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Syntheses and biological activities of novel 2-methoxyestradiol analogs, 2-fluoroethoxyestradiol and 2-fluoropropanoxyestradiol, and a radiosynthesis of 2-[^{18}F]fluoroethoxyestradiol for positron emission tomography

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Abstract

Introduction: 2-Methoxyestradiol (2ME2) is an endogenous metabolite of the human hormone, estrogen, which has been shown to possess anti-tumor activity. 2-Fluoroethoxyestradiol (2FEE2) and 2-fluoropropanoxyestradiol (2FPE2), novel analogs of 2-methoxyestradiol, were designed and synthesized to be utilized as F-18 radiotracers for positron emission tomography (PET), with which the bio-distribution and intratumoral accumulations of 2ME2 could be measured in vivo for potential translation to human use.
Methods: 2FEE2 and 2FPE2 were synthesized from 3,17β-estradiol in five steps respectively. Drug-induced microtubule depolymerization, antiproliferative activity against human cancer cell lines and HIF-1α down-regulation by 2FEE2 and 2FPE2 were investigated to examine whether these molecules possess similar anti-tumor activities as 2-methoxyestradiol. 2-[^{18}F]Fluoroethoxyestradiol was synthesized for PET.
Results: Novel 2ME2 analogs, 2FEE2 and 2FPE2, were synthesized in 29% and 22% overall yield, respectively. 2FEE2 and 2FPE2 showed microtubule depolymerization and cytotoxicities against the human ovarian carcinoma cell line, 1A9, and the human glioma cell line, LN229. HIF-1α was down-regulated by 2FEE2 and 2FPE2 under hypoxic conditions. 2FEE2 was chosen as an F-18 radiotracer candidate, since it showed stronger antiproliferative activity than 2ME2 and 2FPE2. 2-[^{18}F]Fluoroethoxyestradiol (2[^{18}F]FEE2) was prepared in 8.3% decay-corrected yield in 90 min, based on a production of H[^{18}F]F with more than 98% radiochemical purity.
Conclusions: 2FEE2 and 2FPE2 showed similar activity as 2ME2. 2[^{18}F]FEE2 was synthesized to be utilized as a PET radiotracer to measure the biological efficacy of 2ME2 and its analogs in vivo.

Keywords: 2-Fluoroethoxyestradiol; 2-Fluoropropanoxyestradiol; 2-Methoxyestradiol; PET; 2-[^{18}F]Fluoroethoxyestradiol

1. Introduction

2-Methoxyestradiol (2ME2) (**1**) is an endogenous metabolite of the human estrogen, estradiol, which has drawn a lot of attention because of its anti-tumor activity against a wide variety of human tumors such as head and neck squamous cell carcinoma, glioma, prostate cancer and metastatic breast cancer [1–4]. At the cellular level, 2ME2 binds to tubulin, depolymerizes microtubules (MTs) and exerts antiangiogenic activity [5]. Mechanistically, we have previously shown that the antiangiogenic activity of 2ME2 in vivo stems from the ability of the molecule to inhibit the protein levels and transcriptional activity of the hypoxia-inducible factor 1 (HIF-1α) [6]. More importantly, we showed that inhibition of HIF-1α occurs downstream of the drug-induced MT disruption [6]. Phase I trials of 2ME2 in patients with metastatic breast cancer have been reported and showed that this orally available small molecule is well tolerated unlike

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other MT-targeting chemotherapy drugs [7–9]. Currently, 2ME2 is in Phase I/II oncology clinical trials.

To monitor the bio-distribution and intratumoral accumulations of 2ME2 in vivo, we have recently reported an improved synthesis of 2-[^{11}C]methoxyestradiol (2[^{11}C]ME2) [10]. Carbon-11 labeling does not change the chemical structure, so the pharmacokinetics and bio-distribution of 2ME2 can be investigated by positron emission tomography (PET), using 2[^{11}C]ME2. A significant limitation in the application of carbon-11 tracers for human use is the short 20-min half-life of carbon-11. The 20-min half-life requires an on-site particle accelerator for production of carbon-11. In addition, only a single or relatively few doses can be generated from each batch production of carbon-11 tracers. Therefore, carbon-11 tracers are poor candidates for regional distribution for widespread human use.

In order to overcome the physical half-life limitation of carbon-11, we have recently focused on the development of several new analogs of 2ME2 which contain an intrinsic F-18 labeling site. These analogs were designed both as radiotracers for PET and as anti-tumor drugs. Fluorine-18 is a more desirable PET radionuclide for radiolabeling because its 110-min half-life allows substantially more time for radiochemical synthesis and for purification of the final product for human administration. Also, the 110-min half-life allows sufficient time for distribution to hospitals without on-site particle accelerators. Thus, fluorine-18 2ME2 analogs have significant advantages over 2[^{11}C]ME2 for widespread human use.

Structure designs of F-18 analogs were based on 2-ethoxyestradiol (**2**), 2-(2′,2′,2′-trifluoroethoxy)estradiol (**3**) and 2-(2′-hydroxyethoxy)estradiol (**4**), published by Cushman et al. (Fig. 1) [11–13]. The substitution of 2-methoxy group with 2-ethoxy group in 2-ethoxyestradiol induced stronger inhibition of tubulin polymerization and cytotoxicities against various cancer cell lines than 2ME2. 2-(2′,2′,2′-Trifluoroethoxy)estradiol and 2-(2′-hydroxyethoxy)estradiol showed less potency as anti-tumor drugs than 2ME2; however, they retained high activity enough to show cytotoxicities against various cancer cell lines.

Based on these results, the 2-methoxy group of 2ME2 was replaced with a 2-(2′-fluoroethoxy) group and a 2-(3′-fluoropropanoxy) group, to afford F-18 radiotracers, 2-fluoroethoxyestradiol (2FEE2) (**5**) and 2-fluoropropanoxyestradiol (2FPE2) (**6**), respectively (Fig. 2).

The syntheses of 2FEE2 and 2FPE2 were performed to investigate whether these newly synthesized analogs share similar biological activities as their parent compound. We performed MT depolymerization and cytotoxicity assays against human ovarian cancer cell lines and human glioma cell lines. We have further investigated the ability of 2FEE2 and 2FPE2 to down-regulate HIF-1α. A synthesis of 2-[^{18}F]fluoroethoxyestradiol (2[^{18}F]FEE2) is also reported.

2. Materials and methods

2.1. General

All reagents used were obtained from commercially available source. Solvents used in reactions were purchased from Sigma-Aldrich Corporation (Milwaukee, WI, USA), while solvents for chromatography were obtained from VWR Scientific Products (West Chester, PA, USA).

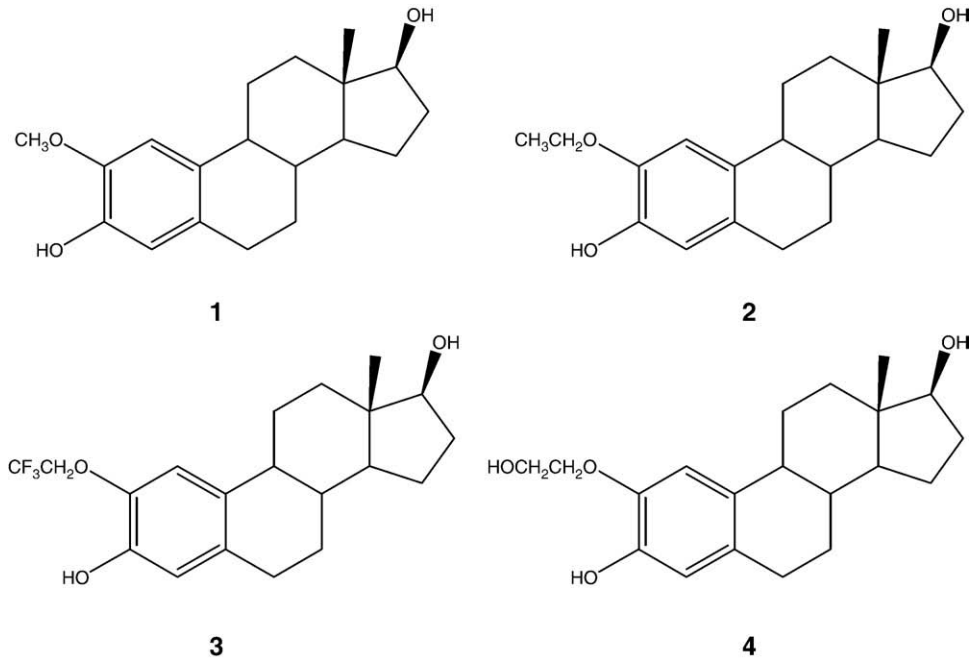

Fig. 1. The structures of 2-methoxyestradiol and its analogs.