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Discovery and Development of the Aryl O-Sulfamate Pharmacophore for Oncology and Women's Health

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Abstract

In 1994, following work from this laboratory, it was reported that estrone-3-*O*-sulfamate irreversibly inhibits a new potential hormone-dependent cancer target steroid sulfatase (STS). Subsequent drug discovery projects were initiated to develop the core aryl *O*-sulfamate pharmacophore that, over some twenty years, have led to steroidal and non-steroidal drugs in numerous pre-clinical and clinical trials, with promising results in oncology and women's health, including endometriosis. Drugs have been designed to inhibit STS e.g. Irosustat, as innovative dual-targeting aromatase-steroid sulfatase inhibitors (DASIs) and as multi-targeting agents for hormone-independent tumors, such as the steroidal STX140 and non-steroidal counterparts, acting *inter alia* through microtubule disruption. The aryl sulfamate pharmacophore is highly versatile, operating *via* three distinct mechanisms of action and imbues attractive pharmaceutical properties. This *Perspectives* article gives a personal view of the work leading both to the therapeutic concepts and these drugs, their current status and how they might develop in the future.

Introduction

Many cancers are hormone-dependent, with tumor growth stimulated by estrogens and androgens. This is particularly true in female breast cancer where the majority of sufferers are postmenopausal and about three-quarters of the cancers are hormone-dependent.¹ Such tumors are categorized as hormone responsive if there is expression of either estrogen receptors (ER+), progesterone receptors, or both. In the adult human there are three main A-ring aromatic estrogens, estrone (E1), estradiol (E2) and estriol (E3). The non-aromatic androgen androstenediol also has estrogenic effects, but is about 100-fold weaker than estradiol, albeit produced in relatively large amounts in the postmenopausal setting.² Androstenediol potently stimulates the growth of MCF-7 breast cancer cells *in vitro* and

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Notes

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Dedication

This article is dedicated to Professor Michael John Reed, B.Sc., M.Sc., Ph.D., D.Sc., FRCPATH (1944-2009), Imperial College London, UK, without whose enthusiastic and dedicated collaboration much of this work would not have been possible.

mammary tumors in rats. One approach to preventing the action of estrogens that stems from the success of the antiestrogen tamoxifen, which for decades has been considered the first line therapy for hormone-responsive breast cancer, is to block the estrogen receptor with a selective estrogen receptor modulator (SERM) or down-regulator (SERD), e.g. raloxifene, ospemifene, fulvestrant, etc.³ A second approach is to prevent the production of estrogen by inhibiting the biosynthetic enzymes involved in estrogen production, and the mainstay of this approach has been the development of aromatase inhibitors (AIs). Clinical trials have found AIs to be superior to tamoxifen both in terms of disease-free survival and adverse side effects, although ultimately tumor progression will occur.

The estrogens are synthesized from androgen precursors, estradiol from testosterone and estrone from androstenedione (Figure 1). In both cases the enzyme catalysing the reaction is aromatase, which is the only enzyme able to catalyze the aromatization of the A-ring and, therefore, the only enzyme capable of synthesizing estrogens. If aromatase is inhibited in the postmenopausal setting, where most ovarian estrogen production has ceased, by e.g. the clinically successful so-called third generation aromatase inhibitors anastrozole (Arimidex), letrozole (Femara) or exemestane (Aromasin), then the production of estrogens should be stopped. In postmenopausal women, peripheral conversion through aromatase is the major source of estrogen and AIs have been found to reduce systemic estrogen levels by as much as 98%. In postmenopausal women levels of estrone sulphate (E1S), formed by the action of a sulfotransferase, are much higher than those of E1 and the half-life of E1S in plasma is long. Thus, this large circulatory E1S may act as a reservoir of E1.⁴ Since the body is able to store estrogens peripherally in the form of E1S, steroid sulfatase (STS) can convert E1S *in situ* in tumor cells to estrone which can then be converted to estradiol by 17 β -hydroxysteroid dehydrogenase type 1 (17 β -HSD1) ^{5, 6} thus producing the hormone in an intracrine fashion. In cancerous breast tissue this may be the main route of estrogen production as the levels of STS and 17 β -HSD1 are reported to be higher in these tissues than in normal tissues.^{7–10} The importance of intracrinology as a concept has been recently reviewed ¹¹ and its key role in breast cancer in particular.¹²

STS mRNA expression is significantly higher in breast cancer tissue than in non-malignant breast tissue ¹³ and is also a predictor of recurrence.¹⁴ High levels are thus a significant predictor of reduced relapse-free survival. Using immunohistochemistry and a specific STS antibody STS was located in the cytoplasm of malignant breast tissue cells, and therefore may play a significant role in regulating estrone or estrogen levels within the tumor.¹⁴ While mRNA for the sulfotransferase was found to be localized in both carcinoma and intratumoral stromal cells, that of STS was detected only in carcinoma cells and STS immunoreactivity was significantly correlated with tumor size.¹⁵ Therefore, STS inhibitors might be particularly beneficial for tumors expressing high levels of STS and this would in principle also allow desirable patient stratification by means of a diagnostic test.

Circulating E1S entering tumor cells *via* organic anion transporters can be hydrolyzed *in situ* to E1 by STS and then converted to E2 to drive tumor cell proliferation. This process will clearly be unaffected by aromatase inhibition. Also, since aromatase has no role to play in the synthesis of the androgen androstenediol, inhibition of aromatase should have no influence on the estrogenic effects of androstenediol.¹⁶ Nevertheless, aromatase inhibitors

have proven to be highly effective treatments for many post-menopausal breast cancers.¹⁷ Indeed, a very recent study, the IBIS-II trial, investigated the use of anastrozole for prevention of breast cancer in healthy postmenopausal women at high risk of the disease and showed that it significantly reduced the risk of invasive ER+ cancer developing.¹⁸ By contrast, androstenediol is formed from androstenediol sulfate, itself derived from dehydroepiandrosterone sulfate (DHEAS) and both of these are substrates for STS. STS was found to hydrolyze readily both DHEAS and estrone sulphate, thus indicating that only one sulfatase exists for both estrogen and androgen pathways.¹⁹ This suggests that inhibition of STS should not only affect the *in situ* generation of E1, in an intracrine fashion as above, but also should block the other pathway of estrogenic stimulation *via* androstenediol, both aromatase-independent pathways. It was thus reasoned that additional blockade of the sulfatase-dependent pathways may improve the therapeutic response to endocrine therapy, either alone or in combination.

The structural biology and enzymology of the enzymes of estrogen metabolism have been recently reviewed²⁰ and there are multiple reviews that cover various aspects of the biology and chemistry of aromatase and STS and their inhibitors^{21–27}, as well as the intellectual property status of the STS field.²⁸ This *Perspectives* article illustrates the development of series of inhibitors that have been or could be still further developed into drugs to treat cancer. All are based around the aryl sulfamate pharmacophore, the chemistry and therapeutic potential of which have been the subject of several extensive reviews.^{29–33} *N*-Sulfamate derivatives such as cyclamate, and including aryl *N*-sulfamate derivatives, are long known as sweeteners.³⁴ Also, *O*-sulfamates when attached to nucleosides, as esters of sulfamic acid, have found roles in the inhibition of bacterial aminoacyl-tRNA synthetases³³, and as antivirals, antibiotics and antitrypanosomals, e.g. nucleosidin (Figure 2)³⁵ and, when attached to a modified monosaccharide, the anticonvulsant fructopyranose derivative, Topiramate (Topomax, Figure 2) as a treatment for epilepsy³⁶ as well as the related analogue RWJ-37497³⁷, both carbonic anhydrase inhibitors. Intriguingly and most recently, the sulfamate-based nucleoside analogue MLN4924³⁸ (Figure 2) is a first-in-class selective inhibitor of the proximal regulator of the NEDD8-activating enzyme (NAE), a protein homeostatic pathway essential for cancer cell growth and survival³⁸ and is currently in clinical trials for the treatment of cancer. It is a mechanism-based inhibitor of NAE and creates a covalent adduct with NEDD8 catalyzed by the enzyme.³⁹ However, at the inception of our work in the early 1990s, although earlier patents had been filed on dialkyl aryl sulfamate esters and some work in the anticonvulsant field claimed aryl sulfamates, we were aware of little, if any, formal work with polycyclic aryl *O*-sulfamate esters as drug candidates; the surprisingly very clean patent landscape then in this regard verified this and enabled an initial 1991 priority filing and subsequent grant of very broad composition of matter patent coverage around this pharmacophore.⁴⁰ Around this time also the first work on imidazolylphenyl sulfamate-based aryl *O*-sulfamates as potential topical anti-glaucoma agents was published.⁴¹ Later, the *N*-substituted aryl sulfamate ester derivative Avasimibe (Figure 2), a selective acyl-coenzyme A:cholesterol *O*-acyltransferase inhibitory lipid-regulating agent reaching Phase III trials, but later withdrawn, was under development by Pfizer up to 2004 for the potential treatment of hyperlipidemia and atherosclerosis.⁴²

Estrone 3-O-sulfamate (EMATE)

The isoxazole derivative of 17- α -ethinyltestosterone, danazole, was the first compound shown to inhibit STS 43 and a small human study later concluded that it had *in vivo* effects on steroid sulfatase, but was weak.⁴⁴ Initial work in our group to develop highly potent inhibitors of STS was published in 1993 with the development of structural analogues of estrone sulfate (E1S). This work was based upon exploring structural surrogates of the sulfate group and led to reversible inhibitors. An early success was the synthesis of the diastereoisomeric estrone 3-methylthiophosphonate, **1**, (and other 3-*O*-phosphonate and thiophosphonate analogues) that was a good inhibitor of STS in MCF-7 breast cancer cells (96% inhibition at 10 μ M) and in placental microsomes (IC_{50} = 43 μ M), with the *S_p*-isomer being the more potent diastereoisomer.^{45, 46} The following year the inhibitory activities of a different set of E1S analogues, **2-4**, were reported, the most potent of which was estrone 3-*O*-sulfamate, **2** (EMATE).⁴⁷ In MCF-7 breast cancer cells **2** inhibited STS activity by more than 99% at 0.1 μ M with the level of inhibition declining with increasing methylation of the amino group, and in placental microsomes the IC_{50} for STS inhibition by **2** was 80 nM. By treating MCF-7 cells with **2**, **3** and **4**, washing the cells and then assaying the STS activity, **2** was characterized as a time- and concentration-dependent irreversible active-site directed inhibitor. Cells treated with **3** and **4** by contrast recovered 50% and 80% of their STS activity, respectively.⁴⁷ The hydrolysis of both E1S and DHEAS by human placental STS expressed in COS-1 cells was also effectively inhibited by **2**, it not making any difference whether the cells were intact or broken.¹⁹ Kinetic studies showed that **2** inactivates STS in a time-, dose- and pH-dependent manner.⁴⁸

The *in vivo* inhibition of E1S and DHEAS hydrolysis in the liver was tested by subcutaneous (s.c.) injection of rats for seven days with 10 mg/kg **2**.⁴⁹ Both activities were almost completely abolished. A dose-response study found no effect of 0.01 mg/kg **2** on liver STS activity, but 87% inhibition at 0.1 mg/kg and almost complete inhibition at 1 mg/kg and 10 mg/kg when **2** was administered by s.c. injection.⁴⁹ A single dose of **2** (10

mg/kg, s.c.) was sufficient to abolish STS activity for three days with a 10-15% recovery of activity after seven days in liver, uterine and brain tissues, but still no activity in ovarian or adrenal tissues. Treatment for ten days (10 mg/kg/day, s.c.) resulted in inhibition for ten days after the last dose, with up to 30% recovery after fifteen days.^{49, 50} The growth of mammary tumors was significantly reduced in animals receiving **2**.⁴⁹ Although clearly inactive *in vitro* as an irreversible STS inhibitor the dimethyl analogue of **2** was nevertheless orally active and highly potent *in vivo* in inhibiting NMU-induced mammary tumor growth⁵¹, demonstrating that this compound is likely demethylated *in vivo* to **2**.

Also in 1996 the development of a self-emulsifying formulation of **2** suitable for oral administration^{51, 52} and the results of a pharmacokinetics study⁵³ were reported. A dose-response study with oral administration to rats produced results almost identical to those obtained following s.c. injection.⁴⁹ **2** was rapidly absorbed and readily detected in rat plasma after oral administration, with plasma concentrations correlating with the size of the administered dose over the 10-40 mg/kg range. Rat liver STS was almost completely inhibited within 30 minutes of oral or intravenous (i.v.) administration of **2**. The following year the development of an assay of STS activity in white blood cells (WBC) was reported.⁵⁴ This was necessary because, if patients with breast cancer were to be treated, a simple method to monitor the extent and duration of STS inhibition is essential. Two hours after the oral administration of 10, 20 or 40 mg/kg **2** to rats no difference in STS activity between samples taken from the liver and WBC was observed, both being inhibited by over 98% at all doses. Plasma concentrations of **2** after a single oral dose of 0.5 mg/kg to two human male volunteers peaked at 12.3 nmol/L after four hours in one, and at 10.9 nmol/L after six hours in the other: in both cases **2** appeared to be rapidly absorbed. It was not possible to measure E1 or E1S concentrations in these volunteers, but twelve hours after administration of **2** dihydroxyepiandrosterone (DHEA) levels had fallen by 41% and 72% and remained below pre-treatment levels for more than 72 hours. In keeping with **2** inhibiting STS the levels of DHEAS increased, by 50% and 34% after 24 hours with this increase being maintained for at least 72 hours.⁵⁴

Several analogues of **2** at the sulfamate group were synthesized to try to illuminate the mechanism of STS inhibition.^{55, 56} None of the compounds **5-8** was a good inhibitor, the best of them, **5**, achieving 53% inhibition at 50 μ M (compared with >99% inhibition at 1 μ M for **2**) with **6** inhibiting by 12% at 50 μ M, **7** failing to inhibit at 100 μ M, and **8** inhibiting by 19% and 45% at 60 and 300 μ M, respectively. Both **5** and **6** failed to show irreversible time-dependent inhibition of the enzyme suggesting that the bridging oxygen in the sulfamate moiety of **2** is essential for irreversible inhibition. The X-ray crystal structure of **2** was also determined.⁵⁶ Based on these results two possible mechanisms of action for inhibition by **2** were proposed, one *via* an electrophilic aminosulphene intermediate, and another *via* a nucleophilic attack by an amino acid residue in the active site^{55, 56} (*vide infra*).

Additional analogues of **2** were synthesised to determine the structure-activity relationship (SAR), with modifications of the sulfamate (**9-21**).⁵⁷ The *N*-acylated derivatives (**14, 15, 18**) were highly potent.⁵⁸ Despite the initial promising findings, however, **2** was not chosen for clinical development at this stage as an STS inhibitor in oncology as the estradiol sulfamate

version (E2MATE, **22**) was found to be a highly potent estrogen in rats, with increased systemic, but reduced hepatic, estrogenicity upon oral administration ⁵⁹ despite (like E1S) not binding to the estrogen receptor.⁶⁰ This results from its sequestration and transport through the liver within red blood cells where it is bound to carbonic anhydrase. A uterine weight gain assay in ovariectomized rats showed that a dose of only 2 µg of **22** was required to double the weight of the uterus, compared with 10 µg of ethinyl estradiol and 200 µg of estradiol.⁵⁹ Thus, **22** is approximately five times more potent than ethinyl estradiol, which is widely used in HRT and oral contraception. This surprising finding led to the clinical evaluation of the estradiol version of **2** (**22**, also known as STX68 and J995) under licence, as a prodrug for hormone replacement therapy applications.^{60, 61} In later work we showed that STS is able to hydrolyze estrogen sulfamates as a result of its mechanism of irreversible inhibition and that, therefore, **2** is indeed a prodrug for estrone ⁶² or, in principle, **22** for estradiol. *In vitro* studies, using [³H]estrone sulfamate, revealed that its uptake ($95.9 \pm 2.4\%$) by red blood cells is much higher than that for the non-sulphamoylated estrogen. Results from these studies demonstrated convincingly that STS is the enzyme responsible for the removal of the sulphamoyl group and it therefore has a crucial role in regulating the estrogenicity associated with this class of drug. To date, the pro-drug approach for **22** has been investigated clinically up to Phase II and some limited clinical data are available in the public domain.⁶³

- 5** R = NHSO₂NH₂
- 6** R = SSO₂NH₂
- 7** R = SSO₂NME₂
- 8** R = SO₂NH₂
- 9** R = OSO₃H
- 10** R = OSO₂CH₃
- 11** R = OSO₂C₂H₅
- 12** R = OSO₂C₄H₉
- 13** R = OSO₂N(CH₂Ph)₂
- 14** R = OSO₂NHCOMe
- 15** R = OSO₂NHCOPh
- 16** R = OSO₂NHEt
- 17** R = OSO₂NHCH₂Ph
- 18** R = OSO₂NHCOEt

COUMATES

Because the estrogenicity of **2** precluded its originally anticipated application in oncology a search for an orally active, nonsteroidal, nonestrogenic, Lipinski-compliant STS inhibitor was undertaken, primarily based upon steroidal A/B ring surrogates. One of the first compounds developed was 4-methylcoumarin-7-*O*-sulfamate (**23**, COUMATE), which inhibits STS irreversibly in a time- and concentration-dependent manner, has an IC₅₀ of 380 nM in MCF-7 cells, and in rats has no estrogenic activity.⁶⁴ Oral dosing of rats for seven days (10 mg/kg/day) inhibited liver STS activity by 85% with enzyme activity returning to normal over seven days.⁶⁵ An initial series of derivatives of **23** was synthesized of which the most potent was 3,4-dimethylcoumarin-7-*O*-sulfamate (**24**), which inhibited STS in MCF-7 cells with IC₅₀ = 30 nM.⁶⁶ This suggested that there was room for larger moieties at the coumarin 3- and 4-positions, so a series of tricyclic compounds was synthesized (**25-34**) (Table 2) that proved more potent.⁶⁷⁻⁷⁰ The most potent of these compounds were **29** and **30**, but it was **27**, known as Irosustat (also known as 667 COUMATE, STX64 and subsequently BN83495),⁶⁸ that was selected for extensive *in vivo* studies, because it was thought that the lower hydrophobicity of the smaller ring size would result in the compound having better pharmacokinetic and pharmacodynamic properties.⁶⁸ Note that tricyclic **34**, with a ring size of 15, although significantly weaker *in vitro* was the most potent *in vivo*. The greater activity of these non-steroidal compounds relative to **2** was attributed to the enhanced “sulfamoyl transfer potential” as a result of the lower phenolic p*K*_a of the parent.⁶⁷ This class of non-steroidal compound importantly was also shown to irreversibly inhibit STS. Single or multiple doses of **27** inhibited rat liver STS by >90%, and oral or subcutaneous doses prevented the growth of nitrosomethylurea-induced mammary tumors in rats.⁶⁸ The synthetic route to **27** and its congeners is very straightforward, comprising only two steps, a Pechmann reaction between resorcinol and the corresponding cyclic β-ketoester, and a sulfamoylation⁶⁷, making them attractive candidates for industrial scale-up.

An investigation of the pharmacokinetics of **27** following a single p.o. or i.v. dose (10 mg/kg) revealed a bioavailability of 95% and detectable levels in plasma for eight hours.⁷¹ In plasma *ex vivo* **27** is rapidly degraded but *in vivo* degradation is prevented by sequestration inside red blood cells where it binds to (and inhibits) carbonic anhydrase II (CAII, IC₅₀ = 22 nM), a feature, also, of **2** (IC₅₀ = 23 nM) and its derivatives.⁷² The X-ray crystal structures of both **2**⁷³ and **27**⁷⁴ in complex with CAII were determined. The ability of red blood cells to prevent metabolic degradation and facilitate transport to tissues may account for the high bioavailability of **27**.⁷¹ Moreover, this sequestration allows the drug to evade first pass metabolism.^{75, 76}

The structure of one of the COUMATE class, **29**, is shown below drawn in a folded fashion to show how it could potentially mimic a steroid structure in binding to STS. When one also notes that sulfamate esters, like sulphonamides, are weak acids with the first p*K*_a in the 7-9 region, depending upon solvent, it becomes clear that there will be a significant amount present in the anionic form as drawn, at physiological pH, thus making the analogue (and indeed **2** itself) a good mimic of the normal STS substrate. Added to this is the fact that the sulfamate ester can also be substantially present as the neutral form which will aid permeability, tissue penetration and presumably sequestration in red blood cells.

The working hypothesis adopted for the mechanism of action of aryl sulfamates in inactivation of STS is generally that the sulfamoyl group is likely transferred to the protein active site in some fashion and several mechanisms have been postulated, although none is conclusively proven as yet.^{48, 55, 67} In eukaryotic sulfatases a critical formylglycine (FGly) residue is generated by post-translational modification of an active site cysteine residue. This FGly, in its hydrated form as a *gem*-diol, is thought to be generally involved in the catalytic mechanism of all sulfatases and is a potential target for irreversible modification by sulfamate-based inhibitors.⁶⁷ In 2003 an X-ray crystal structure of human STS became available illustrating its “mushroom-like” structure ⁷⁷ and structural aspects of sulfatases have been reviewed.⁷⁸ Using the STS crystal structure docking experiments indicated the likely correct positioning of the sulfamate group of **27** in the active site is opposite the crucial hydrated FGly residue ⁷⁰, suggesting that the sulfamate group, as a good steric and electronic sulfate mimic, might be transferred to this residue during inhibition. The idea that inhibition of STS by sulfamate esters is facilitated by some kind of sulfamoyl group transfer to the active site of STS is intrinsic to the ideas presented in this review and is the currently accepted mode of action for such drugs, even if the precise mechanism is as yet unknown. Further ideas on this subject have been recently discussed ⁷⁹ but are beyond the scope of the present review.

27 Was the first STS inhibitor to enter phase I clinical trials in post-menopausal women with estrogen receptor-positive breast cancer, who had failed on other treatment regimens.⁸⁰ An initial oral dose of either 5 mg or 20 mg was followed a week later by three fortnightly cycles, each comprising of five days dosing and nine days off treatment. Systemic uptake after oral administration was monitored using the WBC assay.⁵⁴ At the end of the dosing period STS activity in biopsied breast tumor tissue was inhibited by 99% and serum concentrations of estrone, estradiol, androstenediol and DHEA had, as expected, fallen significantly from pre-treatment levels. Unexpectedly, the levels of the aromatase substrates testosterone and androstenedione also fell significantly implying that these were also linked to STS-active precursor cleavage, at least in part. This could in principle augment STS inhibitor effectiveness as a monotherapy and androstenedione is of course the substrate for aromatase. The drug was well-tolerated with only minor adverse effects recorded. Four patients who had previously progressed on aromatase inhibitors showed stable disease for up to seven months ⁸⁰; some of these patients continued to receive compassionate dosing after the end of the trial. Results of the first trial have been summarized.⁸¹ A subsequent phase I dose escalation study determined the optimal dose of **27** as 40 mg/day. In Phase I/II trials clinical observation of stable disease was observed in a cohort of 35 estrogen receptor-positive metastatic breast cancer patients at this dose (one patient stable for 13 months, one for 8 months, one for 7 months and 3 for *ca.* six months). Biopsy-validated erythematous

skin infiltration in one patient was no longer visible after one month of treatment. Virtually complete inhibition of STS was observed with all doses between 5 and 80 mg/day, achieving 95% STS inhibition and the maximum tolerated dose was not reached.^{82, 83} Excellent safety and tolerability was observed. However, for unknown reasons, relative bioavailability decreases with increasing dose.⁸⁴ Clinical progress has been reviewed.⁸⁵ Thus, the well tolerated treatment showed STS inhibition associated with a significant reduction in the levels of some circulating steroid hormones and within some patients, prolonged clinical partial responses, demonstrating the clinical interest of STS inhibition and importantly validating the underpinning scientific concepts. Further trials in breast cancer are ongoing *vide infra*.

Endometrial cancer, developing from the inner uterine lining, is the most common cancer found in the female reproductive system and there is a strong medical need for new agents. Of *ca* 40,000 new cases of endometrial cancer diagnosed during 2008 in the US *ca* 7,500 women died from this disease. A multicentre phase II study of **27** as a treatment for estrogen receptor-positive endometrial cancer in women with recurrent or advanced disease was undertaken, against the current standard of care (megestrol acetate) with, as primary endpoint, the proportion of patients who neither progressed nor died after 6 months of treatment. Progression free survival, clinical benefits, overall survival and safety were evaluated as secondary endpoints. Although the primary endpoint was not reached, the study found clinical benefit from **27**, with 47.2% of patients having stable disease, 36.1% alive without progression at six months, and 11.1% having a complete or partial response.⁸⁶ Again, the treatment was well tolerated and STS inhibition was associated with a significant reduction in the levels of some circulating steroid hormones and, in some patients, prolonged clinical partial responses.

Male patients suffering from prostate cancer often undergo castration to reduce hormone levels, with a further period of remission following adrenalectomy.⁸⁷ This is probably because the adrenal production of androgens (DHEA and DHEAS) is stopped. In the prostate these androgens can be converted to testosterone and dihydrotestosterone that drive tumour growth, analogous to the *in situ* cleavage of E1S in breast tumour cells. Significant STS activity has been detected in prostate tissue and in LNCaP prostate cancer cells, suggesting that here also STS could facilitate the intracrine production of hormones to stimulate tumour growth. Organic anion transporting polypeptides can transport DHEAS into human prostate cancer cells⁸⁸ to be converted *via* endogenous STS action into DHEA and then into testosterone by hydroxysteroid dehydrogenases and into DHT *via* 5 α reductase. Knockdown of the transporter reduced DHEAS-stimulated cell growth as did the administration of **27**.⁸⁸ The effect of **27** on hormone levels in male patients suffering from castration-resistant prostate cancer was studied in a further US clinical trial⁸⁹ and a phase I dose escalation study of **27** was conducted in chemo-naïve, castration resistant prostate cancer patients showing evidence of disease progression. Aims were to evaluate the safety, tolerability, pharmacokinetic and endocrine parameters (DHEA, androstenediol and testosterone) consequent upon STS inhibition resulting from 28 days of oral drug administration. In all the patients the concentration of testosterone, androstenediol, DHEA and DHEAS fell. **27** demonstrated nearly complete STS inhibition and was well tolerated,

with dry skin as the most common related adverse event. Pharmacodynamic proof of concept was thus demonstrated leading to an increase in DHEAS level and significant suppression of relevant non-sulphated androgens. The DHEA:DHEAS ratio was decreased by 338%.

Further development of **27** in monotherapy for the above hormone-dependent cancers is not currently ongoing, substantially based on the observation that **27** is not superior to megestrol acetate, the current standard of care, in a phase II trial of endometrial cancer. However, on the basis of the excellent STS inhibition observed in this trial, and the phase I/II clinical study results obtained in the metastatic prostate and breast cancer trials, the good clinical safety profiles noted, associated with the reduction of hormonal parameters and the potentially encouraging clinical efficacy seen, the development focus is currently moving towards exploration of **27** in combination with other hormonal therapies. Thus, two further UK clinical trials with **27** were initiated in 2012 in breast cancer; these trials aim to explore the benefit of combination dosing with the aromatase inhibitor anastrozole (the IRIS trial) 90, 91 and also to examine the effects of **27** in breast cancer patients using PET scanning (the IPET Trial).⁹²

The ectopic growth of endometrial tissue outside the uterus is a characteristic of endometriosis, a non-malignant gynaecological disease leading to chronic pelvic pain and infertility. In addition to production of estrogens in the ovaries, there is compelling evidence that local synthesis of estrogens in endometriotic lesions promotes progression of the disease and resistance to endocrine therapy. The disease is rarer in post-menopausal women suggesting that it is estrogen-dependent. With an estimated 80 million patients worldwide, the disease is still poorly understood, most treatments have unpleasant side effects and current therapies are grossly inadequate. Aromatase and STS activity have been detected in both eutopic and ectopic endometrial tissue with the level of STS activity, but not aromatase activity, correlating with the severity of the disease.⁹³ **27** almost completely blocked STS activity in homogenized endometrial tissue suggesting that STS inhibition may be a useful treatment for endometriosis.^{93, 94} Positive *in vivo* preclinical data using our original STS inhibitor **22** were also reported independently. In induced endometriotic lesions **22** inhibited STS *in vivo*, with no effect on the number of lesions, though the lesions were smaller than those in untreated animals.⁹⁵ Clinical trials have taken place in endometriosis using **22** (also known as PGL2001).⁹⁶ **22** entered phase I clinical trials in Germany in 2008 as an STS inhibitor in healthy pre-menopausal women to advance this compound towards a novel, once-a-week, oral medication. In this randomized, double-blind, placebo-controlled phase I study of **22**, either alone or in combination with an inhibitor of ovarian estrogen production, 24 healthy women of reproductive age were treated for four weeks.⁹⁶ **22** reduced endometrial STS activity by 91% and both drugs together reduced activity by 96%: in both cases the activity levels remained low for at least a month after cessation of treatment.⁹⁶ The drug continues in clinical trials against endometriosis, and multicentre phase IIa studies to investigate its efficacy, safety, pharmacokinetics and pharmacodynamics started in Hungary, Poland and Romania in 2012.⁹⁷

27 undergoes desulfamoylation to yield the sufamoyl-free derivative, 667-coumarin.⁹⁸ Metabolism by hepatocytes and liver microsomes was extensive, and *in vitro* studies showed

similar metabolic profiles in rats, dogs, monkeys and both human sexes. The main metabolites were sulphated and glucuronidated derivatives of 667-coumarin and some of its monohydroxylated analogues.⁹⁸ CYP1A2 was competitively inhibited by 667-coumarin with a K_i of 0.77 μ M, but CYP2A6, CYB2B6, CYP2C8, CYP2C9, CYP2D6, CYP2E1, CYP3A4/5 and several UDP-glucuronosyltransferases were not inhibited.⁹⁹ However, in the *in vivo* setting where the drug is sequestered inside red blood cells, the significance of any such CYP inhibition may be significantly reduced.

27, R = H, Irosustat, BN83495,
STX64, 667 COUMATE

35, R = Me, STX289

The topical application of **27** or its *N,N*-dimethyl analogue (STX289, **35**) to the dorsal neck region of nude mice resulted in the inhibition of skin and liver STS.¹⁰⁰ Desulfamoylation of **27** occurred in both the presence and absence of cells but *N,N*-demethylation of **35** did not occur in the absence of cells suggesting that **35** may be suitable for development as a topically applied STS inhibitor.¹⁰¹

A number of potent steroid sulfatase inhibitors have been developed, originally and subsequently in women's health and also in oncology for the treatment of hormone sensitive diseases such as breast cancer, extending to endometrial cancer, prostate cancer and endometriosis. STS inhibition was also earlier shown to have potential in immune-modulation. Thus, DHEA and DHEAS have an immunostimulatory role *in vivo*.¹⁰² However, **2** blocked the ability of DHEAS, but not DHEA, to act as an immunostimulant. Since conditions such as rheumatoid arthritis may result from an inappropriate immune response and increased production of Th1 cytokines STS inhibition could be of therapeutic benefit. **2** markedly altered progression using a model of arthritis¹⁰³; in dermatology¹⁰¹, and in memory and cognitive function. **2** was administered alone and in combination with DHEAS to rats which were then tested for the reversal of scopolamine-induced amnesia. **2** enhanced the reversal of amnesia by DHEAS, suggesting that STS inhibition can potentiate the memory-enhancing properties of DHEAS and that so increasing the levels of endogenous sulfated neurosteroids may enhance learning and/or memory function.¹⁰⁴ Interestingly, a recent report details that pharmacological modulation of the STS axis using **23** results in enhanced response control in models of attention deficit hyperactivity disorder (ADHD) comparable to that with commonly used ADHD therapeutics such as methylphenidate and atomoxetine.¹⁰⁵ More recently, there are indications of STS involvement¹⁰⁶ and therapeutic potential in ovarian cancer¹⁰⁷, lung cancer¹⁰⁸, colon cancer¹⁰⁹ and bladder cancer.¹¹⁰ STS occurs in bone, but little is known about its role in bone function. STS is present in human pre-osteoblastic bone cells and can influence bone

cell growth *via* conversion of sulfated steroids. Pre-osteoblastic MG-63 cells and microsomes both possess STS activity that was blocked by **2** and **27**; **27** inhibited cell growth.¹¹¹ Such wider topics are outside the scope of this review and the earlier work has been more widely discussed ²⁴, but demonstrate that the scope for application of the concept of STS inhibition and the inhibitors developed is very wide and still substantially unexplored. The broadly positive results observed in the initial clinical trials serve to validate the original scientific concepts put forward. Whether the future lies in monotherapy or combination therapy, however, still remains to be established.

Steroid sulfamate derivatives, leading to microtubule disruptors

Alongside the development of **23** and **27**, and given the powerful estrogenicity of **22**, a program was initiated to develop a non-estrogenic derivative of **2** based on the steroidal core. A first series of compounds involved modification of the A ring (**36–38**) alongside, for some of the structures, converting the estrone core to estradiol (**39**, **40**) (Table 3).^{112–116}

In placental microsomes **2** inhibits STS with $IC_{50} = 199$ nM.^{112–116} The 2-methoxy derivative, **36**, inhibits STS with $IC_{50} = 30$ nM.^{112–116} Converting the estrone core of **2** to an estradiol core yields **22**. In placental microsomes **22** inhibits STS with $IC_{50} = 16$ nM.¹¹⁴ **22** is also able to inhibit STS *in vitro* in human endometrial tissue and *in vivo* in murine liver, leukocytes and uterine tissue.⁹⁵ Note also the activity of **22** in models of endometriosis *vide supra*.

KW-2581, 17-diisopropylcarbamoyl-1,3,5(10),16-estratetraen-3-yl sulfamate (**41**), a potent and non-estrogenic steroidal STS inhibitor based on **2**, was evaluated in a range of *in vivo* tumor models. **41**, given daily orally at 1 mg/kg, inhibited the growth of human breast cancer ZR-75-1 xenografts stimulated with E1S ¹¹⁷, ¹¹⁸ and further work demonstrated the inhibitory effect of **41** in E1S-stimulated growth of NMU-induced rat mammary tumors.¹¹⁷, ¹¹⁸ These studies importantly also demonstrated that tumors with an elevated STS activity had a greater final tumor volume as also noted earlier.¹¹⁹ A more recent development is ESE-16, **42**, which has been shown to cause both apoptotic and autophagic cell death. ¹²⁰, ¹²¹

41 (KW-2581) $R_1 = H$, $R_2 = CON(CH(CH_3)_2)_2$

42 (ESE-16) $R_1 = CH_2CH_3$, $R_2 = H$

A development of the 17-position-reduced version of **36**, but also sulfamoylated on the D ring (**43**, known as STX140), inhibited MCF-7 cell proliferation and *in vivo* STS activity.¹²² Both **43** and **44** (STX243), the 2-ethyl variant of **43**, inhibited the proliferation of

MCF-7 cells and doxorubicin- and mitoxantrone-resistant variants, causing arrest in the G₂/M phase of the cell cycle.¹²³ Treatment of adult human fibroblasts with 0.1 μ M **43** caused a reversible change in cell morphology and induced G₂/M arrest but not apoptosis.¹²⁴ The same treatment when applied to human umbilical vein endothelial cells (HUVECs) had no effect until the coadministration of TNF- α which induced apoptosis.¹²⁴ CAL51 cells are resistant to TRAIL-induced apoptosis but **43** is able to stimulate the activation of caspases downstream of TRAIL and thus overcome the TRAIL resistance.¹²⁵ When tested for their ability to inhibit the proliferation of estrogen receptor positive (MCF-7) and negative (MDA-MB-231) cells **43** inhibited the growth of both cell lines (IC₅₀ = 0.25 μ M and 0.29 μ M, respectively) as did the 17-cyanomethyl derivative (STX641) **45** (IC₅₀ = 0.07 μ M and 0.08 μ M, respectively).¹²⁶ SAR studies looking at the effects of a range of alkyl and alkoxy substituents at the 2-position in combination with various substituents (sulfamates and their derivatives, acetates and amides) at the 17-position yielded a large amount of data pertaining to both *in vitro* and *in vivo* activity (Table 4, compounds **40** and **43-45**).¹²⁶⁻¹²⁸ Although derived from considerations linked to a desire to decrease the estrogenicity of **2**, it was also realized that **43** is in effect a derivative of 2-methoxyestradiol (2MeOE2, Panzem) a natural endogenous metabolite of estradiol that binds poorly to the estradiol receptor. 2MeOE2 is a microtubule inhibitor, antiproliferative, antiangiogenic and induces apoptosis in some cancer cell lines ^{129, 130}; it inhibits tubulin polymerization by interacting at the colchicine site.¹²⁹ While several phase I/II cancer clinical trials have been undertaken with this compound, progress was hindered by its very poor bioavailability and its extensive metabolism *in vivo*. Indeed, one such trial was terminated due to the complete absence of 2MeOE2 detected in patient plasma, despite patients being dosed with 3 g per day.¹³¹ Extensive efforts have been made to synthesize analogues of this agent ¹³² and **43** in particular is a promising derivative and clinical trial candidate *vide infra*.

2-Methoxy-EMATE (**36**), at a concentration of 1 μ M, inhibited the growth of MCF-7 breast cancer cells by 52% by inducing apoptosis: the cells arrested in the G₂/M phase.¹³³ This was not totally unexpected as 2MeOE2 (**40**) inhibits tubulin polymerisation ¹²⁹, cell adhesion, migration and motility ¹³⁴, induces cells to undergo mitotic arrest and apoptosis ^{135, 136}, and has anti-angiogenic and anti-tumor properties.^{130, 135, 137} Over an eleven-day period **36** induced the *in vivo* regression of two out of three tumors in rats when given orally at a dosage of 20 mg/kg/day.¹³³ **36** and its close analogue **37** (2-ethyl-EMATE) function as anti-microtubule agents, inhibiting the ability of paclitaxel to promote tubulin assembly *in vitro* ¹³⁸, a finding that is a possible explanation for the marked effect on the morphology of MDA-MB-231 cells and breast tumor-derived fibroblasts which adopt a more rounded shape than usual.¹³⁹ The same two compounds were tested for their ability to inhibit the growth of two prostate cell lines, LNCaP (androgen receptor positive) and PC3 (androgen receptor negative), and an ovarian cell line (A2780) and adriamycin- and cisplatin-resistant derivatives of it (A2780cis and A2780adr).¹⁴⁰ Both **36** and **37** inhibited the growth of all five cell lines with IC₅₀ values between 1.3 μ M and less than 0.1 μ M. Several of the compounds of this class were tested against the NCI 55 cell panel and **37** was found to be consistently the most potent inhibitor of cell growth.¹¹⁵ Furthermore, **37** was also an effective inhibitor of angiogenesis.^{115, 141}

Nude mice were inoculated with MCF-7 cells and tumor growth was stimulated using subcutaneously implanted estradiol pellets before oral administration of 20 mg/kg of **43** or **45** for five days a week. After three weeks tumor volumes had decreased by 52% (**43**) and 22% (**45**).¹²⁶ Similarly, **38** caused a dramatic reduction in tumor growth or tumor regression at three different dosing regimens, with the effects being sustained after cessation of treatment.¹²⁷ A pharmacokinetic study of **40** and its bis-sulfamoylated analogue **43** found that in mice after a single oral dose of 10 mg/kg the bioavailability of **43** was 85% with significant amounts being detectable after 24 hours: there were insignificant amounts of metabolites of **43**, and no **40** was detectable.¹⁴² In nude mice the growth of xenografts (derived from MDA-MB-435 (estrogen receptor negative) melanoma cancer cells) was almost totally inhibited by **43** given orally for 28 days at a dosage of 20 mg/kg/day with inhibition of tumor growth being maintained for 28 days after cessation of dosing: **40**, at the same dose had no effect on tumor growth.¹⁴² The action of **43** was assessed in a P-glycoprotein-over-expressing tumor cell line both *in vitro* and *in vivo* and efficacy determined in xenograft models. **43** showed excellent efficacy in both wild-type and resistant breast cancer xenograft models, in contrast to taxol and 2-methoxyestradiol. Its potential was further highlighted by its efficacy in xenografts derived from patients who had failed on taxane therapy, such as a docetaxel-resistant xenografts, including a metastatic triple-negative tumor.¹⁴³ **43** also proved to be synergistic when dosed *in vivo* in MCF-7 (breast) and LNCaP (prostate) xenograft models with the metabolic inhibitor 2-deoxyglucose (2DG). The idea was to combine the antiangiogenic-microtubule disruptor **43**, with an inhibitor of glycolysis. Glycolysis is inhibited by 2DG. Angiogenesis inhibition by **43** may increase hypoxia and make a solid tumor even more reliant on glycolysis, in theory sensitising it to 2DG. In this first study showing the benefit of combining a microtubule disruptor with 2DG in the two most common solid tumors, daily oral administration of **43** resulted in a 46% reduction of tumour volume, while the combination of **43** and 2DG reduced tumour volume by 76%.¹⁴⁴ 2DG alone had no significant effect on tumor growth.

Despite paclitaxel's clinical success in hormone-refractory breast cancer it has a poor pharmacological profile, characterized by a low therapeutic index (TIX) and severe dose limiting toxicities, such as neutropenia and peripheral neuropathy. **43** was compared to paclitaxel *in vivo* and the TIX and pharmacological profile determined in three breast cancer models.¹⁴⁵ In a murine metastatic 4T1 orthotopic model **43** significantly inhibited primary tumor growth and lung metastases. While paclitaxel induced significant peripheral neuropathy and neutropenia no **43**-induced neuropathies were observed in MF-1 female nude mice. C3(1)/SV40 T/t-antigen transgenic mice develop hormone-independent breast cancer: this animal model closely mimics the human condition. An oral dose of **43** at 20 mg/kg/day for fifteen weeks starting at age 12 weeks significantly attenuated the growth of mammary tumors: all control animals developed palpable mammary tumors by age 23 weeks, but 47% of treated animals remained tumor free throughout the study.¹⁴⁵ A late intervention study, with treatment (20 mg/kg/day, p.o. for eight weeks) commencing when tumor volume reached 100-300 mm³ resulted, after three weeks of dosing, in an 80.4% reduction in tumor growth compared to controls and, after seven weeks, in a 35% reduction, with treated animals surviving 1-2 weeks longer than control animals.¹⁴⁵ Mice bearing MDA-MB-231 xenografts were treated with **43** oral doses of 10-80 mg/kg/day. Doses from

25 mg/kg/day upwards induced tumor regression, but also an initial weight loss. The lowest dose to cause mortality was 30 mg/kg/day. A thrice weekly dose of 80 mg/kg induced tumor regression. Daily dosing established the therapeutic index (LD_{50} (30 mg/kg) / ED_{50} (10 mg/kg)) as 3, with that for intermittent dosing (LD_{50} = 80 mg/kg, ED_{50} = 20 mg/kg) as 4. No plasma accumulation of **43** was observed.¹⁴⁵ Thus, **43** has a greater anti-cancer efficacy, TIX, and reduced neurotoxicity compared to paclitaxel and may be of significant benefit to patients with breast cancer.

Both **43** and **44** compete with colchicine for tubulin binding and disrupt interphase microtubules leading to cell cycle arrest and apoptosis *in vitro* and *in vivo*.^{140, 146, 147} and both inhibit angiogenesis *in vitro* and *in vivo*.^{141, 142} It has been proposed that the anti-angiogenic activities of microtubule disruptors are mediated by the inhibition of hypoxia inducible factor 1 (Hif-1).¹⁴⁸ This protein is a transcription factor which, under hypoxic conditions, activates the transcription of about 60 target genes, and which, in an O_2 -independent manner, is regulated through the phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) signaling pathways.^{149, 150} Intra-tumoral hypoxia results in increased levels of Hif-1. These higher levels result in increased expression of carbonic anhydrase IX (CAIX).^{151, 152} CAIX helps regulate intracellular pH in the more extreme than normal environment found under hypoxic conditions, stimulates cancer cell migratory pathways, and is associated with tumor aggressiveness and invasiveness. We have found that under hypoxic conditions *in vitro* both **43** and **44** inhibit Hif-1 accumulation in the nucleus but, *in vivo*, of the five genes examined the expression of only CAIX is down-regulated (Potter *et al*, unpublished data). This suggests that the anti-tumor activity of these two compounds is probably independent of Hif-1 and may be mediated by a more direct inhibition of CAIX expression and/or activity. Sulfamates and their bioisosteres (sulfonamides and sulfamides) are known carbonic anhydrase inhibitors. **44** inhibits carbonic anhydrase II (CAII) with K_i = 232 nM, and we have reported the co-crystal structure of the CAII-**44** complex (PDB code 2X7T), as well as with other steroid sulfamates.¹⁵⁴ (As with **27** the binding of **44** to CAII inside red blood cells may contribute to the pharmacokinetic properties of the compound). This study also uncovered the presence of a second binding site on CAII. Given the high structural similarity between CAII and CAIX **44** would be expected to bind to and inhibit CAIX. Non-steroidal sulfamates have been shown to inhibit CAIX ¹⁵⁶ and to inhibit metastasis, and using the recently published crystal structure of the CAIX catalytic domain ¹⁵⁷ and computational docking we have shown that **43** and **44** can dock effectively into the CAIX substrate binding site (Potter *et al*, unpublished data).

Building on the observation of the potency of **45**, the SAR of C-17 cyano-substituted estratrienes was explored (Table 5, compounds **46-51**).¹⁵⁸ Compounds **45**, **46** and **47** had potent antiproliferative effects against human cancer cells *in vitro* with the SAR suggesting that a sterically unhindered hydrogen bond acceptor attached to C-17 may be responsible for the enhanced activity of these compounds. The sulfamate derivatives inhibited STS, and **47** was shown to act as a microtubule disruptor and to have significant *in vitro* anti-angiogenic activity. The *in vivo* effects of compounds **45** and **46** on the growth of MDA-MB-231 xenografts in female nude athymic mice following oral dosing for 28 days at 40 and 80

mg/kg (**45**) and 120 mg/kg (**46**) were studied. Both inhibited tumor growth with no observable toxic effects. 158 Compounds **48** and **49** achieved greater than 50% growth inhibition in a majority of eight cancer cell lines at concentrations below 10nM: **50** and **51** were poorer inhibitors (mean growth inhibition ~3 μ M). 158

D-Ring Modified Estrogen Derivatives

A third approach adopted to find a non-estrogenic derivative of **2** was to retain the A-, B- and C-rings of the estrogen core, but replace the D ring with an *N*-substituted piperidinedione moiety to create a series of 16,17-*seco*-estra-1,3,5(10)-triene-16,17-imide derivatives (Table 6, compounds **52-64**). 159, 160 The most potent of these, inhibiting STS in placental microsomes with $IC_{50} = 1$ nM, were **55** and **61**. 159 Both compounds, when given to ovariectomized rats for five days at 10 mg/kg/day, inhibited liver STS activity by 99%, and had no effect on uterine growth, so are not estrogenic. 160

The *in vivo* activity of **55**, also known as STX213, has been compared with that of **27**. 119 MCF-7 cells stably expressing STS (MCF-7_{STS}) were generated and, along with wild-type MCF-7 cells (MCF-7_{WT}), were implanted into ovariectomized female nude mice receiving estradiol sulphate (E2S) s.c. injections. **55** or **27** were given orally for 49 days followed by a 35 day recovery period during which the mice received only E2S. After 49 days tumor size in control mice receiving only E2S had increased 5.5-fold (MCF-7_{WT}) and 3.8-fold (MCF-7_{STS}). **55** (10 mg/kg) reduced MCF-7_{WT} tumor growth by 56% over the dosing period, with growth during the recovery period being significantly retarded. **27** (20 mg/kg) reduced MCF-7_{WT} tumor growth by 39% over the dosing period, but during the recovery period did not significantly affect tumor growth. The MCF-7_{STS} tumors responded even better: **55** (10 mg/kg) caused a 39% regression of tumor growth with no significant growth during the recovery period, and **27** (20 mg/kg) inhibited growth by 79%. Both compounds completely inhibited liver and tumor STS activity. 119

If the *N*-propyl group of **55** is replaced with an *N*-3,3,3-trifluoropropyl group, designed to block a potential site of metabolism, the resultant compound, **65** (STX1938), is 5-fold more potent *in vitro*, inhibiting STS with $IC_{50} = 35$ pM, and is non-estrogenic. 161 After a single oral dose of 0.5 mg/kg **65** completely inhibits rat liver STS, and, compared with **55**, has a significantly longer duration of inhibition. 161 We have attributed the greater potency of **65** (compared to **55**) to the increased lipophilicity imparted by the trifluoropropyl moiety and the ability of the fluorine atoms to participate in C-F...H-O and C-F...H-N interactions in the STS binding site. An *in vivo* study found that both **55** and **65** at a once weekly dose of 1 mg/kg to rats carrying MCF-7 cells stably expressing STS significantly inhibited tumor growth, completely inhibited liver and tumor STS activity, and significantly reduced plasma E2 levels. 162

55 (STX213) **65 (STX1938)**

Non-Fused Ring Sulfatase Inhibitors

All of the compounds mentioned so far have had fused ring cores. To attempt to break away from this constraint diethylstilbestrol-*bis*-sulfamate (**66**) was synthesized.

66

This inhibited STS in MCF-7 cells with $IC_{50} = 10$ nM.¹⁶³ However, this compound is likely to be desulfamoylated to yield the estrogenic diethylstilbestrol so was not developed further, but it did show that a fused ring system is not necessary for inhibition of STS. Consequently, we developed a series of benzophenone-based STS inhibitors (**67-86**, Table 7).¹⁶⁴ From Table 7 it can be seen that **67** is a potent inhibitor: *in vivo* evaluation in rats showed liver STS activity to be inhibited by 84% and 93% 24 hours after a single oral dose of 1 or 10 mg/kg, respectively.¹⁶⁴

Other polycyclic sulfamates that inhibit STS include chromenone-based compounds (**87**) with potencies of up to 0.34 nM¹⁶⁵ and 2-phenylindole-based compounds (**88**) with potencies of up to 2 nM.¹⁶⁶ Reversible STS inhibitors are also known: these do not contain a sulfamate moiety and are not cleaved as part of the inhibition mechanism.^{167, 168}

87

X = O, S

R = large hydrophobic moiety

88

n = 1-10

R₁ = H, CH₃R₂ = H, CN, CONH₂, CONH-alkaneR₃ = H, OSO₂NH₂

Non-steroidal tubulin polymerization inhibitors

As described above, **36** and **43** inhibit the growth of cancer cells, at least in part, by inhibiting tubulin polymerization. Having established the SAR for anti-proliferative activity in the steroidal series the translation of the key elements of the steroidal three-point pharmacophore, namely the 2-methoxy group, 17-position H bond acceptor and the 3-*O*-sulfamate motif, into alternate non-steroidal series was envisaged. The expectation was to engineer in better pharmaceutical properties and promote more chemical diversity, to develop new series of microtubule disruptors optimized hopefully to surpass, in many respects, the steroidal template from which their design was inspired. Thus, the two key substituents of the steroidal A-ring were incorporated into a heterocyclic mimic of the A,B-ring system and then projected, through attachment at *N*-2, onto a hydrogen bond acceptor into the appropriate area of space to address the third interaction. The *N*-2 nitrogen offers an achiral site of attachment and allows for a convergent approach with a good degree of synthetic flexibility. The tetrahydroisoquinoline (THIQ) template appeared ideal for this purpose (Figure 3).

We also applied this template to the generation of a chimeric microtubule disruptor class. Colchicine (**89**) is a potent and well-established natural product microtubule disruptor, of which its trimethoxyaryl motif is an important part of the active pharmacophore. In a non-steroidal chimeric tubulin polymerization inhibitor the aryl sulfamate pharmacophore was combined with the trimethoxyaryl motif in a THIQ-based structure (**90**, STX2484): this compound inhibited the growth of DU-145 and MDA-MB-231 cells with GI₅₀ values of 0.297 μM and 0.329 μM, respectively.¹⁶⁹ Consequently, over two hundred compounds were synthesized based on core compound **91** with a range of substituents at various positions to

determine the SAR.103–107 Antiproliferative results for some of the more potent compounds (**92–101**) are shown in Table 8.

91 The nature of the R groups are shown below.
Core structure for compounds **90** and **92–101** (Table 8)

R₁	R₂	R₃	R₄	X	R₅	R₆	R₇
OH	H	H	H	CH ₂	H	H	H
OSO ₂ NH ₂	Et	Me	Me	CO	Me	Me	OMe
	OMe	Ph	Et	SO ₂	OMe	Et	OEt
					F	F	
					Cl	Cl	
						CN	
						NHAc	
						NHSO ₂ Me	
						Ac	
						OH	
						OMe	
						OCF ₃	
						OEt	
						O- <i>i</i> Pr	
						OPh	
						OAc	
						OSO ₂ NH ₂	
						NO ₂	
							R₈
							H
							OMe
							R₉
							H
							OMe

R₃ and **R₄** are racemates.
Also at **R₄** *gem*-dimethyl
compounds were synthesised.

Experiments to determine the mode of action of this novel class revealed that **90** is a tubulin disruptor which induces p53 expression, Bcl2 phosphorylation, caspase-3 cleavage, cell cycle arrest and apoptosis.¹⁷⁰ It is also a potent anti-angiogenic agent, both *in vitro* and *in vivo*. In breast cancer xenografts in mice **90**, when dosed orally at 20 mg/kg/day for 35 days, suppressed tumor growth by 84%, with limited toxicity.¹⁷⁰ Similarly, **92** (20 mg/kg p.o.) greatly inhibited the growth of MDA-MB-231 xenografts in athymic female nude mice.¹⁶⁹

Crucially, and in stark contrast to paclitaxel, the efficacy of **90** was unchanged in two clinically relevant drug-resistant models.

One compound (**93**) inhibited the growth of DU-145 and MDA MB-231 cells with GI_{50} = 0.222 μ M and 0.234 μ M, respectively, and in mice displayed good *in vivo* activity against RPMI-8226 multiple myeloma xenografts at a dosage of 40 mg/kg/day for 28 days.¹⁷³

Not surprisingly, this general class of non-steroidal sulfamate-substituted compounds also behaves as STS inhibitors (Potter *et al*, unpublished data), similarly to their steroidal counterparts. Even though the sulfamate group in all of these multi-targeting microtubule disruptors, designed to target hormone-independent tumours, is acting not to engender STS inhibition but as a structural component to enhance the overall recognition pharmacophore, it is possible that the additional property of irreversible STS inhibition might confer further advantages. Others have taken our sulfamoylated THIQ core in modified form (e.g. **102**), confirmed that it inhibits STS (IC_{50} = 0.3 μ M) and shown that both **102** and the desulfamoylated product of the STS inhibition reaction (**103**) function as SERMs binding to human ER α with IC_{50} = 3.1 μ M (**102**) and IC_{50} = 24.6 μ M (**103**).¹⁷⁵

Our focus of THIQ-based compounds was also extended to their dihydroisoquinolinone-based equivalents, **104**.¹⁷⁶ Many of the compounds with linkers other than a carbonyl were inactive but the most potent, **105** (surprisingly with a hydroxyl group rather than a sulfamate), inhibited the growth of DU-145 and MDA-MB-231 cells with GI_{50} values of 51 nM and 71 nM, respectively.¹⁷⁶ The sulfamate derivatives of **105**, **106** still possessed sub-micromolar activity and both exhibited anti-angiogenic activity.¹⁷⁶ Moreover, both also inhibited tubulin polymerization as well as colchicine binding with potencies comparable to the clinically used microtubule disruptor combretastatin A4.¹⁷⁶ An X-ray structure of one analogue with X = C=O showed how steric and electronic factors can in principle produce a conformation to mimic a steroid.

104

The nature of the R groups are shown below.

R₁	X	R₅	R₆	R₇	R₈
OH	CH ₂	H	H	H	H
OSO ₂ NH ₂	CO	OMe	OMe	OMe	OMe
OBn	SO ₂	CO ₂ Me	CN		
			Cl

105 R = OH**106** R = OSO₂NH₂

Multi-targeting Agents, including Dual Aromatase-Sulfatase Inhibitors (DASIs)

As stated in the Introduction the major route of estrogen production is by the aromatization of the A-ring of androgens, a reaction catalysed by aromatase. Inhibition of aromatase lowers estrogen levels and has resulted in an effective endocrine therapy for many breast cancers.¹⁷ It is to be expected that chronic treatment with an aromatase inhibitor will lead to the development of compensatory mechanisms to increase estradiol levels: indeed, a recent paper showed that the administration of an aromatase inhibitor to breast cancer patients increased levels of both STS and 17 β -HSD1 leading to increased estradiol levels.¹⁷⁷ The administration of an STS inhibitor alongside an aromatase inhibitor should, in a multi-targeting fashion, lower estrogen levels further but, importantly, should also address the *in situ* production of estrogen (including androstenediol) from the sulfate in tumor cells by STS in an intracrine fashion. Trials are currently ongoing to explore the effects of co-administration of aromatase inhibitors and 27.^{90–92} An alternative to administering two drugs, one to inhibit each enzyme, is to develop a single agent that inhibits both aromatase and STS, i.e. a *Dual Aromatase-Sulfatase Inhibitor* (DASI). Pharmaceutical arguments in favour of using single molecule multi-targeted agents have been advanced¹⁷⁸ and there are numerous reports of approaches now emerging using dual- and even triple-targeted agents, particularly to address the development of resistance. For example, cancer cells that have acquired resistance to single-target EGFR inhibitors through upregulation of AXL or loss of E-cadherin remain sensitive to the triple-targeted single molecule agent CUDC-101, now in clinical trials, suggesting that coupling HDAC and HER2 inhibitory activities to an EGFR

inhibitor is effective in overcoming drug resistance and preventing cancer cell migration.
179, 180

One attractive approach to achieve a DASI prototype was to take advanced or clinically used aromatase inhibitors and to build in the aryl sulfamate pharmacophore to imbue potential dual activity. The molecule 4-[(4-bromobenzyl)(4-cyanophenyl)amino]-4*H*-1,2,4-triazole, **107** known as YM511, is an inhibitor of aromatase with $IC_{50} = 0.12$ nM in human placental microsomes.¹⁸¹ This was transformed into a prototype DASI by replacing the bromobenzyl moiety with an aryl sulfamate yielding an initial series of potent dual activity compounds (**108**, **109**, Table 9).¹⁸² In order to validate the concept *in vivo* female Wistar rats were given a single oral dose (10 mg/kg) of either **107** or **108** ($IC_{50} = 227$ nM_(STS), 100 nM_(aromatase)) three days after hormonal stimulation of estradiol production. Three hours after the drug dosing plasma estradiol levels in the **107**-treated rats were reduced by 70% compared to controls, and in the **108**-treated rats the level was down by 39%, implying that aromatase was being inhibited. **107** itself had no significant effect on liver STS inhibition, as expected, but **108** showed almost complete inhibition (98%). This demonstrated that the DASI concept is viable.

A significant attraction of this overall approach is that irreversible targeting through low nM potency STS inhibition is combined with reversible low nM potency aromatase inhibition. Lead compounds have already been developed that possess IC_{50} values for aromatase inhibition that are at least comparable to or often even better than those clinical aromatase inhibitors in widespread use *vide infra*. Thus, in a clinical setting for a DASI there will only effectively be the need to titrate the dose required for aromatase inhibition, since STS will be rapidly and irreversibly inhibited. This is likely to be the case even with dual agents potentially with weaker STS inhibitory potency; inhibition will still be targeted and irreversible, but slightly less rapid.

The initial series of compounds was extended with further variants to establish the SAR (**110**, Table 9).^{183, 184} This showed that moving the sulfamate group from the *para*- to the *meta*-position of the aryl ring generally improves the aromatase inhibitory activity but weakens the STS inhibitory activity. We evaluated compounds **109** ($IC_{50} = 39$ nM_(STS), 0.82 nM_(aromatase)) and **110** *in vivo* in the same manner as **108** (described above). Three hours after dosing with **109** there was almost complete inhibition of both aromatase and STS: after 24 hours the percentage inhibition of aromatase and STS had fallen to 85% and 72%, respectively. Compound **110** was initially as successful as **109** in inhibiting STS but the level of inhibition fell to only 33% after 24 hours. However, the aromatase inhibition was much worse than **109**, achieving 82% inhibition after 3 hours and falling to only 20% after 24 hours.

The activities of **27** (a STS inhibitor), Letrozole (**111**, an aromatase inhibitor) and **109**, a DASI¹⁸⁵ were compared. MCF-7 cells stably expressing either aromatase (MCF-7_{aromatase}) or STS (MCF-7_{STS}) were implanted into MF-1 ovariectomized female nude mice receiving both hormonal injections to stimulate tumor growth and one of the three drugs for 28 days. **111** inhibited the growth of MCF-7_{aromatase} tumors but not MCF-7_{STS} tumors: the inhibition pattern of **27** was the reverse of this. However, **109** completely inhibited the growth of both

tumor types, completely inhibited both aromatase and STS activity, and caused a significant reduction in plasma estradiol levels.¹⁸²

A further 53 compounds based around core structure **112** with various combinations of X, R₁ and R₂ moieties were synthesized, and evaluated: some of these are exemplified (**113-119**) and the activity data for these compounds are shown in Table 10.¹⁸⁶ All 53 compounds have the 4-(4*H*-1,2,4-triazol-4-ylamino)benzonitrile moiety for aromatase inhibition and most have the aryl sulfamate moiety for STS inhibition – those that don't are the phenolic DASI precursors, the crystal structures of two of which (**113** and **119**¹⁸⁶) have been solved, as has the crystal structure of the DASI **118**.¹⁸⁶ Most of the compounds are better aromatase inhibitors than **109**, but worse STS inhibitors. With IC₅₀ = 0.26 nM **114** is the best aromatase inhibitor, and the best DASI is **116** with aromatase IC₅₀ = 0.45 nM and STS IC₅₀ = 1200 nM.¹⁸⁶ The SAR of these compounds shows that those containing a thioether or alkylene linker are more potent aromatase inhibitors than those containing an ether, sulfone or sulphonamide linker, and that beyond linkers of two atoms the length of the linker has a limited effect on aromatase inhibition. Compounds **115-117** were administered to female Wistar rats at a dose of 10 mg/kg. **116** was the most potent, reducing aromatase activity by 92% and STS activity by 98%.¹⁸⁶

In a further series of compounds the single ring aryl sulfamate was replaced with a bicyclic aryl sulfamate, seven examples of which are exemplified, **120-126** (Table 11).¹⁸⁷ All displayed good aromatase inhibition *in vitro* but STS inhibition was variable. The most potent DASI was **122** with a substituted naphthalene ring. In female Wistar rats an oral dose of 10mg/kg resulted, after three hours, in 93% inhibition of both aromatase and STS.¹⁸⁷

With a DASI successfully based on the **107** core a series of DASIs based around the Anastrozole (**127**), Letrozole (**111**) and Vorozole (**128**) core structures was synthesized: all of the precursor compounds are potent aromatase inhibitors with IC₅₀ values of 1.5 nM, 0.89 nM, and 29 nM, respectively.

127 was modified by replacing one of the 2-cyanopropyl moieties with aryl sulfamate derivatives connected to the core by a variety of linkers (**130-139**, Table 12).¹⁸⁸ Two control compounds were also synthesized, one lacking the triazole moiety necessary for aromatase inhibition (**130**), and the other lacking the sulfamate moiety necessary for STS inhibition (**131**). With $IC_{50} = 3.5$ nM **132** was the most potent aromatase inhibitor but, in common with all the compounds of this class, had little activity against STS *in vitro*. However, when tested *in vivo*, **132** reduced aromatase activity by 70% and, surprisingly, STS activity by 83%.¹⁸⁸ This difference between *in vitro* and *in vivo* activities may possibly be related to DMPK (drug metabolism and pharmacokinetic) factors. It is also possible that even a weak *in vitro* sulfamate-based STS inhibitor could still induce time-dependent irreversible STS inhibition *in vivo*, albeit more slowly. Thus, while a reasonable target profile was low nM inhibition *in vitro* for both activities, this is most critical for the aromatase inhibitory component but may not be essential for the STS inhibitory component.

Derivatives of **111** and **128** were synthesized. The first compound, **140**, *in vitro* inhibited aromatase with $IC_{50} = 3044$ nM and STS with $IC_{50} > 10000$ nM.¹⁸⁹ However, *in vivo* it reduced aromatase activity by 60% and STS activity by 88%. This demonstrated that using **111** as a DASI template could also work. A series of compounds based on the **111** core (**140**, **141**, Table 13) 190, 191 was thus developed, along with two compounds based on the **128** core (**142**, Table 13).¹⁹¹ The sulfamates are likely to be substantially desulfamoylated *in vivo* so the phenolic parent compounds were also tested for their ability to inhibit aromatase. Note that compound **141** is a racemate. Of the DASIs the most potent aromatase inhibitor was **141**. When the separate enantiomers of this compound were synthesized the *R*-enantiomer proved to be the more potent aromatase inhibitor and the *S*-enantiomer the more potent STS inhibitor. Desulfamoylation resulted in greater inhibition of aromatase. The **128**-

based sulfamate, **142**, had very poor activity against STS so this core structure was not developed any further.

It was also attractive to develop a new series of aromatase inhibitor with the structural potential to be converted into a DASI. Thus, compounds were synthesized employing Suzuki chemistry based around a biphenyl core (**143**) with a triazole to interact with the iron in the aromatase heme group and a variety of other substituents designed.¹⁹² These compounds inhibited aromatase in JEG-3 cells with IC_{50} values as low as 0.12 nM. They were then further developed by modification of one of the phenyl groups to imbue STS inhibitory activity, thus incorporating the aryl sulfamate pharmacophore.¹⁹³ Almost fifty compounds were synthesized based around this core, the most potent of which as a dual inhibitor was **144** with aromatase IC_{50} = 0.5 nM and STS IC_{50} = 5.5 nM in JEG-3 cells: the non-sulfamoylated equivalent inhibited aromatase with IC_{50} = 0.15 nM. Compound **144** also inhibits CAII (IC_{50} = 86 nM), is non-estrogenic and, in rats, following an oral dose of 1 mg/kg, inhibits aromatase activity by 60% and STS activity by 92%.¹⁹³

143

R_2 = CN, $C(CH_3)_2CN$, H

R_3 = OSO_2NH_2 , OH, F, Cl, CN, $COCH_3$, H

R_4 = OSO_2NH_2 , OSO_2NMe_2 , OH, Cl, H

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Examination of the docked poses of aromatase and STS inhibitors suggested there was space in both enzyme substrate binding sites to further develop this pharmacophore. Consequently, several hybrid compounds were synthesized based around core structure **145**.^{193, 194} These represented a combination of the most successful biphenyl-based structures coupled with the proven aryl triazole motif, from other series and linked to the substituted biphenyl moiety through nitrogen. Impressively, the most potent of these inhibited both enzymes in JEG-3 cells with IC_{50} values in the low picomolar range (Table 14), making such compounds currently the best in class, worthy of further development and *in vivo* studies.

Another attempt at designing a dual-targeting drug using steroid sulfamate-based technology, and based upon the idea behind our original pro-drug work on **22**, has led to a

molecule possessing both anti-estrogenic and STS inhibitory components.¹⁹⁵ Thus, SR16157 (**155**) was designed as an STS inhibitor that should also release a selective estrogen receptor modulator (SERM) SR16137 (**156**) upon sulfamate cleavage.¹⁹⁵ **155** is not itself a SERM: it inhibits E1S-stimulated MCF-7 cell proliferation and displays a 10-fold higher anti-estrogenic potency than other SERMs such as tamoxifen. **155** is active *in vivo* and shows low toxicity with a good pharmacokinetic profile.¹⁹⁶ As yet, no clinical studies appear to be planned. See also the recent extension of this idea to the non-steroidal realm, as discussed above.^{175, 197} This approach is different from the DASI approach outlined above, where the sulfamate-based STS inhibitor is also itself an aromatase inhibitor without any necessary metabolism. Moreover, in many cases the sulfamate-cleaved phenolic moiety is also itself a potent aromatase inhibitor. What limits the “pro-drug” approach, as already discussed here ⁶², as elsewhere, is that enzymatic cleavage is accompanied by irreversible inhibition of STS, the cleavage facilitator. The effectiveness of the dual STS-SERM approach *in vivo* will thus be dictated both by the extent of STS-mediated cleavage achievable as well as overall pharmacodynamic properties.

155 SR16157, R = SO₂NH₂

156 SR16137, R = H

Conclusion

There are compelling reasons to explore potential new mechanisms for endocrine therapy working *via* biosynthetic principles, and renewed interest in doing so. These are reinforced by the clinical success of the now very well established aromatase inhibitors, as an endocrine therapy in cancer together with their recently demonstrated potential as preventative agents ¹⁸ and the more recent and still ongoing success story exemplified by the 17 α -hydroxy lyase/C17,20 lyase inhibitor abiraterone (Zytiga) as a hormone therapy in prostate cancer.¹⁹⁸

The past twenty years or so has seen a greater appreciation of the mechanisms of “intracrinology” ¹¹, whereby e.g. circulating DHEAS can be converted peripherally to sex steroids. Also, in this intracrine fashion stimulation of cell growth can still occur *via* mechanisms thought to involve import of E1S locally into tumors *via* organic anion transporters ¹⁹⁹ followed by further *in situ* enzymatic transformations, although the relevant transporters have yet to be conclusively identified. Of the various enzymes implicated in intracrine processes the STS-mediated pathway has emerged as a significant player not only in breast cancer ¹², but in multiple other hormone-dependent disease states, and the therapeutic value of pharmacological intervention at STS is worthy of clinical exploration. It

is also clear that measurement of tumor STS expression can be a biomarker for progression-free and overall survival and could be used for patient stratification.

The discovery of aryl-*O*-sulfamates as the first potent and irreversible inhibitors of STS has stimulated the generation of a large body of preclinical and clinical work through “first-in-class” drugs **22** and **27** (Irosustat) that has substantially validated the scientific and therapeutic concepts originally proposed. These irreversible sulfamate-based drugs have cemented their value as the leading agents to block STS and have not been surpassed in terms of potency. Indeed, the reversible derivatives so far developed have not progressed beyond early *in vitro* pre-clinical studies. Only one sulfamate derivative, **27**, has been explored clinically in three types of hormone-dependent cancer to date, but there are still emerging targets becoming available - ovarian cancer is a good example 200 and very recently there are interesting preliminary implications for bladder cancer.¹¹⁰ Interestingly also, **22** was originally developed as an estradiol prodrug for HRT and reached phase II trials but is now being actively developed as a STS inhibitor for treating endometriosis.⁹⁶

The aryl-*O*-sulfamate pharmacophore has proven to be highly versatile and in active compounds features in three different roles: as a prodrug cleavable by STS; as a moiety that is most likely actively transferred in some fashion to a residue in the STS active site when employed as an STS inhibitor; and finally, simply as a structural augmentation enhancing potency in the steroidal and non-steroidal multi-targeting microtubule disruptors. It seems clear that the sulfamate group not only offers versatility and high potency but also, by virtue of such compounds being sequestered in red blood cells, also confers *in vivo* stabilization, resistance to metabolism and excellent pharmacokinetic and bioavailability properties. This could possibly be more widely exploited in drug design.

So far, no aryl sulfamate derivative targeting hormone-dependent disease has reached phase III clinical trials. It remains to be seen whether this will be achieved in some indication as monotherapy or whether it might better be achieved *via* a combination approach, e.g. with an aromatase inhibitor, as is currently being pursued for breast cancer. In terms of such multi-targeting it seems clear that the DASI approach offers great potential in cases where there is expected to be a synergy between STS and aromatase inhibition and that such an approach could address acquired resistance mechanisms. Additionally, compounds such as **43** or their non-steroidal counterparts offer huge potential as multi-targeting agents for either hormone-independent tumors, where the pre-clinical potential is strongly proven, or perhaps even as a still highly potent STS inhibitor with added cytotoxic/anti-angiogenic activity in a hormone-dependent setting. Compared with the parent endogenous 2-methoxyestradiol that is very poorly bioavailable and extensively metabolized, the *bis*-sulfamate derivative is highly bioavailable and strongly protected against metabolism, whilst maintaining or even enhancing the core activities of the parent. This is a model that could be exploited elsewhere.

Interestingly, there have been reports focusing upon the design of ¹¹C-labelled radiopharmaceuticals both for **43** to study angiogenesis²⁰¹ and related to the DASI concept to study aromatase and STS expression using PET.^{202, 203} These will be of particular utility once these other classes of compound enter formal clinical trials.

Thus, after some 20 years of research and development it is clear that aryl-*O*-sulfamates offer considerable promise and to date have still perhaps not yet demonstrated their full potential. The therapeutic value of STS inhibition is, however, now firmly established clinically, but the optimal setting for an STS inhibitor still needs to be found, both in terms of clinical pathology and whether as monotherapy, classical combination therapy or (even in certain relevant applications) using a single molecule dual agent such as a DASI.

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Biographies

Mark Thomas has a B.Sc. degree (biochemistry, 1989, Wales), a Ph.D. degree (protein chemistry and enzymology, 1992, London) and a M.Sc. degree (computer science, 2000, Bristol). He has academic experience in protein chemistry and enzymology (cysteine proteinases, HIV integrase) and crystallization and crystallography (restriction endonucleases), and both academic and industrial experience in computational drug discovery, design and development (kinases, steroid-metabolising enzymes, proteins involved in calcium signalling, and opioid receptors). He is a named author on thirty-eight published papers and five patents.

Barry Potter studied Chemistry at Oxford, completing a DPhil and a DSc. After postdocs in Oxford and at the Max-Planck-Institute for Experimentelle Medizin, he became Lecturer at Leicester and Lister Institute Fellow, moving to the chair of Medicinal Chemistry at Bath and in 2015 to Oxford. Research interests are in signal transduction and anticancer drug discovery. He was CSO of *Sterix*, acquired by major pharma. He has published over 500 papers and is inventor of 45 granted US patents. He has won four RSC medals, the GlaxoSmithKline International Achievement Award, the RSC-BMCS Medicinal Chemistry Lectureship and was “Investigator of the Year” at the 2012 European Life Science Awards. He is a Wellcome Trust Senior Investigator and a Fellow of the UK *Academy of Medical Sciences*.

Abbreviations Used

3β-HSDI	3 β -hydroxysteroid dehydrogenase C5,C4-isomerase
17β-HSD	17 β -hydroxysteroid dehydrogenase
CAII	Carbonic anhydrase II
CAIX	Carbonic anhydrase IX
Cyp₄₅₀	Cytochrome P ₄₅₀
DASI	Dual aromatase-sulfatase inhibitor
DHEA	Dehydroepiandrosterone

DHEAS	Dehydroepiandrosterone-3- <i>O</i> -sulfate
DMPK	Drug metabolism and pharmacokinetics
E1	Estrone
E2	Estradiol
E3	Estriol
E1S	Estrone-3- <i>O</i> -sulphate
E2S	Estradiol-3- <i>O</i> -sulphate
EMATE	Estrone 3- <i>O</i> -sulfamate
E2MATE	Estradiol-3- <i>O</i> -sulfamate
ER	Estrogen Receptor
Hif-1	Hypoxia inducible factor 1
HRT	Hormone replacement therapy
HUVEC	human umbilical vein endothelial cell
i.v.	intravenous
MAPK	Mitogen-activated protein kinase
NAE	NEDD8-activating enzyme
PET	Positron Emission Tomography
PI3K	Phosphatidylinositol 3-kinase
PM	placental microsomes
p.o.	by mouth
s.c.	subcutaneous
SAR	Structure-activity relationship
SERD	Selective estrogen receptor down-regulator
SERM	Selective estrogen receptor modulator
STS	Steroid sulfatase
TNF-α	tumor necrosis factor- α
WBC	White blood cells
2MeOE2	2-methoxyestradiol
SERD	selective estrogen receptor down-regulator

SERM selective estrogen receptor modulator

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The origin of estrogenic steroids in post-menopausal women with hormone-dependent breast cancer. Also shown is the steroid ring labeling and atom numbering system. Cyp₄₅₀ – Cytochrome P₄₅₀. ER – Estrogen Receptor. 3 β -HSDI - 3 β -hydroxysteroid dehydrogenase C5,C4-isomerase. 17 β -HSD - 17 β -hydroxysteroid dehydrogenase – the numeral after the HSD denotes the α -subtype of this enzyme.

Figure 2. Some sulfamate-based drugs and drug candidates.

Figure 3. The three-point pharmacophore.

Table 2

Activities of tricyclic coumarin sulfamates against STS in human placental microsomes

R

H **23** (COUMATE)

CH₃ **24**

n n

3 **25** 8 **30**

4 **26** 9 **31**

5 **27** 10 **32**

6 **28** 11 **33**

7 **29** 13 **34**

Compound	IC ₅₀ (nM)	Compound	IC ₅₀ (nM)
25	200	30	1
26	70	31	13
27	8	32	60
28	30	33	75
29	2.4	34	370

Table 3

Activities of A-ring-modified derivatives of **2** against STS using placental microsomes (PM) and MCF-7 cells. Data taken from references 112–116. Data for many other compounds with different substituents at R₁, R₂ and R₃ are not shown.

Core structure 'A' for compounds **2** and **36-38** (Table 3)

The range of compounds synthesized are illustrated by the list of substituents below.

R_1	R_2	R_3
H	F	OH
Me	Cl	OSO ₂ NH ₂
Et	Br	OSO ₂ NMe ₂
OMe	I	OSO ₂ NHAc
OEt	CHF ₂	2-Propenyl
SMe	NO ₂	
SEt	CN	
S(O)Me	n-Pr	R_1 -NH-SO ₂ -O- R_2
SO ₂ Me	2-Propenyl	R_1 -NMe-SO ₂ -O- R_2
		R_2 -O-SO ₂ -NH- R_3

Core structure 'B' for compounds **39-40** (Table 3)

The range of compounds synthesized are illustrated by the list of substituents below.

R_1	R_2	R_3
H	OH	H
Et	OSO ₂ NH ₂	
OMe		
OEt		
SMe		
SEt		
CHF ₂		

Compound	R_1	R_2	R_3	Sulfatase IC ₅₀ (nM) in PM	MCF-7 GI ₅₀ (μM)
	Core Structure 'A'				
2	H	OSO ₂ NH ₂	H	199	22.8
36	OMe	OSO ₂ NH ₂	H	30	0.3
37	Et	OSO ₂ NH ₂	H	-	0.34
38	H	OSO ₂ NH ₂	Br	1,200	0.042

	Core Structure 'B'				
39	H	OSO ₂ NH ₂	H	16	-
40	OMe	OH	H	-	2.35

Table 4

Inhibitory and antiproliferative activities of estradiol derivatives. PM – placental microsomes. Data taken from references 126–128. Data for many other compounds with different substituents at R₁, R₂ and R₃ are not shown.

Core structure for compounds
40 and **43-45** (Table 4)

The range of compounds synthesized are illustrated by the list of substituents below.

R₁	R₂	R₃
H	OH	OH
Me	OSO ₂ NH ₂	OSO ₂ NH ₂
Et	OSO ₂ NMe ₂	OSO ₂ NMe ₂
OMe		OSO ₂ NPent ₂
OEt		OSO ₂ NBn ₂
SMe		OSO ₂ NHAc
n-Pr		OCONH ₂
i-Pr		OCOCH ₃
n-Bu		CH ₂ CN
t-Bu		

Compound	R ₁	R ₂	R ₃	Sulfatase IC ₅₀ (nM) in PM	GI ₅₀ (μM)		
					MCF-7	MDA-MB-231	DU145
40	OMe	OH	OH	-	2.35	0.94	1.22
43	OMe	OSO ₂ NH ₂	OSO ₂ NH ₂	39	0.25	0.29	0.34
45	OMe	OSO ₂ NH ₂	CH ₂ CN	-	0.07	0.071	0.062
44	Et	OSO ₂ NH ₂	OSO ₂ NH ₂	1000	0.07	0.21	0.21

Table 5

Antiproliferative activities of estradiol derivatives. Data taken from reference 158. Data for many other compounds with different substituents at R₁, R₂ and R₃ are not shown.

Core structure 'C' for compounds
45 - 50 (Table 5)

Core structure 'D'

Core structure 'E' for
compound **51** (Table 5)

The range of compounds synthesized are illustrated by the list of substituents below.

R₁
OMe
Et

R₂
OH
OSO₂NH₂

R₃
CH₂CN
CH(CN)₂
CHMeCN
CH₂CCH
CN

R₁
OMe
Et

R₂
OH
OSO₂NH₂

R₃
(*E*)=CHCN
=C(CN)₂

R₁
Et

R₂
OH
OSO₂NH₂

R₃
CN

Compound	R ₁	R ₂	R ₃	GI ₅₀ (μM)		
	Core Structure 'C'			MCF-7	MDA-MB-231	DU145
46	OMe	OH	CH ₂ CN	0.3	0.117	0.485
45	OMe	OSO ₂ NH ₂	CH ₂ CN	0.07	0.071	0.062
47	Et	OSO ₂ NH ₂	CH ₂ CN	0.06	0.141	0.054
48	Et	OSO ₂ NH ₂	CH(CN) ₂	0.33	0.249	0.159
49	Et	OH	CHMeCN	29.1	7.5	>100
50	Et	OSO ₂ NH ₂	CN	0.324	0.342	<0.5
	Core Structure 'E'					
51	Et	OSO ₂ NH ₂	CN	>100	75.4	–

Table 6

Inhibitory activities of estradiol derivatives. PM – placental microsomes. Data taken from reference 159.

<p>Core structure for compounds 52-64 (Table 6)</p>		
Compound	R	Sulfatase IC ₅₀ (nM) in PM
52	H	20
53	Me	12
54	Et	52
55	(CH ₂) ₂ CH ₃	1
56	(CH ₂) ₃ CH ₃	382
57	(CH ₂) ₄ CH ₃	150
58	(CH ₂) ₅ CH ₃	288
59	(CH ₂) ₄ Br	12
60		74
61		1
62		23
63		3
64	CH ₂ CH=CH ₂	75

Table 7

Inhibition of STS activity. PM – Placental microsomes. ND – Not determined. Data taken from reference 164.

<div style="display: flex; justify-content: space-around; align-items: flex-start;"> <div style="text-align: center;"> <p>Core structure for compounds 67-76 (Table 7)</p> </div> <div style="text-align: center;"> <p>77</p> </div> </div> <div style="display: flex; justify-content: space-around; align-items: flex-start; margin-top: 20px;"> <div style="text-align: center;"> <p>78</p> </div> <div style="text-align: center;"> <p>79</p> </div> </div> <div style="display: flex; justify-content: space-around; align-items: flex-start; margin-top: 20px;"> <div style="text-align: center;"> <p>Core structure for compounds 80 and 81 (Table 7)</p> </div> <div style="text-align: center;"> <p>82</p> </div> </div> <div style="display: flex; justify-content: space-around; align-items: flex-start; margin-top: 20px;"> <div style="text-align: center;"> <p>Core structure for compounds 83 and 84 (Table 7)</p> </div> <div style="text-align: center;"> <p>Core structure for compounds 85 and 86 (Table 7)</p> </div> </div>				
Compound	X	R	Sulfatase % Inhibition in MCF-7 Cells	Sulfatase % Inhibition in PM
			0.1μM	10μM
67	C=O	OSO ₂ NH ₂	71.4	98.2
68	C=O	OSO ₂ N=CHNMe ₂	46.3	85.3
69	C=O	OH	34.8	62.1
70	C=O	H	<10	76.7
71	SO ₂	OSO ₂ NH ₂	<10	65.0
72	S	OSO ₂ NH ₂	67.2	65.3
73	S	OH	26.6	33.1
74	CH ₂	OSO ₂ NH ₂	22.2	22.1
75	CH ₂	OH	<10	11.7
76	C(OH)C ₆ H ₅	OSO ₂ NH ₂	<10	<10
77	–	–	<10	47.4

78	–	–	<10	71.3
79	–	–	21.3	28.8
80	–	OSO ₂ NH ₂	93.1	91.3
81	–	OH	99.3	86.1
82	–	–	46.6	ND
83	–	OSO ₂ NH ₂	16.1	31.4
84	–	OH	<10	<10
85	–	OSO ₂ NH ₂	75.3	51.1
86	–	OH	<10	18.8

Table 8

Antiproliferative activities of THIQ derivatives (based on **91**). In all cases **R**₁ = OSO₂NH₂, **X** = CH₂ and **R**₉ = H. Data taken from references 169, 171–174. Data for many other compounds with different substituents at **R**₂, **R**₃, **R**₄, **R**₅, **R**₆, **R**₇ and **R**₈ are not shown.

Cmp	R2	R3	R4	R5	R6	R7	R8	GI ₅₀ (μM)	
								DU-145	MDA-MB-231
90	OMe	H	H	H	OMe	OMe	OMe	0.297	0.329
92	Et	H	H	H	OMe	OMe	OMe	0.041	0.052
93	OMe	H	Me	H	OMe	H	H	0.222	0.234
94	OMe	H	Et	H	OMe	H	H	0.286	0.281
95	OMe	H	Me	F	H	H	OMe	0.299	0.269
96	OMe	H	Me	OMe	H	H	OMe	0.189	0.16
97	OMe	Me	H	H	OMe	OMe	OMe	0.36	0.3
98	OMe	H	H	H	OMe	OMe	Cl	0.04	0.04
99	OMe	H	H	H	OMe	OMe	Br	0.03	0.03
100	Et	H	H	H	OMe	OMe	Br	0.02	0.03
101	OMe	H	H	H	OMe	OEt	OMe	0.1	0.07

Table 9

In vitro inhibition of aromatase and STS in JEG-3 cells. ND – Not determined. Data taken from references 182–184. Data for many other compounds with different substituents at R₁, R₂, R₃, R₄ and R₅ are not shown.

107 (YM511)

109 (STX681)

111, Letrozole

Core structure for compounds
107-110 (Table 9)

R₁	R₂	R₃	R₄	R₅
H	H	H	H	H
F	F	F	F	F
Cl	Cl	Cl	Cl	Cl
Br	Br	Br		
	I	OH		
	OH	OMe		
	OMe	OSO ₂ NH ₂		
	OSO ₂ NH ₂	CF ₃		
	CN			
	CHF ₂			

Compound	R ₁	R ₂	R ₃	R ₄	R ₅	Aromatase IC ₅₀ (nM)	Sulfatase IC ₅₀ (nM)
107	H	H	Br	H	H	0.5	ND
108	H	H	OSO ₂ NH ₂	H	H	100	227
109	H	Br	OSO ₂ NH ₂	H	H	0.82	39
110	H	OSO ₂ NH ₂	F	H	H	0.77	590

Table 10

In vitro inhibition of aromatase and STS in JEG-3 cells. ND – Not determined. Data taken from reference 186. Data for many other compounds with different substituents at R₁, R₂ and X are not shown.

112

The variety of substituents on core structure **112** and the nature of the linker (X) between the (boxed) aromatase-interacting moiety and the sulfatase-interacting moiety

R₁
H
OH
OSO₂NH₂

R₂
H
F
Cl
Br
OH
OSO₂NH₂

X
CH₂
(CH₂)₂
(CH₂)₃
(CH₂)₂O
(CH₂)₃O
(CH₂)₄O
CH₂S
(CH₂)₂S
(CH₂)₃S
(CH₂)₄S
(CH₂)₅S
(CH₂)₁₀S
(CH₂)₂SCH₂
(CH₂)₃SO₂
(CH₂)₂NMeSO₂

113 n = 2, R₁ = OH, R₂ = H
114 n = 2, R₁ = OH, R₂ = Cl
115 n = 2, R₁ = OSO₂NH₂, R₂ = H
116 n = 2, R₁ = OSO₂NH₂, R₂ = Cl
117 n = 3, R₁ = OSO₂NH₂, R₂ = H
118 n = 5, R₁ = OSO₂NH₂, R₂ = H

119

Compound	Aromatase IC ₅₀ (nM)	Sulfatase IC ₅₀ (nM)	Compound	Aromatase IC ₅₀ (nM)	Sulfatase IC ₅₀ (nM)
108	100	227	116	0.45	1200
113	0.28	ND	117	0.51	>10000
114	0.26	ND	118	0.73	1000
115	0.42	3100	119	15	ND

Table 11

In vitro inhibition of aromatase and STS in JEG-3 cells. Data taken from reference 187.

<p>Core structure for compounds 120-126 (Table 11)</p> <p>R</p> <div style="display: flex; flex-wrap: wrap; justify-content: space-around;"> <div style="text-align: center;"> <p>120</p> </div> <div style="text-align: center;"> <p>122</p> </div> <div style="text-align: center;"> <p>121</p> </div> <div style="text-align: center;"> <p>123</p> </div> <div style="text-align: center;"> <p>124</p> </div> <div style="text-align: center;"> <p>125</p> </div> <div style="text-align: center;"> <p>126</p> </div> </div>					
Compound	Aromatase IC ₅₀ (nM)	Sulfatase IC ₅₀ (nM) or % inhibition at 10μM	Compound	Aromatase IC ₅₀ (nM)	Sulfatase IC ₅₀ (nM) or % inhibition at 10μM
120	5.2	42.6%	124	2.7	<10%
121	31	367	125	2.3	4%
122	2.1	26	126	4.0	44.9%
123	0.25	205			

Table 12

In vitro inhibition of aromatase and STS in JEG-3 cells. Data taken from reference 188.

<div style="text-align: center;"> <p>129</p> <p>The core structure for compounds 127 and 131-139 (Table 12)</p> <p>130</p> </div>					
Compound	R ₁	R ₂	R ₃	Aromatase IC ₅₀ (nM) or % inhibition at 1μM	Sulfatase IC ₅₀ (nM) or % inhibition at 10μM
127 (Anastrozole)	C(CH ₃) ₂ CN	CH ₃	CH ₃	1.5	-
130	-	-	-	<10%	38.5%
131		CH ₃	CH ₃	2.3	-
132		CH ₃	CH ₃	3.5	10.7%
133		CH ₃	CH ₃	4.2	3000
134		CH ₃	CH ₃	5.0	73.6%
135		-CH ₂ CH ₂ CH ₂ -		20.7	21.4%

136		-CH ₂ CH ₂ CH ₂ -		97.1%	75.3%
137		-CH ₂ CH ₂ -		3.4	<10%
138		CH ₃	CH ₃	5.5	<10%
139		CH ₃	CH ₃	25.5	21.5%

Table 13

In vitro inhibition of aromatase and STS in JEG-3 cells. Data taken from references 189–191.

<p>Core structure for compounds 111, 128 and 140-142 (Table 13)</p>						
Compound	R ₁	R ₂	R ₃	R ₄	Aromatase IC ₅₀ (nM)	Sulfatase IC ₅₀ (nM)
111 (Letrozole)	CN	H	CN	H	0.89	-
140	OSO ₂ NH ₂	H	OSO ₂ NH ₂	H	3044	>10000
141	OSO ₂ NH ₂	Br	CN	H	3.0	2600
141a <i>R</i> -(+)	OSO ₂ NH ₂	Br	CN	H	3.2	4633
141b <i>S</i> -(-)	OSO ₂ NH ₂	Br	CN	H	14.3	553
128 (Vorzole)	Cl	H	-N=N-N(Me)-		29	>10000
142	OSO ₂ NH ₂	H	-N=N-N(Me)-		29	>10000

Table 14

In vitro inhibition in JEG-3 cells of aromatase and STS activity. Data taken from references 193, 194.

<p style="text-align: center;">145</p>					
	R₁		R₂		
146	H		4-OSO ₂ NH ₂		
147	Br		4-OSO ₂ NH ₂		
148	<i>p</i> -OSO ₂ NH ₂		H		
149	<i>p</i> -OSO ₂ NH ₂ , <i>m</i> -F		H		
150	<i>p</i> -OSO ₂ NH ₂ , <i>m</i> -Cl		H		
151	<i>p</i> -OSO ₂ NH ₂ , <i>m</i> -Br		H		
152	<i>p</i> -OSO ₂ NH ₂		4-OMe		
153	<i>p</i> -OSO ₂ NH ₂		4-F		
154	<i>p</i> -OSO ₂ NH ₂		4-CN		

Compound	Aromatase IC ₅₀ (nM)	STS IC ₅₀ (nM)	Compound	Aromatase IC ₅₀ (nM)	STS IC ₅₀ (nM)
146	0.1	2900	151	0.018	0.13
147	0.05	2900	152	0.015	22
148	0.21	17	153	0.47	55
149	0.15	2.3	154	0.75	240
150	0.015	0.83			