

Therapeutic Promises of 2-Methoxyestradiol and Its Drug Disposition Challenges

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Abstract: 2-Methoxyestradiol (2MeO-E2) is an endogenous metabolite of estrogen which was initially considered to be inactive. During the last few decades it has been shown that 2MeO-E2 is a promising anticancer drug. *In vitro* experiments have demonstrated that it has several anticancer activities, and potential to alleviate hypertension, glomerulosclerosis, hypercholesterolemia, and other disorders. However, due to its low solubility and extensive glucuronidation, to achieve effective concentrations large doses of 2MeO-E2 would be required. Clinical studies reflected very high inter- and inpatient variability and oral bioavailability of 1 to 2%. Thus, this review paper highlights the origin of this compound, its therapeutic promises, and possible mechanisms of action. It also discusses the pharmacokinetic properties of 2MeO-E2 as well as current developments to overcome low drug solubility and its extensive first pass metabolism.

Keywords: 2-Methoxyestradiol; anticancer activity; glucuronidation; metabolism; pharmacokinetics; dosage form; structural modifications; sulfamates

Introduction

2-Methoxyestradiol (2MeO-E2) is endogenously formed from estradiol, and was previously thought to be inactive. However, 2MeO-E2 may have potential as an anticancer drug; thus interest in this molecule has been renewed. As a result, herein we review the endogenous formation and metabolism of 2MeO-E2, its pharmacology and toxicity, and its pharmacokinetics in rodents and humans, as well as the associated biopharmaceutical challenges. Furthermore, we discuss attempts to overcome these challenges, including new formulations and synthesis of analogues or prodrugs of 2MeO-E2.

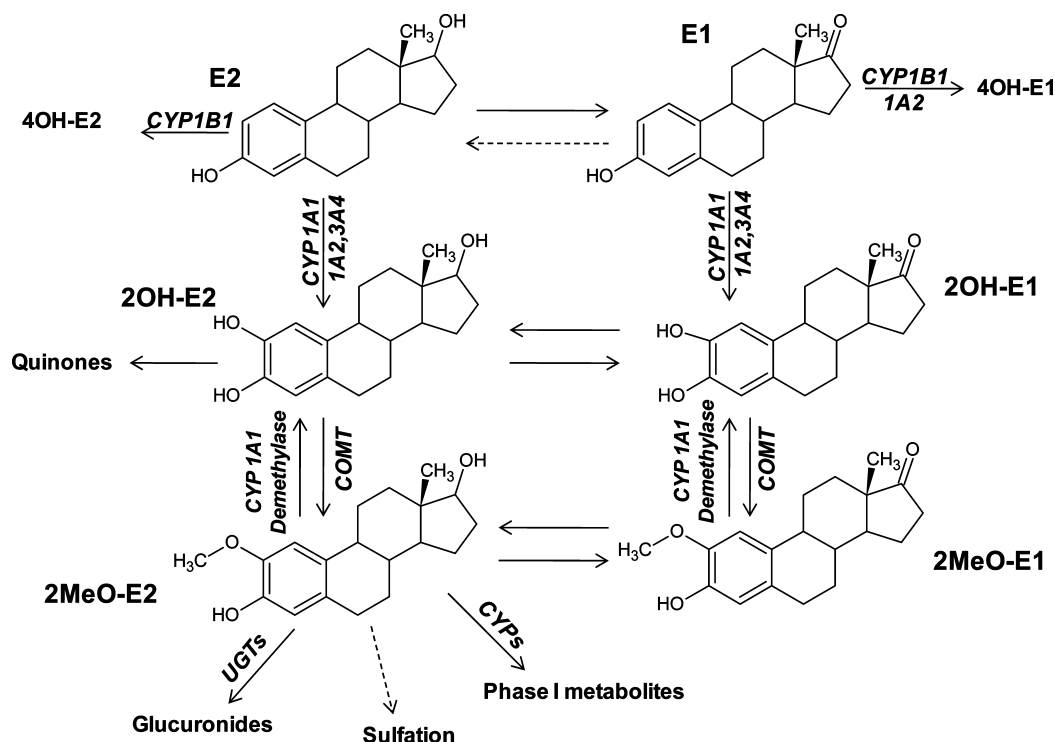
Endogenous Origin of 2MeO-E2

2-Methoxyestradiol (2MeO-E2) is an endogenous compound and a nonpolar metabolite of estradiol (E2). Generally, formation and metabolism of estrogens are rather complex;

thus, Scheme 1 illustrates only 2MeO-E2 formation and its possible pathways of elimination.^{1–4} Estrone (E1) and E2 are substrates for the phase I enzymes, CYP1A1 and 3A4. Oxidative metabolism by these enzymes leads mainly to the formation of 2-hydroxyestrogens with 4-hydroxyestrogens being minor products of CYP-mediated metabolism.⁵ Catechol-O-methyltransferase or (COMT) subsequently O-methylates the 2-hydroxyl group to produce 2MeO-E2 or 2-meth-

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Scheme 1. Pathways of 2MeO-E2 Formation and Elimination^a

^a Compounds and enzymes are previously defined. Estrogen metabolism is notoriously complex; readers should note that other phase I metabolites, glucuronides, and sulfates can be formed from any of these. However, the emphasis of this scheme is to highlight the endogenous formation and metabolism of 2MeO-E2.

oxyestrone (2MeO-E1). In the presence of CYP enzymes⁶ methoxyestrogens can be transformed back to the catechol. It is believed that glucuronidation is a major pathway of 2MeO-E2 elimination. The presence of hydroxylated and dimethylated metabolites of 2MeO-E2 was also detected *in vitro*, but as a minor fraction.⁷

Many tissues can actively produce 2MeO-E2, since COMT is a ubiquitous enzyme found in different organs such as liver, kidney, intestine, stomach, spleen, brain, pancreas, lung, etc.⁸ The exact endogenous 2MeO-E2 concentrations in tissues are not known, and even the serum concentrations of 2MeO-E2 were frequently reported in combination with 2-methoxyestrone.⁹ Recently, Xu et al. (2007) have developed an analytical method to detect different methoxyestrogens and reported total concentration of 2MeO-E2 in the

blood serum to be about 9.4 ± 1.34 , 10.6 ± 7.91 , and 2.5 ± 0.57 pg/mL in luteal, follicular and postmenopausal women, respectively.¹⁰ The concentrations of unconjugated form of 2MeO-E2 were about half of the above values. 2MeO-E2 concentrations, however, might increase by 1000-fold during the last months of pregnancy.⁹

Historically 2MeO-E2 was considered an inert metabolite and the action of COMT was believed to be a secondary pathway of E2 inactivation. However, the activity of COMT was found to be greater in tumor tissues.⁶ Thus, the amount of 2MeO-E2 formed in benign breast tumor was up to 2.9 times higher than in normal tissues and up to 5.4 times higher in malignant tumor tissues. Furthermore, lower levels of 2MeO-E2 as the consequence of low activity of COMT were found in pregnant women with preeclampsia in comparison with normotensive controls.^{11,12} This suggests an important role of COMT and 2MeO-E2 in preventing preeclampsia.

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Pharmacologic Activities of 2MeO-E2

During the past decade 2MeO-E2 has received attention due to its anticancer activities. Phase I and II clinical trials revealed that orally administered 2MeO-E2 is well tolerated by patients with only grade 2 and 3 toxicities observed.^{13–16} No maximum tolerated dose, however, was determined. It was also observed that during the treatment 2MeO-E2 concentrations in plasma remained in the nanogram per milliliter range, partially due to its extensive presystemic metabolism. As for *in vitro* antiproliferative properties, the majority of 60 cell lines from NCI are found to be sensitive to 2MeO-E2 with inhibitory concentrations between 0.08 and 5.0 μM .^{17,18} The list of tumor cells that respond to treatment with 2MeO-E2 continues to increase. For instance, recent studies on esophageal (WHCO3), nasopharyngeal (CNE2) carcinoma cells, and tumor-derived uterine leiomyoma cell lines (ELT3 and huLM) revealed 2MeO-E2 to be an effective antiproliferative agent with inhibitory concentrations between 1 and 20 μM .^{19–21}

It is believed that 2MeO-E2 acts on tumor growth directly by reducing cell proliferation or by inducing apoptosis, and also by inhibiting angiogenesis.¹⁷ Apoptosis induced by 2MeO-E2 occurs by upregulating death receptor 5 (DR 5), increasing expression of STAT1 protein, and activating p53 and caspases 9, 8, and 3.^{22–24,17} The increase in STAT1 expression and activation of caspase are required as apoptosis is often blocked by caspase inhibitor Z-VAD-FMK. The interference in tumor microtubule formation takes place via inhibiting the tubulin polymerization rate by competitively inhibiting colchicine-binding sites. In comparison with other competitive inhibitors of colchicine-binding sites, 2MeO-E2 is atypical since its IC_{50} was found to be one or two orders of magnitude lower than other inhibitors.¹⁷ The K_i value for 2MeO-E2 inhibition of tubulin polymerization is 22 μM .¹⁸ Thus, by binding to the colchicine-binding site, 2MeO-E2 inhibits the tubule formation or alters its stability. Generally, apoptosis requires much lower 2MeO-E2 concentrations than those to attain interference in tubulin polymerization.²²

The antiproliferative activity of 2MeO-E2 is apparently time- and concentration-dependent, whereas it is estrogen receptor (ER) independent. The affinity of 2MeO-E2 for binding to ER α and β was established to be about 0.5% and 0.008% of estradiol, respectively.^{17,18,25} However, Lui and Zhu (2004) observed that 2MeO-E2 affinity to both ER might be much higher than what was estimated earlier, about 1 to 2% of estradiol.²⁶ Moreover, they detected both mitogenic and antiproliferative properties of 2MeO-E2. In agreement with previous findings antiproliferative properties were ER independent, whereas mitogenic ones were ER dependent. The latter were attributed to the residual 2MeO-E2 estrogenic activity which was observed only at very low 2MeO-E2 concentrations, up to 750 nM, and when 2MeO-E2 antiproliferative activity was not yet observed.

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Besides antitumor properties, 2MeO-E2 possesses other biological activities. As such, it was reported that 2MeO-E2 induces the expression of endothelial nitric oxide synthase and production of nitric oxide.^{27,28} Besides being a vasodilator, nitric oxide is also known to have antithrombogenic properties, thereby preventing platelet aggregation and adhesion, and it also enhances endothelial barrier properties via decreasing infiltration of monocytes and macrophages. Therefore, 2MeO-E2 may alleviate the symptoms of atherosclerosis via decreasing plaque formation.²⁹ In addition to barrier-improving properties, 2MeO-E2's ability to decrease proliferating cells and inhibit collagen synthesis may attenuate hypertension, pulmonary hypertension, glomerulosclerosis, brain injury, and possibly other conditions.^{3,30}

Toxicology of 2MeO-E2

Several studies with oral administration of 2MeO-E2 to humans have been reported, and the typical toxicities associated with anticancer drugs such as myelosuppression appear to be absent. Dahut et al. reported that, upon oral dosing of 3 g twice daily, no dose-limiting toxicities were observed, and a maximum tolerated dose was not reached.¹³ They reported some evidence of estrogenic effects including hot flashes, thrombosis, and fluid retention. More recently, Tevaarwerk et al. reported that the maximum tolerated dose of a nanocrystalline dispersion of 2MeO-E2 with a higher bioavailability was 1 g orally every 6 h.⁴⁸ Dose limiting toxicities in this study were fatigue, muscle weakness, hypophosphatemia, hyponatremia, increased alanine aminotransferase and γ -glutamyltransferase, and anorexia. Overall, 2MeO-E2 appears to have minimal toxicities even upon administration of high doses, especially in the context of cancer treatment.

2MeO-E2 Pharmacokinetics

2MeO-E2 PK in Rodents. Tests in mice revealed that a large portion of orally administered 2MeO-E2 (100 mg/kg) was absorbed by both male (85%) and female (76%) mice. Low plasma concentrations of 2MeO-E2 and, therefore, low bioavailability (1.5%) were observed and attributed to extensive metabolic transformations rather than its poor

absorption.³¹ From tissue analysis 60 min after intravenous (iv) bolus injection of ¹¹C-2MeO-E2, this compound was highly accumulated in the liver, followed by lung and kidneys of the mice, consistent with hepatic uptake and elimination.³² However, no determinations of 2MeO-E2 distribution in mouse intestine were performed, although this organ could be vital in 2MeO-E2 metabolism. PK analysis has shown that 2MeO-E2 elimination followed a biexponential pattern with a terminal half-life ($t_{1/2}$) of 19 min, which was in agreement with the findings of other investigators, $t_{1/2}$ of 14 and 20.2 min.^{33,34} 2MeO-E2 clearance (CL) and volume distribution (V_d) were estimated to be 0.36 or 14.3 mL/min and volume of distribution of 52.9 or 427.5 mL for mice and rats, respectively. Lee et al. (2007) also have indicated that AUC and 2MeO-E2 plasma concentration were dose proportional between intravenous doses of 0.18 to 8.5 μ g, corresponding to 0.025–0.3 mg/kg body weight.³²

2MeO-E2 PK in Humans. To achieve limited positive response on tumor treatment with 2MeO-E2, large orally administered doses were required, up to 3000 mg bid.^{13–15} Even at these doses, the plasma concentration of 2MeO-E2 remained in the lower nanogram per milliliter range, and unphysiologically high apparent CL and V_d were estimated with median $t_{1/2}$ of about 1 to 2 days. Such a long half-life in comparison to $t_{1/2}$ obtained in rodents may be attributed to a much higher extent of binding of 2MeO-E2 to plasma proteins. However, the plasma protein binding in rats has not been reported in the literature. Additionally, it has been suggested that the long half-life may be due to extensive enterohepatic recycling in humans,³⁵ but this remains to be established.

Consequently, the drug binding to plasma proteins in humans was $97.4 \pm 0.22\%$, which was also concentration independent at 2MeO-E2 concentrations of 3.3 to 100 nM.³⁵ In this study, the binding association constant for 2MeO-E2 was highest for albumin ($4.29 (\pm 0.420) \times 10^4$ /mol/L), then α_1 -acid glycoprotein ($4.41 (\pm 0.139) \times 10^4$ /mol/L) and sex-hormone-binding globulin ($1.5 (\pm 0.26) \times 10^6$ /mol/L). Taking their reciprocals provides dissociation constants of

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approximately 23.3 μM , 22.7 μM , and 0.67 μM for albumin, α 1-acid glycoprotein, and sex-hormone binding globulin, respectively. Furthermore, at clinically relevant protein concentrations, the fraction unbound for albumin (4 g/dL), α 1-acid glycoprotein (0.25 g/dL), and sex-hormone binding globulin (20 $\mu\text{g/mL}$) was 3.75%, 27.6%, and 73.3%, respectively, indicating that, despite its low apparent affinity, albumin is the greatest contributor to plasma protein binding of 2MeO-E2.

In addition, clinical trials revealed that a linear relationship was not found between C_{max} or AUC and the 2MeO-E2 dose in doses ranging from 400 mg to 3000 mg administered twice daily, or 400 mg to 1200 mg administered once daily.^{13,14} The doses administered in these studies (approximately 6–40 mg/kg) were much larger and given by a different route; thus they cannot be readily compared to the results of Lee et al.³² in which linear kinetics were reported in mice upon a much smaller intravenous dose.

Lakhani et al. (2003) found less than 0.01% of the unchanged 2MeO-E2 in urine and about 1% as glucuronides after oral administration of 800 or 2200 mg bid.² The latter indicates that glucuronidation might be a major pathway of 2MeO-E2 metabolism. The estimated hepatic clearance of 2MeO-E2 could be as high as 712 mL/min, about half of hepatic blood flow.⁷ This value includes known phase I and II metabolism of 2MeO-E2 but not biliary excretion. Hence, 2MeO-E2 clearance could be as high as hepatic blood flow, which makes 2MeO-E2 a high extraction ratio drug. This leads to the conclusion that first pass metabolism is also major player in 2MeO-E2 disposition.^{1,7} Lakhani (2005) also reported that 2MeO-E2 solubility in phosphate buffer (pH 6–7) is only about 2.3 to 3.3 $\mu\text{g/mL}$, whereas its high apparent permeability (P_{app}) of $28.8 \pm 4.9 \times 10^{-6}$ cm/s allows the drug to easily penetrate gastrointestinal wall.³⁶ This makes 2MeO-E2 fall into class 2 of the Biopharmaceutical Classification System (BCS): a drug with high permeability but poor GI solubility.

2MeO-E2 Metabolism. 2MeO-E2 contains two hydroxyl groups at the positions C-3 and C-17 and thus, it is a potential substrate for UDP-glucuronosyltransferases (UGTs). Using transfected cell lines, Basu et al. (2004) and Lépine et al. (2004) have reported that several UGT 1A and some 2B isoforms extensively transformed 2MeO-E2 to the glucuronidated forms, as summarized in Table 1.^{37,38} The relative contribution to the metabolism of 2MeO-E2 by some UGTs is somewhat controversial. The exact reason is unclear; however, the assay methods they used differed in terms of

Table 1. Rates of 2-Methoxyestradiol (2MeO-E2) Glucuronidation by Human UGT Isoforms

UGTs	pmol/h/mg protein	
	Basu et al., 2004 ³⁷	Lépine et al., 2004 ³⁸
1A1	160 \pm 30	1306–2612
1A3	621 \pm 21	261–1305
1A7	2504 \pm 95	
1A8	2045 \pm 77	1306–2612
1A9	~52.5	261–1305
1A10	7933 \pm 267	261–1305
2B4		131–260
2B7		
2B17		26–130

the expression system, buffer and cofactor concentrations, detergents, as well as substrate concentrations and incubation times. For example, Basu et al. expressed the enzymes in COS-1 cells, using 200 μM substrate concentrations and a 4 h incubation, whereas Lépine et al. used HEK293 cells, 25 μM substrate, and 16 h incubation. These different assay methods may have led to differing results. Basu et al. (2004) implied that UGT1A10 is the main isozyme contributing to 2MeO-E2 glucuronidation. However, Lépine et al. (2004) reported catalytic efficiencies for 2MeO-E2 3-glucuronidation for UGT1A8, 1A1 and 1A3 of 15.9, 4.5, and 3.1 $\mu\text{L/min} \cdot \text{mg}$ protein, respectively. Although K_m (12 μM) for UGT1A10 implies that this isozyme has a high affinity for 2MeO-E2, its intrinsic clearance was estimated to be quite low.³⁸ Glucuronidation of 2MeO-E2 took place at higher rates than methoxylated or hydroxylated estrones.^{37,38} Both groups agreed that UGT2B7 was significantly not involved in the formation of 2MeO-E2 glucuronides, and there was no detectable glucuronidation occurring at the C-17 position. Even for primary estrogens like E2, the involvement of UGT2B7 or other isozymes in the formation of estradiol-17 β -glucuronide is not quite clear.^{38–40} On the other hand, incubation with pooled human liver microsomal proteins resulted in formation of two types of glucuronides, presumably 2MeO-E2-17- and -3-glucuronides.⁷ Therefore, the mechanism of formation, conditions, and type of enzymes participating in the formation of 17-glucuronide still need to be clarified.

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In vitro experiments have shown that glucuronidation of 2MeO-E2 might not be limited to the liver. Hence, UGT1A1 mRNA is expressed (mRNA) in duodenum:ileum:stomach at relative levels of approximately 10:4:1.³⁷ UGT1A7 is also present in nearly every tissue with high levels in esophagus, thyroid and adrenal glands, with the highest being in esophagus.⁴¹ UGT1A8 was found at moderate to high expression levels in esophagus, duodenum ileum, colon, and stomach. UGT1A9 appeared to be highly expressed in distal parts of the GI tract such as jejunum, ileum, colon and rectum. The highest amounts, however, were found in kidney and liver.⁴¹ UGT1A10 appeared to be present in duodenum:ileum:colon:stomach:esophagus at relative levels of 9:8:3:2:1.³⁷ Therefore, orally administered 2MeO-E2 can theoretically undergo extensive metabolism starting in esophagus by UGT1A7, followed by UGT1A8 in stomach (empty), then in duodenum by UGT1A1, 1A7, 1A9, and 1A10, in ileum by UGT1A1, 1A9 and 1A10, colon by UGT1A7, 1A9, and 1A10, and of course, in liver by UGT1A1, 1A3, 1A9. Of course, the actual contribution of these enzymes to 2MeO-E2 metabolism would be strongly influenced by tissue surface area, residence time of the drug in the organ, enzyme expression level, and drug concentration, all of which determine the exposure of the drug to the enzyme(s). Lakhani et al. (2007) have also emphasized that phase I metabolism can play an important role in metabolism of 2MeO-E2, but not as extensively as glucuronidation.⁷ Although no oxidative metabolites were found in urine samples of patients taking 2MeO-E2, *in vitro* experiments showed the presence of five phase I metabolites such as 2MeO-E1, 2-hydroxyestradiol, 2-hydroxyestrone, 2,3-dimethoxyestrone, and 2,3-dimethoxyestradiol. The incubation of 2MeO-E2 with different recombinant CYP450 enzymes demonstrated that the following isoforms are responsible for hepatic oxidation of the drug: CYP1A1, 1A2, 3A4, 3A5 and 2E1.³⁶ Neither the urine samples nor *in vitro* studies showed the presence of sulfated metabolites of 2MeO-E2 indicating that 2MeO-E2 metabolism by sulfotransferase is limited or does not take place. However, it was reported that 2MeO-E2 can undergo sulfonation catalyzed by SULT1A1.⁴² Being a low-capacity and high-affinity enzyme, SULT1A1 may be effective in 2MeO-E2 biotransformation in nanomolar range; at very high concentrations UGTs become the major route of 2MeO-E2 biotransformation. The additional bulk of the C-2 methoxy group may also inhibit SULT activity.⁷

2MeO-E2 Transport. To our knowledge, no studies have been published so far to reveal the role of transporters, especially ATP-binding cassette (ABC) transporters, on the disposition of 2MeO-E2. Some research, however, was done to identify an ABC transporter that might be responsible for the movement of E2 through the lipid bilayer. Considering

the fact that E2 and 2MeO-E2 are structurally similar, one might also apply the results obtained with E2 to 2MeO-E2. Because E2 is a very hydrophobic compound, it would be reasonable to assume that E2 is a substrate for Pgp (ABCB1), known to transport a variety of hydrophobic compounds.⁴³ However, Fujise et al. (2002) have shown via *in vitro* experiments that estradiol is not a P-glycoprotein substrate, whereas uptake experiments with inside-out membrane vesicles and BCRP-transduced cells showed that E2 is a competing substrate for breast cancer resistance protein (BCRP), known also as ABCG2.^{44–46} In any case, diffusional permeabilities for these compounds would likely overwhelm any contribution of ABC efflux transporters to overall transport of E2 or 2MeO-E2. However, they may result in inhibition of certain transporters if sufficiently high local concentrations of the parent compounds or their metabolites are achieved.

Pharmacokinetic–Pharmacodynamic Relationships

The pharmacokinetic–pharmacodynamic relationships for 2MeO-E2 have not been clearly established in humans, since phase three clinical trials have not yet been published. However, as clinical trials proceed, one may expect more data to be published to elucidate this issue. In phase 1 clinical trials of 2MeO-E2, one ovarian cancer patient showed a partial response despite low circulating 2MeO-E2 concentrations, leading Lakhani et al. to suspect an active metabolite of 2MeO-E2.³⁶ This possibility remains to be investigated. However, an alternative explanation could be reversible metabolism. In such a case, 2MeO-E2-3-glucuronide could enter a tissue and be cleaved by cellular glucuronidases, releasing “free” 2MeO-E2. Glucuronidase activity in breast cancer tissue was demonstrated long ago by Fishman and Anlyan.⁴⁷ More recently the presence of such glucuronidases

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in lung cancer tissue inspired the design of a glucuronidase-activated prodrug of doxorubicin which would attempt to selectively target cancer tissues in which this enzyme was highly expressed.⁴⁸ Thus the release of 2MeO-E2 from its glucuronide within the tumor, if it occurs, could explain the observation by Lakhani et al. Further research is needed to fully relate the pharmacokinetics of 2MeO-E2 (and its metabolites) to their pharmacodynamics in humans.

Attempts To Conquer Challenges

New Formulations. There are several approaches to solve the problem of low solubility of 2MeO-E2, including improved formulations. For instance, encapsulation of 2MeO-E2 within biodegradable and biocompatible layers of polyelectrolyte led to an increase in the drug solubility and stability for up to 9 months in aqueous solution.⁴⁹ This multilayered matrix easily released the active ingredient and was shown to produce a slightly better or similar effect as free 2MeO-E2 in *in vitro* experiments. These microcrystals, however, need to be further tested *in vivo* to determine their efficacy.

Generally, particles of smaller diameter allow a drug to dissolve at higher rates. Thus, Tevaarwerk et al. (2009) have conducted phase I clinical trials for the purpose of evaluating the efficacy of nanocrystal dispersion of 2MeO-E2.⁵⁰ It was observed that there was a dose dependent increase in the systemic exposure within the dose range of 250–1000 mg, and minimal increase at higher doses. The maximally tolerated dose was found to be 1000 mg every 6 or 8 h. Based on the published data, this nanocrystal drug formulation led to an increase in 2MeO-E2 bioavailability to approximately 3–4%, which is 3- to 4-fold higher than those observed with “typical” formulations, but the bioavailability remains very low.

Coadministration with Inhibitors. It may be possible to formulate 2MeO-E2 with other agents in an attempt to decrease its extensive presystemic elimination, thus enabling more of the drug to reach systemic circulation. Such inhibitors would need to be effective at inhibiting the major enzymes metabolizing 2MeO-E2 including several UGT isoforms, CYP1A1, and SULT1A1 as discussed above, but without significant toxicities of their own. Such an approach, if effective, may increase the toxicity of 2MeO-E2 and may have problems with drug–drug interactions. We are currently investigating a novel, proprietary combination approach.

Alternative Routes of Administration. In addition to oral formulations, parenteral formulations may be explored. These nonoral routes of administration would diminish problems with the extensive presystemic metabolism of 2MeO-E2, although solubility remains problematic. For example, intravenous dosing would almost certainly require a nonaqueous solvent such as propylene glycol or polyethylene glycol, or pharmaceutical surfactants such as Cremophor EL. Also, long-term intravenous dosing would be difficult to maintain. Nonaqueous depot injection of 2MeO-E2 directly into the area of the tumor is a possibility. For transdermal administration, which is commonly used for many contraceptives containing estrogens and progestins, it may be difficult to achieve desired plasma concentrations of 2MeO-E2.

Synthesis of Active Analogues or Prodrugs. Syntheses of 2MeO-E2 analogues with similar or better anticancer activities and lower rates of metabolism or prodrugs with improved solubility are another way to resolve low bioavailability of 2MeO-E2. An introduction of various functional groups at different locations on the structure of 2MeO-E2 structure yielded a variety of compounds.^{51,52} However, it is of interest to synthesize a compound with other than hydroxyl and amine (NH₂) groups at C-3 and C-17 which would still possess similar or improved antitumor properties.

The anticancer activity of 2MeO-E2 is primarily associated with the methoxy moiety at C-2. Therefore, any changes at this location resulted in the reduction in efficacy to inhibition of tubulin polymerization.⁵³ The compounds with different functional groups at C-2 are listed in Table 2, compounds **2** through **10**. These drugs were tested in several cancer cell lines, but for the sake of comparison between analogues only a few cell lines were selected, and the results are listed in Table 2. Antiproliferative properties (IC₅₀) or growth inhibition (GI₅₀) were reported as IC₅₀ (or GI₅₀) ratio of 2MeO-E2 to 2MeO-E2 analogues. Values larger than 1 are indicative of higher potency of 2MeO-E2 analogue than 2MeO-E2. The analogues with bulky side groups or electronegative atoms at C-2 lost their anticancer activities; therefore only drugs with small and nonpolar moieties such as propynyl (compound **9**) and 3-hydroxy-1-propenyl (compound **2**) possessed comparable properties to 2MeO-E2. Hence, modifications at C-3 or C-17 would be more desirable.

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Table 2. 2MeO-E2 Analogues and Their Relative Anticancer Activities

Compound	Position			Effect/Cell Line	Relative Potency	Reference
	C-2	C-3	C-17			
2MeO-E2	CH ₃ O	OH	OH		1	
2	C ₃ H ₄ OH	OH	OH		1.53	
3	EtO-OH	OH	OH		0.01	
4	CHNC ₂ H ₄ N(Me) ₂	OH	OH		<0.01	
5	CHNC ₂ H ₄ OH	OH	OH		<0.01	⁵³ Cushman et al., 2002
6	(MeO) ₃ Ph-C ₂ H ₂	OH	OH	MDA-MB-435 Cytotoxicity	0.02	
7	Ph-C ₂	OH	OH		0.01	
8	C ₂ H	OH	OH		0.04	
9	CH ₃ C ₂	OH	OH		0.62	
10	CN	OH	OH		<<0.01	
11	CH ₃ O	PO ₃ H ₂	OH		0.73	
12	CH ₃ O	OH	PO ₃ H ₂		<0.01	⁵⁹ Edsall et al., 2007
13	CH ₃ O	PO ₃ H ₂	PO ₃ H ₂		<<0.01	
14	CH ₃ O	NHCN	OH		1.44	⁵⁴ Suwandi et al., 2009
15	CH ₃ O	NHCONH ₂	OH		1.15	
16	CH ₃ O	NHCOH	OH		9.7	
17	CH ₃ O	OH	H		0.64	⁵⁵ Shah et al., 2009
18	CH ₃ O	C ₂ H ₃	H		1.17	
19	CH ₃ O	CONHEt	H	HUVEC Antiproliferation	0.02	
20	CH ₃ O	CONH ₂	H		0.57	⁵⁶ Agoston et al., 2009
21	CH ₃ O	NH ₂	H		0.14	
22	CH ₃ O	NHCOH	H		0.12	
23	CH ₃ O	NHCOMe	H		0.4	
24	CH ₃ O	OH	CH ₂		4.42	⁵⁵ Shah et al., 2009
25	CH ₃ O	CONH ₂	CH ₂		2.72	
26	CH ₃ O	NH ₂	CH ₂		0.68	⁵⁶ Agoston et al., 2009
27	CH ₃ O	NHCOH	CH ₂		3.09	
28	CH ₃ O	OH	CHCH ₃		3.23	⁵⁵ Shah et al., 2009
29	CH ₃ O	NH ₂	CHCH ₃		0.94	⁵⁶ Agoston et al., 2009
30	CH ₃ O	NHCOH	CHCH ₃		1.17	
31	CH ₃ O	OH	CHCH ₂ CH ₃		0.26	
32	CH ₃ O	OH	CHCH ₂ CH ₂ CH ₃		0.24	⁵⁵ Shah et al., 2009
33	CH ₃ O	OH	CH ₃		0.35	
34	CH ₃ O	NH ₂	CH ₃		0.26	⁵⁶ Agoston et al., 2009
35	CH ₃ O	NHCOH	CH ₃		1.17	
36	CH ₃ O	OH	CH ₂ CH ₃		0.23	
37	CH ₃ O	OH	CH ₂ CH ₂ CH ₃		0.02	⁵⁵ Shah et al., 2009
38	CH ₃ O	OH	CH ₂ CH ₂ CH ₂ CH ₃	HUVEC Antiproliferation	0.07	
39	CH ₃ O	OH	N ₂ HSO ₂ Ph-CH ₃		0.011	
40	CH ₃ O	OH	NH ₂		0.06	
41	CH ₃ O	OH	NH-Pr		0.04	
42	CH ₃ O	OH	NOH		0.2	⁵⁵ Shah, et al., 2009
43	CH ₃ O	OH	COOH		0.02	
44	CH ₃ O	OH	CONH ₂		0.18	
45	CH ₃ O	OH	16,17-Olefin		1.18	
46	CH ₃ O	CONH ₂	16,17-Olefin		5.23	⁵⁶ Agoston et al., 2009
47	CH ₃ O	NH ₂ SO ₃	OH		10	⁵⁸ Newman et al., 2004
48	CH ₃ O	NH ₂ SO ₃	NH ₂ SO ₃		60	
49	CH ₃ CH ₂	OH	OH		<0.3	⁵⁸ Newman et al., 2004
50	CH ₃ CH ₂	NH ₂ SO ₃	OH		>300	
47	CH ₃ O	NH ₂ SO ₃	OH		6.3	
48	CH ₃ O	NH ₂ SO ₃	NH ₂ SO ₃	MCF7 Antiproliferation	6.3	⁵⁸ Newman et al., 2004
48	CH ₃ O	NH ₂ SO ₃	NH ₂ SO ₃		6.25	⁵⁷ Raobaikady et al., 2003

Table 2 also contains a list of compounds and their antiproliferative properties where a new group was introduced to C-17, C-3 or at both locations simultaneously. Only 3 out of 25 compounds (compounds **14**, **15** and **16**) with

substituted group at C-3 were added to the table because they seemed to be more potent than 2MeO-E2 itself.⁵⁴ All three compounds are H-donors, not bulky and contained π electrons as a part of nitrile or carbonyl. When the hydroxyl

Table 3. Pharmacokinetic Summary of Selected 2MeO-E2 Analogues

compd	C _{max} (nM)	AUC (nM h)	BA (%)	reference
1	BLOQ ^a	BLOQ		Shah et al., 2009 ⁵⁵
24	40	572	32.8	
25	62	467	22.8	Agoston et al., 2009 ⁵⁶
27	127	1703	39.5	
28	80	1163	84.2	Shah et al., 2009 ⁵⁵
30	83	659	24.7	Agoston et al., 2009 ⁵⁶
35	73	673.2	20.6	
46	42	320	14.3	

^a BLOQ: below the limit of quantification.

group at C-17 was substituted by other moieties such as methylene and ethylene (compounds **24** and **28**), the potency of the drug increased in comparison with 2MeO-E2. However, as previously discussed, the substitution with groups of longer chains caused antiproliferative properties to decline (e.g., compounds **33** vs **36–38**). An introduction of amine (**40**), carboxamide (**44**), and hydroxyl amide (**42**) decreased the potency of the 2MeO-E2 analogues.⁵⁵

Double modification at C-3 and C-17 tended to decrease the potency not only in comparison to 2MeO-E2⁵⁶ but, in some cases, also in comparison to the compounds with only one modified group at C-17 (Table 2), despite the fact that each group substituted at a single location showed improvement in antiproliferative properties (e.g., compounds **16**, **24**, and **27**). The only exception is compound **46**, the potency of which is actually increased 5-fold in comparison to 2MeO-E2.

The drugs with improved therapeutic properties should also have better pharmacokinetic properties to be more advantageous than 2MeO-E2. Some PK parameters of the selected 2MeO-E2 analogues measured via cassette dosing in rats are listed in Table 3.^{55,56} Bioavailabilities of all compounds were estimated to be between 14 and 84%. C_{max} and AUC values were possible to estimate unlike for 2MeO-E2. Compound **27** had the highest C_{max}. However, this drug and also **30** and **35** have formamides at C-3 which might have aniline-like toxicity if they are cleaved⁵⁶ and thus might be not suitable as anticancer drugs. The addition of ethylene or methylene groups to C-17 affected the compound solubility and glucuronidation rate by reducing them both. Instead of a single first order

reaction, *in vitro* glucuronidation of 2MeO-E2 analogues occurred via a slow initial phase and a faster second phase.⁵⁵ Perhaps due to the slow initial metabolic phase the AUC for compounds **24** and **28** was larger than that for 2MeO-E2; however their metabolic rate during the second phase was much faster than for 2MeO-E2. Thus, compounds **46** and **25** could be potential anticancer drugs. Compound **46** is currently in phase I clinical trials.⁵⁶

The introduction of other groups to C-17 or C-3 had variable success. For instance, the addition of sulfamate to C-17 (compound **47**) or to both C-17 and C-3 (compound **48**) resulted in drastic improvement of the antiproliferative properties of the compound, and it was attributed to the compound itself rather than its actions as a prodrug.^{57,58} On the other hand, the addition of phosphates to the 2MeO-E2 structure (compounds **11–13**, Table 2) diminished the therapeutic properties in most of the tested cancer cell lines, and it was concluded to be inefficient as prodrugs.⁵⁹ The most effective phosphate prodrug, however, was determined to be 3-phosphate-2-MeO-E2, but it possessed high toxicity in comparison to 2MeO-E2 itself. Sulfamoylation at either C-3 alone or C-3 and C-17 (compounds **47** and **48**, respectively) substantially improved the antiproliferation potency.^{57,58} Finally, although replacing the 2-methoxy group with an ethyl group (compound **49**) had decreased potency as discussed earlier, sulfamoylation (compound **50**) had drastically improved potency.^{57,58}

Conclusions

2MeO-E2 is an active metabolite of estrogen which is found to possess anticancer activities on several cancer cell lines. *In vivo* studies have revealed that 2MeO-E2 is well tolerated, but due to its extensive first pass metabolism and low solubility, subtherapeutic plasma concentrations of 2MeO-E2 were observed despite large orally administered doses. Pharmacokinetic analysis revealed nonlinear dose proportionality in addition to the low oral bioavailability of 2MeO-E2. 2MeO-E2 is extensively metabolized not only in the liver but also in the GI tract. Although glucuronidation is considered to be a major route of 2MeO-E2 inactivation, 2MeO-E2 is also largely converted into 2MeO-E1 and is also metabolized by several CYPs. The attempts to tackle the problems related to 2MeO-E2 low solubility and/or extensive metabolism did not always result in

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the desired improvement. Thus, new formulations only modestly improved the oral bioavailability. Several different 2MeO-E2 analogues have been synthesized that would be more metabolically stable than the parent drug, but only a few of them have shown some promise. Although 2MeO-E2 is a promising anticancer drug, the resolution of its current problems associated with solubility and metabolism are far from being completed for a successful launch of 2MeO-E2 into the market. Further studies will be required to identify the receptors targeted by 2MeO-E2 and mediating its therapeutic effects. Additional compounds can be designed to bind these receptors.

Abbreviations Used

2MeO-E1, 2-methoxyestrone; 2MeO-E2, 2-methoxyestradiol; ABCB1, ATP-binding cassette transporter isoform B1; ABCG2, ATP-binding cassette transporter isoform G2; AUC,

area under the plasma drug concentration–time curve; C_{\max} , maximum drug concentration; BCRP, breast cancer resistance protein; CL, clearance; COMT, catechol-*O*-methyltransferase; CYP or CYP450, cytochrome P450; E1, estrone; E2, estradiol; ER, estrogen receptor; Pgp, P-glycoprotein; SULT, sulfotransferase; $t_{1/2}$, half-time; V_d , volume of distribution; UGT, UDP-glucuronosyltransferase.

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