulated in a solution containing NaCl 0.9%/phosphate buffer 25 mM pH = 7.4/ethanol (v/v/v 80/10/10) and were sacrificed at 1, 2.5, 5, 10 and 20 min post injection (n = 3 per time). Whole brain was quickly removed and analysis were carried out on half-brain crushed in acetonitrile containing 0.01% Et_3N (2.5 mL; UltraTurrax T25, Janke and Kunkel) and centrifuged at 2000 g for 10 min. The radioactivity of the precipitate was measured to quantify the acetonitrile extraction efficiency. Supernatants were filtered and injected onto HPLC (Macherey Nagel nucleosil 100–5 protect1 column 4.6 × 250 mm, 1 mL/min, λ = 254 nm, $CH_3CN/H_2O/Et_3N$ 40:60:0.01, [^{11}C]**4a** : t_R = 3.5 min, [^{11}C]**1a** : t_R = 12.5 min).

Biological investigations of 1b in mice

Animals. Male CD1 mice with body masses of 30–40 g were housed in groups of 15–20 in Makrolon cages (38 × 24 × 18 cm), with free access to water and food and kept in a ventilated room at temperature of 21° ± 1 °C, under 12 h light/dark cycles (light on between 7 a.m. and 7 p.m.). Experiments were carried out between 9 a.m. and 7 p.m. The animals were isolated in small individual cages (27 × 13 × 13 cm) for 15 min prior testing. In these assays, 8 mice were used in each group (N = 8).

Drugs. Dihydroquinoline-GABA derivative **1b** (50–150 mg/kg) was dissolved in dimethylsulfoxide and then diluted in Cremophor EL and NaCl 0.9% (final concentration: 20% DMSO; 10% Cremophor; 70% NaCl 0.9%). The solutions of drugs were prepared fresh and injected i.p. in a volume of 10 mL kg^{-1}.

Locomotor activity. Locomotor activity was measured with a Digiscan Animal Activity Monitor system (Ommitech Electronics Inc., Columbus, OH, USA) which monitored horizontal (locomotion) and vertical (rearing) movements of the animals. The digiscan analyzer was interfaced with a IBM-PC compatible computer using Digipro software. The individual compartments (L = 20; W = 20; H = 30 cm) were put in a dimly lit and quiet room. Horizontal *i.e.* locomotion and vertical movements *i.e.* rearing were expressed as a number of beams crossed over four 15 min periods of testing. Mice were injected with increasing doses of dihydroquinoline **1b** (50–150 mg/kg i.p.) or its vehicle and placed in the actimeters 30 min after injection, for a 60 min test.

In vitro experiments

Evaluation of the binding of 1b and 4b to $GABA_A$ receptors. The incubation mixture consisted of [^{3}H]-muscimol (94 Ci/mmol, 3 nM final concentration, 100 μL), rat cortical membrane suspension as a $GABA_A$ receptor source (2 g/L, 250 μL), phosphate buffer (550 μL) and a solution of compound **1b** or **4b** (100 μL). After a 60.0 min incubation time at room temperature, the reaction was stopped by rapid vacuum filtration through Whatman GF/C filter paper (pre-soaked in a solution of polyethylenimine (0.1%) to reduce binding to filters). Filters were subsequently washed with ice-cold phosphate buffer (3 × 1.5 mL) and placed overnight in 3 mL of Ready-Safe scintillation cocktail (Beckman Coulter, Inc.). Radioactivity was measured using a Wallac liquid scintillation counter. Each experiment was carried out two times. Two concentration points of compounds **1b** and **4b** were evaluated: 10^{-5} and 10^{-6} M.

The specific binding was determined by subtracting non-specific binding (in the presence of 1 mM muscimol) from the total binding (in the absence of 1 mM muscimol and compounds **1b** and **4b**). The occupancy percentage (or percentage of [^{3}H]-muscimol displacement) was determined by subtracting non-specific binding from binding with compounds **1b** and **4b** at 10^{-5} or 10^{-6} M divided by the specific binding. [^{3}H]-muscimol displacement induced by

Table 1

Solutions	Total	Blank	10^{-5} M	10^{-6} M
Phosphate buffer	650 µL	550 µL	550 µL	550 µL
GABA membrane	250 µL	250 µL	250 µL	250 µL
Sample	0	0	100 µL (10^{-4}M)	100 µL (10^{-5} M)
Muscimol 10–3 M	0	100 µL	0	0
[^{3}H]-muscimol	100 µL	100 µL	100 µL	100 µL

compound **1b**: 19% at 10^{-5} M and 6% at 10^{-6} M. [^{3}H]-muscimol displacement induced by compound **4b**: 11% at 10^{-5} M and 8% at 10^{-6} M.

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Notes and references

1 (*a*) For leading reviews on Bodor's chemical delivery systems, see: N. Bodor and P. Buchwald, *Adv. Drug Deliv. Rev.*, 1999, **36**, 229; (*b*) L. Prokai, K. Prokai-Tatrai and N. Bodor, *Med. Res. Chem.*, 2000, **20**, 367; (*c*) N. Bodor and P. Buchwald, *Burger's Medicinal Chemistry and Drug Discovery*, **2**, 534 chapiter 5; (*d*) N. Bodor and L. Prokai, *Drug Discov. Today*, 2002, **7**, 766.

2 (*a*) N. Bodor, H. Farrag and F. Omar, *Bull. Pharm. Sci.*, 1993, **16**, 175; (*b*) E. Pop, T. Loftsson and N. Bodor, *Pharm. Res.*, 1991, **8**, 1044.

3 (*a*) M. Sheha, A. Al-Tayeb, H. El-Sherief and H. Farag, *Bioorg. Med. Chem.*, 2003, **11**, 1865; (*b*) Magda A. El-Sherbeny, Huda S. Al-Salem, Maha A. Sultan, MAhasen A. Radwan, Hassan A. Farag and Hussein I. El-Subbagh, *Arch. Pharm. Pharm. Med. Chem.*, 2003, 445; (*c*) H. A. Hassan, M. Abdel-Aziz, Gamal El-Din A. A. Abuo-Rahma and Hassan H. Farag, *Bioorg. Med. Chem.*, 2009, **17**, 1681.

4 (*a*) S. Gaillard, C. Papamicael, F. Marsais, G. Dupas and V. Levacher, *Synlett*, 2005, 441; (*b*) J-L. Vasse, V. Levacher, J. Bourguignon and G. Dupas, *Chem. Comm.*, 2002, 2256; (*c*) J-L. Vasse, V. Levacher, J. Bourguignon and G. Dupas, *Tetrahedron*, 2003, **59**, 4911; (*d*) C. Vitry, J-L. Vasse, G. Dupas, V. Levacher, G. Quéguiner and J. Bourguignon, *Tetrahedron*, 2001, **57**, 3087; (*e*) V-L. Vasse, P. Charpentier, V. Levacher, G. Dupas, G. Quéguiner and J. Bourguignon, *Synlett*, 1998, **10**, 1144.

5 C. Patteux, L. Foucout, P. Bohn, G. Dupas, J. Leprince, M-C. Tonon, B. Dehouck, F. Marsais, C. Papamicael and V. Levacher, *Org. Bio. Chem.*, 2006, **4**, 817.

6 N. Bodor, H. Farag, M. D. C. Barros, W-M Wu and P. Buchwald, *J. Dug Targ.*, 2002, **10**, 63.

7 (*a*) For previous *in vivo* evaluations of 1,4-dihydropyridine-GABA derivatives able to cross the BBB, see: P. A. Woodard, D. Winwood, M. E. Brewster, K. S. Estes and N. Bodor, *Drug Des. Deliv.*, 1990, **6**, 15; (*b*) K. Matsuyama, C. Yamashita, A. Noda, S. Goto, H. Noda, Y. Ichimaru and Y. Gomita, *Chem. Pharm. Bull.*, 1984, **32**, 4089; (*c*) V. Carelli, F. Liberatore, L. Scipione, G. Giorgioni, A. Di Stefano, M. Impicciatore, V. Ballabeni, F. Calcina, F. Magnanini and E. Barocelli, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 3765.

8 For correlation between alteration of locomotor activity and central GABAergic activity, see: Sk. Jamaluddin and M. K. Poddar, *Neurochemical Research*, 2001, **26**, 439.

9 Prior to log P calculation, the geometry of the molecule was fully optimized at the PM3 level of theory as implemented in the Cerius2 package (Accelrys inc.). The volume of the molecule was then computed and used in the formula published by: N. Bodor and P. Buchwald, *J. Phys. Chem. B.*, 1997, **101**, 3404.

10 P. Charpentier, V. Lobrégat, V. Levacher, G. Dupas, J. Bourguignon and G. Quéguiner, *Tetrahedron. Lett.*, 1998, **39**, 4013.

11 M. E. Brewster, A. Simay, K. Czako, D. Winwood, H. Farag and N. Bodor, *J. Org. Chem.*, 1989, **54**, 3721.

12 D. M. Jewett, *Int. J. Rad. Appl. Instrum. [A]*, 1992, **43**, 1383.

13 (*a*) For previous work related to the active transport of permanently charged pyridinium salts to the brain by means of the BBB choline transporter, see: Z. Zhang, P. R. Lockman, R. K. Mittapalli, D. D. Allen, L. P. Dwoskin and P. A. Crooks, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 5622; (*b*) D. D. Allen, P. R. Lockman, K. E. Roder, L. P. Dwoskin and P. A. Crooks, *Jpet*, 2003, **304**, 1268.

14 P. Bohn, N. Le Fur, G. Hagues, J. Costentin, N. Torquet, C. Papamicaël, F. Marsais and V. Levacher, *Org. Biomol. Chem.*, 2009, **7**, 2612.

15 Thesis F. Gourand, University of Caen, 2007.