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Synthesis and antitumor activities of 3-modified 2-methoxyestradiol analogs

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ABSTRACT

The syntheses of 2-methoxyestradiol analogs with modifications at the 3-position are described. The analogs were assessed for their antiproliferative, antiangiogenic, and estrogenic activities. Several lead substituents were identified with similar or improved antitumor activities and reduced metabolic liability compared to 2-methoxyestradiol.

2-Methoxyestradiol (2ME2) is a naturally occurring metabolite of estradiol which has been shown to have antitumor and antiangiogenic activities in in vivo and in vitro models with little toxicity.[1] It is believed that 2ME2 exerts its antiproliferative activity by inhibiting tubulin polymerization and inhibition of HIF-1α.[2]

2ME2 has been evaluated in several clinical trials under the name Panzem®. The clinical results showed that 2ME2 has low bioavailability due to rapid metabolism via oxidation of the 17-hydroxyl group to estrone and conjugation of both 3- and 17-hydroxyl moieties to form glucuronides.[3] These data highlight the need for modifications of 2ME2 that could reduce metabolic liability at these sites and potentially increase the bioavailability of 2ME.

Numerous studies have reported structure–activity relationships (SAR) of 2ME2, to increase antiproliferative activity and inhibition of tubulin polymerization.[4] These analogs do not address the metabolism issue identified in our clinical studies. Our analog program focused on modification of the 3 and 17 positions of 2ME2 to specifically block known sites of metabolism while maintaining or improving the in vitro antiproliferative activity profile. This Letter reports the SAR of 3-modified analogs and identification of lead substituents at this position. SAR at the 17-position are being reported in a separate publication.[5] The optimal 3- and 17-substituents from these two studies have been combined and their antitumor activity and PK values are discussed in an accompanying Letter.[6]

The analogs synthesized for this study were prepared as shown in Schemes 1–3. 2,3-Dimethoxy analog (**1**) was generated from 2ME2 by selective methylation of the 3-hydroxyl group of 2ME2 with potassium carbonate and methyl iodide in refluxing acetone (90% yield). The 3-hydroxyl group of 2ME2 could also be selectively converted to the triflic ester (**2**) using 4-nitrotrifluoromethane sulfonate and potassium carbonate (98%),[7] which was used for synthesis of compounds **3** to **8**.

Compound **2** was converted to nitrile **3** (70%) using potassium cyanide via Pd-mediated catalysis.[8] Pd-catalyzed CO insertion with potassium acetate led to the formation of the carboxylic acid **4** (60%). Esterification of **4** under acidic conditions in methanol provided methyl ester **5** (73%), and its subsequent reduction with lithium aluminum hydride produced hydroxy methyl analog **6**. Vinyl analog **7** (33%) was prepared using vinyl tributyl tin and Pd catalyst,[8] and catalytic hydrogenation of **7** yielded ethyl analog **8** (quantitative).

Compounds **10**–**13** were prepared from 2-methoxyestrone (**9**) as shown in Scheme 2. Oppenauer oxidation of 2ME2 generated **9** (85%),[4b] which was converted to thiol analog **10** through the formation of *O*-aryl thiocarbamate, followed by Newman–Kwart rearrangement, ester cleavage, and LAH reduction (12%, three steps).[9] Triflic ester **11** was prepared from **9** using triflic anhydride in pyridine (98%). Carboxamide analogs were synthesized by Pd-mediated CO insertion reaction with HMDS (**12a**),[10] Me_2NH (**12b**),[11] or $MeNH_2$ (**12c**) as the nitrogen source, and selective 17-ketone reduction with sodium borohydride (50–70%, two steps). 3-Amine **13** (74%) was prepared from **11** by Pd-catalyzed benzophenone imine formation followed by acid hydrolysis.[12]

Scheme 3 depicts subsequent reactions of 3-amine **13**. Sandmeyer reactions and 17-ketone reduction lead to the formation of halogenated analogs **14a**,[13] **14b**, and **14c** (20–40%).[14] Aminonitrile **15** (30%) was generated from **13** using cyanogen bromide,[15] and sodium borohydride reduction. Additionally, **13** was converted to sulfamide with sulfamoyl chloride, and to trifluoromethyl

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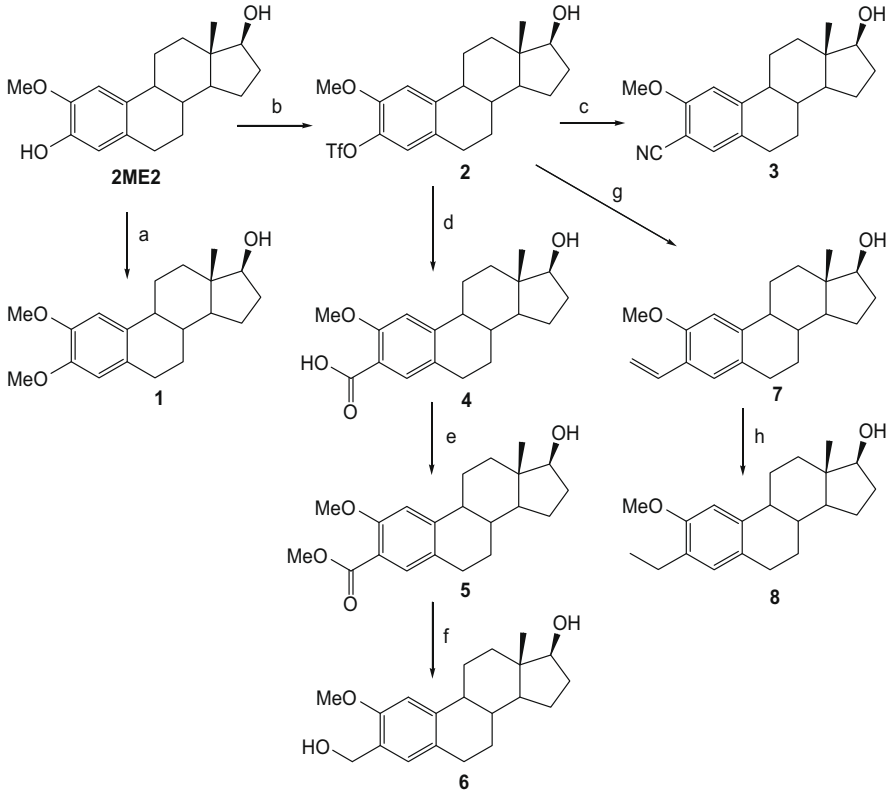
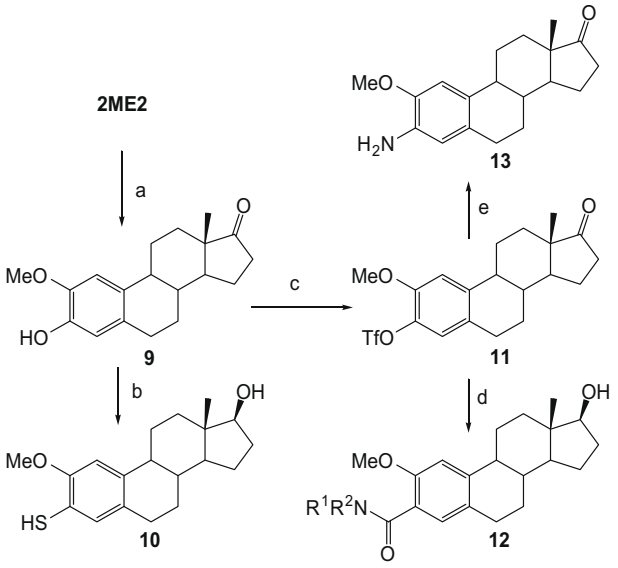


Scheme 1. Reagents and conditions: (a) K_2CO_3, MeI, acetone, reflux; (b) *p*-nitrophenyl triflate, K_2CO_3, DMF; (c) KCN, dppf, 1-methyl-2-pyrrolidinone, tris(dibenzylideneacetone)dipalladium(0)–chloroform adduct; (d) $Pd(OAc)_2$, dppf, KOAc, CO, DMSO, 60 °C; (e) concd H_2SO_4, MeOH, reflux; (f) $LiAlH_4$, THF, 50 °C; (g) $Pd(PPh_3)_2Cl_2$, $Bu_3SnCHCH_2$, DMF, 90 °C; (h) Pd/C, H_2, 2:1 MeOH:dioxane.



Scheme 2. Reagents and conditions: (a) Al(*i*-PrO)$_3$, cyclohexanone, toluene, reflux; (b) (1) NaH, dimethylthiocarbamoyl chloride, DMF; (2) mineral oil, 280 °C; (3) NaOH, EtOH, reflux; (4) LAH, THF, −78 °C; (c) Tf_2O, pyridine, DCM, 0 °C to rt; (d) (1) $Pd(OAc)_2$, *rac*-BINAP, Cs_2CO_3, dppp, HMDS, DMF, CO, 100 °C; (2) MeOH then acidic workup; (3) $NaBH_4$, MeOH for R^1 = H, R^2 = H (**12a**) or (1) $PdCl_2$, dppp, Me_2NH, DMF, CO, 57 °C; (2) $NaBH_4$, MeOH for R^1 = Me, R^2 = Me (**12b**) or (1) $PdCl_2$, dppp, DMF, $MeNH_2$ (in THF), CO, 70 °C; (2) $NaBH_4$, MeOH, rt for R^1 = Me, R^2 = H (**12c**); (e) (1) $Pd(OAc)_2$, *rac*-BINAP, benzophenone imine, Cs_2CO_3, toluene, reflux; (2) 2 M HCl, THF, rt.

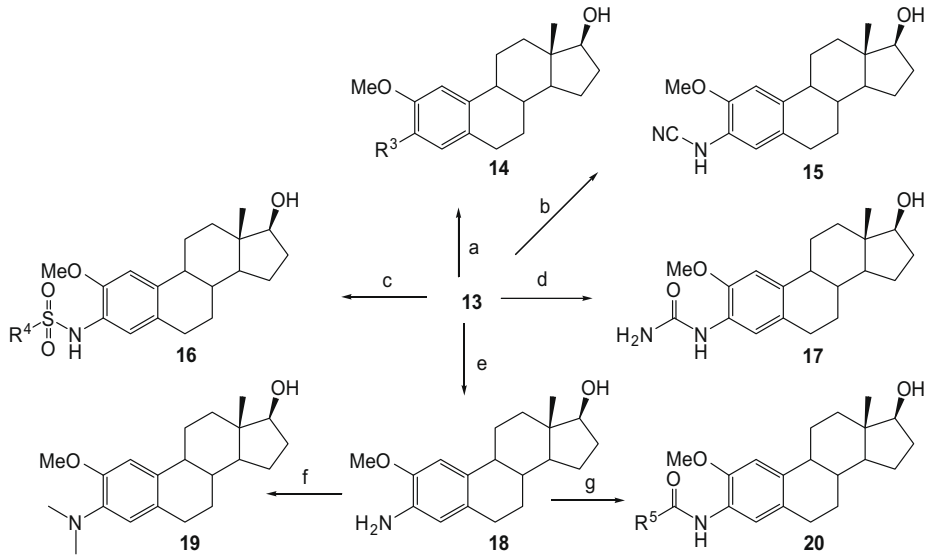
sulfonamide with triflic anhydride and triethylamine. Reduction of the ketone at 17-position resulted in **16** (54%, two steps).

Reaction of amine **13** with sodium cyanate under acidic conditions gave urea **17** (58%),[16] while LAH reduction generated analog **18**, which was converted to **19** (71%) using formic acid and formaldehyde.[17] Formamide **20a** (86%) was prepared by amide coupling of amine **18** with activated formyl imidazolidine generated in situ from formic acid and carbonyl diimidazole.[17] Amine **18** was converted to acetamide **20b** (82%) with acetic anhydride under basic conditions, or to methyl carbamate **20c** (65%) with methyl chloroformate and DMAP.[18] Also, compound **21** (3-deoxy-2ME2, structure not shown) was purchased from a commercial source.

All 3-modified 2ME2 analogs were screened in vitro for antiproliferative activity using human breast carcinoma MDA-MB-231 cells, for antiangiogenic activity using human umbilical vein endothelial cells (HUVEC), and by stimulation of proliferation of estrogen-dependant MCF-7 cells as a surrogate for estrogenic activity.[19] Results are listed in Table 1, sorted by MDA-MB-231 antiproliferative activity.

Generally the most active substituents for antiproliferative activity are hydrogen donors. Analogs with activity <1 μM, such as aminonitrile **15**, urea **17**, formamide **20a**, amine **18**, carboxamide **12a**, and acetamide **20b** are all H-donors.

Hydrogen donor ability appears to be critical for antiproliferative activity. For example, isosteric substitution of the hydroxyl group of 2ME2 with thiol (**10**) leads to substantial loss of activity, which may be explained by thiol's diminished ability to hydrogen bond. Alkylation of an amine or a hydroxyl group, and subsequent loss of H-donor ability, significantly decreases antitumor activities. 2ME2, for example, shows good activity against MDA-MB-231 (IC_{50} of 0.69 μM). However, when the 3-hydroxyl group is methylated (**1**) there is a significant drop in antitumor activity. A similar trend can be observed in compounds **12a**, **12b**, and **12c** and amine series **18** and **19**. Unsubstituted carboxamide **12a** and amine 18 are ac-



Scheme 3. Reagents and conditions: (a) (1) $NaNO_2$ in H_2O, HCl, EtOH, 0 °C; (2) CuCl, 0 °C to rt; (3) LAH, THF, −78 °C for R^3 = Cl (**14a**) or (1) $NaNO_2$ in H_2O, HBr, EtOH, 0 °C; (2) CuBr, 0 °C to rt; (3) LAH, THF, −78 °C for R^3 = Br (**14b**) or (1) $NaNO_2$ in H_2O, HBF_4, EtOH, 0 °C; (2) isolate; (3) 80 °C, vacuum oven; (4) LAH, THF, −78 °C for R^3 = F (**14c**); (b) (1) BrCN, Et_2O, DCM, 0 °C to rt; (2) $NaBH_4$, MeOH, rt; (c) (1) sulfamoyl chloride, Et_3N, THF, rt; (2) $NaBH_4$, MeOH, rt; (3) 1 N HCl for R^4 = NH_2 (**16a**) or (1) Tf_2O, Et_3N, DCM, 0 °C; (2) $NaBH_4$, MeOH, rt; (3) 1 N HCl for R^4 = CF_3 (**16b**); (d) NaOCN, H_2O, AcOH; (e) $LiAlH_4$ (1 M in THF), THF, −78 °C; (f) formic acid, formaldehyde (36% aqueous), toluene, 80 °C; (g) (1) formic acid, 80 °C; (2) NaOH, MeOH, rt for R^5 = H (**20a**) or Ac_2O, NaOH (10 M aqueous), 0 °C to rt for R^5 = Me (**20b**) or methyl chloroformate, Et_3N, DCM, 0 °C to rt for R^5 = OMe (**20c**).

Table 1
In vitro activity of 3-modified 2ME2 analogs, sorted by activity against MDA-MB-231

#	3-Substituent	MDA-MB-231 IC_{50} (μM)	HUVEC IC_{50} (μM)	MCF7 SI relative to 2ME2
15	NHCN	0.62 ± 0.23	0.47 ± 0.21	0.92 ± 0.16
17	$NHCONH_2$	0.65[b]	0.59[b]	0.32 ± 0.12
2ME2	OH	0.79 ± 0.08	0.68 ± 0.15	1.00
20a	NHCOH	0.78 ± 0.01	0.07 ± 0.02	0.15 ± 0.04
12a	$CONH_2$	2.36 ± 0.24	2.59 ± 0.08	0.85 ± 0.04
18	NH_2	2.48 ± 0.50	2.32 ± 0.38	1.60 ± 0.29
14c	F	2.94 ± 0.80	2.10 ± 0.23	2.13 ± 0.02
20b	NHCOMe	3.95 ± 1.06	1.65 ± 0.13	0.93 ± 0.16
7	$CH{=}CH_2$	8.93 ± 0.61	1.96 ± 0.05	0.84 ± 0.04
3	CN	8.98 ± 3.17	2.65 ± 0.25	1.37 ± 0.06
16a	$NHSO_2NH_2$	9.47[b]	3.88[b]	na[a]
20c	NHCOOMe	17.61[b]	5.92[b]	na[a]
2	OSO_2CF_3	21.35 ± 0.81	3.27 ± 2.21	0.43 ± 0.13
14a	Cl	26.93 ± 3.96	7.45 ± 1.01	2.19 ± 0.21
14b	Br	27.49 ± 1.99	9.13 ± 0.37	2.74 ± 0.16
1	OMe	43.68 ± 1.14	31.47 ± 5.11	0.73 ± 0.12
8	Et	46.88 ± 1.98	5.44 ± 0.18	0.73 ± 0.23
6	CH_2OH	55.59 ± 17.31	28.25 ± 5.46	1.26 ± 0.23
10	SH	56.19 ± 3.47	12.87 ± 7.36	1.85 ± 0.38
21	H	62 ± 0.82	22.1 ± 0.7	1.80 ± 0.28
19	NMe_2	103.03 ± 11.55	2.82 ± 1.71	0.63 ± 0.07
12b	$CONMe_2$	>10	>10	na[a]
16b	$NHSO_2CF_3$	>100	46.19 ± 5.26	na[a]
12c	CONHMe	>100	>100	na[a]
4	COOH	>100	>100	na[a]
5	COOMe	>100	>100	0.99 ± 0.23

[a] na = not assayed.
[b] Standard deviation not available where results are reported from a single experiment.

tive against MDA-MB-231 proliferation. However when **12a** and **18** are methylated to give carboxamides **12b** and **12c** and dimethyl amine **19**, antitumor activity diminishes significantly in each case.

Not all hydrogen donors show good antiproliferative activities. Methyl alcohol **6**, for example, is a hydrogen donor but is weakly active against MDA-MB-231 proliferation (IC_{50} of 56 μM). Though the ability to donate a proton is important, there are other factors which determine antiproliferative activities for this compound series. For example, most of the best analogs contain π electrons, either as part of carbonyl or a nitrile. Size of the 3-substituent is also important. In the 3-halogen series, 3-fluoro analog **14c** is the most potent while bromo **14b** and chloro **14c** are roughly equipotent.

HUVEC cell proliferation was used as an in vitro surrogate for antiangiogenic activity. In general, HUVEC antiproliferative activity follows the trends discussed above for antitumor activity. The most active substituents in MDA-MB-231 were also active in HUVEC. There were differences in sensitivities between assays. Vinyl **7** and nitrile **3** substituents have modest antitumor activities on MDA-MB-231 (IC_{50} of 8.9 μM), but are more active against HUVEC (IC_{50} values of 2–3 μM). Ethyl **8** and dimethylamino **19**, on the other hand, have antiangiogenic activity (5.4 μM and 2.8 μM, respectively) but show little antitumor activity (46.9 μM and 103.0 μM, respectively). These differences may be driven by variations in the cellular composition of tubulin isoforms, as has recently been reported for other antitubulin agents.[20]

Similar to the effect seen on antitumor activity, methylation of the 3-hydroxyl group of 2ME2 resulted in 40-fold decrease in antiangiogenic activity. Additionally, alkylated carboxamide **12b** showed a substantial drop in activity compared to unsubstituted amide **12a**. In contrast, dimethylation of the amine **18** to **19** did not result in significant changes to antiangiogenic activity (1.4-fold change, compared to 41-fold change in antitumor activity). Following the general SAR observed for antitumor activity, in addition to being hydrogen donors, the most antiangiogenic analogs also have π electrons.

Studies have shown that 2ME2 is significantly less estrogenic than estradiol.[21] Given the known pro-carcinogenic effect of estrogens it is desirable for new analogs to maintain or have further reduced estrogenicity compared to 2ME2. The estrogen-dependent MCF-7 cell line was used as an in vitro surrogate for estrogenicity, expressed as a simulation index relative to 2ME2 (SI). Values less than 1.0 are desirable, representing less estrogenicity than 2ME2.

The results in Table 1 show that for this series of analogs estrogenicity does not follow the same trend seen for antiproliferation or antiangiogenic activity. The data suggest that substituent size is an

important factor in estrogenicity. Smaller substituents at position 3 in general were more estrogenic than larger substituents. All the halides (**14a**–**c**), 3-deoxy (**21**), thiol (**10**), and amine (**18**), for example, had a SI value >1. Comparison of primary amine **18** and tertiary amine **19** show that the smaller, unsubstituted amine is more estrogenic. It seems that there is a steric constraint in the estrogen receptor binding site,[22] and the undesirable effect of estrogenicity can thus be reduced by introducing a relatively bulky substituent.

These data indicate that by modification of the 3-position we were able to enhance antitumor and antiangiogenic activity while further reducing undesirable estrogenicity. The most active 3-position analogs for antiproliferation are generally less estrogenic than 2ME2. The fluoro analog (**14c**), with MCF7 SI of 2.13, was removed from further consideration due to increased estrogenicity. Taking into account the antitumor, antiproliferative, and estrogenic activities, there are several potential lead substituents for position 3 that warrant further investigation. They include aminonitrile **15**, urea **17**, formamide **20a**, carboxamide **12a**, amino **18** and acetamide **20b**. Further work on these lead substituents is described in the accompanying Letter.[6]

In conclusion, 25 novel analogs of 2ME2 modified at the 3-position have been synthesized and screened for their antiproliferative, antiangiogenic, and estrogenic activities. Structure–activity relationships of these compounds led to the identification of several lead substituents, which maintain the antitumor activity of 2ME2 while blocking the 3-position towards conjugation. The lead substituents reported here have been combined with 17-position analogs reported separately,[5] and activity and PK data are reported in the accompanying paper.[6] The results of these three studies led to the identification of ENMD-1198, which is currently in a Phase 1 clinical trial for oncology.[19]

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