Steroids 75 (2010) 967–973

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Synthesis of carbon-11-labeled casimiroin analogues as new potential PET agents for imaging of quinone reductase 2 and aromatase expression in breast cancer

Min Wang [a], Mingzhang Gao [a], Kathy D. Miller [b], George W. Sledge [b], Gary D. Hutchins [a], Qi-Huang Zheng [a,*]

[a] Department of Radiology and Imaging Sciences, Indiana University School of Medicine, 1345 West 16th Street, L3-208, Indianapolis, IN 46202, USA
[b] Department of Medicine, Indiana University School of Medicine, Indianapolis, IN 46202, USA

ARTICLE INFO

Article history:
Received 16 April 2010
Received in revised form 27 May 2010
Accepted 7 June 2010
Available online 15 June 2010

Keywords:
Positron emission tomography (PET)
Quinone reductase (QR) 2
Aromatase
Casimiroin analogues
Radiotracers
Cancer imaging

ABSTRACT

Carbon-11-labeled casimiroin analogues were first designed and synthesized as new potential PET agents for imaging of quinone reductase (QR) 2 and aromatase expression in breast cancer. [^{11}C]casimiroin (6-[^{11}C]methoxy-9-methyl-[1,3]dioxolo[4,5-*h*]quinolin-8(9*H*)-one, [^{11}C]**11**) and its carbon-11-labeled analogues 5,6,8-trimethoxy-1-[^{11}C]methyl-4-methylquinolin-2(1*H*)-one ([^{11}C]**17**), 8-methoxy-1-[^{11}C]methyl-4-methylquinolin-2(1*H*)-one ([^{11}C]**21a**), 6,8-dimethoxy-1-[^{11}C]methyl-4-methylquinolin-2(1*H*)-one ([^{11}C]**21b**), and 5,8-dimethoxy-1-[^{11}C]methyl-4-methylquinolin-2(1*H*)-one ([^{11}C]**21c**), were prepared from their corresponding precursors with [^{11}C]methyl triflate ([^{11}C]CH_3OTf) under basic conditions (NaH) through either *O*- or *N*-[^{11}C]methylation and isolated by semi-preparative HPLC method in 40–50% radiochemical yields decay corrected to end of bombardment (EOB), based on [^{11}C]CO_2, and 111–185 GBq/μmol specific activity at the end of synthesis (EOS).

1. Introduction

Breast cancer is the most common cancer diagnosed in women and is the second leading cause of death in women after lung cancer in the United States [1]. Approximately 75% of breast cancers test positive for estrogen receptor (ER), the progesterone receptor (PgR), or both, and estrogen stimulation of these receptors is a significant factor in the development and growth of breast cancer [2,3]. The enzyme quinone reductase (QR), a detoxifying phase II antioxidant enzyme, is a flavoprotein that reversibly catalyzes the oxidation of the reduced form of nicotinamide adenine dinucleotide or nicotinamide adenine dinucleotide phosphate (NADH or NADPH) by various quinones and oxidation-reduction dyes [4]. There are two main QRs reported in the literature, QR1 and QR2. QR1, a FAD (flavin adenine dinucleotide)-binding protein that forms homodimers and reduces quinones to hydroquinones, has its activity up regulated by antiestrogens in an ER-dependent manner in breast cancer cells [5,6]. QR2, a cytosolic FAD-dependent flavoenzyme that catalyzes metabolic reduction of quinones with unexpected cosubstrate specificity, may actually transform certain quinone substrates into more highly reactive species that are capable of causing more cellular damage in cancer, and cancer is characterized by the uncontrolled growth and spread of abnormal cells [7–13]. The enzyme aromatase, a key cytochrome P450 enzyme, is responsible for a key step in the biosynthesis of estrogens and catalyzes the rate-limiting aromatization step in the conversion of androgens to estrogens [2,7]. The enzymes QR and aromatase are key enzymes in sex steroid production related to ER, and provide the attractive molecular targets for the development of anticancer drugs for breast cancer. The inhibition of estrogen synthesis by aromatase inhibitors (AIs) and inhibition of estrogen action by compounds interacting with ERs (antiestrogens) are two strategies for the design of drugs that can be used to ameliorate the growth effects of estrogens on ER positive (ER^+) tumor cells [14]. AIs have been extensively studied as effective hormonal therapeutic drugs in the treatment of ER^+ breast cancer. They are equivalent or superior to antiestrogen tamoxifen in women with metastatic diseases. In addition, AIs provide an effective treatment in some patients relapsing from tamoxifen [14–16]. The up-regulation of QR may contribute to the beneficial effects of tamoxifen and other antiestrogens in breast cancer prevention and treatment [4]. A next logical step to improve cancer therapeutic benefit would be to develop dual inhibitors to target both enzymes simultaneously [17]. Recently a new series of casimiroin analogues has been developed as new QR2 and aromatase inhibitors, potential chemopreventive or chemotherapeutic agents [7]. The

* Corresponding author at: Department of Radiology and Imaging Sciences, Indiana University School of Medicine, 1345 West 16th Street, L3-208, Indianapolis, IN 46202-2111, USA. Tel.: +1 317 278 4671; fax: +1 317 278 9711.
E-mail address: qzheng@iupui.edu (Q.-H. Zheng).

0039-128X/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.steroids.2010.06.004

enzymes QR and aromatase also provide the attractive targets for the development of enzyme-based breast cancer imaging agents for use in the biomedical imaging technique positron emission tomography (PET). Casimiroin analogues labeled with a positron-emitting radionuclide such as carbon-11 may enable non-invasive monitoring QR and aromatase expression in breast cancer and breast cancer response to QR and aromatase inhibitor therapy using PET imaging technique. Significant efforts in the field of brain aromatase imaging with PET using carbon-11-labeled aromatase inhibitors such as [^{11}C]vorozole and [^{11}C-cyano]letrozole have been reported [18–21], and we have previously published the synthesis and preliminary biological evaluation of carbon-11-labeled aromatase inhibitors, carbon-11-labeled sulfonanilide analogues as new PET breast cancer aromatase imaging agents [3]. In cancer molecular imaging, different molecular targets, enzyme-based imaging agents, radionuclides, and imaging modalities have their own advantages and disadvantages, and cancer is a complicated group of diseases involving multiple targets, and thus there is great interest in development of dual cancer targets, dual enzyme-based imaging agents or dual radiolabeled ligands and dual imaging modalities [1,17,22,23]. These simultaneously 'mixed' agents or modalities would be advantageous over single agent or modality. We are interested in the development of radiolabeled dual enzyme inhibitors as enzyme-based PET breast cancer imaging agents. We focus on the development of carbon-11-labeled non-steroidal analogues that are inhibitors of steroid biosynthetic enzymes or ligands for steroid hormone receptors. In our previous work, we have developed carbon-11-labeled dual aromatase-steroid sulfatase inhibitors [1] based on their unlabeled compounds [24]. In this ongoing study, to further develop therapeutic agent for diagnostic use, we have first designed and synthesized [^{11}C]casimiroin (6-[^{11}C]methoxy-9-methyl-[1,3]dioxolo[4,5-*h*]quinolin-8(9*H*)-one, [^{11}C]**11**) and its carbon-11-labeled analogues 5,6,8-trimethoxy-1-[^{11}C]methyl-4-methylquinolin-2(1*H*)-one ([^{11}C]**17**), 8-methoxy-1-[^{11}C]methyl-4-methylquinolin-2(1*H*)-one ([^{11}C]**21a**), 6,8-dimethoxy-1-[^{11}C]methyl-4-methylquinolin-2(1*H*)-one ([^{11}C]**21b**), and 5,8-dimethoxy-1-[^{11}C]methyl-4-methylquinolin-2(1*H*)-one ([^{11}C]**21c**), as new potential PET agents for imaging of QR 2 and aromatase expression in breast cancer.

2. Experimental

2.1. General

All commercial reagents and solvents from Sigma–Aldrich and Fisher Scientific were used without further purification. [^{11}C]Methyl triflate ([^{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 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). Thin-layer chromatography (TLC) was run using Analtech silica gel GF uniplates ($5 \times 10\,cm^2$). Plates were visualized under UV light. Preparative TLC was run using Analtech silica gel UV 254 plates ($20 \times 20\,cm^2$). 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 high performance liquid chromatography (HPLC) was performed using a Prodigy (Phenomenex) 5 μm C-18 column, 4.6 mm × 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 mm × 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.

2.2. Synthesis of casimiroin and its analogues

2.2.1. Methyl 2,3-dihydroxybenzoate (*2*)

To a solution of 2,3-dihydroxybenzoic acid **1** (18.0 g, 117 mmol) in MeOH (150 mL) was added thionyl chloride (12.8 mL, 175 mol) dropwise at 0 °C. After the reaction mixture was heated to reflux for 16 h, the solvent and excess thionyl chloride was evaporated *in vacuo*. The residue was dissolved in EtOAc, and washed with saturated $NaHCO_3$. The organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated *in vacuo*, and the crude product was purified over a short pad of silica gel using CH_2Cl_2 as eluent with to afford **2** (19.5 g, 99%) as a white solid, m.p. 70–72 °C. ^{1}H NMR ($CDCl_3$) δ: 10.89 (s, 1H, OH), 7.37 (dd, J = 1.5, 8.5 Hz, 1H, Ph-H), 7.12–7.10 (m, 1H, Ph-H), 6.80 (t, J = 8.0 Hz, 1H, Ph-H), 5.66 (s, 1H, OH), 3.96 (s, 3H, OCH_3).

2.2.2. Methyl 2,3-methylenedioxybenzoate (*3*)

A mixture of compound **2** (12.0 g, 71 mmo), KF (20.6 g, 355 mmol) in DMF (150 mL) was stirred at room temperature for 30 min. CH_2Cl_2 (5.94 mL, 85.2 mmol) was added and the reaction mixture was heated at 120 °C for 3 h. After cooling to room temperature, the mixture was poured into water and extracted with Et_2O. The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated *in vacuo*, and the crude product was purified by column chromatography, eluting with hexanes/EtOAc (2:1) to afford **3** (9.95 g, 77%) as a white solid, m.p. 63–64 °C. ^{1}H NMR ($CDCl_3$) δ: 7.42 (dd, J = 1.5, 8.5 Hz, 1H, Ph-H), 6.97 (dd, J = 1.0, 8.0 Hz, 1H, Ph-H), 6.87 (t, J = 8.0 Hz, 1H, Ph-H), 6.10 (s, 2H, OCH_2O), 3.93 (s, 3H, OCH_3).

2.2.3. 2,3-Methylenedioxybenzoic acid (*4*)

A mixture of compound **3** (9.6 g, 53 mmol), 2N KOH (53 mL, 106 mmol) in MeOH (150 mL) was stirred at room temperature for 3 h. After evaporating MeOH *in vacuo*, the solution was cooled in an ice bath and acidified to pH 3.5 with 2N HCl. The white precipitate was collected by filtration, washed with water and Et_2O, dried *in vacuo* to afford **4** (8.08 g, 91%) as a white solid, m.p. 234–235 °C. ^{1}H NMR (DMSO-d_6) δ: 12.97 (s, 1H, OH), 7.27 (dd, J = 1.5, 8.0 Hz, 1H, Ph-H), 7.11 (d, J = 7.5 Hz, 1H, Ph-H), 6.89 (t, J = 8.0 Hz, 1H, Ph-H), 6.11 (s, 2H, OCH_2O).

2.2.4. Benzyl benzo[d][1,3]dioxol-4-ylcarbamate (*5*)

A mixture of compound **4** (7.78 g, 47 mmol), diphenylphosphonyl azide (DPPA) (11.2 mL, 52 mmol), triethylamine (7.25 mL, 52 mmol) in benzene (100 mL) was heated to reflux for 3 h. Benzyl alcohol (10 mL) was added, and the reaction mixture was heated at reflux for another 5 h. After cooling to room temperature, the mixture was washed successively with cold 1N HCl, saturated $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4, and filtered. The solvent was evaporated *in vacuo*, and the residue was purified by column chromatography, eluting with hexanes/EtOAc (2:1) to afford **5** (9.36 g, 74%) as a white solid, m.p. 92–94 °C. ^{1}H NMR ($CDCl_3$) δ: 7.49 (br s, 1H, NH), 7.46–7.32 (m, 5H, Ph-H), 6.81(t, J = 8.0 Hz, 1H, Ph-H), 6.63 (s, 1H, Ph-H), 6.59 (dd, J = 1.0, 8.0 Hz, 1H, Ph-H), 5.94 (s, 2H, OCH_2O), 5.21 (s, 2H, CH_2Ph).

*2.2.5. Benzyl benzo[d][1,3]dioxol-4-yl(methyl)carbamate (**6**)*

NaH (60% dispersion in mineral oil, 1.86 g, 46.5 mmol) was added to a solution of compound **5** (8.44 g, 31 mmol) in DMF (40 mL). After the mixture was stirred at room temperature for 1 h, CH_3I (5.8 mL, 93 mmol) was added. The resulting mixture was continued to stir at room temperature for 4 h, then it was poured into ice water and extracted with CH_2Cl_2. The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated *in vacuo*, and the crude product was purified by column chromatography, eluting with hexanes/CH_2Cl_2 (1:2) to afford **6** (8.44 g, 95%) as colorless oil. ^{1}H NMR ($CDCl_3$) δ: 7.32–7.30 (m, 5H, Ph-H), 6.81–6.71 (m, 3H, Ph-H), 5.90 (s, 2H, OCH_2O), 5.17 (s, 2H, CH_2, CH_2Ph), 3.27 (s, 3H, NCH_3).

*2.2.6. N-Methylbenzo[d][1,3]dioxol-4-amine (**7**)*

A solution of compound **6** (8.4 g, 29.4 mmol) in EtOH (150 mL) was hydrogenated at a pressure of 45 psi in the presence of 10% Pd/C (840 mg) for 6 h. The catalyst was filtered off through Celite. The solvent was evaporated *in vacuo*, and the residue was purified by column chromatography with hexanes/CH_2Cl_2 (1:1.5) to afford **7** (4.45 g, 92%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 6.76 (t, J = 8.0 Hz, 1H, Ph-H), 6.34 (dd, J = 1.0, 8.0 Hz, 1H, Ph-H), 6.32 (dd, J = 1.0, 8.0 Hz, 1H, Ph-H), 5.90 (s, 2H, OCH_2O), 2.89 (s, 3H, NCH_3).

*2.2.7. Ethyl 3-(benzo[d][1,3]dioxol-4-yl(methyl)amino)-3-oxopropanoate (**8**)*

A mixture of compound **7** (4.0 g, 26.5 mmol) and excess diethyl malonate (40 mL) was heated and stirred at 110 °C for 3 h under a nitrogen atmosphere. The excess diethyl malonate was removed *in vacuo,* and the residue was purified by column chromatography with hexanes/EtOAc (2:1) to afford **8** (7.02 g, 70%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 6.86 (t, J = 8.0 Hz, 1H, Ph-H), 6.82 (dd, J = 1.5, 7.5 Hz, 1H, Ph-H), 6.72 (dd, J = 1.5, 8.0 Hz, 1H, Ph-H), 6.03 (s, 2H, OCH_2O), 4.12 (q, J = 7.0 Hz, 2H, CH_2CH_3), 3.30 (s, 2H, $C(O)CH_2C(O)$), 3.27(s, 3H, NCH_3), 1.23 (t, J = 7.0 Hz, 3H, CH_2CH_3).

*2.2.8. 3-(Benzo[d][1,3]dioxol-4-yl(methyl)amino)-3-oxopropanoic acid (**9**)*

A mixture of compound **8** (3.0 g, 11.3 mmol) and 1N NaOH (40 mL) was heated and stirred at 70 °C for 4 h. The solution was cooled in an ice bath and acidified to pH 3.0 with 2N HCl. The mixture was extracted with CH_2Cl_2. The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated *in vacuo*, and the residue was triturated under hexanes/EtOAc (2:1) to afford **9** (2.68 g, 84%) as a pale brown solid, m.p. 112–114 °C (lit. 115–117 °C [7]). ^{1}H NMR ($CDCl_3$) δ: 6.92 (t, J = 8.0 Hz, 1H, Ph-H), 6.88 (dd, J = 1.0, 8.0 Hz, 1H, Ph-H), 6.67 (dd, J = 1.5, 8.0 Hz, 1H, Ph-H), 6.06 (s, 2H, OCH_2O), 3.33 (s, 3H, NCH_3), 3.26 (s, 2H, CH_2, $C(O)CH_2C(O)$).

*2.2.9. 6-Hydroxy-9-methyl-[1,3]dioxolo[4,5-h]quinolin-8(9H)-one (**10**)*

To a preheated polyphosphoric acid (PPA) (20 g) was added compound **9** (2.0 g, 8.4 mmol) at 100 °C. After stirring at the same temperature for 2 h, the reaction mixture was poured into ice water. After PPA had dissolved, the pale yellow precipitate was collected by filtration, washed with water and Et_2O, dried *in vacuo* to afford **10** (1.65 g, 89%) as a pale yellow solid, m.p. 309 °C (dec.) (lit. 310 °C (dec.) [7]). ^{1}H NMR (DMSO-d_6) δ: 11.30 (br s, 1H, OH), 7.46 (d, J = 8.5 Hz, 1H, Ph-H), 6.89 (d, J = 8.5 Hz, 1H, Ph-H), 6.10 (s, 2H, OCH_2O), 5.72 (s, 1H, C=CH), 3.66 (s, 3H, NCH_3).

*2.2.10. Casimiroin (6-methoxy-9-methyl-[1,3]dioxolo[4,5-h]quinolin-8(9H)-one, **11**)*

To a suspension of compound **10** (300 mg, 1.37 mmol) in Et_2O (1 mL) was added TMS-diazomethane (2 M in hexanes, 15 mL) at 0 °C, and the mixture was stirred for 30 min. Anhydrous MeOH (15 mL) was added dropwise, and the reaction mixture was stirred for 6 h. Aqueous acetic acid (0.5 mL) was added to decompose the excess TMS-diazomethane. The solvent was evaporated *in vacuo*, and the residue was purified by column chromatography hexanes/acetone (1:1) to afford **11** (117 mg, 37%) as an orange solid, m.p. 197–198 °C (lit. 199–201 °C [7]). ^{1}H NMR ($CDCl_3$) δ: 7.55 (d, J = 8.5 Hz, 1H, Ph-H), 6.80 (d, J = 8.0 Hz, 1H, Ph-H), 6.05 (s, 2H, OCH_2O), 6.01 (s, 1H, C=CH), 3.92 (s, 3H, OCH_3), 3.86 (s, 3H, NCH_3).

*2.2.11. Benzyl 2,4,5-trimethoxyphenylcarbamate (**13**)*

A mixture of 2,4,5-trimethoxybenzoic acid **12** (8.0 g, 37.7 mmol), DPPA (8.96 mL, 41.5 mmol), triethylamine (5.78 mL, 41.5 mmol) in benzene (100 mL) was heated to reflux for 2 h. Benzyl alcohol (10 mL) was added, and the reaction mixture was heated at reflux for another 5 h. After cooling to room temperature, the mixture was washed successively with cold 1N HCl, saturated $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4, and filtered. The solvent was evaporated *in vacuo*, and the crude product was purified by column chromatography, eluting with hexanes/EtOAc (1:1) to afford **13** (9.67 g, 81%) as a white solid, m.p. 98–99 °C (lit. 99–100 °C [7]). ^{1}H NMR ($CDCl_3$) δ: 7.85 (br s, 1H, NH), 7.43–7.32 (m, 5H, Ph-H), 7.14 (s, 1H, Ph-H), 6.54 (s, 1H, Ph-H), 5.20 (s, 2H, CH_2Ph), 3.86 (s, 6H, OCH_3), 3.82 (s, 3H, OCH_3).

*2.2.12. 2,4,5-Trimethoxyaniline (**14**)*

A solution of compound **13** (6.0 g, 18.9 mmol) in THF (50 mL) and MeOH (15 mL) was hydrogenated at a pressure of 40 psi in the presence of 10% Pd/C (60 mg) for 7 h. The catalyst was filtered off through Celite. The solvent was evaporated *in vacuo*, and the residue was purified by column chromatography with hexanes/EtOAc (1:1) to afford **14** (3.15 g, 91%) as a white solid, m.p. 83–84 °C (lit. 84–86 °C [7]). ^{1}H NMR ($CDCl_3$) δ: 6.54 (s, 1H, Ph-H), 6.41 (s, 1H, Ph-H), 3.83 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3).

*2.2.13. 3-oxo-N-(2,4,5-Trimethoxyphenyl)butanamide (**15**)*

A mixture of compound **14** (3.0 g, 16.4 mmol) and methyl acetoacetate (1.77 mL, 16.4 mmol) in toluene (50 mL) was stirred and refluxed using a Dean-Stark apparatus for 24 h. The solvent was evaporated *in vacuo*, and the crude product was purified by column chromatography, eluting with CH_2Cl_2/MeOH (100:3) to afford **15** (3.25 g, 74%) as a tan solid, m.p. 88–89 °C (lit. 90–92 °C [7]). ^{1}H NMR ($CDCl_3$) δ: 9.10 (br s, 1H, NH), 8.07 (s, 1H, Ph-H), 6.56 (s, 1H, Ph-H), 3.89 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.59 (s, 2H, $C(O)CH_2C(O)$), 2.34 (s, 3H, CH_3).

*2.2.14. 5,6,8-Trimethoxy-4-methylquinolin-2(1H)-one (**16**)*

To a preheated PPA (20 g) was added compound **15** (1.5 g, 5.6 mmol) at 100 °C. After stirring at the same temperature for 2 h, the reaction mixture was poured into ice water and neutralized with 5N NaOH. The mixture was extracted with CH_2Cl_2. The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated *in vacuo*, and the crude product was purified by column chromatography, eluting with CH_2Cl_2/MeOH (100:3) to afford **16** (290 mg, 21%) as a purple solid, m.p. 149–150 °C (lit. 149–151 °C [7]). ^{1}H NMR ($CDCl_3$) δ: 9.40 (br s, 1H, NH), 6.78 (s, 1H, Ph-H), 6.49(d, J = 0.5 Hz, 1H, C=CH), 3.97 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 2.68 (d, J = 1.0 Hz, 3H, CH_3).

*2.2.15. 5,6,8-Trimethoxy-1,4-dimethylquinolin-2(1H)-one (**17**)*

NaH (60% dispersion in mineral oil, 20 mg, 0.50 mmol) was added to a suspension of compound **16** (100 mg, 0.4 mmol) in DMF (3 mL). After the mixture was heated and stirred at 40 °C for 1 h, it was cooled to room temperature, and CH_3I (0.1 mL, 1.60 mmol)

was added. The reaction mixture was continued to stir at room temperature for 0.5 h, and then heated at 80 °C overnight. The mixture was poured into ice water and extracted with CH_2Cl_2. The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated *in vacuo*, and the crude product was purified by preparative TLC plate, eluting with CH_2Cl_2/MeOH (100:3) to afford **17** (50 mg, 47%) as a pale brown solid, m.p. 129–130 °C (lit. 128–130 °C [7]). ^{1}H NMR ($CDCl_3$) δ: 6.83 (s, 1H, Ph-H), 6.57 (s, 1H, C=CH), 3.96 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.82 (d, J = 2.0 Hz, 3H, NCH_3), 2.63 (s, 3H, CH_3).

*2.2.16. N-(2-Methoxyphenyl)-3-oxobutanamide (**19a**)*

A mixture of *o*-anisidine **18a** (3.0 g, 24.4 mmol) and methyl acetoacetate (2.63 mL, 24.4 mmol) in toluene (50 mL) was stirred and refluxed using a Dean-Stark apparatus for 24 h. The solvent was evaporated *in vacuo*, and the crude product was purified by column chromatography, eluting with hexanes/EtOAc (2.5:1) to afford **19a** (1.3 g, 26%) as a yellow solid, m.p. 82–83 °C (lit. 82–84 °C [7]). ^{1}H NMR ($CDCl_3$) δ: 9.23 (br s, 1H, NH), 8.32 (dd, J = 1.5, 8.0 Hz, 1H, Ph-H), 7.06 (dt, J = 1.5, 8.0 Hz, 1H, Ph-H), 6.95 (dt, J = 1.0, 8.0 Hz, 1H, Ph-H), 6.89 (dd, J = 1.0, 8.5 Hz, 1H, Ph-H), 3.91 (s, 3H, OCH_3), 3.60 (s, 2H, C(O)CH_2C(O)), 2.33 (s, 3H, CH_3).

*2.2.17. N-(2,4-Dimethoxyphenyl)-3-oxobutanamide (**19b**)*

According to the procedure for preparation of **19a**, compound **19b** (9.2 g, 59%) was prepared from 2,4-dimethoxyaniline **18b** (10.0 g, 65.0 mmol) and methyl acetoacetate (7.05 mL, 65.0 mmol) as a beige solid, m.p. 91–92 °C (lit. 92–44 °C [7]). ^{1}H NMR ($CDCl_3$) δ: 9.02 (br s, 1H, NH), 8.18 (d, J = 9.0 Hz, 1H, Ph-H), 6.49–6.45 (m, 2H, Ph-H), 3.88 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 3.58 (s, 2H, C(O)CH_2C(O)), 2.33 (s, 3H, CH_3).

*2.2.18. N-(2,5-Dimethoxyphenyl)-3-oxobutanamide (**19c**)*

According to the procedure for preparation of **19a**, compound **19c** (11.7 g, 75%) was prepared from 2,5-dimethoxyaniline **18c** (10.0 g, 65.0 mmol) and methyl acetoacetate (7.05 mL, 65.0 mmol) as a pale yellow solid, m.p. 68–70 °C (lit. 70–72 °C [7]). ^{1}H NMR ($CDCl_3$) δ: 9.25 (br s, 1H, NH), 8.06 (d, J = 3.0 Hz, 1H, Ph-H), 6.80 (d, J = 8.5 Hz, 1H, Ph-H), 6.59 (dd, J = 3.0, 9.0 Hz, 1H, Ph-H), 3.87 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 3.59 (s, 2H, CH_2, C(O)CH_2C(O)), 2.33 (s, 3H, CH_3).

*2.2.19. 8-Methoxy-4-methylquinolin-2(1H)-one (**20a**)*

To a preheated PPA (10 g) was added compound **19a** (800 mg, 3.9 mmol) at 100 °C. After stirring at the same temperature for 2 h, the reaction mixture was poured into ice water and neutralized with 5N NaOH. The mixture was extracted with CH_2Cl_2. The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated *in vacuo*, and the crude product was purified by column chromatography, eluting with CH_2Cl_2/MeOH (100:2.5) to afford **20a** (200 mg, 27%) as a white solid, m.p. 189–190 °C (lit. 188–190 °C [7]). ^{1}H NMR ($CDCl_3$) δ: 9.33 (br s, 1H, NH), 7.28 (d, J = 1.0, 1H, Ph-H), 7.17 (t, J = 8.0 Hz, 1H, Ph-H), 7.00 (dd, J = 0.5, 8.0 Hz, 1H, Ph-H), 6.56 (d, J = 1.0 Hz, 1H, C=CH), 3.98 (s, 3H, OCH_3), 2.49 (d, J = 1.5 Hz, 3H, CH_3).

*2.2.20. 6,8-Dimethoxy-4-methylquinolin-2(1H)-one (**20b**)*

According to the procedure for preparation of **20a**, compound **20b** (320 mg, 23%) was prepared from PPA (20 g) and **19b** (1.5 g, 6.3 mmol) as a yellow solid, m.p. 230–231 °C (lit. 232–234 °C [7]). ^{1}H NMR (DMSO-d_6) δ: 10.48 (br s, 1H, NH), 6.80 (d, J = 2.5 Hz, 1H, Ph-H), 6.72 (d, J = 2.5 Hz, 1H, Ph-H), 6.42 (d, J = 1.0 Hz, 1H, C=CH), 3.88 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 2.40 (d, J = 1.0 Hz, 3H, CH_3).

*2.2.21. 5,8-Dimethoxy-4-methylquinolin-2(1H)-one (**20c**)*

According to the procedure for preparation of **20a**, compound **20c** (640 mg, 46%) was prepared from PPA (20 g) and **19c** (1.5 g, 6.3 mmol) as a pale yellow solid, m.p. 186–187 °C (lit. 187–189 °C [7]). ^{1}H NMR ($CDCl_3$) δ: 9.38 (br s, 1H, NH), 6.89 (d, J = 8.5 Hz, 1H, Ph-H), 6.53 (d, J = 9.0 Hz, 1H, Ph-H), 6.42 (s, 1H, C=CH), 3.92 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 2.65 (d, J = 1.0 Hz, 3H, CH_3).

*2.2.22. 8-Methoxy-1,4-dimethylquinolin-2(1H)-one (**21a**)*

To a suspension of compound **20a** (120 mg, 0.63 mmol) in DMF (2 mL) was added NaH (60% dispersion in mineral oil, 31.6 mg, 0.79 mmol). The reaction mixture was stirred and heated at 40 °C for 1 h. The mixture was cooled to room temperature, and CH_3I (0.16 mL, 2.52 mmol) was added. After stirring at room temperature for 0.5 h and at 60 °C overnight, the reaction mixture was poured into ice water and extracted with CH_2Cl_2. The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated *in vacuo*, and the crude product was purified by preparative TLC plate, eluting with hexanes/EtOAc (1:1) to afford **21a** (65 mg, 50%) as a yellow solid, m.p. 71–72 °C (lit. 72–74 °C [7]). ^{1}H NMR ($CDCl_3$) δ: 7.30 (dd, J = 1.5, 8.0 Hz, 1H, Ph-H), 7.18 (t, J = 8.0 Hz, 1H, Ph-H), 7.09 (dd, J = 1.5, 8.0 Hz, 1H, Ph-H), 6.62 (s, 1H, C=CH), 3.93 (s, 3H, OCH_3), 3.90 (s, 3H, NCH_3), 2.43 (s, 3H, CH_3).

*2.2.23. 6,8-Dimethoxy-1,4-dimethylquinolin-2(1H)-one (**21b**)*

According to the procedure for preparation of **21a**, compound **21b** (139 mg, 65%) was prepared from **20b** (200 mg, 0.91 mmol), NaH (60% dispersion in mineral oil, 45.6 mg, 1.14 mmol), and CH_3I (0.23 mL, 3.64 mmol) as a yellow solid, m.p. 158–160 °C (lit. 157–159 °C [7]). ^{1}H NMR ($CDCl_3$) δ: 6.71 (d, J = 3.0 Hz, 1H, Ph-H), 6.68 (d, J = 3.0 Hz, 1H, Ph-H), 6.66 (s, 1H, C=CH), 3.91 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 3.87 (s, 3H, NCH_3), 2.41 (d, J = 1.0 Hz, 3H, CH_3).

*2.2.24. 5,8-Dimethoxy-1,4-dimethylquinolin-2(1H)-one (**21c**)*

According to the procedure for preparation of **21a**, compound **21c** (121 mg, 57%) was prepared from **20c** (200 mg, 0.91 mmol), NaH (60% dispersion in mineral oil, 45.6 mg, 1.14 mmol), and CH_3I (0.23 mL, 3.64 mmol) as a yellow solid, m.p. 186–187 °C (lit. 187–189 °C [7]). ^{1}H NMR ($CDCl_3$) δ: 7.03 (d, J = 9.0 Hz, 1H, Ph-H), 6.63 (d, J = 9.0 Hz, 1H, Ph-H), 6.50 (s, 1H, C=CH), 3.84 (s, 6H, OCH_3), 3.82 (s, 3H, NCH_3), 2.60 (d, J = 0.5 Hz, 3H, CH_3).

*2.3. Synthesis of [^{11}C]casimiroin ([^{11}C]**11**) and its carbon-11-labeled analogues [^{11}C]**17** and [^{11}C]**21a–c***

[^{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 precursor **10**, **16**, **20a**, **20b**, or **20c** (0.1–0.3 mg) was dissolved in CH_3CN (300 μL). To this solution was added NaH (0.5–1 mg). 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 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 $NaHCO_3$ (0.1 M, 1 mL), 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]**11**, [^{11}C]**17**, [^{11}C]**21a**, [^{11}C]**21b**, or [^{11}C]**21c**, 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

Table 1
Inhibition (IC_{50}) of QR2 and aromatase by casimiroin and its analogues [7].

Compound	QR2 IC_{50} (μM)	Aromatase IC_{50} (μM)	Aromatase/QR2 ratio
Casimiroin (**11**)	54.1 ± 6.7	3.92 ± 0.67	13.8
17	10.8 ± 1.3	0.10 ± 0.01	108
21a	5.8 ± 0.9	2.02 ± 0.1	2.87
21b	1.9 ± 0.2	0.96 ± 0.02	1.98
21c	4.1 ± 0.6	0.96 ± 0.01	4.27

formulation time was 25–30 min from end of bombardment (EOB). 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 **10** = 2.52 min, t_R **11** = 3.47 min, t_R $[^{11}C]$**11** = 3.47 min; t_R **16** = 2.27 min, t_R **17** = 3.31 min, t_R $[^{11}C]$**17** = 3.31 min; t_R **20a** = 2.02 min, t_R **21a** = 2.95 min, t_R $[^{11}C]$**21a** = 2.95 min; t_R **20b** = 2.23 min, t_R **21b** = 3.15 min, t_R $[^{11}C]$**21b** = 3.15 min; and t_R **20c** = 2.11 min, t_R **21c** = 3.17 min, t_R $[^{11}C]$**21c** = 3.17 min. Retention times in the semi-preparative HPLC system were: t_R **10** = 5.11 min, t_R **11** = 7.23 min, t_R $[^{11}C]$**11** = 7.23 min; t_R **16** = 4.58 min, t_R **17** = 6.76 min, t_R $[^{11}C]$**17** = 6.76 min; t_R **20a** = 4.23 min, t_R **21a** = 6.48 min, t_R $[^{11}C]$**21a** = 6.48 min; t_R **20b** = 4.65 min, t_R **21b** = 6.91 min, t_R $[^{11}C]$**21b** = 6.91 min; and t_R **20c** = 4.47 min, t_R **21c** = 6.88 min, t_R $[^{11}C]$**21c** = 6.88 min.

3. Results and discussion

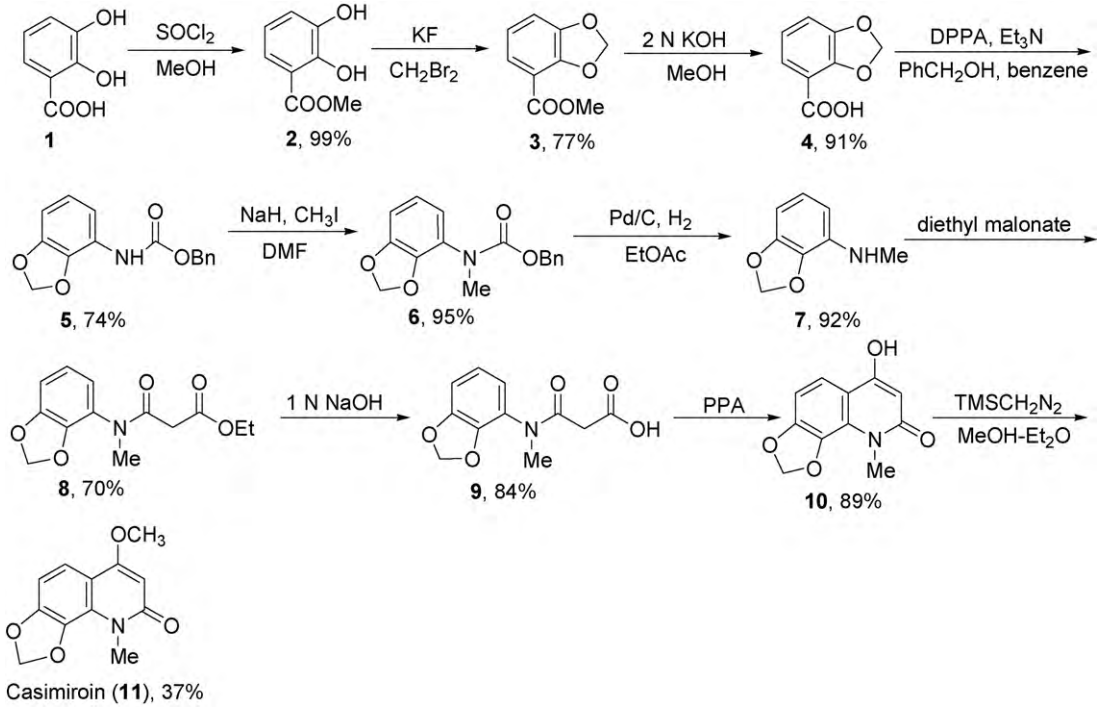
3.1. In vitro data and radiotracer design

Five title compounds casimiroin (**11**) and its analogues 5,6,8-trimethoxy-1,4-dimethylquinolin-2(1*H*)-one (**17**), 8-methoxy-1,4-dimethylquinolin-2(1*H*)-one (**21a**), 6,8-dimethoxy-1,4-dimethylquinolin-2(1*H*)-one (**21b**), and 5,8-dimethoxy-1,4-dimethylquinolin-2(1*H*)-one (**21c**) were served as the reference standards and selected for radiolabeling. These casimiroin analogues are potent QR2 and aromatase inhibitors, and their *in vitro* data from previous report are summarized in Table 1, [7]. The most active compound (**21b** versus QR2 and **17** versus aromatase) has resulted from previous structural modification of parent natural product casimiroin [7]. The relative affinity of casimiroin analogues for aromatase versus QR2 is calculated and also listed in Table 1. These *in vitro* data indicate some interesting information about the structure–activity relationship (SAR). Overall, [1,3]dioxolo ring-opened analogues **17**, **21a–c** displayed superior inhibitory potency for both QR2 and aromatase compared with the parent compound **11**, the replacement of the 6-methoxy group of compound **11** by a methyl group increased the inhibitory activity, and the trimethoxy compound **17** is most selective aromatase inhibitor (aromatase/QR2 ratio 108) in comparison with the dimethoxy or methoxy compounds **21a–c**. These compounds possess an *O*-methyl and/or *N*-methyl position amenable to labeling with a positron-emitting radioisotope such as carbon-11 as PET agents. Therefore, these compounds can be proposed as tools for PET experiments. Previous work [7] indicated *N*-methylated analogues showed better potency than non-*N*-methylated quinolinones in inhibiting QR2. This combined with other SAR information and the impact of the structural modification of casimiroin analogues suggested that the *N*-methyl position is the better isotope incorporation site than the *O*-methyl position. Therefore, we designed and synthesized carbon-11-labeled casimiroin analogues by labeling casimiroin **11** at the *O*-methyl position and labeling its analogues **17** and **21a–c** at the *N*-methyl position.

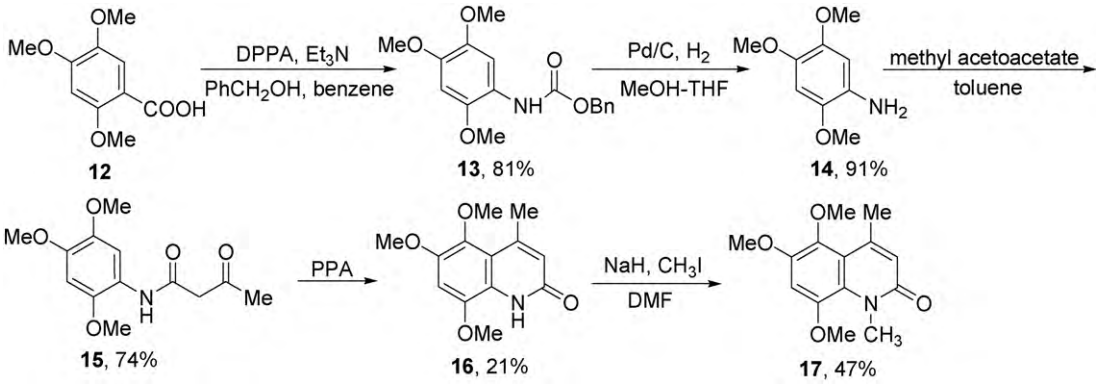
3.2. Chemistry

The precursors **10**, **16**, **20a-c** for radiolabeling and their standards **11**, **17**, **21a–c** were synthesized based on the literature method with modification [7,26]. The improvements included modified synthetic approaches with moderate to excellent chemical yields, more complete experimental procedures and detailed spectral data.

As shown in Scheme 1, 2,3-dihydroxybenzoic acid (**1**) was esterified using MeOH in the presence of thionyl chloride to afford methyl ester **2** in 99% yield. The yield via this method was much higher



Scheme 1. Synthesis of casimiroin (**11**) and its precursor (**10**).

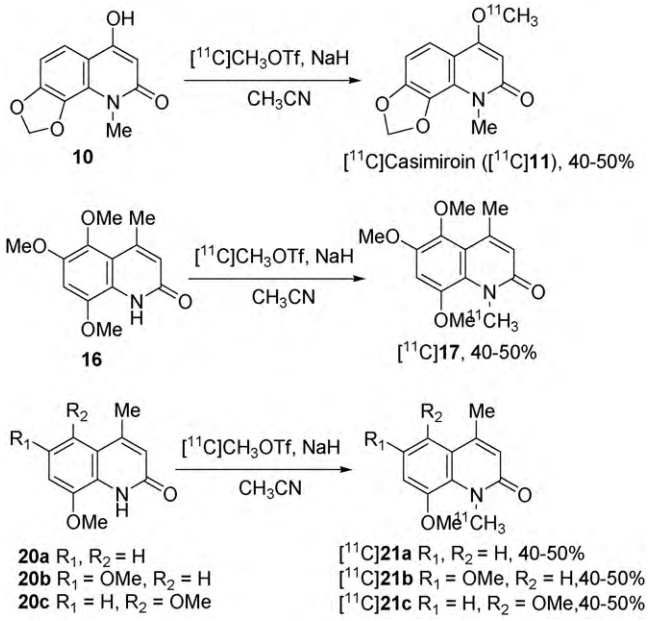


Scheme 2. Synthesis of a casimiroin analogue (**17**) and its precursor (**16**).

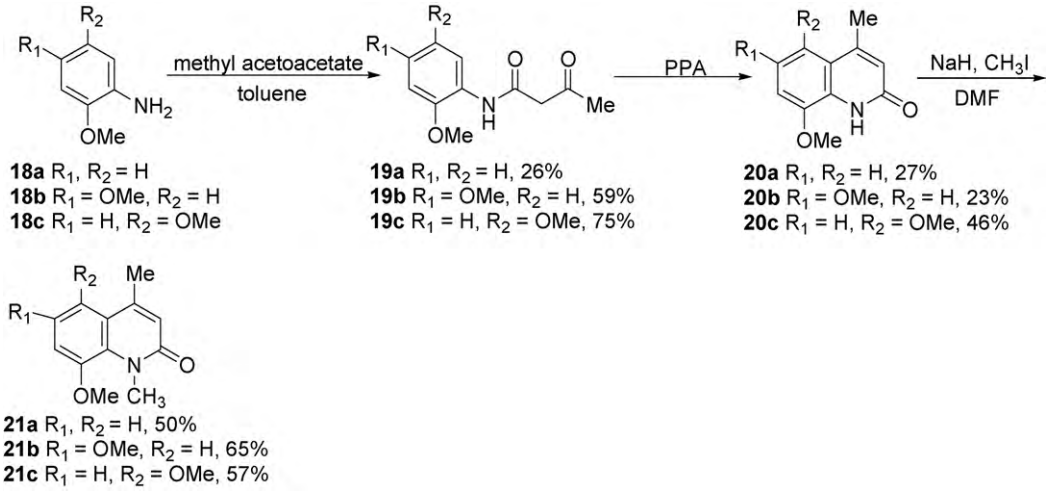
than that obtained using catalystic amount of sulfuric acid, which yield was 40%. The cyclization of catechol **2** was accomplished with dibromomethane to give benzodioxole **3** in 77% yield, which was saponified with 2N KOH in MeOH to provide dioxole benzoic acid **4** in 91% yield. Direct transformation of carboxylic acid **4** into carbamate **5** was accomplished through Curtius rearrangement using DPPA and Et_3N in benzene, followed by benzyl alcohol, in 74% yield. *N*-Methylation of **5** using NaH and CH_3I in DMF provided **6** in 95% yield, which was hydrogenated using Pd/C as catalyst to give the secondary amine **7** in 92% yield. Acylation of amine **7** with diethyl malonate gave β-keto ester **8** in 70% yield, followed by saponification with 1N NaOH to provide the acid **9** in 84% yield. Precursor **10** was obtained by the cyclization of **9** with PPA in 89% yield. *O*-Methylation of **10** with TMS-diazomethane involving the addition of MeOH as a cosolvent afforded standard **11** in 37% yield [27].

As depicted in Scheme 2, Curtius rearrangement of commercially available 2,4,5-trimethoxyl benzoic acid (**12**) gave carbamate **13** in 81% yield. Hydrogenation of **13** yielded amine **14** in 91% yield. Acylation of **14** using methyl acetoacetate provided β-keto amide **15** in 74% yield [28]. Cyclization of **15** with PPA afforded precursor **16** in 21% yield. *N*-Methylation of **16** using NaH and CH_3I in DMF generated its corresponding standard **17** in 47% yield.

Similarly, as indicated in Scheme 3, acylation of commercially available free amines **18a–c** provided β-keto amides **19a–c** in 26–75% yield [28], which were cyclized with PPA to afford precursors **20a–c** in 23–46% yield. *N*-Methylation of **20a–c** gave their corresponding standards **21a–c** in 50–65% yield.



Scheme 4. Synthesis of [^{11}C]casimiroin ([^{11}C]**11**) and its carbon-11-labeled analogues [^{11}C]**17** and [^{11}C]**21a–c**.



Scheme 3. Synthesis of casimiroin analogues (**21a–c**) and their precursors (**20a–c**).

3.3. Radiochemistry

Synthesis of [^{11}C]casimiroin ([^{11}C]**11**) and its carbon-11-labeled analogues, [^{11}C]**17** and [^{11}C]**21a–c** is outlined in Scheme 4. The precursors **10**, **16** and **20a–c** were labeled by [^{11}C]CH_3OTf [25,29] through either *O*- or *N*-[^{11}C]methylation and isolated by reversed-phase HPLC method [1,3] to produce the corresponding pure radiolabeled compounds [^{11}C]**11**, [^{11}C]**17** and [^{11}C]**21a–c** in 40–50% radiochemical yields, based on [^{11}C]CO_2, decay corrected to EOB. Overall synthesis time was 25–30 min from EOB. The radiosynthesis was performed in an automated multi-purpose ^{11}C-radiosynthesis module, allowing measurement of specific activity during synthesis [30,31]. The specific activity was in a range of 222–370 GBq/μmol at EOB measured by the on-the-fly technique [31] and 111–185 GBq/μmol at the end of synthesis (EOS) measured by analytical HPLC [32], respectively. Chemical purity and radiochemical purity were determined by analytical HPLC [32]. 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.

3.4. Conclusion

In conclusion, an efficient and convenient synthesis of [^{11}C]casimiroin and its carbon-11-labeled analogues has been developed. The synthetic methodology employed classical organic chemistry such as esterification, cyclization, saponification, Curtius rearrangement, hydrogenation, acylation, and methylation to produce precursor and standard compounds. Carbon-11-labeled casimiroin analogues were prepared by either *O*- or *N*-[^{11}C]methylation of their corresponding precursors using a reactive [^{11}C]methylating agent, [^{11}C]CH_3OTf, and isolated by HPLC purification procedure in high radiochemical yields, short overall synthesis time, and high specific activity. These chemistry results combined with the reported *in vitro* biological data [7] encourage further *in vivo* biological evaluation of carbon-11-labeled casimiroin analogues as new potential PET agents for imaging of QR2 and aromatase expression in breast cancer.

Acknowledgments

This work was partially supported by the Breast Cancer Research Foundation and Indiana Genomics Initiative (INGEN) of Indiana University, which is supported in part by Lilly Endowment Inc. The authors would like to thank Dr. Bruce H. Mock and Barbara E. Glick-Wilson for their assistance in production of [^{11}C]CH_3OTf. ^{1}H NMR spectra were recorded on a Bruker Avance II 500 MHz NMR spectrometer in the Department of Chemistry and Chemical Biology at Indiana University Purdue University Indianapolis (IUPUI), which is supported by a NSF-MRI Grant CHE-0619254. The referees' criticisms and editors' comments for the revision of the manuscript are greatly appreciated.

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