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Synthesis and in vitro biological evaluation of carbon-11-labeled quinoline derivatives as new candidate PET radioligands for cannabinoid CB2 receptor imaging

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ABSTRACT

Cannabinoids have been recently proposed as a new family of potential antitumor agents, and cannabinoid receptor 2 (CB2) is believed to be over-expressed in tumor cells. This study was designed to develop new radioligands for imaging of CB2 receptor in cancer using biomedical imaging technique positron emission tomography (PET). Carbon-11-labeled 2-oxoquinoline and 2-chloroquinoline derivatives, [^{11}C]**6a**–**d** and [^{11}C]**9a–d**, were prepared by *O*-[^{11}C]methylation of their corresponding precursors using [^{11}C]CH_3OTf under basic conditions and isolated by a simplified solid-phase extraction (SPE) method in 40–50% radiochemical yields based on [^{11}C]CO_2 and decay corrected to end of bombardment (EOB). The overall synthesis time from EOB was 15–20 min, the radiochemical purity was >99%, and the specific activity at end of synthesis (EOS) was 111–185 GBq/μmol. Radioligand binding assays indicated compounds **6f**, **6b**, and **9f** display potent in vitro binding affinities with nanomolar K_i values and at least 100–2000-fold selectivity for CB2.

1. Introduction

The endogenous cannabinoid system consists of cannabinoid receptors, cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2), and they are G protein-coupled receptors. CB1 is found predominantly in the brain, CB2 is expressed mainly on immune tissues and cancer cells, and thus cannabinoid receptors are associated with various brain, cardiovascular, and cancer diseases.[1,2] Cannabinoid receptors provide an attractive target for the development of therapeutic agents, and many CB1 antagonists and CB2 agonists have been developed and described in the literature.[3] Recent attention has turned to CB2 receptors, since CB2-selective inverse agonists have been proposed as a new family of potential antitumor agents.[4] Cannabinoid receptors also provide a particularly attractive target for the development of imaging agents, and numerous papers have reported the synthesis and evaluation of radioligands for imaging of CB1 receptor in the brain using the biomedical imaging technique positron emission tomography (PET).[5–14] However, only a few papers described ligands for imaging of CB2 receptor in cancer.[15–17] We are interested in the development of enzyme-based and/or receptor-based PET cancer imaging agents. Several CB2 receptor agonists were found to induce apoptosis in different tumor cells.[18–21] Therefore, CB2 receptor has become a valuable clinical target for treating cancer diseases. Recently a novel series of 2-oxoquinoline derivatives have been developed as potent CB2 receptor inverse agonists.[22] To radiolabel therapeutic agents as diagnostic agents for noninvasive imaging of cancer and monitoring of therapeutic efficacy, we report the design, synthesis, labeling, and in vitro biological evaluation of quinoline derivatives, 7-[^{11}C]methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxylic acid (2-phenylethyl)amide ([^{11}C]**6a**), 7-[^{11}C]methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxylic acid [2-(4-chlorophenyl)ethyl]amide ([^{11}C]**6b**), 7-[^{11}C]methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxylic acid (3-phenylpropyl)amide ([^{11}C]**6c**), 7-methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxylic acid [2-(4-[^{11}C]methoxyphenyl)ethyl]amide ([^{11}C]**6d**), 2-chloro-7-[^{11}C]methoxy-8-pentyloxy-1-quinoline-3-carboxylic acid (2-phenylethyl)amide ([^{11}C]**9a**), 2-chloro-7-[^{11}C]methoxy-8-pentyloxy-1-quinoline-3-carboxylic acid [2-(4-chlorophenyl)ethyl]amide ([^{11}C]**9b**), 2-chloro-7-[^{11}C]methoxy-8-pentyloxy-1-quinoline-3-carboxylic acid (3-phenylpropyl)amide ([^{11}C]**9c**), and 2-chloro-7-methoxy-8-pentyloxy-1-quinoline-3-carboxylic acid [2-(4-[^{11}C]methoxyphenyl)ethyl]amide ([^{11}C]**9d**), as new +candidate PET radioligands for CB2 receptor imaging.

2. Results and discussion

2.1. Chemistry

Synthesis of 2-oxoquinoline derivative precursors and standards was accomplished using a modification of the previously

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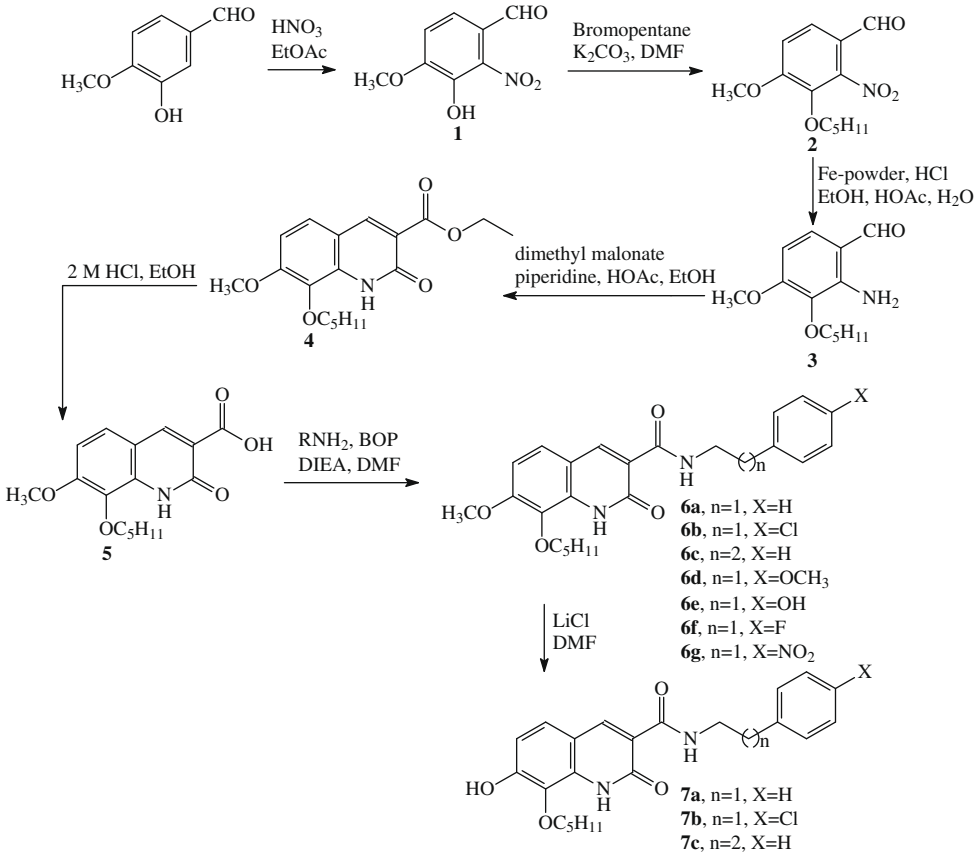
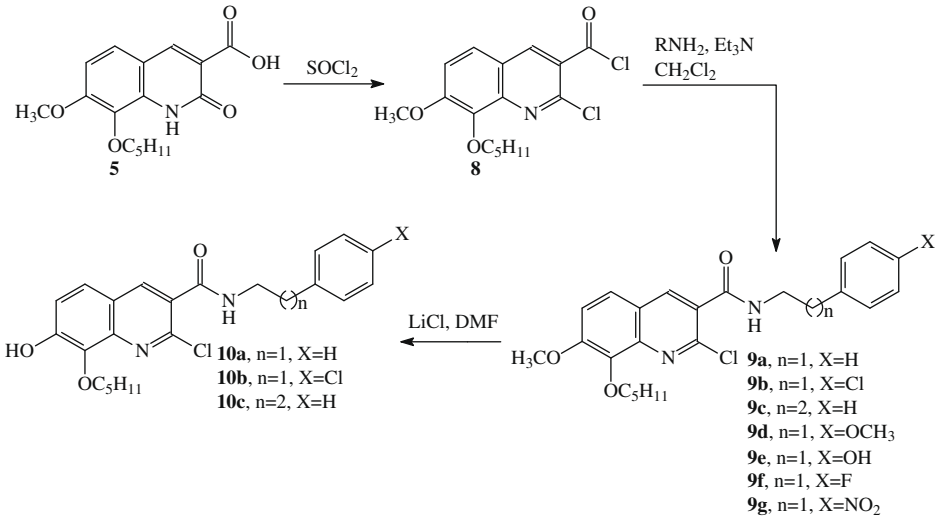
reported procedures.[22] As depicted in Scheme 1, nitration of isovanillin was directly carried out with HNO_3/EtOAc to give compound **1** in 62% yield, instead of expensive nitration reagent nitronium tetrafluoroborate used in the literature,[22] and other typical nitration reagent (HNO_3/H_2SO_4) used in the patent,[23] which only gave **1** as a side product with approximately 10% yield. Nitration of isovanillin with HNO_3 mainly gave **1** (2-position nitration) in ester solvent (EtOAc), whereas it mainly gave 4-position nitration product in acid solvent (H_2SO_4 or HOAc). Alkylation of **1** with bromopentane using the Williamson ether synthesis[23] provided ether **2** in 80% yield. Reduction of **2** with iron powder afforded compound **3** in 85% yield. Transesterification reaction of **3** with dimethyl malonate in the presence of piperidine and HOAc using EtOH as solvent in 2 days produced ethyl ester **4** in 86% yield. The literature procedure (overnight reaction)[22] gave a mixture of ethyl ester **4** and a methyl ester analog. Thus we extended the reaction time to 2 days, since the longer reaction time would favor complete transesterification to form ethyl ester product **4**. Hydrolysis of **4** in HCl formed acid **5** in 84% yield. Then, the two-step literature procedure[22] was modified to a one-step reaction, and acid **5** was directly reacted with amines in the presence of the coupling reagent, (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP), and *N*,*N*-diisopropylethylamine (DIEA) to produce amides **6a–g** in 88–93% yield. Different coupling reagents including BOP, 1-[3-(dimethylamino)propyl]3-ethylcarbodiimide (EDC) and *N*,*N*′-dicyclohexylcarbodiimide (DCC) were tested in different coupling reactions, and BOP was identified as the best coupling reagent. This modification significantly improved the yields and simplified the work-up procedures. Following the literature procedure,[22] we have converted **5** into corresponding acid chloride with $SOCl_2$, which was then reacted with amines in the presence of triethylamine, but we obtained 2-oxoquinoline derivative **6** in very poor yield. Desmethylation of **6a–c** with LiCl in DMF produced phenolic hydroxyl precursors **7a–c** in 80–83% yield. In comparison with the results reported in the literature,[22] several improvements in the synthetic methodology for preparation of 2-oxoquinoline derivatives have been made. They included inexpensive nitration reagent system for regioselective nitration, longer reaction time for complete transesterification, modified synthetic approaches with moderate to excellent chemical yields, and synthesis of new 2-oxoquinoline derivatives **6d**, **6f**, and **7a–c**.

The unsuccessful attempt to prepare 2-oxoquinoline derivatives using the literature method[22] has resulted in the synthesis of new 2-chloroquinoline derivatives. As indicated in Scheme 2, acid **5** was reacted with $SOCl_2$ in toluene[22] to give a minor product of 2-oxoquinoline acid chloride, and the major product was 2-chloroquinoline acid chloride **8**. The direct reaction of acid **5** with $SOCl_2$ without solvent toluene easily formed **8** in 99% yield. Compound **8** was reacted with amines to give 2-chloroquinoline derivatives **9a–g** in 87–93% yield. Likewise, desmethylation of **9a–c** with LiCl in DMF yielded phenolic hydroxyl precursors **10a–c** in 78–83% yield.

Compounds **7a–c** and **6e** are the precursors for positron emitting radionuclide carbon-11 labeling at 7-methoxy position and methoxyphenyl position to prepare 2-oxoquinoline carbon-11 radioligands, and the corresponding reference standards are **6a–c** and **6d**. Compounds **10a–c** and **9e** are the precursors for carbon-11 labeling at 7-methoxy position and methoxyphenyl position to prepare 2-chloroquinoline carbon-11 radioligands, and the corresponding reference standards are **9a–c** and **9d**. Compounds **6g** and **9g**, and **6f** and **9f** can be used as nitro-precursors and fluoro-standards for another positron emitting radionuclide fluorine-18 labeling to prepare corresponding 2-oxoquinoline and 2-chloroquinoline fluorine-18 radioligands. Although the nitrobenzene



Scheme 1. Synthesis of 2-oxoquinoline derivative precursors and standards.

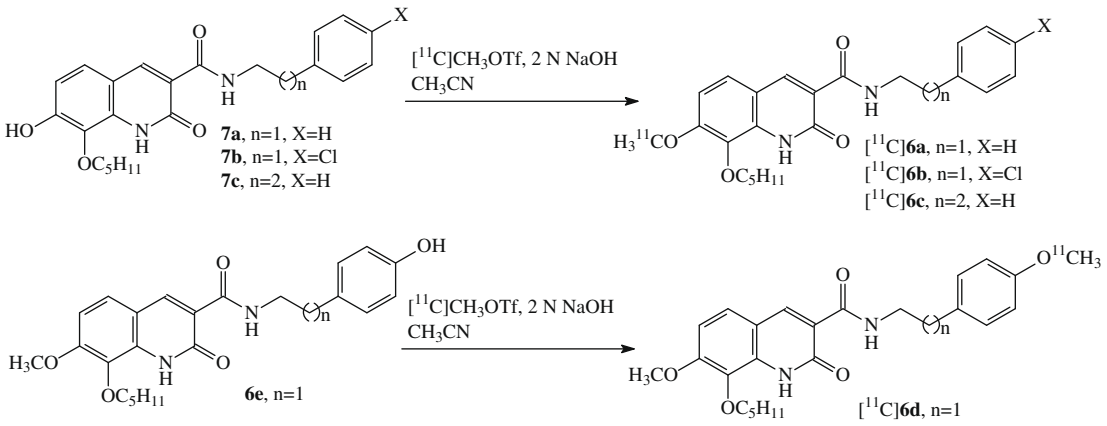


Scheme 2. Synthesis of 2-chloroquinoline derivative precursors and standards.

group is not activated, it is possible to substitute the nitro group by fluorine-18. Recently an effective nucleophilic aromatic [^{18}F]fluorination of nitrobenzene without activating (electron-withdrawing) groups has been developed by using the fluorine-18 labeling reagent Cs[^{18}F]F or TBA[^{18}F]F in TBAOMs solvent instead of K[^{18}F]F/Kryptofix2.2.2 in acetonitrile.[24]

2.2. Radiochemistry

Synthesis of carbon-11 labeled 2-oxoquinoline derivatives [^{11}C]**6a–c** and [^{11}C]**6d** is indicated in Scheme 3. Phenolic hydroxyl precursors **7a–c** and **6e** were labeled by a reactive [^{11}C]methylating agent, [^{11}C]methyl triflate ([^{11}C]CH_3OTf)[25,26] prepared from [^{11}C]CO_2, under basic conditions (2 N NaOH) in acetonitrile through the *O*-[^{11}C]methylation and isolated by a simplified solid-phase extraction (SPE) method[27] to provide target tracers [^{11}C]**6a–d** in 40–50% radiochemical yields, decay corrected to end of bombardment (EOB), based on [^{11}C]CO_2. The large polarity difference between the sodium salt of the phenolic hydroxyl precursor and the labeled *O*-methylated ether product permitted the use of SPE technique for purification of the labeled product from the radiolabeling reaction mixture. Either a C-18 Plus Sep-Pak cartridge or a semi-prep C-18 guard cartridge column was used in SPE purification technique. The crude reaction mixture was treated with sodium bicarbonate and loaded onto the cartridge by gas pressure. Any non-reacted precursor was actually converted into the corresponding sodium salt, which would not stick to the C-18 Sep-Pak. The cartridge was washed with water to remove non-reacted [^{11}C]CH_3OTf, phenolic hydroxyl precursor and reaction solvent, and then the final labeled product was eluted with ethanol. Overall synthesis time was 15–20 min from EOB. SPE technique is fast, efficient and convenient and works very well for the *O*-methylated tracer purification in this case using a free phenolic hydroxyl group in the precursor for radiolabeling.[27] The radiosynthesis was performed in an automated multi-purpose ^{11}C-radiosynthesis module, allowing measurement of specific activity during synthesis.[28,29] The specific activity was estimated in a range of 111–185 GBq/μmol at the end of synthesis (EOS) based on other radiotracers produced using the same targetry conditions in our PET chemistry facility which have been measured by the on-the-fly technique.[29,30] The specific activity is dependent on the production of [^{11}C]CO_2. The cyclotron we used was Siemens radionuclide delivery system (Eclipse RDS-111). The typical proton-beam current and irradiation time for the production of [^{11}C]CO_2 were 55 μA × 30 min. The actual measurement of specific activity at EOS by analytical HPLC[31] is in agreement with this estimation. Chemical purity and radiochemical purity were determined by analytical HPLC.[31] The chemical purity of the precursors and reference standards was >96%. The radiochemical purity of the target tracers was >99% determined by radio-HPLC through γ-ray (PIN diode) flow detector, and the chemical purity of the target tracers was >95% determined by reversed-phase HPLC through UV flow detector.



Scheme 3. Synthesis of carbon-11-labeled 2-oxoquinoline derivatives.

Synthesis of carbon-11 labeled 2-chloroquinoline derivatives [^{11}C]**9a–c** and [^{11}C]**9d** is indicated in Scheme 4, according to the same procedure for preparation of [^{11}C]**6a–c** and [^{11}C]**6d**. The decay corrected radiochemical yields were 40–50%. The specific activity was in a range of 111–185 GBq/μmol at EOS. The chemical purity of the precursors and reference standards was >97%. The radiochemical purity of the target tracers was >99%, and the chemical purity of the target tracers was >95%.

2.3. Lipophilicity

The measured HPLC lipophilicity coefficient (Log *P*) is an important parameter in selecting PET ligand candidates for further evaluations. We calculated Log *P* values of compounds **6a–d**, **6f** and **9a–d**, **9f** based on their retention times that were measured by C-18 HPLC method.[32,33] Retention times in the analytical HPLC system for compounds **6a–d**, **6f** and **9a–d**, **9f** were 5.90, 7.77, 7.16, 5.32, 6.48, 5.45, 5.60, 6.25, 3.62, and 5.49 min, respectively. The calculation results showed the Log *P* values for compounds **6a–d**, **6f** and **9a–d**, **9f** were 2.68, 3.04, 2.94, 2.53, 2.81, 2.57, 2.60, 2.76, 1.88, and 2.58, respectively.

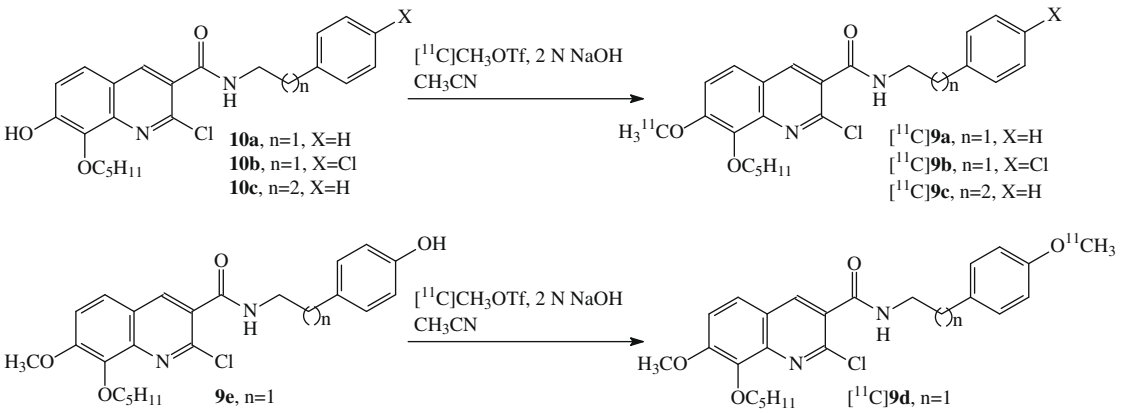
2.4. In vitro binding studies

The CB1 and CB2 receptors activities of the compounds **6a–d**, **6f** and **9a–d**, **9f** were determined by using [^{3}H]CP55940 binding assay.[17] The biological data are summarized in Table 1. These in vitro data indicate some interesting information about the structure–activity relationship (SAR). Overall, 2-oxoquinoline derivatives (nM K_i values) are superior to 2-chloroquinoline derivatives (μM K_i values for compounds **9b**, **9c**, and **9d**). In comparison with the data of compounds **6a**, **6c**, **9a**, and **9c**, the shorter chain length is better for high affinity. Compared with the data of compounds **6b**, **6f**, **9b**, and **9f**, the fluoro-containing compounds are better for high affinity and selectivity than the chloro-containing compounds. The biological data suggest that compounds **6f** (K_i value of 5.4 nM for CB2 and CB1/CB2 ratio of K_i > 1851.8), **6b** (K_i value of 9.37 nM for CB2 and CB1/CB2 ratio of K_i > 1067.2) and **9f** (K_i value of 23.80 nM for CB2 and CB1/CB2 ratio of K_i > 120.79) are the best candidates for in vivo imaging in terms of their affinity and selectivity, since they are potent (nM K_i values) and selective (at least 100–2000-fold)[22] CB2 ligands.

3. Materials and methods

3.1. General

All commercial reagents and solvents from Sigma–Aldrich and Fisher were used without further purification. [^{11}C]CH_3OTf was prepared according to a literature procedure.[25] Melting points were determined on a MEL-TEMP II capillary tube apparatus and were uncorrected. ^{1}H NMR spectra were recorded on Varian



Scheme 4. Synthesis of carbon-11-labeled 2-chlorooquinoline derivatives.

Table 1
CB1 and CB2 binding affinity and selectivity of quinoline derivatives

Compounds	CB1		CB2		CB1/CB2 ratio of K_i[c]
	Primary binding[a] (%)	Secondary binding K_i[b] (nM)	Primary binding[a] (%)	Secondary binding K_i[b] (nM)	
6a	63.4	196.0 ± 15	102.6	6.92 ± 0.14	28.32
6b	−9.0	—	102.0	9.37 ± 0.64	>1067.2
6c	51.4	323.0 ± 17	104.1	10.32 ± 0.46	31.29
6d	68.7	733.0 ± 47	102.0	27.95 ± 1.18	26.22
6f	26.3	—	100.6	5.4 ± 0.22	>1851.8
9a	73.7	>10,000	86.3	496.7 ± 27.66	>20.13
9b	34.4	—	74.1	1008.0 ± 107.21	>9.92
9c	66.3	>10,000	59.2	1068.0 ± 114.18	>9.36
9d	71.0	>10,000	65.9	1330.0 ± 135.33	>7.51
9f	64.8	2875 ± 220	103.6	23.80 ± 1.24	120.79

[a] For primary assays, the PDSP has a low threshold for 'hits' at primary assays. We perform a large number of secondary assays using a relatively low threshold in order not to miss potential high-affinity compounds. The likelihood of an actual, high-affinity, 'hit' is still very low. Thus, one should not over-interpret results from primary assays. Data represent mean% inhibition (N = 4 determinations) for compound tested at receptor subtypes. Significant inhibition is considered >50%. In cases where negative inhibition (−) is seen, this represents a stimulation of binding. Occasionally, compounds at high concentrations will non-specifically increase binding. The default concentration for primary binding experiments is 10 μM.

[b] For secondary assays, data represent K_i (nM) values obtained from non-linear regression of radioligand competition binding isotherms. K_i values are calculated from best fit IC_{50} values using the Cheng–Prusoff equation.

[c] CB1 K_i values of compounds **6b**, **6f**, and **9b** be regarded as >10,000.

Gemini 2000 200 MHz FT-NMR and Bruker Avance II 500 MHz NMR spectrometers using tetramethylsilane (TMS) as an internal standard. Chemical shift data for the proton resonances were reported in parts per million (ppm, δ scale) relative to internal standard TMS (δ 0.0), and coupling constants (J) were reported in hertz (Hz). Low resolution mass spectra (LRMS) were obtained using a Bruker Biflex III MALDI-Tof mass spectrometer, and high resolution mass spectra (HRMS) were obtained using a Thermo MAT 95XP-Trap spectrometer. Chromatographic solvent proportions are indicated as volume: volume ratio. Thin-layer chromatography (TLC) was run using Analtech Silica Gel GF uniplates (5×10 cm^2). Plates were visualized under UV light. Preparative TLC was run using Analtech Silica Gel UV 254 plates (20×20 cm^2). Normal phase flash column chromatography was carried out on EM Science Silica Gel 60 (230–400 mesh) with a forced flow of the indicated solvent system in the proportions described below. All moisture- and/or air-sensitive reactions were performed under a positive pressure of nitrogen maintained by a direct line from a nitrogen source.

Analytical HPLC was performed using a Prodigy (Phenomenex) 5 μm C-18 column, 4.6×250 mm; 3:1:1 CH_3CN:MeOH: 20 mM, pH 6.7 phosphate (buffer solution) mobile phase; flow rate 1.5 mL/min; and UV (254 nm) and γ-ray (PIN diode) flow detectors. C-18 Plus Sep-Pak cartridges (WAT020515) were obtained from Waters Corporate Headquarters, Milford, MA. Semi-prep C-18 guard cartridge column 1×1 cm was obtained from E. S. Industries, Berlin, NJ, and part number 300121-C18-BD 10 μm. Sterile Millex-GS 0.22 μm vented filter unit was obtained from Millipore Corporation, Bedford, MA.

3.2. 3-Hydroxy-4-methoxy-2-nitrobenzaldehyde (1)

To a solution of isovanillin (50.0 g, 0.33 mol) in EtOAc (800 mL) was slowly added nitric acid (28.8 g, 90%, 0.41 mol) at 0 °C, and the mixture was stirred at room temperature (rt) for 2.5 h. Then the reaction mixture was added additional EtOAc (600 mL), washed with water, saturated $NaHCO_3$ and brine, and dried over Na_2SO_4. The organic layer was concentrated under reduced pressure, and the crude product was purified by column chromatography (1:1 EtOAc/hexanes) to give **1** (40.1 g, 62%) as a yellow solid, mp 147–148 °C, R_f = 0.40 (1:1 EtOAc/hexanes). ^{1}H NMR (DMSO-d_6): δ 3.99 (s, 3H, OCH_3), 7.32 (d, J = 8.5 Hz, 1H, Ph-H), 7.56 (d, J = 8.5 Hz, 1H, Ph-H), 9.76 (s, 1H, CHO), 10.77 (s, 1H, OH). MS (ESI): 198 ([M+H]$^+$, 100%).

3.3. 4-Methoxy-2-nitro-3-pentyloxybenzaldehyde (2)

1-Bromopentane (34.5 g, 228.4 mol) was added dropwise to a solution of **1** (15.0 g, 76.1 mmol) and K_2CO_3 (31.55 g, 228.3 mmol) in anhydrous DMF (200 mL). The reaction mixture was stirred under N_2 at 100 °C for 3 h, and then followed by filtration. The solid residue was washed with additional DMF. The combined organic layers were washed with water, extracted with EtOAc, washed with brine, dried over $MgSO_4$, and then purified by column chromatography (30% EtOAc/hexanes) to afford **2** (16.3 g, 80%) as a brown solid, mp 26–27 °C, R_f = 0.57 (1:1 EtOAc/hexanes). ^{1}H NMR ($CDCl_3$): δ 0.92 (t, J = 7.0 Hz, 3H, CH_3), 1.34–1.41 (m, 4H, CH_2CH_2), 1.66–1.76 (m, 2H, CH_2), 3.99 (s, 3H, OCH_3), 4.10 (t, J = 6.6 Hz, 2H, OCH_2), 7.08 (d, J = 8.4 Hz, 1H, Ph-H), 7.61 (d, J = 8.6 Hz, 1H, Ph-H), 9.78 (s, 1H, CHO). MS (ESI): 268 ([M+H]$^+$, 70%), 198 (100%).

3.4. 2-Amino-4-methoxy-3-pentyloxybenzaldehyde (3)

Iron powder (6.68 g, 119.7 mmol) and concentrated HCl (37%, 1.5 mL) were added to a solution of **2** (10.0 g, 37.4 mmol) in EtOH/AcOH/H_2O (96 mL/96 mL/48 mL). The reaction mixture was refluxed for 30 min and then stirred at rt for 1 h, filtered through Celite 521, and extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and then purified by column chromatography (20% EtOAc/hexanes) to produce **3** (7.54 g, 85%) as a yellow oil, R_f = 0.50 (1:3 EtOAc/hexanes). ^{1}H NMR ($CDCl_3$): δ 0.93 (t, J = 7.0 Hz, 3H, CH_3), 1.33–1.48 (m, 4H, CH_2CH_2), 1.70–1.84 (m, 2H, CH_2), 3.89 (s, 3H, OCH_3), 3.93 (t, J = 6.6 Hz, 2H, OCH_2), 6.27 (s, 2H, NH_2), 6.34 (d, J = 8.8 Hz, 1H, Ph-H), 7.18 (d, J = 8.8 Hz, 1H, Ph-H), 9.73 (s, 1H, CHO). MS (ESI): 238 ([M+H]$^+$, 100%).

3.5. 7-Methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxylic acid ethyl ester (4)

Dimethyl malonate (9.74 g, 73.7 mmol), piperidine (6.28 g, 73.7 mmol), and acetic acid (0.1 mL) were added to a solution of **3** (7.0 g, 29.5 mmol) in EtOH (80 mL). The reaction mixture was refluxed under N_2 for 48 h. Subsequently the reaction mixture was cooled to rt, treated with water (50 mL) and extracted with EtOAc (3×80 mL). The combined organic layers were washed with water and brine, dried over Na_2SO_4, and purified by column chromatography (20% EtOAc/hexanes) to obtain **4** (8.45 g, 86%) as a colorless solid, mp 116–118 °C, R_f = 0.31 (1:1 EtOAc/hexanes). ^{1}H NMR ($CDCl_3$): δ 0.93 (t, J = 7.2 Hz, 3H, CH_3), 1.37–1.44 (m, 7H, CH_2CH_2 and CH_3), 1.73–1.83 (m, 2H, CH_2), 3.96 (s, 3H, OCH_3), 4.10 (t, J = 6.8 Hz, 2H, OCH_2), 4.38 (q, J = 7.2 Hz, 2H, OCH_2CH_3), 6.85 (d, J = 8.8 Hz, 1H, Ph-H), 7.32 (d, J = 8.8 Hz, 1H, Ph-H), 8.44 (s, 1H, CH=), 9.01 (s, 1H, CONH). MS (ESI): 234 ([M+H]$^+$, 100%).

3.6. 7-Methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxylic acid (5)

HCl (2 N, 125 mL) was added to a solution of **4** (6.3 g, 18.9 mmol) in EtOH (180 mL). The mixture was stirred at 70 °C for 12 h, cooled to rt, and filtered. The solid was dried in air to afford pure **5** (4.85 g, 84%) as a white solid, mp 181–182 °C, R_f = 0.14 (1:19 MeOH/CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 0.90 (t, J = 7.2 Hz, 3H, CH_3), 1.31–1.42 (m, 4H, CH_2CH_2), 1.74–1.83 (m, 2H, CH_2), 3.96 (s, 3H, OCH_3), 3.99 (t, J = 6.8 Hz, 2H, OCH_2), 7.24 (d, J = 8.8 Hz, 1H, Ph-H), 7.78 (d, J = 8.8 Hz, 1H, Ph-H), 8.89 (s, 1H, CH=), 12.3 (s, 1H). MS (ESI): 306 ([M+H]$^+$, 100%).

3.7. General procedure for preparation of compound 6

BOP (265 mg, 0.6 mmol) and DIEA (155 mg, 1.2 mmol) were added to the solution of **5** (153 mg, 0.5 mmol) and an amine (0.5 mmol) in anhydrous DMF (20 mL). The reaction mixture was stirred at rt under N_2 for 15 h. Water (30 mL) was added to the mixture, and the mixture was extracted with dichloromethane (3×60 mL), washed with brine, dried over Na_2SO_4, and then purified by column chromatography (40% EtOAc/hexanes) to give **6** as a white solid in 88–93% yield. R_f = 0.36–0.47 (1:1 EtOAc/hexanes).

3.7.1. 7-Methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxylic acid (2-phenylethyl)amide (6a)

Mp 116–118 °C. ^{1}H NMR ($CDCl_3$): δ 0.94 (t, J = 7.2 Hz, 3H, CH_3), 1.37–1.46 (m, 4H, CH_2CH_2), 1.74–1.84 (m, 2H, CH_2), 2.95 (t, J = 7.2 Hz, CH_2), 3.73 (dd, J = 7.0, 13.5 Hz, 2H, NCH_2), 3.97 (s, 3H, OCH_3), 4.12 (t, J = 7.2 Hz, 2H, OCH_2), 6.92 (d, J = 8.8 Hz, 1H, Ph-H), 7.21–7.24 (m, 1H, Ph-H), 7.27–7.39 (m, 4H, Ph-H), 7.44 (d, J = 8.8 Hz, 1H, Ph-H), 8.86 (s, 1H), 9.16 (s, 1H), 9.70 (s, 1H, CONH). MS (ESI): 409 ([M+H]$^+$, 100%).

3.7.2. 7-Methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxylic acid [2-(4-chlorophenyl)ethyl]amide (6b)

Mp 137–139 °C. ^{1}H NMR ($CDCl_3$): δ 0.94 (t, J = 7.0 Hz, 3H, CH_3), 1.34–1.48 (m, 4H, CH_2CH_2), 1.78–1.84 (m, 2H, CH_2), 2.92

(t, J = 7.0 Hz, CH_2), 3.69 (dd, J = 7.0, 13.6 Hz, 2H, NCH_2), 3.97 (s, 3H, OCH_3), 4.13 (t, J = 6.8 Hz, 2H, OCH_2), 6.91 (d, J = 8.8 Hz, 1H, Ph-H), 7.17–7.30 (m, 4H, Ph-H), 7.43 (d, J = 8.8 Hz, 1H, Ph-H), 8.86 (s, 1H), 9.15 (s, 1H), 9.68 (s, 1H, CONH). MS (ESI): 443 ([M+H]$^+$, 100%).

3.7.3. 7-Methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxylic acid (3-phenylpropyl)amide (6c)

Mp 84–86 °C. ^{1}H NMR ($CDCl_3$): δ 0.94 (t, J = 6.8 Hz, 3H, CH_3), 1.34–1.48 (m, 4H, CH_2CH_2), 1.75–1.85 (m, 2H, CH_2), 1.90–2.05 (m, 2H, CH_2), 2.73 (t, J = 7.8 Hz, CH_2), 3.48 (dd, J = 7.0, 13.0 Hz, 2H, NCH_2), 3.97 (s, 3H, OCH_3), 4.13 (t, J = 6.8 Hz, 2H, OCH_2), 6.90 (d, J = 8.8 Hz, 1H, Ph-H), 7.13–7.32 (m, 5H, Ph-H), 7.42 (d, J = 8.8 Hz, 1H, Ph-H), 8.86 (s, 1H), 9.15 (s, 1H), 9.68 (s, 1H, CONH). MS (ESI): 423 ([M+H]$^+$, 100%).

3.7.4. 7-Methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxylic acid [2-(4-methoxyphenyl)ethyl]amide (6d)

Mp 131–133 °C. ^{1}H NMR ($CDCl_3$): δ 0.94 (t, J = 6.8 Hz, 3H, CH_3), 1.35–1.48 (m, 4H, CH_2CH_2), 1.75–1.85 (m, 2H, CH_2), 2.89 (t, J = 7.0 Hz, CH_2), 3.68 (dd, J = 7.0, 13.2 Hz, 2H, NCH_2), 3.78 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 4.12 (t, J = 6.8 Hz, 2H, OCH_2), 6.83 (d, J = 8.4 Hz, 2H, Ph-H), 6.90 (d, J = 8.8 Hz, 1H, Ph-H), 7.17 (d, J = 8.4 Hz, 2H, Ph-H), 7.42 (d, J = 8.8 Hz, 1H, Ph-H), 8.86 (s, 1H), 9.14 (s, 1H), 9.67 (s, 1H, CONH). MS (ESI): 439 ([M+H]$^+$, 100%). HRMS (ESI) calcd for $C_{25}H_{30}N_2O_5H$ ([M+H]$^+$), 439.2233; found 439.2229.

3.7.5. 7-Methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxylic acid [2-(4-hydroxyphenyl)ethyl]amide (6e)

Mp 143–145 °C. ^{1}H NMR ($CDCl_3$): δ 0.93 (t, J = 7.0 Hz, 3H, CH_3), 1.35–1.48 (m, 4H, CH_2CH_2), 1.76–1.84 (m, 2H, CH_2), 2.87 (t, J = 6.6 Hz, CH_2), 3.69 (m, 2H, NCH_2), 3.97 (s, 3H, OCH_3), 4.12 (t, J = 6.8 Hz, 2H, OCH_2), 6.78 (d, J = 8.2 Hz, 2H, Ph-H), 6.92 (d, J = 8.8 Hz, 1H, Ph-H), 7.08 (d, J = 8.2 Hz, 2H, Ph-H), 7.43 (d, J = 8.8 Hz, 1H, Ph-H), 8.89 (s, 1H), 9.27 (s, 1H), 9.79 (s, 1H, CONH). MS (ESI): 425 ([M+H]$^+$, 100%).

3.7.6. 7-Methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxylic acid [2-(4-fluorophenyl)ethyl]amide (6f)

Mp 106–108 °C. ^{1}H NMR ($CDCl_3$): δ 0.94 (t, J = 6.8 Hz, 3H, CH_3), 1.34–1.48 (m, 4H, CH_2CH_2), 1.75–1.85 (m, 2H, CH_2), 2.92 (t, J = 7.0 Hz, CH_2), 3.69 (dd, J = 6.8, 13.0 Hz, 2H, NCH_2), 3.97 (s, 3H, OCH_3), 4.13 (t, J = 6.8 Hz, 2H, OCH_2), 6.91–7.03 (m, 3H, Ph-H), 7.19–7.26 (m, 2H, Ph-H), 7.43 (d, J = 8.8 Hz, 1H, Ph-H), 8.86 (s, 1H), 9.14 (s, 1H), 9.66 (s, 1H, CONH). MS (ESI): 427 ([M+H]$^+$, 100%). HRMS (ESI) calcd for $C_{24}H_{27}N_2O_4FH$ ([M+H]$^+$), 427.2033; found 427.2017.

3.7.7. 7-Methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxylic acid [2-(4-nitrophenyl)ethyl]amide (6g)

Mp 170–172 °C. ^{1}H NMR ($CDCl_3$): δ 0.94 (t, J = 6.8 Hz, 3H, CH_3), 1.34–1.48 (m, 4H, CH_2CH_2), 1.74–1.84 (m, 2H, CH_2), 3.06 (t, J = 7.0 Hz, CH_2), 3.75 (dd, J = 7.0, 13.2 Hz, 2H, NCH_2), 3.97 (s, 3H, OCH_3), 4.13 (t, J = 6.8 Hz, 2H, OCH_2), 6.92 (d, J = 8.8 Hz, 1H, Ph-H), 7.41–7.47 (m, 3H, Ph-H), 8.15 (d, J = 8.8 Hz, 2H, Ph-H), 8.84 (s, 1H), 9.12 (s, 1H), 9.73 (s, 1H, CONH). MS (ESI): 454 ([M+H]$^+$, 100%).

3.8. General procedure for preparation of compound 7

Lithium chloride (0.34 g, 8.0 mmol) was added to a solution of **6a–c** (1.0 mmol) in DMF (20 mL) under nitrogen, and then the reaction mixture was refluxed for 18 h. The reaction mixture was cooled down to rt, water (30 mL) was added. The mixture was extracted with EtOAc (3 × 50 mL), washed with brine, and dried over $MgSO_4$. The organic layer was evaporated, and purified by column chromatography (40% EtOAc/hexanes) to give **7a–c** as a white solid in 80–83% yield. R_f = 0.30–0.34 (1:1 EtOAc/hexanes).

3.8.1. 7-Hydroxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxylic acid (2-phenylethyl)amide (7a)

Mp 99–101 °C. ^{1}H NMR ($CDCl_3$): δ 0.92 (t, J = 6.8 Hz, 3H, CH_3), 1.32–1.46 (m, 4H, CH_2CH_2), 1.75–1.87 (m, 2H, CH_2), 2.95 (t, J = 7.2 Hz, CH_2), 3.73 (dd, J = 7.0, 13.5 Hz, 2H, NCH_2), 4.07 (t, J = 7.0 Hz, 2H, OCH_2), 6.94 (d, J = 8.8 Hz, 1H, Ph-H), 7.21–7.38 (m, 6H, Ph-H), 7.62 (s, 1H, OH), 8.84 (s, 1H, CH=), 9.11 (s, 1H, CONH), 9.78 (s, 1H, CONH). MS (ESI): 395 ([M+H]$^+$, 100%). HRMS (ESI) calcd for $C_{23}H_{26}N_2O_4H$ ([M+H]$^+$), 395.1971; found 395.1955.

3.8.2. 7-Hydroxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxylic acid [2-(4-chlorophenyl)ethyl]amide (7b)

Mp 121–123 °C. ^{1}H NMR ($CDCl_3$): δ 0.92 (t, J = 6.8 Hz, 3H, CH_3), 1.32–1.46 (m, 4H, CH_2CH_2), 1.76–1.87 (m, 2H, CH_2), 2.88–2.97 (m, 2H, CH_2), 3.70 (dd, J = 6.8, 13.2 Hz, 2H, NCH_2), 4.09 (t, J = 7.0 Hz, 2H, OCH_2), 6.94 (d, J = 8.8 Hz, 1H, Ph-H), 7.16 (d, J = 8.6 Hz, 2H, Ph-H), 7.24 (d, J = 8.6 Hz, 2H, Ph-H), 7.33 (d, J = 8.8 Hz, 1H, Ph-H), 7.93 (s, 1H, OH), 8.83 (s, 1H, CH=), 9.15 (s, 1H, CONH), 9.79 (t, J = 5.6 Hz, 1H, CONH). MS (ESI): 429 ([M+H]$^+$, 100%). HRMS (ESI) calcd for $C_{23}H_{25}N_2O_4ClH$ ([M+H]$^+$), 429.1581; found 429.1583.

3.8.3. 7-Hydroxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxylic acid (3-phenylpropyl)amide (7c)

Mp 128–130 °C. ^{1}H NMR ($CDCl_3$): δ 0.92 (t, J = 6.8 Hz, 3H, CH_3), 1.32–1.47 (m, 4H, CH_2CH_2), 1.77–1.87 (m, 2H, CH_2), 1.91–2.05 (m, 2H, CH_2), 2.73 (t, J = 7.4 Hz, 2H, CH_2), 3.49 (dd, J = 6.8, 13.2 Hz, 2H, NCH_2), 4.10 (t, J = 7.0 Hz, 2H, OCH_2), 6.95 (d, J = 8.6 Hz, 1H, Ph-H), 7.16–7.31 (m, 5H, Ph-H), 7.33 (d, J = 8.8 Hz, 1H, Ph-H), 7.76 (s, 1H, OH), 8.85 (s, 1H, CH=), 9.18 (s, 1H, CONH), 9.82 (t, J = 5.6 Hz, 1H, CONH). MS (ESI): 409 ([M+H]$^+$, 100%); HRMS (ESI) calcd for $C_{24}H_{28}N_2O_4H$ ([M+H]$^+$), 409.2127; found 409.2107.

3.9. 2-Chloro-7-methoxy-8-(pentyloxy)quinoline-3-carbonyl chloride (8)

To **5** (3.05 g, 10 mmol) was added thionyl chloride (20 mL), and the reaction mixture was refluxed for 3 h. The mixture was cooled down to rt, and the excess thionyl chloride was evaporated. The residue was added anhydrous toluene (20 mL), and evaporation was repeated. The residue was dried under vacuum to afford **8** (3.38 g, 99%) as a yellow solid, mp: 44–46 °C. ^{1}H NMR ($CDCl_3$): δ 0.94 (t, J = 7.0 Hz, 3H, CH_3), 1.31–1.60 (m, 4H, CH_2CH_2), 1.79–1.93 (m, 2H, CH_2), 4.06 (s, 3H, OCH_3), 4.28 (t, J = 6.8 Hz, 2H, OCH_2), 7.42 (d, J = 9.0 Hz, 1H, Ph-H), 7.69 (d, J = 9.0 Hz, 1H, Ph-H), 8.95 (s, 1H, CH=).

3.10. General procedure for preparation of compound 9

The solution of **8** (0.34 g, 1.0 mmol) in dichloromethane (10 mL) was added at 0 °C to a solution of an amine (1.0 mmol) and triethylamine (0.20 g, 2.0 mmol) in dichloromethane (20 mL). The reaction mixture was allowed to warm up to rt and stirred for 2 h. Saturated NaCl (30 mL) was added to the mixture, and the aqueous layer was extracted with dichloromethane (3 × 30 mL). The combined organic layers were washed with saturated NaCl, dried over Na_2SO_4, and purified by column chromatography (30% EtOAc/hexanes) to obtain **9** as a white solid in 87–93% yield. R_f = 0.50–0.58 (1:1 EtOAc/hexanes).

3.10.1. 2-Chloro-7-methoxy-8-pentyloxy-1-quinoline-3-carboxylic acid (2-phenylethyl)amide (9a)

Mp 73–74 °C. ^{1}H NMR ($CDCl_3$): δ 0.93 (t, J = 7.0 Hz, 3H, CH_3), 1.33–1.56 (m, 4H, CH_2CH_2), 1.77–1.88 (m, 2H, CH_2), 2.99

(t, J = 7.2 Hz, 2H, CH_2), 3.78 (dd, J = 6.6, 12.8 Hz, 2H, NCH_2), 4.01 (s, 3H, OCH_3), 4.25 (t, J = 6.6 Hz, 2H, OCH_2), 6.66 (s, 1H, CONH), 7.21–7.30 (m, 4H, Ph-H), 7.33 (d, J = 9.0 Hz, 2H, Ph-H), 7.54 (d, J = 9.0 Hz, 1H, Ph-H), 8.47 (s, 1H, CH=). MS (ESI): 427 ([M+H]$^+$, 100%). HRMS (ESI) calcd for $C_{24}H_{27}N_2O_3ClH$ ([M+H]$^+$), 427.1788; found 427.1772.

3.10.2. 2-Chloro-7-methoxy-8-pentyloxy-1-quinoline-3-carboxylic acid [2-(4-chlorophenyl)ethyl]amide (9b)

Mp 105–107 °C. ^{1}H NMR ($CDCl_3$): δ 0.93 (t, J = 7.0 Hz, 3H, CH_3), 1.30–1.56 (m, 4H, CH_2CH_2), 1.78–1.92 (m, 2H, CH_2), 2.96 (t, J = 7.0 Hz, 2H, CH_2), 3.75 (dd, J = 6.8, 13.0 Hz, 2H, NCH_2), 4.01 (s, 3H, OCH_3), 4.25 (t, J = 6.8 Hz, 2H, OCH_2), 6.69 (s, 1H, CONH), 7.18–7.32 (m, 4H, Ph-H), 7.34 (d, J = 9.0 Hz, 1H, Ph-H), 7.55 (d, J = 9.0 Hz, 1H, Ph-H), 8.47 (s, 1H, CH=). MS (ESI): 461 ([M+H]$^+$, 100%). HRMS (ESI) calcd for $C_{24}H_{26}N_2O_3Cl_2H$ ([M+H]$^+$), 461.1399; found 461.1377.

3.10.3. 2-Chloro-7-methoxy-8-pentyloxy-1-quinoline-3-carboxylic acid (3-phenylpropyl)amide (9c)

Mp 85–87 °C. ^{1}H NMR ($CDCl_3$): δ 0.93 (t, J = 7.0 Hz, 3H, CH_3), 1.33–1.56 (m, 4H, CH_2CH_2), 1.78–1.92 (m, 2H, CH_2), 1.98–2.05 (m, 2H, CH_2), 2.77 (t, J = 7.6 Hz, 2H, CH_2), 3.57 (dd, J = 7.0, 13.2 Hz, 2H, NCH_2), 4.01 (s, 3H, OCH_3), 4.26 (t, J = 6.8 Hz, 2H, OCH_2), 6.68 (s, 1H, CONH), 7.18–7.30 (m, 5H, Ph-H), 7.33 (d, J = 9.0 Hz, 1H, Ph-H), 7.53 (d, J = 9.0 Hz, 1H, Ph-H), 8.44 (s, 1H, CH=). MS (ESI): 463 ([M+Na]$^+$, 100%). HRMS (ESI) calcd for $C_{25}H_{29}N_2O_3ClNa$ ([M+Na]$^+$), 463.1765; found 463.1766.

3.10.4. 2-Chloro-7-methoxy-8-pentyloxy-1-quinoline-3-carboxylic acid [2-(4-methoxyphenyl)ethyl]amide (9d)

Mp 83–84 °C. ^{1}H NMR ($CDCl_3$): δ 0.95 (t, J = 7.0 Hz, 3H, CH_3), 1.37–1.42 (m, 2H, CH_2), 1.50–1.3 (m, 2H, CH_2), 1.82–1.87 (m, 2H, CH_2), 2.94 (t, J = 7.0 Hz, 2H, CH_2), 3.75 (dd, J = 7.0, 12.5 Hz, 2H, NCH_2), 3.80 (s, 3H, OCH_3), 4.02 (s, 3H, OCH_3), 4.26 (t, J = 7.0 Hz, 2H, OCH_2), 6.73 (s, 1H, CONH), 6.87 (dd, J = 2.0, 6.5 Hz, 2H, Ph-H), 7.19 (dd, J = 2.0, 6.5 Hz, 2H, Ph-H), 7.35 (d, J = 9.0 Hz, 1H, Ph-H), 7.56 (d, J = 9.0 Hz, 1H, Ph-H), 8.46 (s, 1H, CH=). MS (ESI): 457 ([M+H]$^+$, 100%). HRMS (ESI) calcd for $C_{25}H_{29}N_2O_4ClH$ ([M+H]$^+$), 457.1894; found 457.1880.

3.10.5. 2-Chloro-7-methoxy-8-pentyloxy-1-quinoline-3-carboxylic acid [2-(4-hydroxyphenyl)ethyl]amide (9e)

Mp 150–152 °C. ^{1}H NMR ($CDCl_3$): δ 0.91 (t, J = 7.0 Hz, 3H, CH_3), 1.36–1.40 (m, 2H, CH_2), 1.47–1.51 (m, 2H, CH_2), 1.81–1.87 (m, 2H, CH_2), 2.90 (t, J = 7.0 Hz, 2H, CH_2), 3.74 (dd, J = 6.5, 12.5 Hz, 2H, NCH_2), 4.01 (s, 3H, OCH_3), 4.25 (t, J = 7.0 Hz, 2H, OCH_2), 6.74 (s, 1H, CONH), 6.78 (d, J = 8.5 Hz, 2H, Ph-H), 7.09 (d, J = 8.5 Hz, 2H, Ph-H), 7.35 (d, J = 9.0 Hz, 1H, Ph-H), 7.55 (d, J = 9.0 Hz, 1H, Ph-H), 8.46 (s, 1H, CH=). MS (ESI): 443 ([M+H]$^+$, 100%). HRMS (ESI) calcd for $C_{24}H_{27}N_2O_4ClNa$ ([M + Na]$^+$), 465.1557; found 465.1550.

3.10.6. 2-Chloro-7-methoxy-8-pentyloxy-1-quinoline-3-carboxylic acid [2-(4-fluorophenyl)ethyl]amide (9f)

Mp 69–71 °C. ^{1}H NMR ($CDCl_3$): δ 0.93 (t, J = 7.0 Hz, 3H, CH_3), 1.36–1.41 (m, 2H, CH_2), 1.48–1.54 (m, 2H, CH_2), 1.81–1.87 (m, 2H, CH_2), 2.97 (t, J = 7.0 Hz, 2H, CH_2), 3.76 (dd, J = 7.0, 13.0 Hz, 2H, NCH_2), 4.01 (s, 3H, OCH_3), 4.25 (t, J = 7.0 Hz, 2H, OCH_2), 6.86 (s, 1H, CONH), 6.99–7.03 (m, 2H, Ph-H), 7.22–7.25 (m, 2H, Ph-H), 7.35 (d, J = 9.0 Hz, 1H, Ph-H), 7.56 (d, J = 9.0 Hz, 1H, Ph-H), 8.48 (s, 1H, CH=). MS (ESI): 445 ([M+H]$^+$, 100%). HRMS (ESI) calcd for $C_{24}H_{26}N_2O_3FClH$ ([M+H]$^+$), 445.1694; found 445.1673.

3.10.7. 2-Chloro-7-methoxy-8-pentyloxy-1-quinoline-3-carboxylic acid [2-(4-nitrophenyl)ethyl]amide (9g)

Mp 110–112 °C. ^{1}H NMR ($CDCl_3$): δ 0.95 (t, J = 7.0 Hz, 3H, CH_3), 1.37–1.43 (m, 2H, CH_2), 1.49–1.55 (m, 2H, CH_2), 1.83–1.88 (m, 2H, CH_2), 3.12 (t, J = 7.0 Hz, 2H, CH_2), 3.83 (dd, J = 7.0, 13.0 Hz, 2H, NCH_2), 4.03 (s, 3H, OCH_3), 4.25 (t, J = 7.0 Hz, 2H, OCH_2), 6.86 (s, 1H, CONH), 7.37 (d, J = 9.0 Hz, 1H, Ph-H), 7.45 (d, J = 8.5 Hz, 2H, Ph-H), 7.56 (d, J = 9.0 Hz, 1H, Ph-H), 8.18 (d, J = 8.5 Hz, 2H, Ph-H), 8.48 (s, 1H, CH=). MS (ESI): 472 ([M+H]$^+$, 100%). HRMS (ESI) calcd for $C_{24}H_{26}N_3O_5ClNa$ ([M+Na]$^+$), 494.1459; found 494.1461.

3.11. General procedure for preparation of compound 10

10a–c were prepared from **9a–c** using the same procedure described for **7a–c** as a white solid in 78–83% yield. R_f = 0.72–0.78 (1:1 EtOAc/hexanes).

3.11.1. 2-Chloro-7-hydroxy-8-pentyloxy-1-quinoline-3-carboxylic acid (2-phenylethyl)amide (10a)

Mp 55–57 °C. ^{1}H NMR ($CDCl_3$): δ 0.93 (t, J = 6.8 Hz, 3H, CH_3), 1.35–1.46 (m, 4H, CH_2CH_2), 1.77–1.87 (m, 2H, CH_2), 2.99 (t, J = 7.2 Hz, CH_2), 3.78 (dd, J = 6.8, 12.6 Hz, 2H, NCH_2), 4.46 (t, J = 7.0 Hz, 2H, OCH_2), 6.44 (s, 1H, OH), 6.63 (s, 1H, CONH), 7.24–7.32 (m, 6H, Ph-H), 7.74 (d, J = 9.0 Hz, 1H, Ph-H), 8.45 (s, 1H, CH=). MS (ESI): 413 ([M+H]$^+$, 100%). HRMS (ESI) calcd for $C_{23}H_{25}N_2O_3ClH$ ([M+H]$^+$), 413.1632; found 413.1623.

3.11.2. 2-Chloro-7-hydroxy-8-pentyloxy-1-quinoline-3-carboxylic acid [2-(4-chlorophenyl)ethyl]amide (10b)

Mp 106–108 °C. ^{1}H NMR ($CDCl_3$): δ 0.95 (t, J = 7.2 Hz, 3H, CH_3), 1.37–1.50 (m, 4H, CH_2CH_2), 1.81–1.87 (m, 2H, CH_2), 2.97 (t, J = 7.2 Hz, CH_2), 3.76 (dd, J = 7.0, 13.0 Hz, 2H, NCH_2), 4.46 (t, J = 7.0 Hz, 2H, OCH_2), 6.66 (s, 1H, CONH), 7.20 (d, J = 8.5 Hz, 2H, Ph-H), 9.29 (dd, J = 1.5, 6.5 Hz, 2H, Ph-H), 7.32 (d, J = 8.5 Hz, 1H, Ph-H), 7.50 (d, J = 8.5 Hz, 1H, Ph-H), 8.47 (s, 1H, CH=). MS (ESI): 447 ([M+H]$^+$, 100%). HRMS (ESI) calcd for $C_{23}H_{24}N_2O_3ClH$ ([M+H]$^+$), 447.1242; found 447.1227.

3.11.3. 2-Chloro-7-hydroxy-8-pentyloxy-1-quinoline-3-carboxylic acid (3-phenylpropyl)amide (10c)

Mp 127–129 °C. ^{1}H NMR ($CDCl_3$): δ 0.93 (t, J = 7.0 Hz, 3H, CH_3), 1.38–1.50 (m, 4H, CH_2CH_2), 1.77–1.91 (m, 2H, CH_2), 1.97–2.03 (m, 2H, CH_2), 2.76 (t, J = 7.6 Hz, CH_2), 3.53 (dd, J = 7.0, 13.0 Hz, 2H, NCH_2), 4.47 (t, J = 7.0 Hz, 2H, OCH_2), 6.49 (s, 1H, OH), 6.66 (s, 1H, CONH), 7.15–7.32 (m, 6H, Ph-H), 7.74 (d, J = 8.8 Hz, 1H, Ph-H), 8.40 (s, 1H, CH=). MS (ESI): 449 ([M+Na]$^+$, 100%). HRMS (ESI) calcd for $C_{24}H_{27}N_2O_3ClNa$ ([M+Na]$^+$), 449.1608; found 449.1609.

3.12. General procedure for preparation of target tracers

[^{11}C]CO_2 was produced by the ^{14}N(p,α)^{11}C nuclear reaction in small volume (9.5 cm^3) aluminum gas target (CTI) from 11 MeV proton cyclotron on research purity nitrogen (+1% O_2) in a Siemens radionuclide delivery system. The proton-beam current was 55 μA, and the irradiation time was 30 min. The phenolic hydroxyl precursor **7a**, **7b**, **7c**, **6e**, **10a**, **10b**, **10c**, or **9e** (0.1–0.3 mg) was dissolved in CH_3CN (300 μL). To this solution was added 2 N NaOH (2 μL). The mixture was transferred to a small reaction vial. No-carrier-added (high specific activity) [^{11}C]CH_3OTf that was produced by the gas-phase production method[25] from [^{11}C]CO_2 through [^{11}C]CH_4 and [^{11}C]CH_3Br with silver triflate (AgOTf) column was passed into the reaction vial at rt until radioactivity reached a maximum (~2 min), and then the reaction vial was isolated and heated at 80 °C for 3 min. The contents of the reaction vial were diluted with $NaHCO_3$ (1 mL, 0.1 M). The reaction tube was connected to either a C-18 Plus Sep-Pak cartridge or a semi-prep C-18 guard cartridge column. The labeled product mixture solution was passed onto the cartridge for SPE purification by gas pressure. The cartridge was washed with H_2O (2 × 3 mL), and the aqueous washing was discarded. The product was eluted from the column with EtOH (2 × 3 mL), and then passed onto a rotatory

evaporator. The solvent was removed by evaporation under vacuum. The labeled product [^{11}C]**6a**, [^{11}C]**6b**, [^{11}C]**6c**, [^{11}C]**6d**, [^{11}C]**9a**, [^{11}C]**9b**, [^{11}C]**9c**, or [^{11}C]**9d** was formulated with saline, sterile-filtered through a sterile vented Millex-GS 0.22 μm cellulose acetate membrane and collected into a sterile vial. Total radioactivity was assayed and the total volume was noted for tracer dose dispensing. The overall synthesis time including SPE purification and formulation was 15–20 min. The radiochemical yields decay corrected to EOB, from [^{11}C]CO_2, were 40–50%. Retention times in the analytical HPLC system were: t_R **7a** = 3.90 min, t_R **6a** = 5.90 min, t_R [^{11}C]**6a** = 5.90 min; t_R **7b** = 4.80 min, t_R **6b** = 7.77 min, t_R [^{11}C]**6b** = 7.77 min; t_R **7c** = 4.55 min, t_R **6c** = 7.16 min, t_R [^{11}C]**6c** = 7.16 min; t_R **6e** = 2.71 min, t_R **6d** = 5.32 min, t_R [^{11}C]**6d** = 5.32 min; t_R **10a** = 3.96 min, t_R **9a** = 5.45 min, t_R [^{11}C]**9a** = 5.45 min; t_R **10b** = 2.18 min, t_R **9b** = 5.60 min, t_R [^{11}C]**9b** = 5.60 min; t_R **10c** = 4.50 min, t_R **9c** = 6.25 min, t_R [^{11}C]**9c** = 6.25 min; and t_R **9e** = 2.38 min, t_R **9d** = 3.62 min, t_R [^{11}C]**9d** = 3.62 min.

3.13. Radioligand binding assays

Radioligand binding assays were performed by the National Institute of Mental Health's Psychoactive Drug Screening Program (NIMH/PDSP). The receptors were CB1 and CB2. The radioligand was [^{3}H]CP55940. The reference was CP55940. The assay buffer was cannabinoid binding buffer, 50 mM Tris–HCl, 1 mM EDTA, 3 mM $MgCl_2$, 5 mg/mL fatty acid-free bovine serum albumin (BSA), pH 7.4.

4. Conclusions

An efficient and convenient synthesis of new quinoline derivative radioligands, carbon-11-labeled 2-oxoquinoline and 2-chloroquinoline derivatives, has been well developed. The synthetic methodology employed classical organic chemistry such as nitration, alkylation (Williamson ether synthesis), reduction, transesterification, hydrolysis, coupling reaction, and desmethylation to prepare a series of 2-oxoquinoline and 2-chloroquinoline derivative precursors and standard compounds. The target radioligands were prepared by *O*-[^{11}C]methylation of their corresponding phenolic hydroxyl precursors using a reactive [^{11}C]methylating agent, [^{11}C]CH_3OTf, and isolated by a simplified SPE purification procedure in high radiochemical yields, short overall synthesis time, and great specific radioactivities. In vitro radioligand binding assays indicated the compounds **6f**, **6b**, and **9f** display potent in vitro binding affinities with nanomolar K_i values and at least 100–2000-fold selectivity for CB2. These chemistry and biology results combined with the reported in vitro and in vivo biological data[17,22] encourage further in vivo biological evaluation of new carbon-11-labeled 2-oxoquinoline and 2-chloroquinoline derivatives as candidate PET radioligands for imaging of CB2 in cancer.

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