2-methoxyestradiol represents the prototype of a new anti-cancer agent. In this article, the authors discuss the beneficial and detrimental features of the prototype agent and strategies for the design of improved agents.

2-Methoxyestradiol – a unique blend of activities generating a new class of anti-tumour/anti-inflammatory agents

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The estradiol metabolite, 2-methoxyestradiol (2MEO), is currently being evaluated in Phase II clinical trials for the treatment of solid tumours and is undergoing preclinical evaluation for inflammatory conditions. The anti-proliferative/cytotoxic/pro-apoptotic effects on tumour and endothelial cells have conferred potential on this metabolite for a synergistic impact on tumour growth. Exploitation of this synergy of 2MEO has previously required the combination of well-established cytotoxic agents with newer anti-angiogenic agents. This article reviews the pharmacology of 2MEO and describes the limitations inherent in its residual estrogen receptor affinity. The extent to which the metabolite 2MEO embodies an optimised therapeutic candidate is discussed. The challenges involved in using rational (3D QSAR-based) drug design to optimise the activity profile of analogues of 2MEO to provide additional members of this new class of anti-tumour/anti-inflammatory drug are also outlined.

Introduction

2-Methoxyestradiol (2MEO), formerly regarded as an inactive metabolite of 17β-estradiol (E2), is now known to be an anti-angiogenic agent with anti-proliferative and cytotoxic activity that confers anti-tumour activity in some animal models of solid tumour. Thus, the structure of 2MEO embodies multiple activities that have the potential to synergise to become an efficacious anti-tumour agent. 2MEO is currently being evaluated in Phase II clinical trials for the treatment of solid tumours and is undergoing preclinical evaluation for inflammatory conditions. However, emerging data suggest that the multi-faceted mechanisms of action of 2MEO are not optimised for potency or safety. In the present article, we review the actions of 2MEO and discuss potential strategies for a significant improvement in potency, selectivity and safety of novel 2MEO analogues.

Formation and fate of endogenous 2MEO

E2 metabolism results in the formation of several metabolites that retain various degrees of estrogenic activity (Figure 1). 2MEO is formed from cytochrome P450-dependent conversion of E2 to 2-hydroxyestradiol, which is then methylated by catechol-*O*-methyl transferase (COMT) [1]. The plasma concentrations of 2MEO range from low picomolar concentrations in postmenopausal females to nanomolar concentrations during pregnancy. Higher concentrations (micromolar) in specific tissue compartments such as ovaries and breast tissue that have high local E2 concentrations may occur.

Metabolism of 2MEO is likely to involve hydroxylation at a number of sites and dehydrogenation at C17 to yield the relatively inactive metabolite 2-methoxyestrone [1]. Conjugation on the 3-position or 17-position with sulfate or glucuronic acid is likely to explain the short half-life (less than 0.5 hours) of this compound in rodents [2] and poor bioavailability in humans in whom 2-methoxyestrone is rapidly generated [3]. Conversion of 2MEO to an estrogenic metabolite has been observed in some studies [4], possibly as a result of demethylation. The existence of multiple pathways for metabolism of 2MEO is highly pertinent to the potential development of analogues designed for resistance to transformation at those positions. 2MEO analogues have been designed to improve the efficacy and the pharmacokinetic profile of this drug

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1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2007.05.005

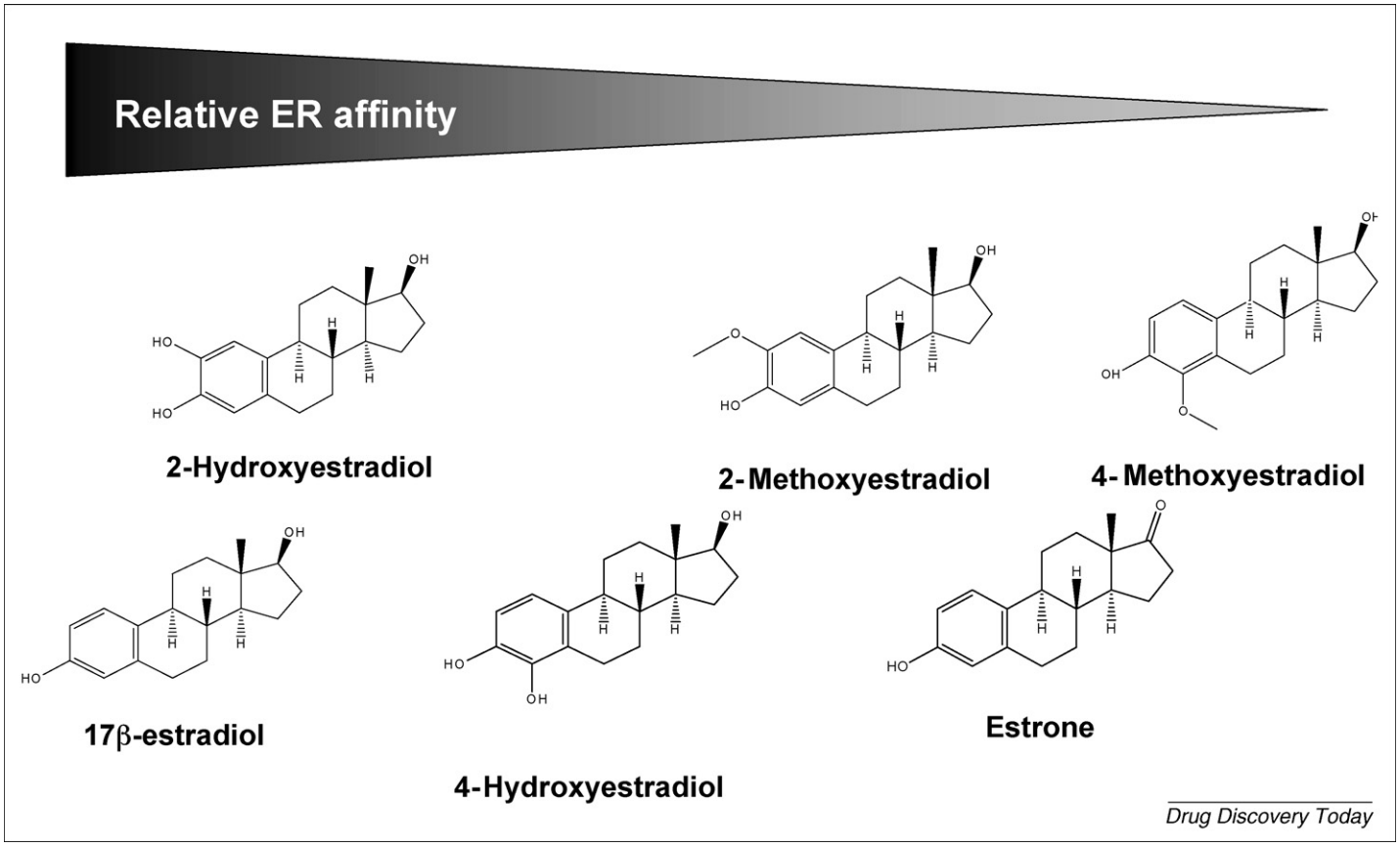

FIGURE 1

Structures and relative estrogen receptor affinities of 2-methoxyestradiol and related compounds.

class by modifying the 2, 3 and 17 positions of the steroid structure. Such an approach has been utilised by several research groups including those at Entremed Inc., the pharmaceutical company developing 2MEO for the treatment of tumour and rheumatoid arthritis (http://www.entremed.com) [5]. Analogues with modifications at the 3 and 17 positions have demonstrated improved metabolic stability and bioavailability associated with enhanced anti-tumour activity [2].

Actions of 2MEO

Anti-proliferative actions

The anti-proliferative effects of 2MEO on tumour cell lines, first reported in the 1980s, have been confirmed and extended to a variety of tumour and endothelial cells, as previously reviewed [1,6]. Cell proliferation is inhibited at a threshold concentration of 0.1–0.3 μM, with maximum inhibitory effects being reached between 10 and 20 μM in most cell types, including endothelial and tumour cells [7], multiple myeloma [8], fibroblasts, smooth muscle [9,10], hepatic stellate cells [11] and glomerula mesangial cells [12], respectively.

The anti-proliferative/cytotoxic activities of 2MEO are not shared by E2, making it unlikely that such actions are estrogen receptor (ER)-dependent or occur through metabolism of 2MEO to estrogenic metabolites. In most cell types, 2MEO treatment results in G1 cell cycle arrest, as a result of DNA synthesis inhibition, and G2/M cell cycle arrest that may be caused by the actions on microtubules [9,13].

Apoptotic activity

The apoptotic activity of 2MEO is specific for actively proliferating cells, such as tumour and activated endothelial cells, as 2MEO does not induce apoptosis in normal mammary epithelial cells [14] or quiescent endothelial cells [15]. Apoptosis induced by 2MEO in bovine pulmonary aortic endothelial cells is associated with transient activation of JNK and an increased expression of Fas and Bcl-2 [16], whereas in prostate tumour cells, a $p38^{MAPK}$-dependent apoptotic pathway has been implicated [17]. 2MEO induces p53-independent apoptosis in leukaemia cells, possibly by inhibition of superoxide dismutase leading to oxidative-stress [18], whereas, apoptosis in non-small cell carcinoma cells is reported to be p53-dependent [19]. In bovine vascular endothelial cells, 2MEO induces an increase in and redistribution of endothelial nitric oxide synthase, resulting in a localised production of NO within the plasma membrane, which is associated with senescence and apoptosis [20]. An increase in nitric oxide levels coincident with an inhibition of superoxide dismutase, as seen in leukaemia cells [18], could generate peroxynitrite, triggering free radical damage and resulting in apoptosis. The diverse and potentially redundant mechanisms through which 2MEO induces apoptosis identifies 2MEO as an anti-cancer agent that may have less risk of inducing the development of tumour cell resistance.

Anti-angiogenic actions

Widespread interest in 2MEO was generated by the recognition of anti-angiogenic actions *in vivo* [15]. Oral administration of 2MEO

in mice suppressed tumour growth and inhibited neovascularisation [15]. Inhibition by 2MEO of tumour cell vascular endothelial growth factor (VEGF) production is likely to contribute to the reduction in tumour neovascularisation [21]. Anti-angiogenic effects have also been observed in non-tumour tissues using the corneal micropocket vascularisation and chick chorioallantoic membrane assays [7,16].

Potential of 2MEO as a therapeutic agent for cancer

Synergy between anti-angiogenic and cytotoxic agents

Cytotoxic therapies are essential for killing malignant tumour cells but are limited in impact by significant adverse effects and the development of resistance. Anti-angiogenic agents limit the size of tumour growth by restricting blood supply and may be useful as adjuvants to standard cytotoxic therapies [22] by synergising with cytotoxic therapies to yield greater maximum effect on tumour growth [23]. Anti-angiogenic therapy does not appear to induce acquired drug resistance in animal models [24], primarily because it targets slowly dividing endothelial cells that have limited capacity for development of resistance through mutation. As 2MEO is both anti-angiogenic and cytotoxic for tumour cells, its development has proceeded with great optimism. The use of one molecule having both cytotoxic and anti-angiogenic activities as opposed to a combination of agents could be expected to have fewer mechanism-independent adverse effects. The uniqueness of 2MEO in combining these activities is now open to some question, as it appears that decreasing HIF-1α and hence VEGF production is a general property of microtubule-destabilising anti-cancer agents, such as taxol [25]. Nevertheless, the combination of 2MEO with paclitaxel demonstrates synergistic anti-cancer actions at doses of 2MEO (30 mg/kg) that have no direct anti-tumour action [26].

Preclinical studies with 2MEO

2MEO administered orally to mice reduced the growth of both Meth A Sarcoma and B16 melanoma, apparently without the toxicity of conventional anti-tumour agents [15]. The decrease in tumour growth was accompanied by a decrease in neovascularisation [15] prompting more extensive preclinical studies. The efficacy of 2MEO was established against breast tumour cells, both *in vitro* and in mouse tumour models accompanied by an apparently benign toxicity profile [7], supporting the development of 2MEO as a novel agent for solid tumour therapy (Table 1). However, weight loss has been observed [14,15,27]. Orally administered 2MEO (75 mg/kg) preserves cancellous bone in ovariectomised rats at non-uterotropic doses [28] by an action that may be linked to apoptosis of osteoclasts [29]. In contrast, long bone growth is inhibited [27]. Uterotropic actions of 2MEO [28,30] are attenuated by treatment with the ER antagonist, ICI 182,780 [30]. *In vitro*, 2MEO activates ER to cause an ICI 182,780-reversible mitogenesis in MCF-7 cells [4]. In mice inoculated with E2-dependent and ER-positive MCF-7 cells, 2MEO treatment sustained tumour growth [30]. 2MEO has demonstrated both ER-dependent and ER-independent adverse effects including hepatotoxicity [31]. The anti-tumour actions of 2MEO appear to only manifest at doses greater than 50 mg/kg, which coincide with doses used in studies documenting adverse effects. The pharmacokinetic/pharmacodynamic (PK/PD) relationship has not been clearly established in published preclinical studies and will prove to be important in assessing the relevance of any particular molecular targets.

Clinical studies with 2MEO

The anti-tumour efficacy, anti-angiogenic actions and tolerability of 2MEO (Panzem®) in preclinical studies led to Phase I/II clinical trials for the treatment of breast and prostate cancer. Oral doses of 2MEO up to 1.2 g daily have shown neither toxicity nor anti-tumour efficacy [3], although the prostate tumour biomarker PSA velocity was significantly reduced. A Phase I study, incremented to doses of 6 g/day without reaching the maximum tolerated dose (MTD) [32]. After an oral dose of 2.2 g maximum plasma concentrations reached less than 10 nM [33], which may well be sub-threshold for anti-tumour actions. A more recent Phase II study in prostate cancer reported a C_{max} of ~25 nM following 1.2 g orally [3]. The continued development of 2MEO has occurred through reformulation in a nanocrystal colloidal dispersion (NCD). 2MEO NCD® was shown to have improved bioavailability (http://www.entremed.com). Despite improved bioavailability through re-formulation, the short half-life of 2MEO, determined by susceptibility to metabolic degradation and conjugation, remains a major influence on C_{max} and AUC.

Other therapeutic indications for 2MEO

The combination of anti-proliferative, apoptotic and anti-angiogenic actions supports the use of 2MEO as an anti-tumour agent, but these properties also indicate that 2MEO has wider therapeutic potential. The cytotoxic profile of anti-tumour agents would normally preclude development for other indications associated with tissue remodelling and angiogenesis such as cardiovascular disease, airway disease [10] and rheumatoid arthritis [34]. However, the apparent safety of 2MEO led to attention in these other areas.

2MEO may be an effective agent in the treatment of airway wall structural changes in asthma, as it is anti-mitogenic for human cultured airway smooth muscle [9]. In murine models of allergen-induced airway hyper-responsiveness, 2MEO reduced the number of inflammatory cells in the bronchoalveolar lavage fluid and airway hyper-responsiveness [35]. The process of angiogenesis, crucial for tumour growth, may also play an important permissive role in tissue remodelling through metabolically supporting an increased tissue mass.

2MEO has been demonstrated to suppress the development of collagen type II-induced arthritis, by a mechanism involving inhibition of endothelial cell proliferation, downregulation of nitric oxide synthase and regulation of lymphocyte proliferation, all being recognised as important factors controlling the inflammatory response [34]. Induction of apoptosis and reduced collagen production by leiomyoma cells suggests utility of 2MEO in uterine fibroids [36].

Interest in potential anti-atherosclerotic actions has been increased by the identification of a dual mechanism of action in the regulation of cell cycle at G_0/G_1 and at G2/M in vascular smooth muscle [37], as is the case in airway smooth muscle [9], though the mechanisms of arrest at G_0 are not yet clear. An inhibitory effect on pulmonary vascular smooth muscle proliferation may underlie the protective effect of 2MEO in a rat model of pulmonary hypertension [38]. 2MEO is protective in a model of

TABLE 1

Preclinical studies evaluating efficacy and toxicity of 2MEO as an anti-tumour agent

Species	Sex	Tumour type	Inoculation parameters	Dose	Vehicle	Treatment period	Effect on tumour	Toxicity	Ref
C3H mice	NR	Meth-A sarcoma	1×10^6 cells in saline s.c. in dorsal skin	100 mg/kg/day p.o.	Olive oil	Day 0–12	76% ↓ of Meth-A sarcoma weight	15% ↓ body weight. No hair loss, intestinal disturbances or infection	[15]
C3H mice	NR	B16 melanoma	1×10^6 cells in saline s.c. in dorsal skin	100 mg/kg/day p.o.	Olive oil		~85% ↓ of B16 melanoma weight	15% ↓ body weight. No hair loss, intestinal disturbances or infection	[15]
SCID	F	MDA-MB-435 (breast tumour)	1×10^6 cells s.c.	75 mg/kg/ day p.o.	0.5% CMC	Day 12–31	60% ↓ tumour volume, tumour weight not reported	No hair loss, diarrhoea, lethargy or body weight loss	[7]
Athymic nude mice	F	MIA PaCa-2 (pancreatic tumour)	3×10^6 cells injected in tail vein	1 mg/day p.o. (~50 mg/kg)	2% DMSO in olive oil	Day 3–21	60% lower incidence of lung colonies.	No body weight loss or altered behaviour	[44]
Athymic nude mice	F	SVR bag 4 cells(angiosarcoma)	1×10^6 cells	100 mg/kg/day p.o.	PBS	Day 7–20	68% ↓ tumour volume, tumour weight not reported.	No body weight loss	[45]
Fisher 344 rats	F	No tumour	–	100 mg/kg/day p.o	Liposomes	13 Days	–	Transient ↓ body weight gain, ↓ serum cholesterol, ↓ longitudinal bone growth rate and thickness	[27]
G gamma T-15 transgenic mice	M	Androgen-independent prostate tumours	Spontaneously arising	75 mg/kg/day p.o. and 75 mg/kg 21-day pellets	Olive oil or placebo	21–60 Days	Tumour volume not reported, 38% ↓ tumour weight	No change in body weight, behaviour, bone marrow and gut	[46]
BNX mice	NR	Multiple myeloma	3×10^7 cells in RPMI-Matrigel s.c. in flank	100 mg/kg/day p.o.	0.5% CMC	11 Days	35–40% ↓ tumour volume, tumour weight not reported	No body weight loss	[8]
Sprague Dawley rats	F	No tumour	–	Increasing doses 4–75 mg/kg/day p.o.	Liposomes	16 days	–	↓ Bone elongation, ↓ proliferation and ↑ apoptosis of chondrocytes	[47]
Copenhagen × Fisher F1 rats	M	Dunning R3327-PAP prostate tumours	Tumour transplanted s.c into flank	12.5 mg/kg/day i.p.	Sesame seed oil	14 Days	82% ↓ tumour volume, tumour weight not reported	9% body weight loss. No hair loss, diarrhoea	[14]
Aythmic NCr-*nu* mice	F	MDA-MB-435 (breast tumour)	30 mg tumours transplanted s.c	37.5 mg/kg/day i.p.	NR	Day 13–43	No decrease in tumour area	No body weight loss, or obvious toxicity	[43]
SCID mice	F	HeLaS3 cervical carcinoma cells	$3–4 \times 10^6$ cells s.c. in flank	75 mg/kg/day p.o.	DMSO in olive oil	15 days	34% ↓ tumour volume, tumour weight not reported.	Changes to liver and spleen	[31]
Nu/nu Balb/c mice	F	MDA-MB-435 (breast tumour)	1×10^6 cells in mammary fat pad	Increasing doses 15–150 mg/kg/day i.p.	DMSO in peanut oil	18 days	47% ↑ in tumour volume and 43% ↑ tumour weight at 150 mg/kg dose	↑ Uterine and liver weight at all doses	[30]
SCID mice	F	MDA-MB-435 (breast tumour)	1×10^6 cells s.c.	75 mg/kg/day p.o.	0.5% CMC	Day 14–31	No effect on tumour volume or weight	↑ Uterine and liver weight	[30]
Nu/nu Balb/c mice	F	MCF-7 (breast tumour)	1×10^6 cells in mammary fat pad	50 mg/kg/day i.p.	DMSO in peanut oil	Day 0–16	92% of 2-Meo-treated mice developed tumours c.f. 33% of vehicle treated mice	↑ Uterine and liver weight	[30]

NR – not reported.

BOX 1

Molecular targets for 2MEO

A number of novel 2MEO-binding proteins have been identified by affinity chromatography (referred to as protein X in Figure 2), but the functional importance of these proteins is yet to be established [48]. In addition, there is extensive literature on the potential role of tubulin and ER in the therapeutic and adverse actions of 2MEO.

Tubulin

Disruption of microtubule function may contribute to the anti-proliferative actions of 2MEO [21]. 2MEO binds to tubulin at high (10–100 μM) concentrations (Table 2). Generation of an MDA-MB-435 subline by culture in increasing concentrations of 2MEO resulted in the emergence of a 2MEO resistance associated with mutations in β-tubulin, supporting a role for this target [49]. Recent studies have established a link between the down regulation of HIF-1α protein levels and microtubule-binding agents, raising the possibility that the two major actions of 2MEO, namely tumour cell apoptosis and reduced tumour vascularity, are mechanistically linked.

Estrogen receptors

The ER affinities of 2MEO for ER (Table 2) indicate that 2MEO has a low, but not negligible affinity, for the ER in cytosolic preparations of rat uterus [30] and human ERα [4]. At concentrations of 2MEO required to inhibit MCF-7 breast tumour cell (ER-positive) proliferation, there was a 75% overlap in gene regulatory activity between 2MEO (3 μM) and E2 (100 nM) at concentrations that saturate ER [30]. Furthermore, 2MEO activates an estrogen response element (ERE)-dependent reporter gene transfected into ER-positive MCF-7 breast tumour cells, demonstrating that 2MEO has agonist actions on nuclear ER [30]. This agonist activity is unlikely to contribute to the anti-angiogenic or pro-apoptotic effects of 2MEO, but it does have implications for potential side effects and confounding actions in E2-dependent tumours.

GPR30, a GPCR, has been cloned and identified in breast tumour cell lines and binds E2 at supra-physiological concentrations [50]. Some nuclear ER-independent actions of E2 occur from interactions with G proteins [51]. Activation of GPR30 is linked directly to an increase in the proliferation of breast tumour cell lines [52]. The capacity of 2MEO to bind to GPR30 or non-nuclear ERα has not been established. However, we have evidence that 2MEO and E2 regulate ER-negative MDA-MB-435 cell growth through a pertussis toxin-sensitive mechanism (Sutherland and Stewart, unpublished observations) also implicated in 2MEO-induced apoptosis in the ERα-negative cell line, MDA-MB-468 cells [14]. At 10–50 nM, 2MEO has rapid (within seconds) actions in breast tumour cell lines [53]. Similar nuclear ER-independent 2MEO actions have been implicated in inhibition of cardiac fibroblast growth [54].

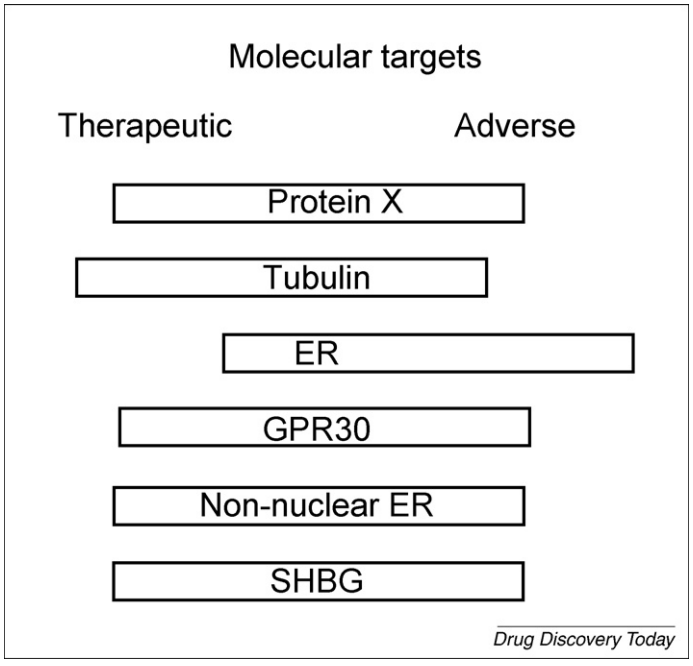

FIGURE 2

A number of molecular targets have been identified as being involved in the therapeutic and adverse actions of 2MEO. However, the extent to which each of these targets contributes to each action remains uncertain.

subarachnoid haemorrhage, in which it is thought that 2MEO acts by microtubule disruption to impair the upregulation of VEGF. Hypoxia induced by cerebral vasospasm is sensed by microtubules upstream of HIF-1α degradation [39]. Thus, disruption of microtubules limits vessel leak by reducing the expression of VEGF, a cytokine originally identified as 'vascular permeability factor'.

2MEO analogues as the way forward

There has been much speculation about the important molecular targets for 2MEO. However, their relative contribution to the actions of 2MEO and whether a single molecular target can account for the anti-proliferative, cytotoxic and anti-angiogenic activity remain to be established (Box 1). 2MEO combines the activities of cytotoxicity and anti-angiogenic actions that have

TABLE 2

ER affinity, tubulin affinity and effects on endothelial cell number of 2MEO, E2 metabolites and 2MEO analogues

Compound	ER affinity pIC_{50} (^{3}H-E2 displacement)	Tubulin affinity (^{3}H-Colchicine)	Endothelial cell number pIC_{50}[a] [15]
17β-Estradiol	9.89[b] [9]	3.83	4.46
Estrone	6.95[c]	n/a	4.58
2-Hydroxyestradiol	9.49[b] [9]	>3.50	4.80
4-Hydroxyestradiol	7.79[c]	n/a	n/a
2-Methoxyestradiol	7.50[b] [9]	4.82	6.87
4-Methoxyestradiol	6.15[c]	NA	5.13

[a] pIC50 for reductions in endothelial cell number – Bovine brain derived capillary endothelial cell were incubated with 2.5 ng/mL bFGF and increasing concentrations of E2 or E2 metabolite and IC_{50} values express the concentration at which half the cells remained in the bFGF-stimulated cells.

[b] ER affinity was measured using rat uterine cytosol as a source of ER and displacement of 0.2 nM ^{3}H-E2 by E2 metabolites.

[c] ER affinity was measured using recombinant ERα and displacement of 10 nM ^{3}H-E2 by E2 metabolites.

BOX 2

Although 2MEO possesses a range of pharmacological actions that confer on it unique potential as an anti-tumour/anti-inflammatory agent, it is possible that optimal candidates for clinical use will need to have had their activity profile 'tweaked'. Teasing out the structural requirements for the various actions of 2MEO and its analogues, with a view to selectively optimise individual activities, poses a considerable challenge to the development of novel 2MEO derivatives with clinical potential.

To guide the design of optimised 2MEO analogues, we are generating three-dimensional quantitative structure activity relationships (3D-QSARs) for individual 2MEO actions, using a comparative molecular field analysis (CoMFA) approach [55]. The CoMFA approach is particularly appropriate for the study of the 2MEO derivatives, in that (i) it can handle a wide range of structural variability; (ii) it performs most reliably with a dataset of conformationally rigid compounds, such as steroids; and (iii) as with all ligand-based QSAR approaches, it can be performed in the absence of knowledge of the receptor(s) for the ligands. We have previously described CoMFA models for DNA synthesis inhibition and ER binding [9]. The models for these two activities of 2MEO differ markedly, indicating that there are distinct structural preferences for these two activities.

More recently, we have extended this approach to develop 3D-QSAR models for 2MEO and its analogues on tubulin-binding. Using an extended dataset of 58 compounds (Figure 3A) and a combination of binding data from our own laboratory and – following normalisation using 2MEO as a standard – additional data from the literature [41,56], a robust CoMFA model for displacement of [^{3}H]-colchicine from tubulin was obtained (cross-validated $r^2 = 0.584$; generally, CoMFA models with a cross validated $r^2 > 0.5$ are considered to have predictive value [55]). The CoMFA model for colchicine displacement generally differs from that of DNA synthesis inhibition or ER binding. For example, the electrostatic field contour for [^{3}H]-colchicine displacement (Figure 3B) contains favoured regions for both electronegativity (red polyhedra, primarily above the plane of the molecule in Figure 3B) and electropositivity (blue polyhedra, primarily below the plane of the molecule) stemming from substitutions in the 2-position. In the steric CoMFA field for [^{3}H]-colchicine displacement (Figure 3C), although there is a disfavoured (yellow) region in the vicinity of the 2-position (largely obscured by a favoured (green) polyhedron in Figure 2C) that partially overlaps with a similar region in the CoMFA for DNA synthesis inhibition, the large sterically favoured region above the plane of the molecule is unique to the [^{3}H]-colchicine displacement CoMFA. Furthermore, the [^{3}H]-colchicine displacement CoMFA does not delineate steric requirements around either the 6-position or the 17-position, although these are present in the DNA synthesis inhibition CoMFA. This orthogonality of the QSAR features for DNA synthesis inhibition, ER binding and tubulin binding strongly suggests that there is scope to use CoMFA analyses to assist in the design of 2MEO analogues with a selectively optimised pharmacological profile.

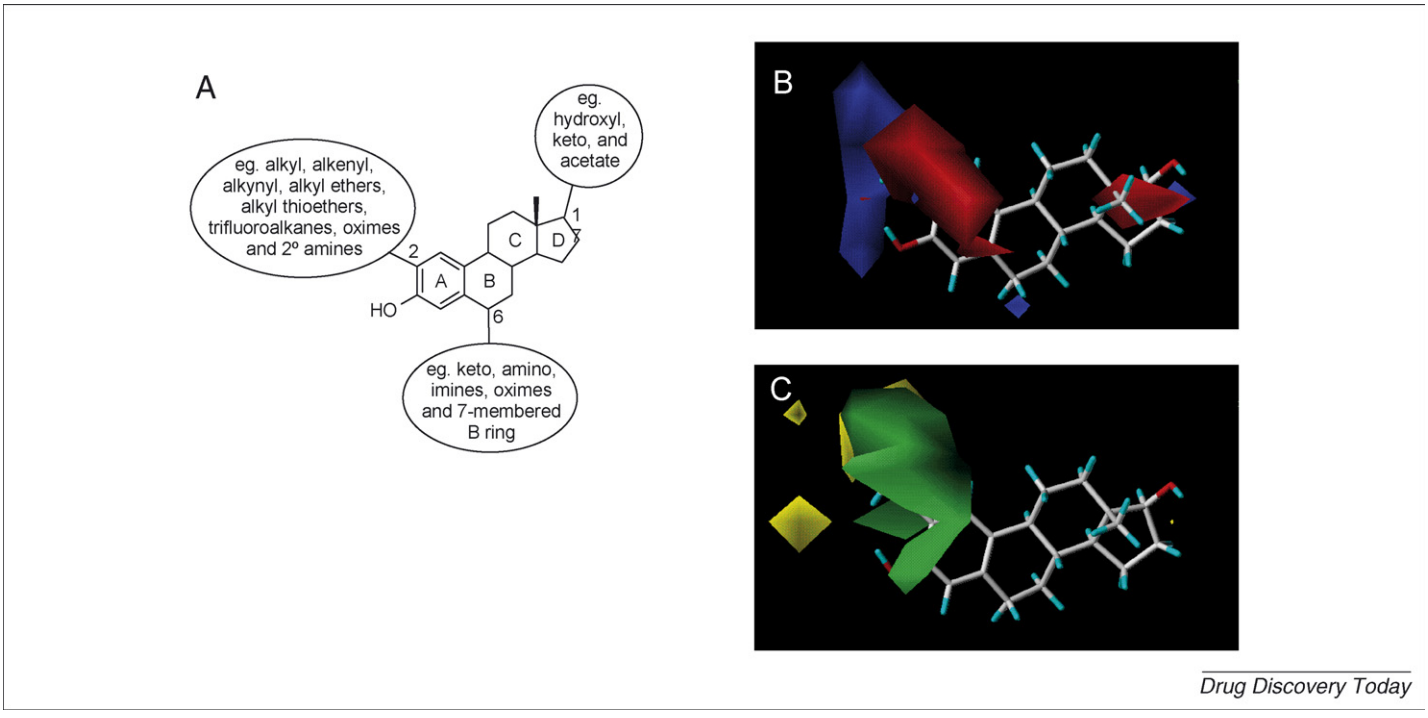

FIGURE 3

Contour plots of (A) electrostatic field contributions and (B) steric contributions for an optimised CoMFA-derived QSAR model for the displacement of [^{3}H]-colchicine from tubulin by 58 2MEO analogues. 2MEO is depicted for orientation. For the electrostatic field contributions, blue polyhedra correspond to regions in which [^{3}H]-colchicine displacement is favored by increased positive charge, whereas red polyhedra define regions in which more negative charge is favorable to [^{3}H]-colchicine displacement. For the electrostatic field contributions, green polyhedra correspond to regions in which [^{3}H]-colchicine displacement is favoured by increased steric bulk, whereas yellow polyhedra define regions in which reduced steric bulk is favorable for [^{3}H]-colchicine displacement. (C) The generic steroid nucleus indicates diversity of the 2MEO analogue dataset used to generate CoMFA-based QSAR model for [^{3}H]-colchicine displacement.

recently been identified as having synergistic effects on tumour progression. Theoretically, completely non-estrogenic 2MEO derivatives optimised structurally for selective inhibition of cell cycle progression and anti-angiogenic action represent the most desirable agents in this class of potential anti-tumour compounds.

2MEO analogues with more desirable properties have been developed using cell enumeration assays to direct iterative structural alterations [9,40–43]. Further initiatives have utilised metabolism to direct synthesis of analogues to improve bioavailability, half-life and hence efficacy [2]. In the absence of definitive knowledge of the molecular target(s) for 2MEO, such an empirical approach remains valuable. Defining the molecular mechanisms of action of 2MEO has proved to be an elusive goal. Nevertheless, identification of further systems influenced by 2MEO provides valuable information for the development of 2MEO derivatives. For example, gene expression analyses may be used to identify other targets for the actions of 2MEO that replace or complement end-stage functional outcomes, such as control of cell proliferation, in the development of 3D-QSARs to guide the synthesis of novel 2MEO analogues (Box 2).

Challenges for future development of 2MEO analogues

Our goal in structurally modifying 2MEO is to achieve better control of tumour mass through the regulation of tumour cell proliferation and survival. The determinants of cell number potentially influenced by 2MEO to different extents in different cell types include arrest at G1 (established in smooth muscle cell cultures) and/or M phase (as shown in tumour cell lines) of the cell cycle, activation of ER to increase tumour cell proliferation in nuclear ER-positive breast cancer, apoptosis and cytotoxicity (as shown in tumour cell lines). Tumour cell proliferation *in situ* is also determined by angiogenesis and blood flow. The extent to which 2MEO anti-tumour actions are independent of microtubule inactivation is an important issue that needs to be resolved to fully rationalise a lead optimisation programme. Several recent studies suggest that both actions have a common mechanism involving microtubule disruption [25].

Strategy to produce novel 2MEO analogues with improved anti-tumour properties

There is only a modest correlation between the IC_{50} values for effects on tubulin polymerisation and anti-proliferative potency [41], suggesting that the effects of 2MEO analogues are unlikely to be mediated solely through the inhibition of tubulin polymerisation. One aim would be to resolve whether it is possible to design compounds that arrest tumour cells in G1 of the cell cycle and inhibit angiogenesis, but do not displace colchicine from its tubulin binding site. Synthetic programmes will be guided by the structure–activity relationships for the multiple (un)desirable activities of 2MEO and will be designed to generate analogues with significant improvements in properties desirable in anti-cancer agents.

Conclusions

The original concept that 2MEO represented a novel and potent anti-proliferative, pro-apoptotic and anti-angiogenic agent, with few adverse effects, underpinned the evaluation of 2MEO in clinical trials for the treatment of breast and other tumours and potentially inflammatory conditions. Moreover, the synergistic combination of anti-proliferative and anti-angiogenic properties was potentially encompassed within the one molecule. These observations suggested that 2MEO would most probably be a superior treatment than utilising a combination of several agents to achieve the breadth of actions of 2MEO. However, poor bioavailability, resultant lack of efficacy and the estrogenic actions of 2MEO show that 2MEO is a suboptimal therapeutic agent in its current formulation. Nevertheless, 2MEO could be considered as a prototype for the development of other compounds that would combine the synergistic properties but it lacks ER affinity. Several analogues have been developed with more potent anti-proliferative and anti-angiogenic actions than 2MEO, and show resistance to metabolism and conjugation and hence a superior pharmacokinetic profile [2]. Further knowledge regarding the molecular target(s) of this class of compounds will advance the design of novel analogues with optimised activity.

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