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Journal of Steroid Biochemistry & Molecular Biology 86 (2003) 423–432

The Journal of Steroid Biochemistry & Molecular Biology

www.elsevier.com/locate/jsbmb

Steroid sulphatase inhibitors for breast cancer therapy☆

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Abstract

In contrast to aromatase inhibitors, which are now in clinical use, the development of steroid sulphatase (STS) inhibitors for breast cancer therapy is still at an early stage. STS regulates the formation of oestrone from oestrone sulphate (E1S) but also controls the hydrolysis of dehydroepiandrosterone sulphate (DHEA-S). DHEA can be reduced to 5-androstenediol (Adiol), a steroid with potent oestrogenic properties. The active pharmacophore for potent STS inhibitors has now been identified, i.e. a sulphamate ester group linked to an aryl ring. This has led to the development of a number of STS inhibitors, some of which are due to enter Phase I trials in the near future. Such first generation inhibitors include the tricyclic coumarin-based 667 COUMATE. Aryl sulphamates, such as 667 COUMATE, are taken up by red blood cells (rbc), binding to carbonic anhydrase II (CA II), and transit the liver without undergoing first-pass inactivation. 667 COUMATE is also a potent inhibitor of CA II activity with an IC_{50} of 17 nM. Second generation STS inhibitors, such as 2-methoxyoestradiol bis-sulphamate (2-MeOE2bisMATE), in addition to inhibiting STS activity, also inhibit the growth of oestrogen receptor negative (ER^-) tumours in mice and are anti-angiogenic. As the active pharmacaphores for the inhibition of aromatase and STS are now known it may be possible to develop third generation inhibitors that are capable of inhibiting the activities of both enzymes. Whilst exploring the potential of such a strategy it was discovered that 667 COUMATE possessed weak aromatase inhibitory properties with an IC_{50} of 300 nM in JEG-3 cells. The identification of potent STS inhibitors will allow the therapeutic potential of this new class of drug to be explored in post-menopausal women with hormone-dependent breast cancer. Second generation inhibitors, such as 2-MeOE2bisMATE, which also inhibit the growth of ER^- tumours should be active against a wide range of cancers.

Keywords: Breast cancer; Steroid sulphatase (STS); Aromatase; Sulphatase inhibitors

1. Introduction

The development of potent steroid sulphatase (STS) inhibitors has made substantial progress in recent years. A number of drugs are shortly due to enter Phase I trials as potential therapies for the treatment of hormone-dependent breast cancer in post-menopausal women. Since the identification of the first STS inhibitor, oestrone-3-*O*-methylthiophosphonate, (E1-MTP) a range of steroid-based and non-steroid based STS inhibitors has been synthesised and tested. All candidates to-date have incorporated the active pharmacophore required for potent inhibition of STS activity, i.e. a sulphamate ester group linked to an aryl ring [1]. In this paper, we review the rationale that led to the development of STS inhibitors and the progress that has been made in identifying first, second and third generation drugs in this class.

2. Rationale

STS inhibitors were originally developed with the specific aim of using them for the treatment of hormone-dependent cancers. It is now apparent that, in addition to breast cancer, STS inhibitors may also have a role in the treatment of certain dermatological and immunological conditions [2,3]. The highest incidence of breast cancer occurs in post-menopausal women at a time when ovarian oestrogen production has ceased. In this group of women, oestrogens are produced exclusively in extraglandular tissues, mainly by the conversion of androstenedione to oestrone, a reaction mediated by the aromatase enzyme complex and forming the aromatase pathway for oestrogen synthesis (Fig. 1). This enzyme is located in stromal cells within adipose tissue but is also found in normal and malignant breast tissues. In breast tumours, aromatase activity can make an important contribution to the

☆ Presented at the VI International Aromatase Conference: AROMATASE 2002, Kyoto, Japan, 26–30 October 2002.

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doi:10.1016/S0960-0760(03)00353-4

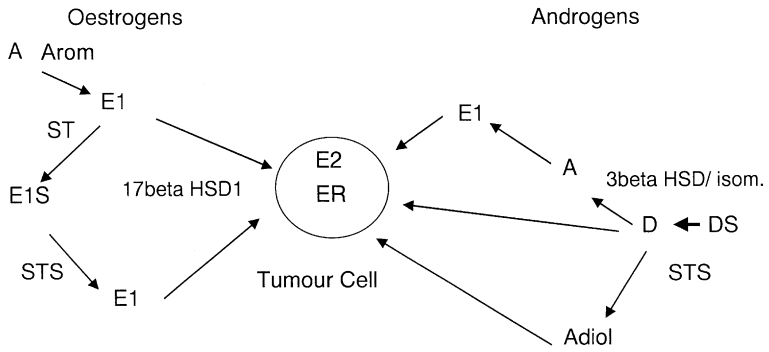

Fig. 1. Origin of oestrogens in post-menopausal women. Oestrogens can originate via the aromatase (arom) pathway (conversion of androstenedione, A, to oestrone, E1) or the steroid sulphatase (STS) route (conversion of oestrone sulphate, E1S, to E1). E1 can be converted to E1S by oestrone sulphotransferase (ST). The STS pathway is also important in the formation of 5-androstenediol (Adiol) which originates from dehydroepiandrosterone (D) which is formed from D-sulphate (DS). Like oestradiol (E2), Adiol can bind to the oestrogen receptor (ER) and stimulate the growth of hormone-dependent tumours. In post-menopausal women, there is evidence that D, via the peripheral action of 3β-hydroxysteroid dehydrogenase/isomerase (3-betaHSD/isom), may be an important source of A for conversion to E1 [59].

high levels of oestrogens that are present in this tissue [4]. There is still some controversy as to whether the aromatase is located in the epithelial or stromal compartment within the breast. Whereas biochemical analysis of aromatase activity has revealed that most activity resides in the stromal compartment, immunohistological studies have provided evidence to support an epithelial or stromal location [5–7].

4-Hydroxyandrostenedione (4-OHA) was one of the first potent aromatase inhibitors to be tested clinically. Using a double-isotopic infusion technique convincing evidence was obtained that peripheral aromatase activity was almost completely inhibited by 4-OHA [8]. Measurements of plasma oestrogen concentrations in several of these early studies with 4-OHA, however, failed to show complete suppression of circulating oestrogens [8,9]. With improvements in the specificity of the anti-sera used for the measurements of oestrone and oestradiol, there is now agreement that 4-OHA, and the later generation of aromatase inhibitors, do reduce circulating oestrogen level towards the limits of detection of these assays [10,11]. In spite of the potent inhibition of aromatase activity, and reduction in circulating oestrogen that results from the use of the latest generation of inhibitors, the objective response rates remain relatively low. In a recent Phase III study in which letrozole (0.5 and 2.5 mg) were compared with megestrol acetate (400 mg), complete response rates were 4.0, 4.5 and 2.0%, respectively with partial response rates of 16.8, 11.6 and 12.9%, respectively [12]. Overall, the use of letrozole offered no survival advantage compared with megestrol acetate. Furthermore, there appears to be no relationship between the clinical response to aromatase inhibitors and the degree of suppression of circulating oestrogen levels achieved with these inhibitors [13]. As a similar degree of suppression of plasma oestradiol concentrations is seen in subjects showing complete regression or disease progression other factors, in addition to the classical oestrogens, must be involved in supporting tumour growth.

The realisation that, in addition to the formation of oestrogens by the aromatase pathway, steroids with oestrogenic properties could also be formed via a sulphatase route stimulated our initial interest in developing potent STS inhibitors. As shown in Fig. 1 much of the oestrone synthesised from androstenedione can be converted to oestrone sulphate (E1S) by oestrone sulphotransferase and phenol sulphotransferase enzymes that are ubiquitously distributed throughout the body [14,15]. Plasma and tissue concentrations of E1S are considerably higher than those of unconjugated oestrogens [16,17]. Furthermore, the half-life of E1S (10–12 h) is considerably longer than that of unconjugated oestrone and oestradiol (20–30 min) [18]. Thus, it was initially postulated that the high tissue and circulating levels of E1S could act as a reservoir for the formation of biologically active oestrogens via the action of STS [19].

It has been known for some time that STS activity in breast tumours is much higher than that of the aromatase complex [20]. There is evidence that, as a result of the increased STS activity, as much as a 10-fold greater amount of oestrone may originate via the sulphatase route than via the aromatase pathway [21]. More recently, studies at the molecular level, have revealed that in 87% of breast cancer patients investigated, STS mRNA levels were higher in malignant than in non-malignant tissues [22]. Furthermore, STS mRNA expression in breast tumours was found to be an independent prognostic indicator in predicting relapse-free survival [23].

As also illustrated in Fig. 1, however, there is a further compelling reason for developing STS inhibitors. 5-Androstenediol (Adiol) is another steroid that circulates in the blood of post-menopausal women at relatively high concentrations [24]. Adiol, although an androgen, can bind to the ER and stimulate the growth of ER^+ breast cancer cells in vitro and carcinogen-induced mammary tumours in ovariectomised rats [25,26]. Using similar isotopic infusion techniques to those used to measure peripheral aromatase in human subjects Thijssen and co-workers [27] found that, in

post-menopausal women, 80–90% of Adiol originates from dehydroepiandrosterone sulphate (DHEA-S). DHEA-S is hydrolysed to DHEA by STS before undergoing reduction to Adiol by 17β-hydroxysteroid dehydrogenase type I. Studies in which a placental STS cDNA was transiently transfected into COS-1 cells revealed that the expressed enzyme could hydrolyse both E1S and DHEA-S [28]. This finding indicates that only one STS is responsible for the hydrolysis of aryl and alkyl steroid sulphates. The ability of the expressed enzyme to hydrolyse both E1S and DHEA-S was blocked by a STS inhibitor. Thus, the STS inhibitors currently being developed should block not only the conversion of E1S to oestrone but also the hydrolysis of DHEA-S, leading to a reduction in Adiol formation.

Two recent studies have provided convincing evidence in support of the sulphatase pathway (i.e. steroid sulphates) giving rise to steroids with oestrogenic properties. Billich et al. [29] confirmed that Adiol, DHEA and DHEA-S, at physiological concentrations, could all stimulate the proliferation of MCF-7 breast cancer cells. The ability of all three adrenal androgens to stimulate MCF-7 cell proliferation was blocked by an anti-oestrogen, showing that they were acting via the ER. The potent aromatase inhibitors 4-hydroxyandrostenedione and letrozole were unable to inhibit the stimulation of MCF-7 cells by these androgens. However, DHEA-S stimulated growth was blocked by a STS inhibitor. From these studies, the authors concluded that the metabolism of the adrenal androgen to aromatic oestrogens was not required for stimulation of MCF-7 cell proliferation and that, in these cells, stimulation by DHEA-S occurred via an aromatase-independent pathway.

It has been known for some time that some adrenal androgens can stimulate the growth of ER^+ breast cancer cells in vitro and induce mammary tumours in rodents. However, it was only recently that convincing clinical evidence was obtained to support a role for DHEA-S in stimulating breast tumour growth in humans. Morris et al. [30] monitored plasma DHEA-S and oestrogen concentrations in women with breast cancer who were treated by oophorectomy and aromatase inhibitor therapy, using anastrozole, letrozole or exemestane. They made the important observation that 12/19 subjects with tumour progression had higher average plasma DHEA-S levels (3.8 μM) than women where disease remained stable (0.6 μM). In both sets of women, plasma oestrone and oestradiol concentrations remained suppressed to minimal detectable levels. The authors concluded that, in patients with progressive disease and suppressed oestrogen levels, elevated DHEA-S levels appeared to stimulate tumour progression. As noted by Morris et al. [30], this clinical finding could have serious implications for the use of aromatase inhibitors on their own. While further studies are required to confirm this important clinical observation, together with other in vitro data, these findings do provide a possible explanation for the relatively low objective response rates that have been detected in subjects receiving aromatase inhibitor therapy.

As STS inhibitors are only just entering Phase I trials it remains to be determined whether it will be possible to use such inhibitors alone or whether combination with an aromatase inhibitor will be required. It is known, however, that the affinity of sulphotransferase enzymes for unconjugated oestrogens is much higher than that of STS for steroid sulphates [31,32]. This suggests that, in the presence of a potent STS inhibitor, any unconjugated oestrogens would be rapidly inactivated by conversion to their sulphated forms. Thus, it is possible that good therapeutic results could be obtained by the use of STS inhibitors alone.

3. First generation STS inhibitors

The realisation that the sulphatase pathway is an important route for the formation of steroids with oestrogenic properties promoted several groups to attempt to develop potent inhibitors. Several steroid sulphates and 2-phenyl-indole derivatives were initially identified as having relatively weak STS inhibitory properties [33,34]. The first compound to be specifically synthesised was E1-MTP (Fig. 2, **1**), a compound which acted as a competitive inhibitor of STS activity [35]. Subsequently, a series of related steroid sulphate surrogates was synthesised and of these oestrone-3-*O*-sulphamate (Fig. 2, **2**, EMATE) emerged as being extremely potent [36,37]. At 1 nM EMATE inhibited STS activity in MCF-7 cells by 95% and had an IC_{50} of 65 pM. EMATE was found to inhibit STS activity in an irreversible manner and enzyme kinetic studies subsequently confirmed that it inhibited activity in a time- and concentration-dependent manner [37,38]. EMATE was active in vivo and inhibited the growth of E1S stimulated nitrosomethylurea-induced mammary tumours in ovariectomised rats [39].

Unexpectedly, EMATE proved to be a potent orally active oestrogen with its oestradiol analogue (Fig. 2, **3**, E2MATE) being five times more potent than ethinyloestradiol on oral administration in rodents [40]. In attempts to develop non-oestrogenic STS inhibitors which were equipotent with EMATE several groups have synthesised and tested a number of 1–3 ringed non-steroid based sulphamates [19,41]. In addition, a number of modifications have been made to the A and D rings of the steroid oestrane nucleus to reduce the oestrogenicity of this type of inhibitor [42,43]. However, all the compounds tested to-date, that have an inhibitory potency in the same range as EMATE, incorporate the active pharmacophore that was discovered by our group for STS inhibition, i.e. a sulphamate ester group linked to an aryl ring [1].

Studies by our group to develop a non-oestrogenic STS inhibitor initially explored the structure–activity relationship of a series of sulphamate derivatives of tetrahydronaphthol and diethylstilboestrol [19]. While these sulphamate derivatives were less potent STS inhibitors than EMATE it was apparent that phenolic compounds other than steroids could be developed as STS inhibitors. A series of coumarin

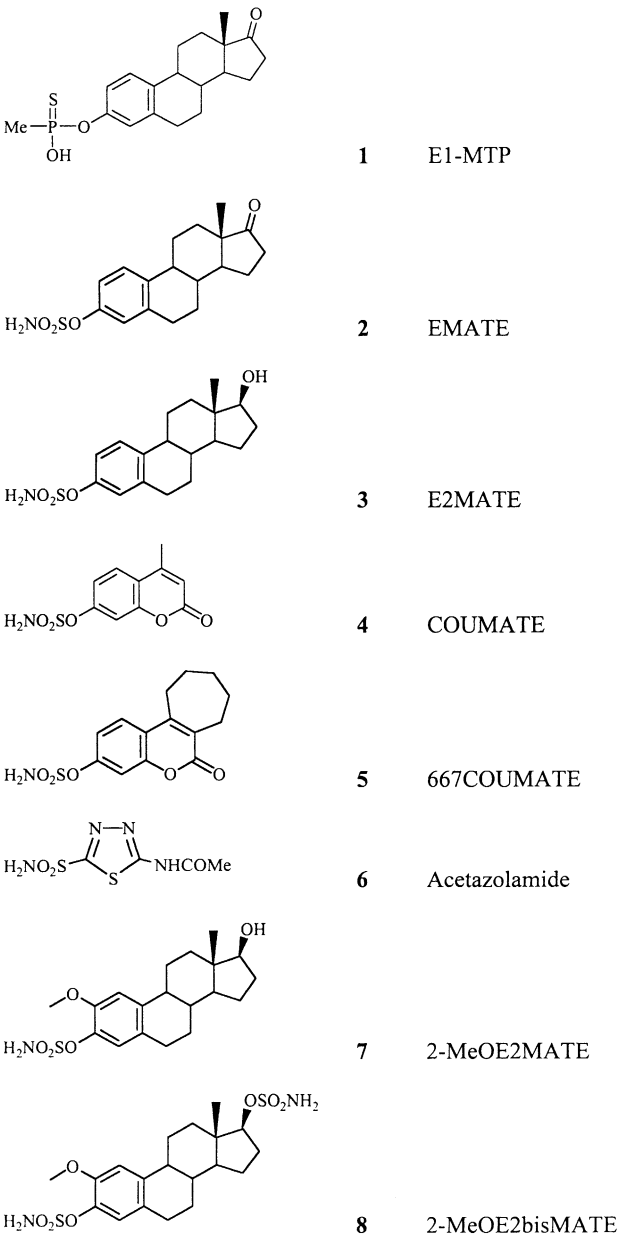

Fig. 2. Structures: compound **1**, oestrone-3-*O*-methylthiophosphonate (E1-MTP); compound **2**, oestrone-3-*O*-sulphamate (EMATE); compound **3**, oestradiol-3-*O*-sulphamate (E2MATE); compound **4**, 4-methylcoumarin-7-*O*-sulphamate (COUMATE); compound **5**, 6-oxo-8,9,10,11-tetrahydro-7*H*-cyclohepta-[c] [1] benzopyran-3-*O*-sulphamate (667 COUMATE); compound **6**, acetazolamide; compound **7**, 2-methoxyoestradiol-3-*O*-sulphamate (2-MeOE2MATE); compound **8**, 2-methoxyoestradiol-3,17-*O*,*O*-bis-sulphamate (2-MeOE2bisMATE).

sulphamates was synthesised as part of this development programme one of which, 4-methylcoumarin-7-*O*-sulphamate (Fig. 2, **4**, COUMATE), inhibited STS activity in MCF-7 breast cancer cells by >90% at 10 μM [44]. Subsequently, a series of tricyclic coumarin sulphamates was synthesised, one of which, 667 COUMATE (Fig. 2, **5**), proved to be more potent than EMATE with an IC_{50} of 8 nM in placental microsomes [4,5]. This compares with an IC_{50} of 25 nM for EMATE in the same assay system. 667 COUMATE was devoid of oestrogenicity when tested in vitro and in vivo [45,46]. It also inhibited the E1S-stimulated growth of carcinogen-induced mammary tumours in ovariectomised rats in a dose-dependent manner. 667 COUMATE has been selected for evaluation in a Phase I trial in post-menopausal women with hormone dependent breast cancer.As STS activity is present in white blood cells it will be possible to monitor the extent and duration of inhibition during such clinical trials [47].

4. Delivery of 667 COUMATE

As previously discussed E2MATE, at low doses (1–20 μg) is a potent orally active oestrogen in rodents. The reason for its enhanced oestrogenicity is thought to result from its rapid partition into red blood cells after ingestion [48]. This results in the drug being able to transit the liver without undergoing first-pass metabolism. This is in contrast to other oestrogens that are administered orally, which undergo substantial metabolism and inactivation, necessitating the use of high doses to achieve biological effectiveness. After transit through the liver, E2MATE is slowly released from rbcs. As E2MATE itself does not appear to bind to the ER, as such, it has to be de-sulphamoylated to yield oestradiol [48]. As yet, there is little information as to how, or where in the body, this process occurs.

5. 667 COUMATE in red blood cells

The partitioning of EMATE into red blood cells is now thought to be due to its reversible binding to carbonic anhydrase II (CA II) within the cell [49]. Many CA II inhibitors, such as acetazolamide (Fig. 2, **6**) have a sulphonamide group. Using radiolabelled 667 COUMATE, we have recently demonstrated that this compound is taken up by rbcs to a similar extent to EMATE (unpublished observation). Like EMATE therefore, 667 COUMATE should transit the liver without being inactivated. The ability of these compounds to inhibit CA II activity was also examined using a modified colorimetric method [50]. Surprisingly, EMATE (IC_{50} = 9 nM) and 667 COUMATE (IC_{50} = 17 nM) had a similar potency to that of acetazolamide (IC_{50} = 14 nM) to inhibit CA II activity. It is possible that these aryl sulphamate drugs, like sulphonamide drugs, may have a wider therapeutic role and could be used in the treatment of conditions such as glaucoma, where inhibition of CA II is effective.

There is currently considerable interest in the role that CAs may have in supporting tumour growth. CA IX is highly expressed in some tumours and acetazolamide has been shown to inhibit the invasion of renal cancer cells in vitro and to produce tumour growth delays in vivo [51,52]. It is possible, therefore, that some of the inhibitory effects that EMATE and 667 COUMATE have on tumour growth

in vivo could result from inhibition of CA II in addition to STS activity. Thus, 667 COUMATE is a potent STS inhibitor that should transit the liver without undergoing first-pass metabolism and also inhibit CA II activity, making it an exciting new drug for development as an anti-cancer agent.

6. Second generation inhibitors

As an alternate approach to synthesising and testing non-steroid based STS inhibitors several groups, including our own, have made a number of modifications to the A or D ring of the oestrane nucleus to render them non-oestrogenic [42,53]. Such inhibitors developed by other groups include a number of 17α-benzyl- or 17β-(*N*-alkylcarbamoyl)-EMATE derivatives [43,54]. Our own research is this area focused on the synthesis of a series of 2-substituted oestrogens including the 2-methyl, 2-nitro, 2-methoxy, 2-allyl and 2-propyl-EMATE-derivatives [42]. Of this series, and related compounds, the 2-methoxyoestradiol-3-*O*-sulphamate (Fig. 2, **7**) proved to have a similar potency to that of EMATE. In vivo it inhibited liver STS activity by >95% at 2 mg/kg and was devoid of oestrogenic activity. 2-MeOE2MATE and 2-methoxyoestradiol-3,17-*O*,*O*-bis-sulphamate (Fig. 2, **8**, 2-MeOE2bisMATE) have proved to be particularly interesting compounds having a number of additional properties. These 2-substituted oestrogen derivatives induce cells to undergo apoptosis and cause an irreversible arrest in the growth of cells at the G_2/M phase of the cell cycle [55]. They also induce phosphorylation of the anti-apoptotic protein BCL-2. These compounds inhibit the in vitro polymerisation of tubulin and are thought to act by binding to the colchicine site on tubulin. These 2-methoxy sulphamate derivatives are also potent inhibitors of angiogenesis. Using human umbilical vein endothelial cells, a widely used model for angiogenesis studies, 2-MeOE2MATE and 2-MeOE2bisMATE inhibit cell proliferation with IC_{50}s of 0.3 and 0.5 μM, respectively [56].

In vivo studies with 2-MeOE2bisMATE have revealed that, like EMATE, a single 10 mg/kg dose almost completely inhibits liver STS activity for at least 5 days with some recovery of activity being detected by day 10 (Fig. 3). 2-MeOE2bisMATE is also resistant to in vivo metabolism on oral application. In plasma from blood samples collected 24 h after the administration of a 10 mg/kg dose p.o., significant concentrations (563 ng/ml) were still detectable (Fig. 4). At this time point, no major metabolic products of 2-MeOE2bisMATE were identified. This finding is compatible with the hypothesis that oestrogen sulphamate derivatives are protected from first-pass metabolism and inactivation by their partitioning into rbcs. The STS enzyme, in addition to clearing steroid sulphates, is also responsible for the de-sulphamoylation of compounds such as 2-MeOE2bisMATE. The inhibition of STS by this compound, which will also prevent de-sulphamoylation, no doubt contributes to its apparent long half-life in blood.

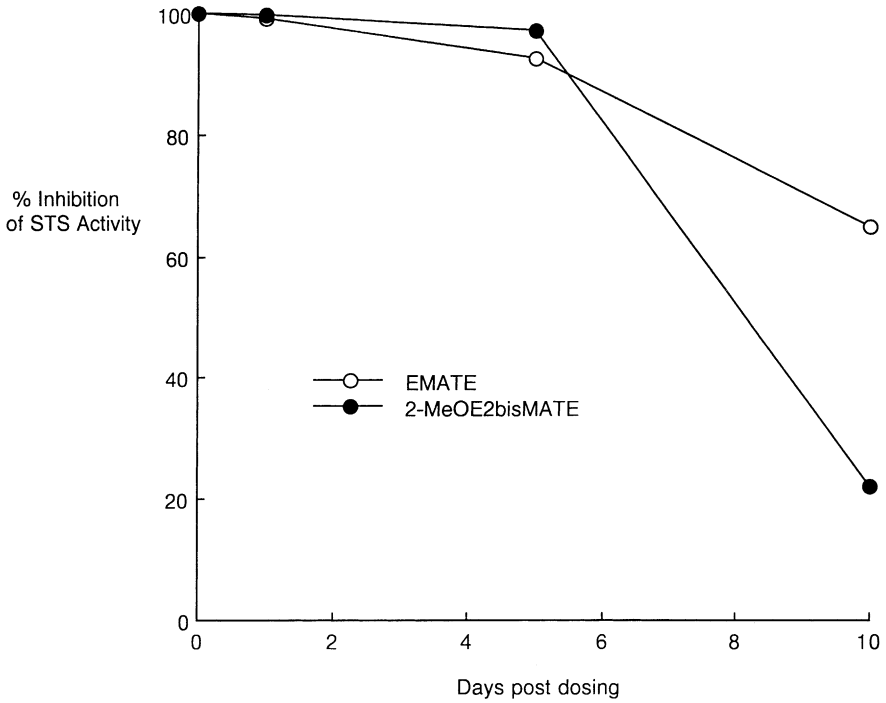

Fig. 3. In vivo inhibition of steroid sulphatase (STS) activity by oestrone-3-*O*-sulphamate (EMATE) and 2-methoxyoestradiol bis-sulphamate (2-MeOE2bisMATE) in rats. Animal received a single oral dose (10 mg/kg) in propylene glycol. Samples of liver tissue were obtained on days 1, 5, and 10 post-dosing and used to assess the extent of inhibition of STS activity (means of replicate measurements for which the coefficient of variation was <10%). Data are re-plotted from reference [60].

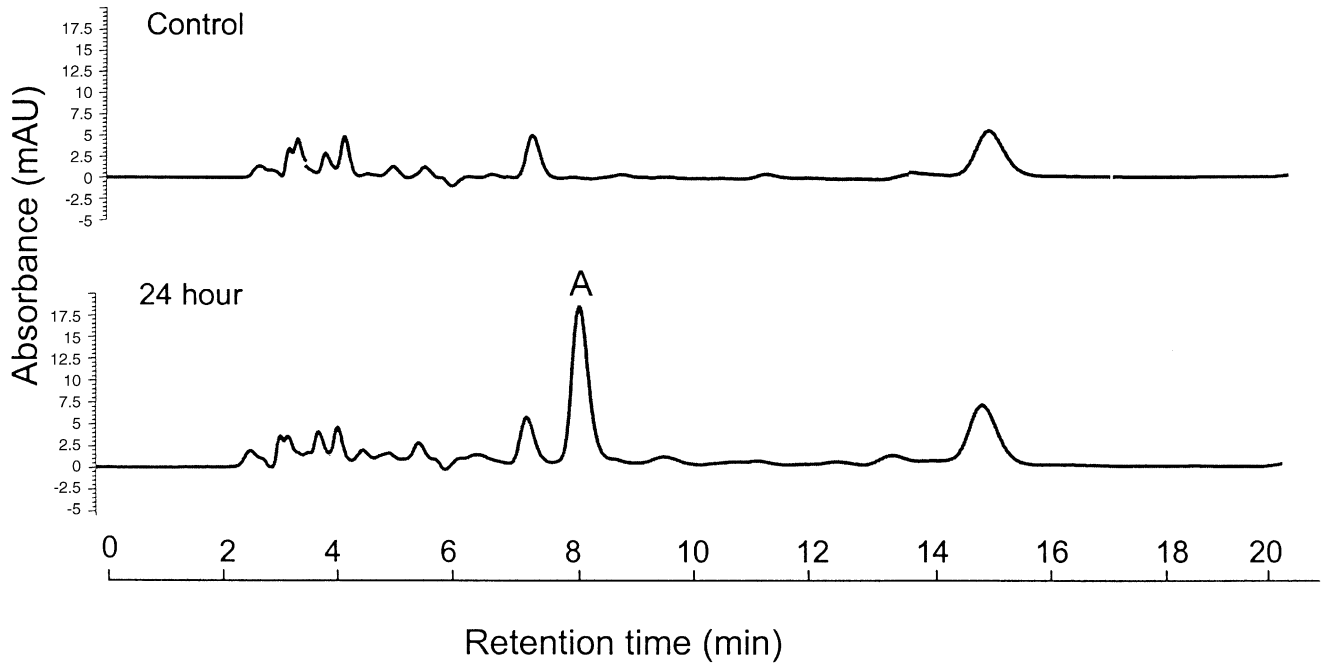

Fig. 4. High-performance liquid chromatography profile of plasma extracts from a control rat (vehicle, 1% tetrahydrofuran/propylene glycol) and 24 h after administration of 2-MeOE2bisMATE (10 mg/kg, p.o.). Only 2-MeOE2bisMATE (peak A) was detected with no other major metabolites identified.

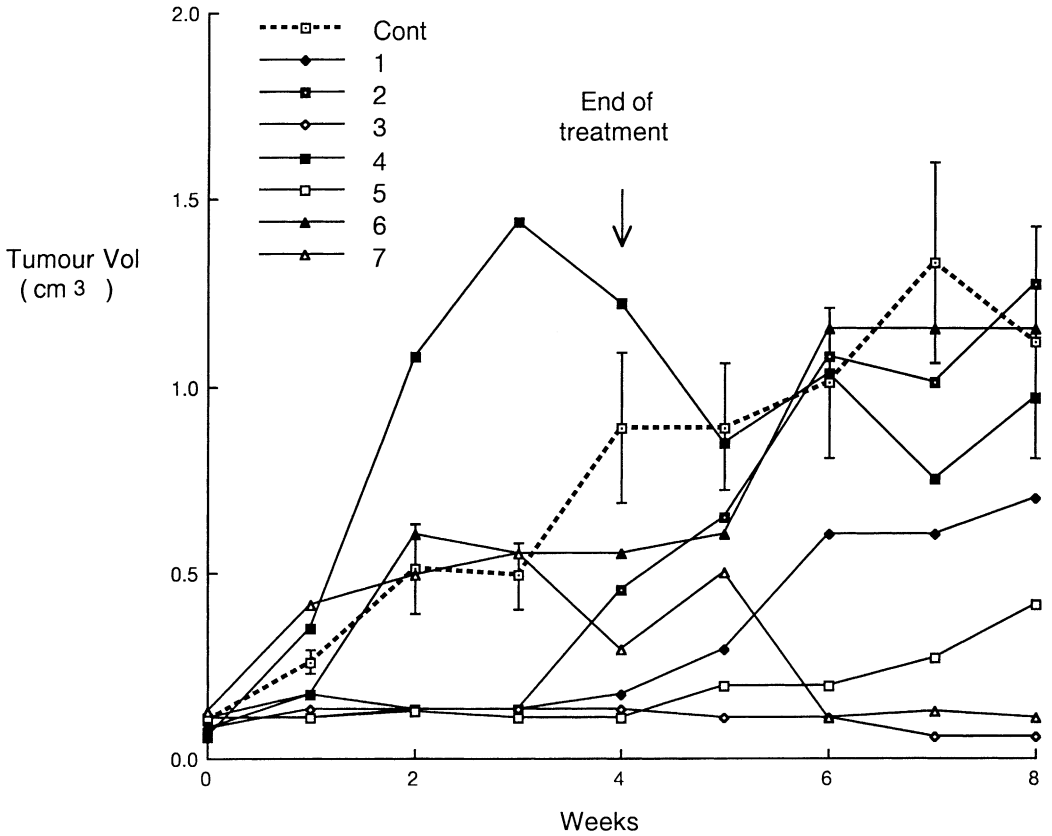

Fig. 5. Effect of 2-methoxyoestradiol-3-*O*-sulphamate (2-MeOE2MATE) on the growth of nitrosomethylurea-induced mammary tumours in intact (i.e. non-ovariectomised) rats. 2-MeOE2MATE was administered orally (20 mg/kg) for 28 days with tumour volumes continuing to be monitored for a further 28 day period. The growth of tumours in animals receiving vehicle only (1% tetrahydrofuran/propylene glycol, controls means ± *S.E.*) are also shown. 2-MeOE2MATE reduce the growth of tumours in 4/7 animals. For two animals (nos. 4 and 7) evidence of tumour regression was seen after treatment for 3 weeks.

Administration of 2-MeOE2MATE (20 mg/kg per day, p.o. for 28 days) reduced the growth of nitrosomethylurea-induced mammary tumours in 4/7 intact (i.e. non-ovariectomised) animals (Fig. 5). In addition, using tumour xenografts derived from inoculated MDA-MB-435 (ER^-) breast cancer cells, 2-MeOE2bisMATE (20 mg/kg per day, p.o. for 28 days) significantly reduced tumour growth compared with that in a control group of nude mice [56]. This class of drug is, therefore, active against both hormone-dependent and -independent tumours. It is apparent that this second generation of STS inhibitors, such as 2-MeOE2bisMATE, has a number of additional properties that make them attractive candidates for use against a wide range of hormone-dependent and hormone-independent cancers.

7. Third generation inhibitors

If STS inhibitors do prove to have therapeutic value it would be logical also to test them in combination with an aromatase inhibitor. This possibility has prompted us to explore the feasibility of developing single molecule compounds which can inhibit both activities (dual aromatase-sulphatase inhibitors (DASIs)). A number of flavonoids are known to possess aromatase inhibitory properties [57,58]. Sulphamoylation of this class of compound could give rise to a dual inhibitor. We have previously sulphamoylated a number of flavonoids, some of which are weak aromatase inhibitors [53]. Compounds examined in this class included 5,7-dihydroxy-isoflavone-4′-*O*-sulphamate and 5-hydroxy-isoflavone-4′,7-bis-sulphamate. At 1 μM, these compounds inhibited STS activity in MCF-7 cells by 90 and 83%, respectively. These compounds were also active in vivo as STS inhibitors but were considerably less potent than EMATE.

As part of our research in this area we have used an in vivo model to test the efficacy of a number of DASIs. For this, intact rats are injected with pregnant mare's serum gonadotrophin and 3 days later receive a single dose of a specific STS, aromatase or dual inhibitor. The ability of drugs to inhibit aromatase or STS activities in this model was assessed by taking samples of blood and liver at 3 h post-dosing with inhibitors. The extent of aromatase inhibition is determined from the reduction in circulating oestradiol concentrations that occurs with STS inhibition being measured in liver biopsy samples. As expected, 667 COUMATE proved to be a potent STS inhibitor but surprisingly also showed some aromatase inhibitory activity (Fig. 6). Further, in vitro studies using JEG-3 cells revealed that 667 COUMATE did have weak aromatase inhibitory properties (Fig. 7). In this assay, letrozole had an IC_{50} value of 1 nM compared with an IC_{50} of 300 nM for 667 COUMATE. Results from other compounds tested in the in vivo dual inhibitor assay, such as STX A, a non-steroidal based compound, have indicated that it should be feasible to develop

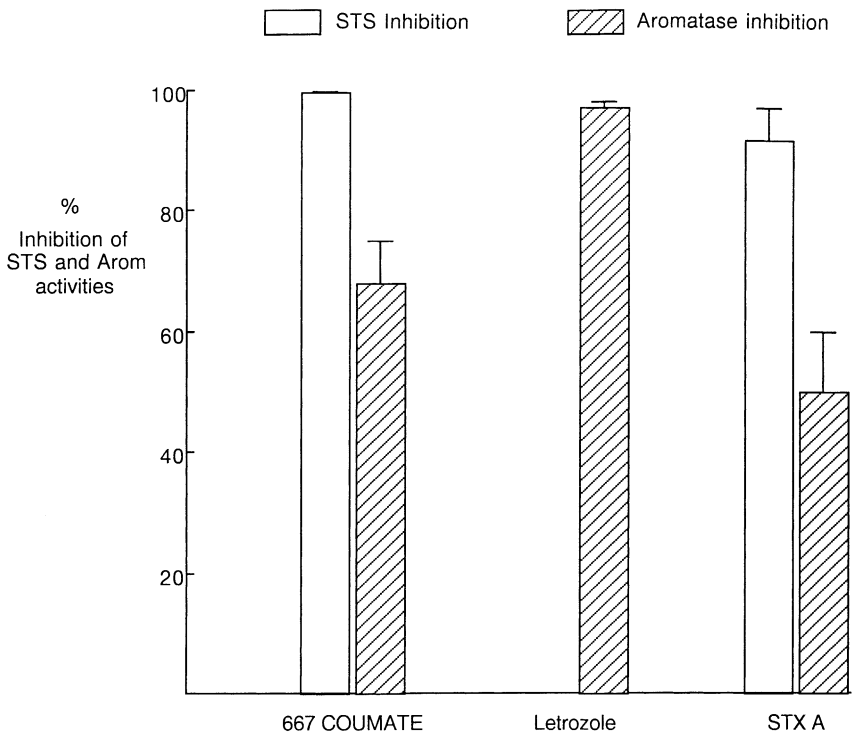

Fig. 6. The in vivo inhibition of aromatase and steroid sulphatase (STS) activities by 667 COUMATE, letrozole and STX A. For this assay, rats were pre-treated with pregnant mare's serum gonadotrophin (200 IU, Sigma, Poole, Dorset, UK). Three days later they received a single 10 mg/kg, p.o. dose of drug. Three hours after drug administration samples of blood and liver were collected. The extent of aromatase inhibition was determined by measuring plasma oestradiol concentrations (Diagnostic Products Corporation) while STS inhibition was measured by assaying liver STS activity (mean ± S.E., $n = 3$).

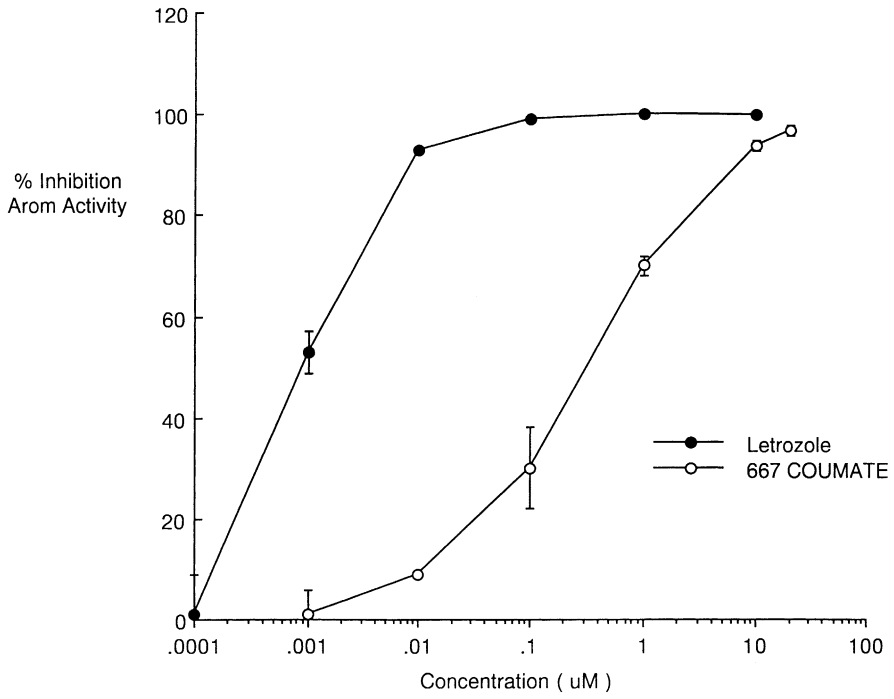

Fig. 7. In vitro inhibition of aromatase activity in JEG-3 cells by 667 COUMATE. Aromatase activity was measured using [1β-^{3}H] androstenedione. In this system, the IC_{50} for the aromatase inhibitor letrozole was 1 nM while that for COUMATE was 300 nM.

other single molecules, different from 667 COUMATE, that are capable of inhibiting both aromatase and STS activities.

8. Conclusions

The last few years has seen the development of a number of potent STS inhibitors, some of which are due to enter Phase I clinical trials in the near future. 667 COUMATE, in addition to inhibiting STS activity also inhibits CA II activity and is a somewhat weaker aromatase inhibitor. It will therefore be of interest to see whether such compounds prove to be potent anti-cancer agents when tested in post-menopausal women with breast cancer. Second generation inhibitors, such as 2-MeOE2bisMATE, in addition to being active against hormone-dependent cancers, should also be effective against a wide range of hormone-independent tumours. The development and testing of DASIs, third generation inhibitors, should resolve the question as to whether inhibition of both aromatase and sulphatase activities is superior to inhibition of only aromatase or STS activity when used for the treatment of hormone-dependent breast cancer.

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