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[^{11}C]Enzastaurin, the first design and radiosynthesis of a new potential PET agent for imaging of protein kinase C

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ABSTRACT

Enzastaurin (LY317615) is a potent and selective protein kinase C (PKC) inhibitor with an IC_{50} value of ~6 nM. [^{11}C]Enzastaurin (3-(1-[^{11}C]methyl-1*H*-indol-3-yl)-4-[1-[1-(2-pyridinylmethyl)-4-piperidinyl]-1*H*-indol-3-yl]-1*H*-pyrrole-2,5-dione), a new potential PET agent for imaging of PKC, was first designed and synthesized in 20–25% decay corrected radiochemical yield and 370–555 GBq/μmol specific activity at end of bombardment (EOB). The synthetic strategy was to prepare a carbon-11-labeled maleic anhydride intermediate followed by the conversion to maleimide.

Protein kinase C (PKC) is a family of enzymes that are involved in controlling the function of other proteins through the phosphorylation of hydroxyl groups of serine and threonine amino acid residues on these proteins. PKC enzymes are activated by signals such as increases in the concentration of diacylglycerol or Ca^{2+}, and thus PKC enzymes play important roles in several signal transduction cascades.[1] PKC expression and activity are associated with various disease processes, such as Alzheimer's disease and cancer.[2] In cancer, PKC activation is directly responsible for the vascular endothelial growth factor (VEGF) signaling that leads to neovascularization.[3] VEGF is the most common and direct acting angiogenic factor in cancer, and VEGF receptors are overexpressed in tumor-associated endothelial cells. The PKC family consists of about 10 isozymes. Enzastaurin (LY317615, 3-(1-methyl-1*H*-indol-3-yl)-4-[1-[1-(2-pyridinylmethyl)-4-piperidinyl]-1*H*-indol-3-yl]-1*H*-pyrrole-2,5-dione) is a potent and selective inhibitor of PKCβ with antiproliferative activity, IC_{50} value ~6 nM, originally developed by Eli Lilly.[4–7] The studies[4–7] indicated Enzastaurin suppresses VEGF-induced angiogenesis in the rat corneal micropocket assay, decreases microvessel density, and prevents VEGF secretion from human tumor cell xenografts in nude mice; in addition, prolonged courses of Enzastaurin increase chemotherapy or radiation tumor growth delay of breast, glioma, and small cell lung cancer xenografts. Enzastaurin is a structurally distinct maleimide, and bisarylmaleimide protein kinase inhibitors have attracted great interest from synthetic and medicinal chemists.[8,9] Carbon-11-labeled Enzastaurin may serve as a new probe for the biomedical imaging technique positron emission tomography (PET), and enable non-invasive monitoring of enzyme PKC in diseases.[8,9] To radiolabel therapeutic agents as diagnostic agents for imaging of PKC and monitoring of therapeutic efficacy of PKC inhibitors, we have first designed and synthesized [^{11}C]Enzastaurin (3-(1-[^{11}C]methyl-1*H*-indol-3-yl)-4-[1-[1-(2-pyridinylmethyl)-4-piperidinyl]-1*H*-indol-3-yl]-1*H*-pyrrole-2,5-dione) as a new potential PET agent.

There is limited synthetic information regarding the target compound Enzastaurin appearing in the literature. Wishing to study this compound in this laboratory, we decided to make our own material by adopting the available literature methods from previous works.[8–11] As illustrated in Scheme 1, Enzastaurin (**5b**) as well as its *N-des*-methylated compound **5a** were synthesized according to the synthetic protocol developed by Faul et al.[10,11] The procedure involved a one-step condensation of substituted (aryl or indolyl) acetamides with substituted (aryl or indolyl) glyoxyl esters in the presence of *t*-BuOK in THF. Treatment indole-3-acetamide with concentrated HCl formed hydrochloride salt, which was reduced with BH_3·pyridine in MeOH to give indolin-3-acetamide (**1**) in 49% yield. Condensation of **1** and 1-pyridin-2-ylmethylpiperidin-4-one, followed by reductive amination with $NaBH(OAc)_3$ in HOAC provided **2** in 86% yield. The indolin ring of **2** was oxidized by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in THF to afford its corresponding indole compound **3** in 60% yield. Indole-3-glyoxylate esters **4a–b** were prepared by the reaction of indoles with oxalyl chloride in Et_2O, followed by quench of the intermediate glyoxyl chloride with NaOMe in MeOH in 73% and 34% yield, respectively. The maleimides **5a** and **5b** were

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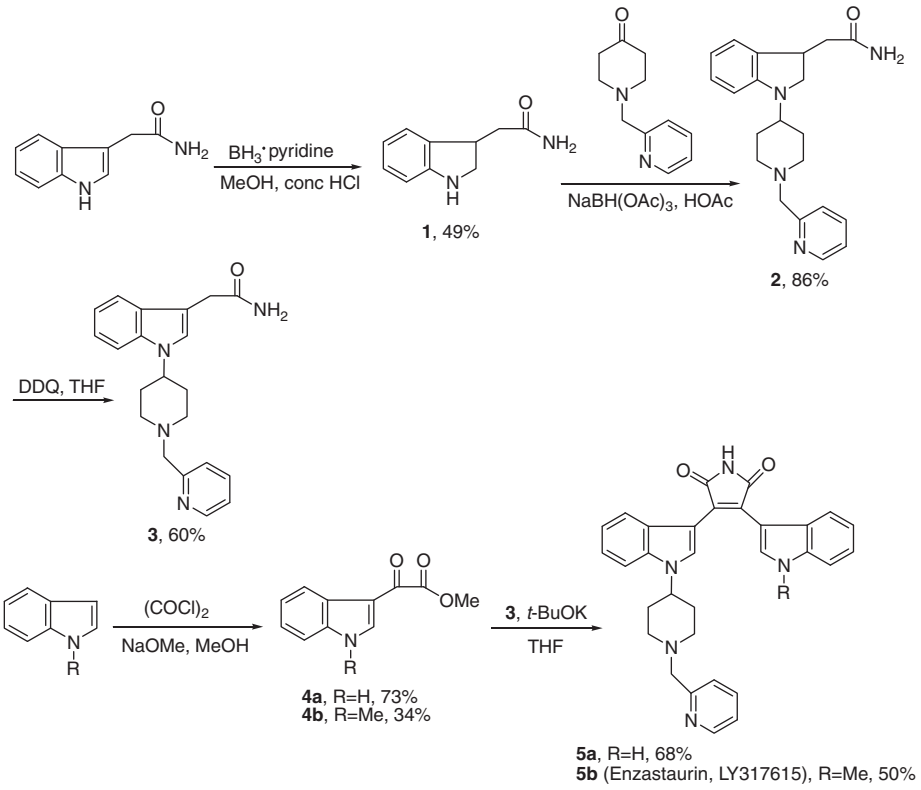
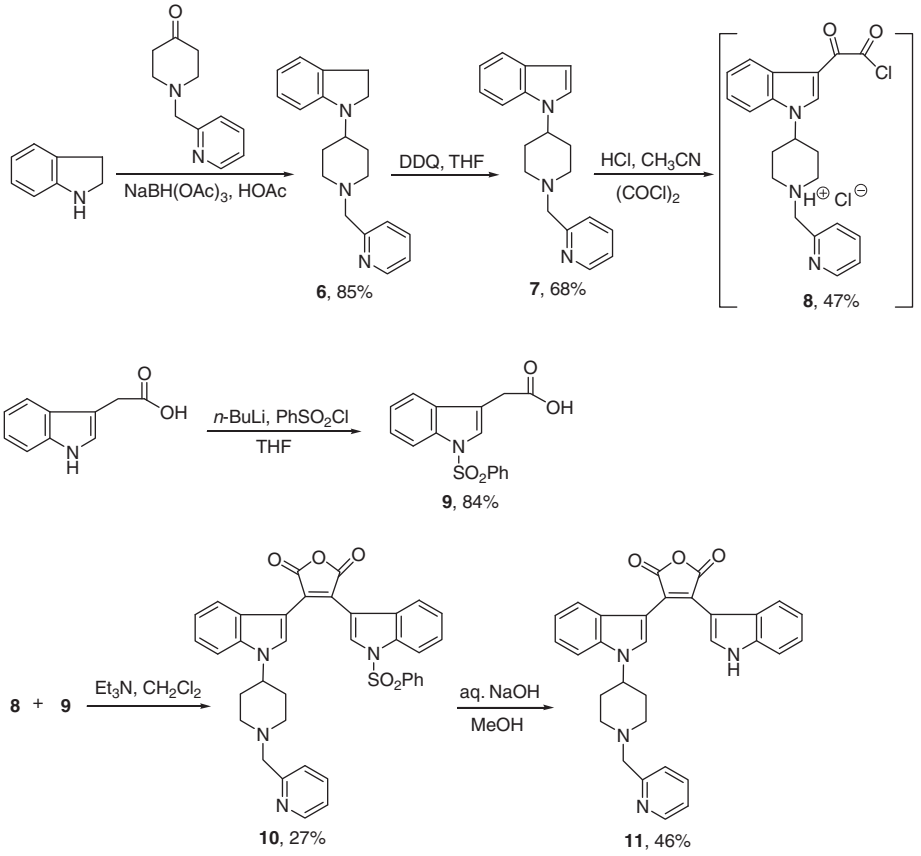


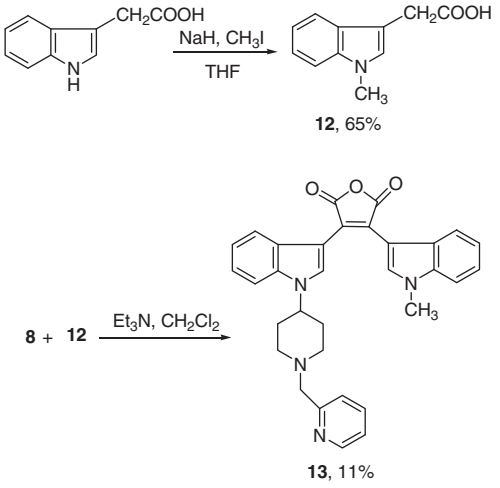
Scheme 1. Synthesis of Enzastaurin and *N-des*-methylated Enzastaurin.



Scheme 2. Synthesis of maleic anhydride precursor.

achieved by condensation of indolyl acetamide **3** with indole-3-glyoxylate esters **4a–b** in the presence of *t*-BuOK in THF in 68% and 50% yield, respectively.

Enzastaurin is an agent with potential utility in the treatment of solid tumors, which is currently in phase II clinical trials. The synthesis of carbon-14-labeled and tritium-labeled Enzastaurin for use in ADME (absorption, distribution, metabolism, elimination) studies in laboratory animals and humans has been reported.[1] Different from the stable isotopes carbon-14 and tritium, carbon-11 only has a short half-life ($T_{1/2}$ = 20 min). Hence, the synthetic strategies employed for the carbon-14 and tritium labeled enzastaurins are unsuitable for the synthesis of carbon-11-labled enzastaurin. The most straightforward procedure to prepare carbon-11-labled enzastaurin would entail the ^{11}C-methylation of *N-des*-methylated Enzastaurin (**5a**) with the most commonly used ^{11}C-methylating reagent, [^{11}C]methy iodide ([^{11}C]CH_3I) or [^{11}C]methyl triflate ([^{11}C]CH_3OTf).[12,13] However, the p*K*a value of maleimide is much lower than that of indole, and methylation would perform mainly on the maleimide nitrogen, not on the indole nitrogen.[1] Considering that maleic anhydride was mild enough to tolerate a wide range of substituent functionalities and can be converted to maleimide in the presence of ammonia or an ammonia source, we selected its maleic anhydride **11** as the precursor to escape the competitive methylation of indole nitrogen with maleimide nitrogen. We have reported a method for preparation of maleic anhydride by hydrolysis of its corresponding maleimide to synthesize the precursor of SB-216763.[9] Initially, this method prompted us to hydrolyze maleimide **5a** with 2 N KOH in EtOH to prepare maleic anhydride **11**. Unfortunately, it failed to give the desired compound **11**. Therefore, we had to resort to other synthetic strategy to obtain the precursor.

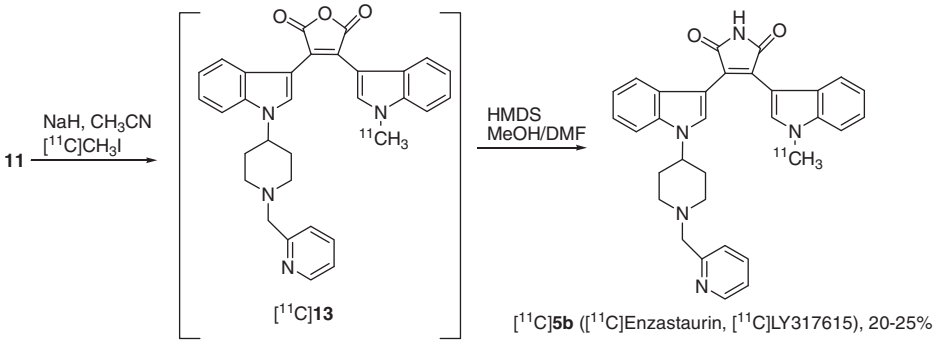


Scheme 3. Synthesis of maleic anhydride intermediate.

As shown in Scheme 2, condensation of indolin and 1-pyridin-2-ylmethylpiperidin-4-one, followed by reductive amination with $NaBH(OAc)_3$ in HOAC afforded **6** in 85% yield. The indolin ring of **6** was oxidized by DDQ in THF to yield its corresponding indole compound **7** in 68% yield. Treatment of **7** in CH_3CN with 1.0 M HCl in Et_2O formed monohydrochloride salt, followed by the reaction with oxalyl chloride in CH_3CN at 0 °C to generate indole-3-glyoxylyl chloride **8** in 47% yield, which was used for next step reaction without further purification. The indole *N*-phenylsulfonyl group was introduced from reaction of indole-3-acetic acid with benzenesulfonyl chloride in THF at −70 °C to achieve **9** in 84% yield. Coupling of *N*-protected indole-3-acetic acid **9** with indolyl-3-glyoxylyl chloride **8** in the presence of Et_3N in CH_2Cl_2 resulted in maleic anhydride **10** in a very low yield (<2%). Surprisingly, the yield was increased to 27% when 4 Å molecular sieves were added to the reaction system, and the reaction time was shortened from overnight to 5 h. The *N*-phenylsulfonyl group of **10** was removed by hydrolysis with 20% NaOH in MeOH to afford the precursor **11** in 46% yield.

As shown in Scheme 3, an intermediate standard compound **13** was prepared in 11% yield by coupling indolyl-3-glyoxylyl chloride **8** with *N*-methyl indole-3-acetic acid **12**,[14] which was obtained by methylation of 3-indolylacetic acid on nitrogen with CH_3I in the presence of NaH in THF in 65% yield.

Synthesis of the target radiotracer [^{11}C]Enzastaurin ([^{11}C]**5b**) is indicated in Scheme 4. Precursor **11** was labeled by [^{11}C]CH_3I prepared from [^{11}C]CO_2,[12,13] in the presence of NaH in acetonitrile through the N-[^{11}C]methylation[8,9] to provide a radiolabeling intermediate [^{11}C]**13**. Without further purification, [^{11}C]**13** was quickly converted to the target tracer [^{11}C]**5b** using hexamethyldisilazane (HMDS) in MeOH/DMF. [^{11}C]**5b** was isolated from the radiolabeling mixture by the semi-preparative reversed-phase high performance liquid chromatography (HPLC) in 20–25% radiochemical yields, decay corrected to end of bombardment (EOB), based on [^{11}C]CO_2. Another more reactive radiolabeled precursor [^{11}C]CH_3OTf[12,13] was also used in the radiolabeling reaction of the precursor **11**, but the radiochemical yield was much lower than that of [^{11}C]CH_3I. This suggested it is better to use [^{11}C]CH_3I for the N-[^{11}C]methylation of indole nitrogen, which is consistent with the results from our previous works.[8,9] The synthesis was performed in an automated multi-purpose ^{11}C-radiosynthesis module, allowing measurement of specific activity during synthesis.[15,16] The specific activity of [^{11}C]**5b** was in a range of 370–555 GBq/μmol at EOB measured by the on-the-fly technique using semi-preparative HPLC during synthesis[16] and 185–278 GBq/μmol at the end of synthesis (EOS) determined by analytical HPLC,[17] respectively. Chemical purity and radiochemical purity were determined by analytical HPLC.[17] The chemical purity of the precursor **11**, intermediate **13** and reference standard **5b** was >95%. The radiochemical purity of the target tracer [^{11}C]**5b** was >99% determined by radio-HPLC through γ-ray (PIN diode) flow detector, and the chemical purity



Scheme 4. Synthesis of [^{11}C]Enzastaurin.

of the target tracers [^{11}C]**5b** was >93% determined by reversed-phase HPLC through UV flow detector.

The synthetic information of Enzastaurin was limited in the literature. Thus, the experimental details and characterization data for compounds **1–13** and for the tracer [^{11}C]**5b** are given.[18]

In summary, [^{11}C]Enzastaurin was first designed and synthesized as a new potential PET agent for imaging of PKC in various diseases. An automated multi-purpose ^{11}C-radiosynthesis module of our own design for fully automated synthesis of [^{11}C]Enzastaurin has been built, featuring the measurement of specific activity by the on-the-fly technique. The radiosynthesis employed a one-pot two-step reaction via N-[^{11}C]methylation radiolabeling on indole nitrogen of the precursor incorporated efficiently with the most commonly used [^{11}C]methylating agent, [^{11}C]CH_3I, produced by gas-phase production of [^{11}C]methyl bromide ([^{11}C]CH_3Br) from our laboratory. The target tracer was isolated and purified by a semi-preparative HPLC procedure in moderate radiochemical yields, short overall synthesis time, and high specific activity. These results facilitate the potential preclinical and clinical PET studies of [^{11}C]Enzastaurin in animals and humans.

Acknowledgments

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18. (a) *General*. All commercial reagents and solvents from Sigma–Aldrich and Fisher Scientific were used without further purification. [^{11}C]CH_3Br was prepared according to a literature procedure,[13] and [^{11}C]CH_3I was prepared from [^{11}C]CH_3Br by passing through a NaI column (NaI mixed with Carbograph™-2 60/80, Grace, 1:1 w/w) at 300 °C. Melting points were determined on a MEL-TEMP II capillary tube apparatus and were uncorrected. ^{1}H NMR spectra were recorded on 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). Liquid chromatography–mass spectra (LC–MS) analysis was performed on an Agilent system, consisting of an 1100 series HPLC connected to a diode array detector and a 1946 D mass spectrometer configured for positive-ion/negative-ion electrospray ionization. The high resolution mass spectra (HRMS) were obtained using a Waters/Micromass LCT Classic 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. Semi-preparative HPLC was performed using a YMC-Pack ODS-A, S-5 μm, 12 nm, 10×250 mm C-18 column; 3:1:1 CH_3CN/MeOH/20 mM, pH 6.7 phosphate (buffer solution) mobile phase; 5.0 mL/min flow rate; UV (254 nm) and γ-ray (PIN diode) flow detectors. Sterile Millex-GS 0.22 μm vented filter unit was obtained from Millipore Corporation, Bedford, MA.
(b) 2-(Indolin-3-yl)acetamide (**1**). A suspension of indole-3-acetamide (7.75 g, 44.5 mmol) in MeOH (60 mL) was cooled to 0 °C. 12 N HCl (21 mL, 252 mmol) was added dropwise, keeping the reaction temperature below 25 °C. After cooling to 0 °C, borane-pyridine complex (15 mL, 150 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 1 h. After cooling to 0 °C, water (75 mL) was added slowly. The mixture was basified to pH 8.0 with 5 N NaOH and then extracted with EtOAc. The combined organic layer was washed with saturated $NaHCO_3$, brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by column chromatography (20:1 EtOAc/MeOH) to afford **1** (3.8 g, 49%) as a white solid: mp 88–90 °C; ^{1}H NMR (DMSO-d_6) δ 7.36 (br s, 1H), 7.00 (d, J = 7.0 Hz, 1H), 6.90 (td, J = 7.5, 0.5 Hz, 1H), 6.85 (br s, 1H), 6.51 (td, J = 7.5, 0.5 Hz, 1H), 6.47 (d, J = 7.5 Hz, 1H), 5.43 ((br s, 1H), 3.55–3.47 (m, 2H), 3.03–3.05 (m, 1H), 2.50–2.45 (m, 1H), 2.25–2.20 (m, 1H).
(c) 2-(1-(1-(Pyridin-2-ylmethyl)piperidin-4-yl)indolin-3-yl)acetamide (**2**). To a stirred solution of **1** (3.15 g, 17.9 mmol) in HOAc (15 mL) was added 1-pyridin-2-ylmethylpiperidin-4-one (3.74 g, 19.7 mmol) in one portion at room temperature. After cooling to 0 °C, $NaBH(OAc)_3$ (5.72 g, 27.0 mmol) was added in portions. The reaction mixture was stirred at room temperature for 3 h. The mixture was cooled, diluted with water and basified to pH 11.0 with 5 N NaOH, then extracted with EtOAc. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The residue was first purified by column chromatography (100:10:1 EtOAc/MeOH/NH_4OH) to obtain a yellow sticky oil, which was triturated with minimal cold 9:1 EtOAc/MeOH to afford **2** (5.41 g, 86%) as a white solid: mp 52–53 °C; ^{1}H NMR (DMSO-d_6) δ 8.48 (d, J = 4.5 Hz, 1H), 7.76 (td, J = 1.5, 7.5 Hz, 1H), 7.45 (d, J = 7.5 Hz, 1H), 7.37 (br s, 1H), 7.25 (t, J = 6.0 Hz, 1H), 6.70–6.96 (m, 2H), 6.88 (br s, 1H), 6.51 (t, J = 7.5 Hz, 1H), 6.45 (d, J = 7.5 Hz, 1H), 3.61 (s, 2H), 3.50–3.47 (m, 2H), 2.99 (t, J = 7.0 Hz, 1H), 2.91 (d, J = 5.5 Hz, 2H), 2.48–2.50 (m, 2H), 2.25–2.15 (m, 3H), 1.66–1.58 (m, 4H).
(d) 2-(1-(1-(Pyridin-2-ylmethyl)piperidin-4-yl)-1*H*-indol-3-yl)acetamide (**3**). A suspension of **2** (3.90 g, 11.1 mmol) in THF (60 mL) was cooled to 0 °C under N_2 atmosphere. A solution of DDQ (2.78 g, 12.2 mmol) in THF (25 mL) was added dropwise. The reaction mixture was stirred at room temperature overnight. The reaction mixture was diluted with water (100 mL) and 5% $NaHCO_3$ (100 mL). THF was removed in vacuo and the solid precipitated from the solution. After collected by filtration, and rinsed with water and EtOAc, the solid was reslurried in 5:1 EtOAc/MeOH at 50 °C for 15 min, cooled to room temperature, filtered, and rinsed with minimal 5:1 EtOAc/MeOH to afford **3** (2.33 g, 60%) as a pale brown solid: mp 202–203 °C; ^{1}H NMR (DMSO-d_6) δ 8.51 (s, 1H), 7.79 (t, J = 7.0 Hz, 1H), 7.55–7.48 (m, 3H), 7.34–7.28 (m, 3H), 7.11 (t, J = 7.5 Hz, 1H), 6.70 (t, J = 7.0 Hz, 1H), 6.84 (br s, 1H), 4.34 (m, 1H), 3.68 (s, 2H), 3.46 (s, 2H), 2.98 (d, J = 9.5 Hz, 2H), 2.32 (m, 2H), 2.04–1.94 (m, 4H); MS (ESI, m/z): 349 ([M+H]$^+$, 100%).
(e) Methyl 2-(1*H*-indol-3-yl)-2-oxoacetate (**4a**). To a stirred solution of indole (2.0 g, 17.1 mmol) in Et_2O (20 mL) was added oxalyl chloride (1.5 mL, 17.2 mmol) dropwise at 0 °C. After the resultant yellow slurry was stirred at 0 °C for 0.5 h, it was cooled to −78 °C. A solution of NaOMe in MeOH (25 wt %, 7.8 mL, 34.1 mmol) was added to this slurry at −78 °C. The reaction mixture was allowed to warm to room temperature and quenched by addition of water (10 mL). The solid was collected by filtration, rinsed with water and dried to give **4a** (2.53 g, 73%) as a yellow solid: mp 160 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 12.42 (s, 1H), 8.45 (d, J = 3.5 Hz, 1H), 8.16 (dd, J = 1.5, 6.0 Hz, 1H), 7.55 (dd, J = 1.5, 6.0 Hz, 1H), 7.32–7.27 (m, 2H), 3.89 (s, 3H).
(f) Methyl 2-(1-methyl-1*H*-indol-3-yl)-2-oxoacetate (**4b**). To a stirred solution of 1-methyl-1*H*-indole (5.0 g, 38.2 mmol) in Et_2O (50 mL) was added oxalyl chloride (3.5 mL, 40.1 mmol) dropwise at 0 °C. After the resultant yellow slurry was stirred at 0 °C for 0.5 h, it was cooled to −78 °C. A solution of NaOMe in MeOH (25 wt %, 22.5 mL, 98.5 mmol) was added to this slurry at −78 °C. The reaction mixture was allowed to warm to room temperature and quenched by addition of water (25 mL). The solid was collected by filtration, rinsed with water, Et_2O and dried to give **4b** (2.83 g, 34%) as a tan solid: mp 80–81 °C; ^{1}H NMR (DMSO-d_6) δ 8.50 (s, 1H), 8.18 (d, J = 7.5 Hz, 1H), 7.62 (d, J = 8.0 Hz, 1H), 7.38–7.31 (m, 2H), 3.92 (s, 3H), 3.90 (s, 3H).

(g) 3-(1*H*-Indol-3-yl)-4-(1-(1-(pyridin-2-ylmethyl)piperidin-4-yl)-1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione (**5a**). To a stirred suspension of **3** (200 mg, 0.57 mmol) and **4a** (168 mg, 0.83 mmol) in THF (5 mL) was added 1.0 M *t*-BuOK in THF(1.15 mL, 1.15 mmol) at 0 °C. The reaction mixture was stirred at room temperature overnight. The mixture was partitioned between EtOAc and 5% $NaHCO_3$. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by preparative TLC plate (100:5 CH_2Cl_2/MeOH) to afford **5a** (194.5 mg, 68%) as an orange solid: mp 259 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 11.68 (s, 1H), 10.91 (s, 1H), 8.51 (d, *J* = 4.0 Hz, 1H), 7.78–7.77 (m, 2H), 7.69 (s, 1H), 7.56 (d, *J* = 8.5 Hz, 1H), 7.48 (d, *J* = 7.5 Hz, 1H), 7.38 (d, *J* = 8.0 Hz, 1H), 7.28–7.26 (m,1H), 7.07–6.96 (m, 3H), 6.75 (t, *J* = 7.5 Hz, 1H), 6.66 (d, *J* = 8.0 Hz, 1H), 6.61 (t, *J* = 7.5 Hz, 1H), 4.43 (m, 1H), 3.67 (s, 2H), 2.94 (d, *J* = 7.5 Hz, 2H), 2.32 (m, 2H), 1.91 (m, 4H); MS (ESI, *m/z*): 502 ([M+H]$^+$, 100%).

(h) 3-(1-Methyl-1*H*-indol-3-yl)-4-(1-(1-(pyridin-2-ylmethyl)piperidin-4-yl)-1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione (Enzastaurin, **5b**). To a stirred suspension of **3** (200 mg, 0.57 mmol) and **4b** (150 mg, 0.69 mmol) in THF (5 mL) was added 1.0 M *t*-BuOK in THF (1.15 mL, 1.15 mmol) at 0 °C. After the reaction mixture was stirred at room temperature overnight, it was partitioned between EtOAc and 5% $NaHCO_3$. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by preparative TLC plate (100:5 CH_2Cl_2/MeOH) to afford **5b** (147 mg, 50%) as a red solid: mp 154–156 °C; ^{1}H NMR (DMSO-d_6) δ 10.93 (s, 1H), 8.50 (d, *J* = 4.0 Hz, 1H), 7.88 (s, 1H), 7.78 (t, *J* = 7.5 Hz, 1H), 7.76 (s, 1H), 7.57 (d, *J* = 8.5 Hz, 1H), 7.46 (d, *J* = 8.0 Hz, 1H), 7.43 (d, *J* = 8.5 Hz, 1H), 7.27 (t, *J* = 6.0 Hz, 1H), 7.08–7.02 (m, 3H), 6.77 (t, *J* = 7.5 Hz, 1H), 6.62 (t, *J* = 7.5 Hz, 1H), 6.56 (d, *J* = 8.0 Hz, 1H), 4.43–4.45 (m, 1H), 3.86 (s, 3H), 3.65 (s, 2H), 2.92 (d, *J* = 10 Hz, 2H), 2.30 (m, 2H), 1.91–1.84 (m, 4H); MS (ESI, *m/z*): 516 ([M+H]$^+$, 100%); HRMS (ESI, *m/z*): Calcd for $C_{32}H_{30}N_5O_2$ ([M+H]$^+$) 516.2400. Found: 516.2389.

(i) 1-(1-(Pyridin-2-ylmethyl)piperidin-4-yl)indoline (**6**). To a stirred solution of indoline (4.0 g, 33.6 mmol) in HOAc (20 mL) was added 1-pyridin-2-ylmethylpiperidin-4-one (7.02 g, 36.9 mmol) in one portion at room temperature. After cooling to 0 °C, $NaBH(OAc)_3$ (10.7 g, 50.3 mmol) was added in portions. The reaction mixture was stirred at room temperature for 5 h. The mixture was cooled, diluted with water, basified to pH 11.0 with 5 N NaOH, and then extracted with EtOAc. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The residue was first purified by column chromatography (500:10:1 EtOAc/MeOH/NH_4OH) to afford **6** (8.37 g, 85%) as a pale brown solid: mp 89–90 °C; ^{1}H NMR (DMSO-d_6) δ 8.48 (dd, *J* = 0.5, 5.0 Hz, 1H), 7.76 (td, *J* = 1.5, 7.5 Hz, 1H), 7.44 (d, *J* = 8.0 Hz, 1H), 7.26–7.25 (m, 1H), 6.98 (d, *J* = 7.5 Hz, 1H), 6.95 (t, *J* = 7.5 Hz, 1H), 6.50 (t, *J* = 7.0 Hz, 1H), 6.43 (d, *J* = 8.0 Hz, 1H), 3.60 (s, 2H), 3.38–3.35 (m, 1H), 3.32 (m, 3H), 2.91 (d, *J* = 7.0 Hz, 2H), 2.85 (t, *J* = 8.0 Hz, 2H), 2.17–2.12 (m, 2H), 1.65–1.62 (m, 4H); MS (ESI, *m/z*): 294 ([M+H]$^+$, 100%).

(j) 1-(1-(Pyridin-2-ylmethyl)piperidin-4-yl)-1*H*-indole (**7**). A solution of **6** (8.36 g, 28.5 mmol) in THF (80 mL) was cooled to 0 °C under N_2 atmosphere. A solution of DDQ (7.18 g, 31.6 mmol) in THF (40 mL) was added dropwise. After stirring at room temperature for 4 h, the reaction mixture was diluted with water, basified to pH 11.0 with 5 N NaOH, and then extracted with EtOAc. Saturated $NaHCO_3$ was added to help the separation of two phases. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The residue was purified was purified by column chromatography (500:10:1–500:20:2 EtOAc/MeOH/NH_4OH) to afford **7** (5.66 g, 68%) as a pale brown solid: mp 106–108 °C; ^{1}H NMR (DMSO-d_6) δ 8.51 (dt, *J* = 1.0, 4.0 Hz, 1H), 7.76 (td, *J* = 2.0, 7.5 Hz, 1H), 7.54–7.50 (m, 4H), 7.28–7.26 (m, 1H), 7.13–7.10 (m, 1H), 7.02–6.99 (m, 1H), 6.44 (d, *J* = 3.0 Hz, 1H), 4.40–4.34 (m, 1H), 3.68 (s, 2H), 2.98 (d, *J* = 12.0 Hz, 2H), 2.32 (t, *J* = 11.0 Hz, 2H), 2.06–1.91 (m, 4H); MS (ESI, *m/z*): 292 ([M+H]$^+$, 100%).

(k) 4-(3-(2-Chloro-2-oxoacetyl)-1*H*-indol-1-yl)-1-(pyridin-2-ylmethyl)piperidinium chloride (**8**). To a stirred suspension of **7** (2.0 g, 6.87 mmol) in CH_3CN (60 mL) was added 1.0 M HCl in Et_2O (7.0 mL, 7.0 mmol) at 0 °C. After the white slurry was stirred at room temperature for 1 h, it was filtered and dried to afford a HCl salt (0.95 g) as an off-white solid. The HCl salt was added to CH_3CN (10 mL), and the suspension was cooled to −10 °C. Oxalyl chloride (0.5 mL, 5.80 mmol) was added dropwise. After the resultant yellow slurry was stirred at 0 °C for 2 h, it was filtered, rinsed with cold CH_3CN and Et_2O, and dried to afford **8** (1.35 g, 47%) as a yellow solid. This compound was used for next step without purification.

(l) 2-(1-(Phenylsulfonyl)-1*H*-indol-3-yl)acetic acid (**9**). To a stirred solution of indole-3-acetic acid (5.26 g, 30.0 mmol) in THF (150 mL) was added 2.5 M *n*-BuLi in hexane (25.6 mL, 64.0 mmol) dropwise under nitrogen atmosphere at −70 °C. After stirring at same temperature for 1 h, a solution of benzenesulfonyl chloride (3.8 mL, 30.0 mmol) in THF (50 mL) was added slowly. The reaction mixture was then allowed to warm to room temperature, and stirred overnight. The solvent was evaporated in vacuo, and the residue was dissolved in CH_2Cl_2, and washed with 1 N HCl. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and filtered. The solvent was evaporated in vacuo, and the crude product was recrystallized from EtOAc/hexanes to give **9** (7.9 g, 84%) as a brown solid: mp 165–167 °C; ^{1}H NMR (DMSO-d_6) δ 12.5 (s, 1H), 7.97–7.92 (m, 3H), 7.74 (s, 1H), 7.70–7.67 (m, 1H), 7.59 (t, *J* = 8.0 Hz, 2H), 7.54 (d, *J* = 8.0 Hz, 1H), 7.37–7.34 (m, 1H), 7.28–7.25 (m, 1H), 3.71 (s, 2H).

(m) 3-(1-(Phenylsulfonyl)-1*H*-indol-3-yl)-4-(1-(1-(pyridin-2-ylmethyl)piperidin-4-yl)-1*H*-indol-3-yl)furan-2,5-dione (**10**). To a stirred solution of **9** (875 mg, 2.78 mmol) and Et_3N (1.16 mL, 8.34 mmol) in CH_2Cl_2 (30 mL) containing 4 Å molecular sieves was added **8** (1.10 g, 2.63 mmol) in portions at 0 °C. After the reaction mixture was stirred at room temperature for 5 h, the solvent was evaporated in vacuo. The residue was purified by column chromatography (1:1.5 hexanes/acetone) to afford **10** (417 mg, 27%) as a red solid: mp 108–109 °C; ^{1}H NMR (DMSO-d_6) δ 8.52 (d, *J* = 4.5 Hz, 1H), 8.14 (s, 1H), 8.04 (dd, *J* = 1.0, 8.5 Hz, 2H), 7.95 (d, *J* = 8.5 Hz, 1H), 7.92 (s, 1H), 7.81 (td, *J* = 1.5, 7.5 Hz, 1H), 7.78–7.75 (m, 1H), 7.64 (t, *J* = 8.0 Hz, 2H), 7.60 (d, *J* = 8.5 Hz, 1H), 7.49 (d, *J* = 7.5 Hz, 1H), 7.30–7.24 (m, 2H), 7.06–7.03 (m, 1H), 6.92–6.88 (m, 1H), 6.84 (d, *J* = 8.0 Hz, 1H), 6.71 (d, *J* = 8.0 Hz, 1H), 6.40 (t, *J* = 7.5 Hz, 1H), 4.49–4.44 (m, 1H), 3.68 (s, 2H), 2.94 (d, *J* = 1.0 Hz, 2H), 2.37–2.32 (m, 2H), 1.99–1.79 (m, 4H); MS (ESI, *m/z*): 643 ([M+H]$^+$, 100%); HRMS (ESI, *m/z*): Calcd for $C_{37}H_{31}N_4O_5S$ ([M+H]$^+$) 643.2015. Found: 643.2009.

(n) 3-(1*H*-Indol-3-yl)-4-(1-(1-(pyridin-2-ylmethyl)piperidin-4-yl)-1*H*-indol-3-yl)furan-2,5-dione (**11**). To a stirred suspension of **10** (280 mg, 0.44 mmol) in MeOH (5 mL) was added 20% NaOH (4 mL). The reaction mixture was heated at reflux for 4 h. MeOH was evaporated in vacuo, and the residue was poured into ice-water, and acidified to pH 2.0 with 2 N HCl. The solid was collected by filtration, and rinsed with EtOAc and Et_2O to afford **11** (101 mg, 46%) as a red solid: mp 268 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 11.95 (d, *J* = 2.5 Hz, 1H), 8.50 (d, *J* = 4.0 Hz, 1H), 7.92 (d, *J* = 3.0 Hz, 1H), 7.80–7.76 (m, 2H), 7.64 (d, *J* = 8.5 Hz, 1H), 7.45 (t, *J* = 8.5 Hz, 2H), 7.28–7.26 (m, 1H), 7.14–7.10 (m, 2H), 7.08–7.04 (m, 1H), 6.85 (t, *J* = 7.5 Hz, 1H), 6.72–6.68 (m, 2H), 4.48–4.44 (m, 1H), 3.65 (s, 2H), 2.94 (d, *J* = 7.5 Hz, 2H), 2.31 (t, *J* = 6.5 Hz, 2H), 1.99–1.84 (m, 4H); HRMS (ESI, *m/z*): Calcd for $C_{31}H_{27}N_4O_3$ ([M+H]$^+$) 503.2083. Found: 503.2079.

(o) 2-(1-Methyl-1*H*-indol-3-yl)acetic acid (**12**). To a stirred suspension of NaH (60% dispersion in mineral oil, 3.0 g, 75 mmol) in THF (60 mL) was added a solution of indole-3-acetic acid (2.63 g, 15 mmol) in THF (25 mL) at 0 °C. After stirring at 0 °C for 0.5 h, a solution of methyl iodide (3.1 mL, 50 mmol) in THF (20 mL) was added. The reaction mixture was stirred at room temperature overnight, and then cooled to 0 °C. MeOH (4 mL) was added slowly to destroy excess hydride with vigorous stirring, followed by cold water until a clear yellow solution resulted. Et_2O (50 mL) was added. The aqueous layer was separated, acidified with 6 N HCl and extracted with CH_2Cl_2. The combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated to about 10 mL. Hexane was added slowly until a brown solid completely precipitated out. The crude solid was recrystallized from EtOH to give **12** (2.11 g, 65%) as a tan solid: mp 121–123 °C (lit.[14] 127–128 °C); ^{1}H NMR ($CDCl_3$) δ 7.58 (d, *J* = 8.0 Hz, 1H), 7.29 (d, *J* = 8.0 Hz, 1H), 7.23–7.21 (m, 1H), 7.14–7.11 (m, 1H), 7.03 (s, 1H), 3.79 (s, 2H), 3.75 (s, 3H).

(p) 3-(1-Methyl-1*H*-indol-3-yl)-4-(1-(1-(pyridin-2-ylmethyl)piperidin-4-yl)-1*H*-indol-3-yl)furan-2,5-dione (**13**). To a stirred solution of **12** (25 mg, 0.13 mmol) and Et_3N (0.05 mL, 0.33 mmol) in CH_2Cl_2 (1 mL) containing 4 Å molecular sieves was added **8** (50 mg, 0.11 mmol) in portions at 0 °C. After the reaction mixture was stirred at room temperature for 4 h, the solvent was evaporated in vacuo. The residue was purified by column chromatography (1:1.5 hexanes/acetone) to afford **13** (6.0 mg, 11%) as a red solid: mp 121–123 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 8.51 (d, *J* = 4.0 Hz, 1H), 8.02 (s, 1H), 7.83–7.78 (m, 1H), 7.76(s, 1H), 7.65 (d, *J* = 8.5 Hz, 1H), 7.50 (d, *J* = 8.5 Hz, 1H), 7.46 (d, *J* = 7.5 Hz, 1H), 7.32–7.28 (m, 1H), 7.17–7.10 (m, 3H), 6.87(t, *J* = 7.5 Hz, 1H), 6.71 (t, *J* = 7.5 Hz, 1H), 6.60 (d, *J* = 8.0 Hz, 1H), 4.50–4.45 (m, 1H), 3.90 (s, 3H), 3.68 (s, 2H), 2.93 (d, *J* = 11.5 Hz, 2H), 2.39–2.32 (m, 2H), 1.92–1.85 (m, 4H); MS (ESI, *m/z*): 517 ([M+H]$^+$, 100%); HRMS (ESI, *m/z*): Calcd for $C_{32}H_{29}N_4O_3$ ([M+H]$^+$) 517.2240. Found: 517.2249.

(q) 3-(1-[^{11}C]Methyl-1*H*-indol-3-yl)-4-[1-[1-(2-pyridinylmethyl)-4-piperidinyl]-1*H*-indol-3-yl]-1*H*-pyrrole-2,5-dione ([^{11}C]Enzastaurin, [^{11}C]**5b**). [^{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 (Eclipse RDS-111). The proton-beam current was 55 μA, and the irradiation time was 30 min. The precursor **11** (0.1–0.3 mg) was dissolved in CH_3CN (300 μL). To this solution was added NaH (1 mg). The mixture was transferred to a small reaction vial. No-carrier-added (high specific activity) [^{11}C]CH_3I that was produced by the gas-phase production method[14] from [^{11}C]CO_2 through [^{11}C]CH_4 and [^{11}C]CH_3Br with NaI column was passed into the reaction vial at 0 °C until radioactivity reached a maximum (~2 min), and then the reaction vial was isolated and heated at 45 °C for 4 min to produce 3-(1-[^{11}C]methyl-1*H*-indol-3-yl)-4-(1-(1-(pyridin-2-ylmethyl)piperidin-4-yl)-1*H*-indol-3-yl)furan-2,5-dione ([^{11}C]**13**). Then, a solution of HMDS (2 μL) and MeOH (2 μL) in DMF (300 μL) was introduced to the reaction vial. The reaction mixture was sealed and heated at 80 °C for 8 min. The contents of the reaction vial were diluted with $NaHCO_3$ (1 mL, 0.1 M), and injected onto the semi-preparative HPLC column with 2 mL injection loop. The product fraction was collected, the solvent was removed by rotatory evaporation under vacuum, and the final product, [^{11}C]**5b**, was formulated in 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 total volume was noted for tracer dose dispensing. The overall synthesis, purification and formulation time was 35–40 min from EOB. Retention times in the analytical HPLC system were: t_R **11** = 5.21 min, t_R **13** = 4.06 min, t_R **5b** = 7.79 min, t_R [^{11}C]**13** = 4.06 min, and t_R [^{11}C]**5b** = 7.79 min. Retention times in the semi-preparative HPLC system were: t_R **11** = 6.18 min, t_R **13** = 5.51 min, t_R **5b** = 11.15 min, t_R [^{11}C]**13** = 5.51 min, and t_R [^{11}C]**5b** = 11.15 min. The radiochemical yields were 20–25% decay corrected to EOB, based on [^{11}C]CO_2.