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2-Alkylsulfanyl estrogen derivatives: synthesis of a novel class of multi-targeted anti-tumour agents

Mathew P. Leese,[a] Simon P. Newman,[b] Atul Purohit,[b] Michael J. Reed[b] and Barry V. L. Potter[a,*]

[a]*Medicinal Chemistry, Department of Pharmacy and Pharmacology and Sterix Ltd, University of Bath, Bath BA2 7AY, UK*
[b]*Endocrinology and Metabolic Medicine and Sterix Ltd, Faculty of Medicine, Imperial College, St. Mary's Hospital, London W2 1NY, UK*

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Abstract—A flexible, direct, high yielding synthesis of 2-alkylsulfanyl estrogens from estrone has been developed. 2-Methylsulfanyl estradiol (2-MeSE2) **7** displays a similar anti-proliferative activity to the established 2-methoxyestradiol (2-MeOE2) **1**, whilst its 3-*O*-sulfamate derivative (2-MeSE2MATE) **9** exhibits greatly enhanced anti-proliferative activity, combined with significant inhibition of steroid sulfatase, an enzyme target for the treatment of hormone-dependent tumours.

2-Methoxyestradiol (2-MeOE2) **1**, an endogenous estrogen metabolite, has emerged as a promising therapeutic candidate for the treatment of a number of conditions. The discovery that 2-MeOE2 inhibits angiogenesis, is anti-proliferative and has in vivo anti-tumour activity[1] has led to its evaluation in phase I/II clinical trials against a range of cancers under the trade name Panzem™. Although the precise mechanism(s) by which 2-MeOE2 exerts its anti-tumour effects remain to be fully elucidated it has been shown, amongst other actions, to interfere with normal microtubule assembly,[2] induce p53[3] and trigger BCL-2 phosphorylation.[4] A recent advance has been the demonstration that 2-MeOE2 inhibits HIF-1α at the post-transcriptional level, prevents HIF-1 target gene expression in tumour cells and inhibits HIF-2α in human endothelial cells.[5] 2-MeOE2 has also been proposed as a potential therapy in a wide range of other areas including arthritis,[6] asthma,[7] atherosclerosis,[8] inflammation and cardiovascular disease.[9]

As part of a programme aimed at the discovery of new anti-tumour agents we synthesised and evaluated a number of 2-alkylsulfanyl estrogen derivatives. We set out in particular to determine if 2-methylsulfanyl estrogen derivatives would demonstrate similar or even enhanced activity to their 2-alkoxy analogues. An efficient entry to the 2-substituted estrone core would also allow a broader exploration of the SAR of these compounds (Fig. 1).

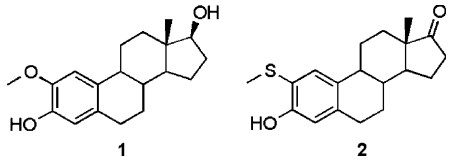

Figure 1. The structure of 2-MeOE2 and 2-MeSE1.

We reasoned that rapid elaboration of 2-alkylsulfanyl estrogens could be achieved by a directed *ortho*-lithiation reaction of suitably protected estrone followed by disulfide quench and final deprotection. Estrone was preferred to estradiol as starting material to afford greater synthetic flexibility, notably for selective functionalisation at the 3- and 17-positions of the estrane nucleus. *ortho*-Lithiation at the 2-position of 3-methoxymethyl protected estradiol has ample precedent;[10] we thus required robust protection of the 17-keto group, which could be installed and removed in an efficient manner and ethylenedioxyketal protection was selected.

Keywords: 2-Methoxyestradiol; Sulfamate; Cancer.
* Correponding author. Tel.: +44-(0)1225-386087; fax: +44-(0)1225-386114; e-mail: b.v.l.potter@bath.ac.uk

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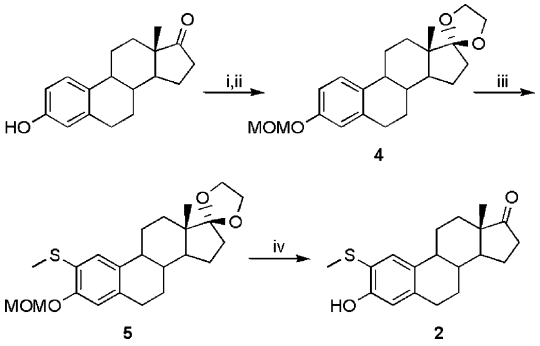


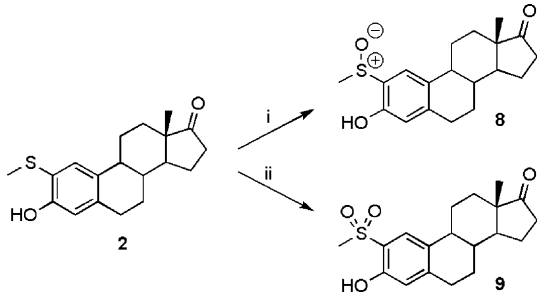
Scheme 1. The synthesis of 2-MeSE1. Reagents and conditions: (i) ethylene glycol, *p*-TsOH, Dean–Stark, PhMe, quant; (ii) NaH, MOMCl, DMF, 95%; (iii) *sec*-BuLi, THF, −78 °C, 1 h then MeSSMe −78 °C to rt, 94%; (iv) 4 M HCl/MeOH, 90%.

Our synthesis of 2-methylsulfanyl estrone (2-MeSE1) **2** is illustrated in Scheme 1. It proved expedient to first introduce ethylenedioxyketal protection under Dean–Stark conditions and then incorporate the methoxymethyl group to give estrone MOM/Ketal **4** in 95% yield over two steps. A sterically controlled selective *ortho*-lithiation with *sec*-butyl lithium was followed by disulfide quench to afford sulfide **5** in excellent yield and selectivity.[11] Cleavage of both protecting groups was then achieved by brief treatment with freshly generated methanolic HCl to give 2-methylsulfanyl estrone **2** in 90% yield. This synthesis proved amenable to scale up with consistently high yields. 2-MeSE1 was thus obtained in 79% yield over four synthetic steps.

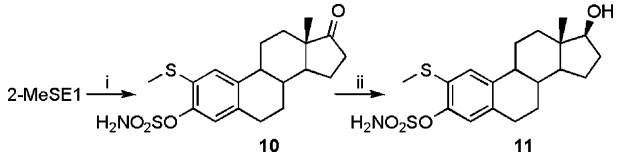
2-Ethylsulfanyl estrone (2-EtSE1) **6** was elaborated in the same manner with the key *ortho*-lithiation step proceeding in 91% yield. We fully expect that this chemistry will allow access to a wide range of 2-sulfanyl estrogens. Interconversion of the estrone derivative to the corresponding 17-β estradiol was then achieved by highly selective sodium borohydride reduction; 2-Methylsulfanyl estradiol (2-MeSE2) **7** and 2-ethylsulfanyl estradiol (2-EtSE2) were thus obtained in near quantitative yield. 2-EtSE2 has previously been synthesised from estradiol in a modest 12% yield by Cushman and co-workers via an iodination/nucleophilic substitution strategy.[12] Clearly, the synthesis reported here is both more flexible and higher yielding.

Oxidation of the sulfide **2** to both sulfoxide **8** (as a mixture of diastereoisomers) and sulfone **9** with *m*-CPBA in dichloromethane proved straightforward (Scheme 2).

An established interest in the synthesis of inhibitors of the steroid sulfatase enzyme led us to investigate the 3-*O*-sulfamate derivatives. Treatment of 2-MeSE1 **2** with sulfamoyl chloride in DMA gave 2-methylsulfanyl estrone 3-*O*-sulfamate (2-MeSEMATE) **10** in 95% yield. Reduction to the estradiol variant (2-MeSE2MATE) **11** was then achieved as above with sodium borohydride (Scheme 3). 2-Ethylsulfanyl estrone 3-*O*-sulfamate (2-EtSE2MATE) **12** was synthesised in the same manner.



Scheme 2. Oxidation of 2-methylsulfanyl estrone. Reagents and conditions: (i) *m*-CPBA (1.7 equiv), DCM, 0 °C, 72%; (ii) *m*-CPBA (3.4 equiv), DCM, rt, 78%.



Scheme 3. The synthesis of 2-methylsulfanyl EMATEs. Reagents and conditions: (i) H_2NSO_2Cl, DMA, 0 °C to rt, 3 h, 95%; (ii) $NaBH_4$, MeOH/THF, 0 °C to rt, 1 h, 97%.

Results obtained from the biological evaluation of the 2-alkyl sulfanyl estrogen compounds against the proliferation of MCF-7 cells, an estrogen dependent (ER+ve) human breast cancer cell line, are presented in Table 1. These data reveal only low activity for the 2-MeSE1 and 2-EtSE1 compounds (>30 μM), highlighting the important effect of the 17-hydroxyl group in this series. In contrast to the 2-alkoxy estradiols where the 2-ethoxy analogue had proved to be optimal for anti-proliferative activity Cushman et al. observed only modest activity for 2-EtSE2.[12] Our study confirms this result (data not shown), which seems likely to arise from the larger steric size of 2-EtS versus 2-EtO. In contrast, 2-MeSE2 was observed to have a similar degree of activity to 2-MeOE2 in this cell line, confirming the validity of the S/O substitution. The greater H-bonding character of the ether compared to the thioether group may explain the slight reduction in activity. The magnitude of this reduction, however, suggests that any H-bond acceptor makes only a small contribution to the overall activity. Clearly, the 2-methylsulfanyl group is small enough to fit into the available binding pocket and is, in this case, a good bioisostere. Whether 2-MeSE2 shows the range of activities attributable to 2-MeOE2 remains to be seen. The oxidised derivatives of 2-MeSE2, sulfoxide **8** and sulfone **9**, proved to be inactive.

Table 1. Anti-proliferative activity of 2-substituted estradiols and their 3-*O*-sulfamate derivatives on MCF-7 human breast cancer cells[16]

Compound no.	Compound name	MCF-7plate assay IC_{50} (μM)
1	2-MeOE2	2.39
2	2-MeSE1	33.4
6	2-EtSE1	31.9
7	2-MeSE2	3.96
10	2-MeSEMATE	0.40
11	2-MeSE2MATE	0.43
12	2-EtSE2MATE	35.3
13	EMATE	22.8

Of this series of results perhaps the most significant is the enhancement of in vitro activity afforded by incorporation of the sulfamoyl group in the 3-position of the 2-alkyl sulfanyl estrogens, with sub-micromolar growth inhibition being observed for both 2-MeSEMATE and 2-MeSE2MATE. This observation is consistent with earlier results obtained in the 2-MeO- and 2-Et-series where the 3-*O*-sulfamate derivatives proved to be significantly more active than the parent estrones and estradiols.[13] These studies also showed that sulfamoylated 2-substituted estrogens exhibit biological activity broadly similar to that of 2-MeOE2, with anti-angiogenic, microtubule disruptor and pro-apoptotic activity being observed.[14] 2-MeOEMATE was, however, shown to cause an irreversible arrest in the G_2M phase of the cell cycle in contrast to 2-MeOE2, which only induces a reversible arrest. Although a full mechanistic characterisation of the effects of 2-MeSEMATE has not yet been carried out it would seem reasonable to suggest that the activity of this molecule would reflect that observed for its 2-MeO- and 2-Et-analogues. Evaluation in the NCI 55 human cancer cell line panel[15] revealed that 2-MeSEMATE exhibits similar potency in a wide range of ER+ve and −ve cells demonstrating that its anti-proliferative effects are independent of the estrogen receptor. The absence of significant anti-proliferative activity for estrone 3-*O*-sulfamate (EMATE) **13** illustrates the necessity of 2-substitution for this property in the sulfamoylated estrogens.

EMATE is a known inhibitor of the steroid sulfatase (STS) enzyme,[17] itself a clinical target for treatment of hormone-dependent cancers.[18] Inhibition of this enzyme reduces the circulatory estrogen derived from estrone sulfate, a major source of estrogen in post-menopausal women, thus arresting tumour growth. We were interested to see whether inhibition of STS could be maintained with introduction of the 2-methyl sulfanyl group and thus 2-MeSEMATE was evaluated alongside EMATE as an inhibitor of steroid sulfatase activity in placental microsomes.[19] IC_{50} values of 120 and 22 nM, respectively, were obtained showing that 2-MeSEMATE maintains significant STS inhibitory activity, albeit slightly less than EMATE (this observation reflects that previously observed for 2-MeOEMATE).[20] However, introduction of functional groups such as MeO at the 2-position has proved a successful strategy for the removal of the estrogenicity shown by EMATE in rodents,[20] an observation which may well also hold for the 2-MeS functionality. EMATE has also proved to possess high oral bioavailability due to its uptake by red blood cells and resultant bypassing of first pass liver metabolism.[21] The operation of such a delivery mechanism would auger well for orally available 2-MeSEMATE derived drugs should this observation prove to be a general one for substituted EMATEs.

These preliminary results suggest that these molecules may be effective against hormone dependent tumours, arresting tumour growth directly by inhibiting cell proliferation and indirectly by reducing circulatory estrogen levels by blocking the steroid sulfatase pathway, together with other multi-targeted effects. Clearly, this combination of biological properties highlights these compounds as exciting lead molecules for the development of new multi-targeted therapeutic agents for the treatment of cancer.

Thus, in summary, a brief and efficient route to the 2-alkylsulfanyl estrogens has been realised affording rapid access to a range of biologically interesting compounds. Work is presently underway in our laboratory to further exploit this chemistry in order to develop new therapeutic candidates for the treatment of cancer and to determine the precise nature by which these molecules inhibit cancer cell proliferation.

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References and notes

1. Pribluda, V. S.; Gubish, E. R., Jr.; LaVallee, T. M.; Treston, A.; Swartz, G. M.; Green, S. J. *Cancer Metastasis Rev.* **2000**, *19*, 173–179.
2. D'Amato, R. J.; Lin, C. M.; Flynn, E.; Folkman, J.; Hamel, E. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 3964–3968.
3. Mukhopadhyay, T.; Roth, J. A. *Oncogene* **1997**, *14*, 379–384.
4. Attalla, H.; Westberg, J. A.; Andersson, L. C.; Adlercreutz, H.; Makela, T. P. *Biochem. Biophys. Res. Commun.* **1998**, *247*, 616–619.
5. Mabjeesh, N. J.; Escuin, D.; LaVallee, T. M.; Pribluda, V. S.; Swartz, G. M.; Johnson, M. S.; Willard, M. T.; Zhong, H.; Simons, J. W.; Giannakakou, P. *Cancer Cell* **2003**, *3*, 363–375.
6. Josefsson, E.; Tarkowski, A. *Arthritis Rheum.* **1994**, *40*, 154–163.
7. Stewart, A. G.; Harris, T.; Guida, E.; Vlahos, R.; Koutsoubos, V.; Hughes, R.; Robertson, A. *Am. J. Respir. Crit. Care Med.* **1999**, *159*, A531.
8. Nishigaki, I.; Sasguri, Y.; Yagi, K. *Atherosclerosis* **1995**, *113*, 167–170.
9. Dubey, R. K.; Gillespie, D. G.; Jackson, E. K.; Keller, P. J. *Hypertension* **1998**, *31*, 522–528.
10. Pert, D. J.; Ridley, D. D. *Aust. J. Chem.* **1989**, *42*, 405–419.
11. Small amounts of 4-SR substituted products occur if tight control of temperature is not maintained.
12. Cushman, M.; He, H.-M.; Katzenellenbogen, J. A.; Lin, C. M.; Hamel, E. *J. Med. Chem.* **1995**, *38*, 2041–2049.
13. Purohit, A.; Hejaz, H. A. M.; Walden, L.; MacCarthy-Morrogh, L.; Packham, G.; Potter, B. V. L.; Reed, M. J. *Int. J. Cancer* **2000**, *85*, 584–589.
14. MacCarthy-Morrogh, L.; Townsend, P. A.; Purohit, A.; Potter, B. V. L.; Reed, M. J.; Packham, G. *Cancer Res.* **2000**, *60*, 5441–5450.
15. Boyd, M. R.; Paull, K. D. *Drug Dev. Res.* **1995**, *34*, 91–104.
16. For assay protocol see Ref. 13.
17. Howarth, N. M.; Purohit, A.; Reed, M. J.; Potter, B. V. L. *J. Med. Chem.* **1994**, *37*, 219–221.

18. Reed, M. J.; Purohit, A.; Woo, L. W. L.; Potter, B. V. L. *Rev. Endrocrine Relat. Cancer* **1993**, *45*, 51.
19. For assay protocol, see: Purohit, A.; Williams, G. J.; Howarth, N. M.; Potter, B. V. L.; Reed, M. J. *Biochemistry* **1995**, *34*, 11508–11512.
20. Purohit, A.; Vernon, K. A.; Wagenaar-Humelinck, A. E.; Woo, L. W. L.; Hejaz, H. A. M.; Potter, B. V. L.; Reed, M. J. *J. Steroid Biochem. Molec. Biol.* **1998**, *64*, 269–275.
21. Elger, W.; Palme, H.-J.; Schwarz, S. *Exp. Opin. Invest. Drugs* **1998**, *7*, 575–589.