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Review

# Recent Advances in chemistry and pharmacology of 2-methoxyestradiol: An anticancer investigational drug

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ABSTRACT

2-Methoxyestradiol ($2ME_2$), an estrogen hormone metabolite is a potential cancer chemotherapeutic agent. Presently, it is an investigational drug under various phases of clinical trials alone or in combination therapy. Its anticancer activity has been attributed to its antitubulin, antiangiogenic, pro-apoptotic and ROS induction properties. This anticancer drug candidate has been explored extensively in last twenty years for its detailed chemistry and pharmacology. Present review is an update of its chemistry and biological activity. It also extends an assessment of potential of $2ME_2$ and its analogues as possible anticancer drug in future.



**Contents**



*Abbreviations:* ROS, reactive oxygen species; CYP450, cytochrome P450; VEGFR, vascular endothelial growth factor; HIF, hypoxia inducible factor; PDT, podophyllotoxin; Cdk, cyclin-dependent kinase; TNF, tumour necrosis factor.

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## 1. Introduction

Estrogen metabolites, considered as inactive excretion products, have now been proved useful products by various findings. A number of studies suggest that the carcinogenesis as well as cardiovascular system can be influenced by the estradiol metabolite i.e. 2-methoxyestradiol. 2-Methoxyestradiol ($2ME_2$) is a potent anticancer clinical candidate currently under evaluation as an investigational drug against various types of human cancers [1]. It is a natural metabolite of endogenous estrogen hormone 17β-estradiol in human and devoid of estrogenic activity. It shows multiple modalities to tackle the cancer with antiproliferative, proapoptotic, antiangiogenic, antitubulin and antimetastatic effects. $2ME_2$ is efficacious in multiple solid tumors and metastatic models [2]. This estradiol entity exhibits potential cytotoxic effects against various types of carcinomas i.e. breast (ER+ve and ER−ve both), ovarian, lung, prostate, colorectal etc. It shows strong cytotoxic effect on estrogen dependent and independent cancerous cells, which is mainly due to disruption of microtubule process and p53 induced apoptosis through caspase, reactive oxygen species (ROS), superoxide dismutase (SOD) and nitric oxide synthase. 2-Methoxyestradiol inhibits tubulin polymerisation by binding to colchicine binding site of the tubulin and arrests cell cycle at G2/M-phase. These promising properties of $2ME_2$ encouraged us to provide a systematic summary and insight on the progress in the field of steroid chemistry. In this review, physiological, pharmacological properties and synthetic methods of the $2ME_2$ are discussed. This review is expected to assist effective study and successful development of $2ME_2$ as a promising therapeutic agent.

## 2. Chemistry of $2ME_2$

2-Methoxy-estra-1,3,5(10) trien-3,17β-diol ($2ME_2$, **1**) is a steroid molecule possessing decahydrocyclopentano[*a*]phenanthrene skeleton. It possesses four fused ring system (A, B, C, & D rings) where first three rings (A, B & C) are six membered, ring A is aromatic in nature, whereas the fourth ring (ring D) is five membered. Ring A bears a methyl ether at 2-position, a phenolic hydroxyl at 3-position and an alcoholic hydroxyl at 17β-position.

### 2.1. Biosynthesis of $2ME_2$

$2ME_2$ is a metabolite of 17β-estradiol (**2**) which is produced by sequential hydroxylation and *O*-methylation at its 2-position. In females, estrogens are mainly produced in ovaries and partly in adrenals. Cholesterol is the main source of steroids in ovaries. Various types of steroid molecules are synthesized biochemically in human body involving conversion of cholesterol (C27) into progestins (C21) followed by androgens (C19) and finally into estrogens (C18) with the help of various enzymes. This multistep process is known as steroidogenesis [3,4]. Enzymes such as aromatase (CYP450arom), 17β-hydroxysteroid dehydrogenase (17β-HSDs) are essentially required in the last step of estrogen biosynthesis while steroid sulfatase (STS) is required for interconversion of the inactive form of estrogen in their active form. Various important steroid hormones are synthesized by this process such as progestins, androgens, estrogens, glucocorticoids and mineralocorticoids etc. Other than estradiol, the ovary secretes estrone, which serves as a source of estradiol. 17β-Estradiol (**2**), estrone (**3**) and estriol (**4**) are the estrogens produced and secreted in the human body. The estrogenic effect is mainly due to estradiol while the estrogenic effect of estrone and estriol are insignificant. Estriol is mainly a peripheral metabolite of estrogens. The metabolism of estradiol primarily involves cytochrome P450 (CYP 450) dependent hydroxylations at C2, C4 and C16 positions to yield 2-hydroxyestradiol, 4-hydroxyestradiol and 16α-hydroxyestradiol (Fig. 1). Cytochrome CYP1A2 and CYP3A enzymes catalyse 2-hydroxylation in the liver [5–10]. Further, catechol-*O*-methyltransferase present in many tissues including uterus [11], liver, kidney, blood [12], breast etc. It catalyses the *O*-methylation of catecholestrogens by transferring a methyl group from the cofactor S-adenosyl methionine to the 2-OH/4-OH groups and produces 2-methoxy/4-methoxy estradiol [13–15]. In normal physiological conditions the plasma level of $2ME_2$ is in picomolar (pM) range, however, during late pregnancy it is increased to nanomolar (nM) range [16]. This conversion was demonstrated through trans-methylation of 2-hydroxyestradiol to 2-methoxyestradiol $C^{14}$ in presence of methionine-methyl-$C^{14}$ and ATP in breis of Guinea pig tissues in *in vitro* conditions [17].

### 2.2. Analytical techniques to determine $2ME_2$

Various analytical methods have been developed to determine $2ME_2$ in different biological samples. Reverse phase HPLC method was developed for the quantification of $2ME_2$ in human plasma by Lakhani et al. (2004) using C18 column and UV detector at 205 nm [18]. Several other groups have also developed similar methods [19,20]. A fluorescent detector based reverse phase HPLC method has been developed by Du et al. for pharmacokinetics study of $2ME_2$ in rat samples [21]. $2ME_2$ and other nine metabolic steroids were determined by Katayama et al. (2007) with enhanced sensitivity by using a diamond electrode in electrochemical and fluorescent detector in HPLC analysis. This dual detector improved peak detection in urine samples of patients and also in biopsy samples [22].

Several other detectors were also used for the quantification of $2ME_2$. Capillary electrophoretic separations of five estrogens and two stereoisomers were performed in biological samples using an anionic sulfobutyl ether β-cyclodextrin [23]. A Liquid chromatography-electrochemical detection method has been developed for the estimation of eight estrogens and their metabolites in serum samples [24]. Zhang et al. developed a luminescence analyser based detection of $2ME_2$ in serum samples. In this method high sensitivity of chemiluminescence of silver nano particle was used [25].