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REVIEW ARTICLE

ESTRONE SULFATASE, A TARGET ENZYME IN THE TREATMENT OF HORMONE-DEPENDENT BREAST CANCER

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SUMMARY

The conversion of estrone sulfate (E1S) to estrone (E1) is catalyzed by the membrane-bound enzyme estrone sulfatase (ES). This provides a non-aromatase-based route to E1 and to the potent estrogen estradiol (E2) in postmenopausal women. Prolonged exposure to these estrogens has been shown to increase the risk of breast cancer, and as such, ES is a major target in the treatment of this disease, as the inhibition of this enzyme could lead to estrogen ablation and thus a decrease in the further stimulation of breast cancer cells. Researchers in the field have directed their efforts towards the design and synthesis of potential inhibitors of ES, and thus far only a limited number of compounds have progressed into clinic trials. Here, we discuss some of the major developments in the research conducted in an effort to develop potential inhibitors of ES. More specifically, the inhibitors are divided into two classes, steroidal and nonsteroidal, and within each of these major subdivisions we consider sulfamate derivatives and nonsulfamate derivatives that have shown promise.

INTRODUCTION

Beatson (1896) (1) demonstrated the relationship between the ovaries and breast cancer when he treated locally recurrent breast cancer involving bilateral oophorectomy, thereby establishing a clear link between estrogens and breast cancer development and progression. The long-term exposure to potential estrogens has been shown to increase the risk of developing hormone-dependent breast cancer (Table I) (2-4); in particular, estradiol (E2) (Fig. 1) has been shown to be the major mutagen responsible for the initiation and progression of estrogen-dependent tissue growth (5).

Table I. *Summary of established breast cancer risk factors (2-4).*

Factor	High risk	Low risk
Gender	Female	Male
Country of birth	N. America, N. Europe	Asia, Africa
Age of menarche/menopause	< 12/> 55 years	> 14/< 45 years
Age of first full-term pregnancy	> 30 years	< 20 years
Age	> 45 years	< 25 years
Relatives diagnosed at early age	Yes	No
History of breast cancer	Yes	No
History of other hormone-dependent cancer (endometrial or ovarian)	Yes	No
BRCA1/2	Yes	No
Family history of disease	Yes	No
Oral contraception/hormone replacement therapy (HRT)	Yes	No
Weight	Obese	Underweight

Estrogens are transported complexed with albumin or sex hormone-binding globulin (SHBG). Once free, estrogens enter the cell via passive diffusion and form a complex with an estrogen receptor (ER). The estrogen–ER complex undergoes dimerization with another estrogen–ER complex and the dimer then enters the nucleus to interact with the DNA at the estrogen-responsive elements, inducing transcription, followed by protein synthesis and subsequent cell division(s).

ER can also be stimulated by androgens, more specifically, androstenediol (ADIOL) (Fig. 2) (6, 7), which possesses much lower affinity for the ER compared to E2; however, due to the high levels of ADIOL present in the plasma (approximately 100 times those of E2), the interaction between ADIOL and the ER results in weak stimulation, but due to the increased concentration of the androgen within the body, an overall strong response is produced (8).

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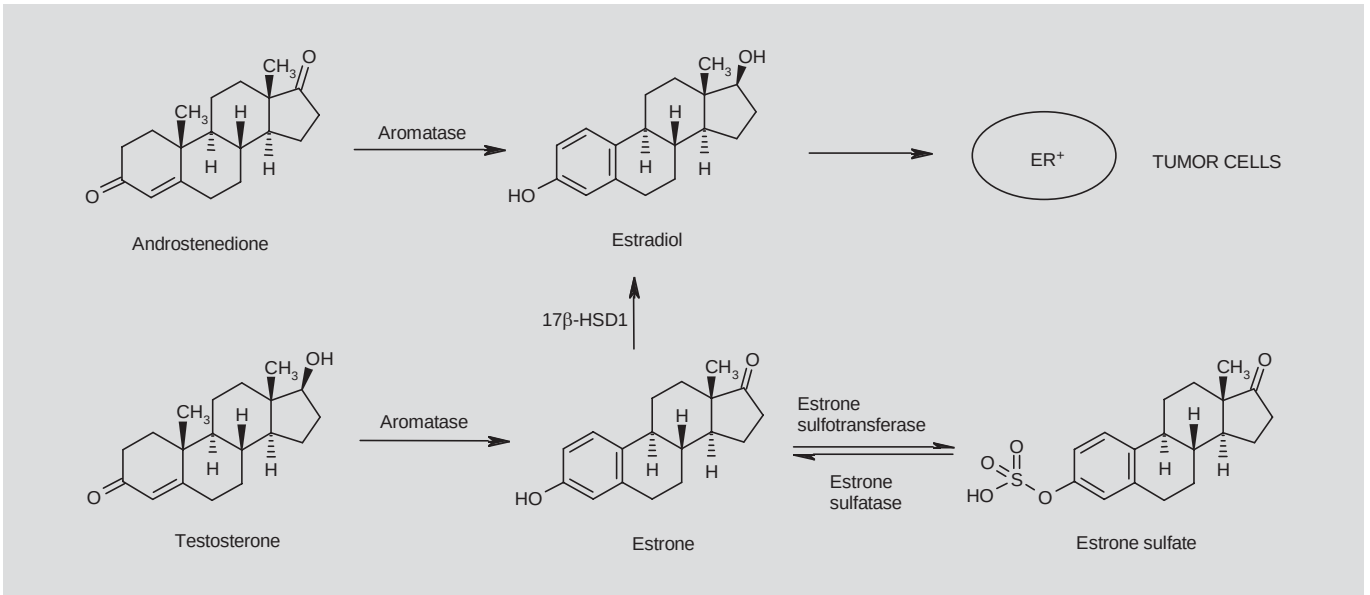


Figure 1. Diagram showing the biosynthetic routes to estrone and estradiol from the appropriate steroidal precursor.

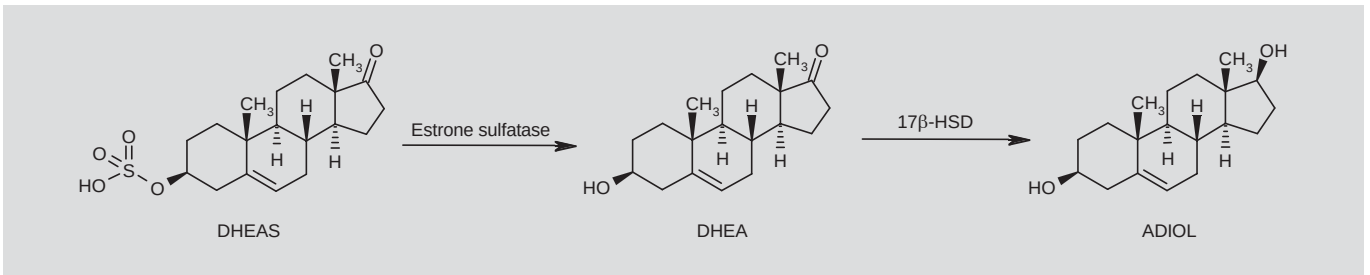


Figure 2. Involvement of estrone sulfatase in the biosynthesis of ADIOL.

The biosynthesis of estrogens involves three main enzymes (Fig. 1) within the overall steroidal cascade, namely: aromatase (AR), which catalyzes the conversion of C_{19} androgens to C_{18} estrogens involving aromatization of the steroid A ring (9, 10); type 1 17β-hydroxysteroid dehydrogenase (17β-HSD1), which catalyzes the conversion of estrone (E1) to E2 (11, 12); and estrone sulfatase (ES), which catalyzes the conversion of steroidal sulfates, in particular estrone sulfate (E1S), to the more potent steroid form, namely E1.

Membrane-bound ES is located in the endoplasmic reticulum (ER) and is produced within various types of tissues of the female body, e.g., liver, breast and placental tissues (13). Prior to the menopause, the ES pathway functions at a minimum, allowing AR to be the major route for the biosynthesis of E1 and E2 via the aromatization of androstenedione (AD) and testosterone (T), respectively (Fig. 1). After the menopause, the ES pathway becomes the major pathway for the biosynthesis of E1, which subsequently leads to the production of E2 involving 17β-HSD1 (Fig. 1). ES has therefore been proposed to be a key enzyme in the continued development of hormone-dependent breast cancer in postmenopausal women (14). ES inhibitors have also been demonstrated to be potentially useful in the treatment of other hormone-dependent diseases in women, such as endometrial cancer (15). Indeed, higher levels of ES activity have been reported in endometrial cancer cells in comparison to breast cancer cells; as such, the use of ES inhibitors may also have potential in decreasing the stimulation of estrogen-dependent endometrial cancer cells.

As previously mentioned, ADIOL has been shown to possess estrogenic properties. In this respect, ES has been found to catalyze the conversion of sulfated forms of androgens to the nonsulfated form; in particular, ES has been shown to catalyze the conversion of androstenediol sulfate (ADIOLS) to ADIOL. ES also plays a part in the conversion of dehydroepiandrosterone sulfate (DHEAS) to dehydroepiandrosterone (DHEA), which can be subsequently converted to ADIOL (16) (Fig. 2).

The desulfatation of androgens catalyzed by ES allows for the potential use of its inhibitors in the treatment of other hormone-dependent diseases, such as prostate cancer. For example, ES activity has been reported in the cells of the prostate, more specifically in LNCaP cells; as such, ES allows prostate cancer cells to biosynthesize DHEA, which can be converted to T and subsequently to dihydrotestosterone (DHT; involving 5α-reductase); the latter has been shown to be the more potent androgen, while prolonged exposure to

T and DHT is suggested to be the major etiological factor in the development and progression of prostatic diseases. As such, while clinical and chemical castration methods have been directed towards the elimination of T, ES offers an alternative biosynthetic route to T and DHT via the initial conversion of DHEAS to DHEA, which, if allowed to be biosynthesized within prostate cancer cells, would result in the continued stimulation of cancer cells. The inhibition of ES could aid the effort towards total androgen ablation and therefore result in a decrease in the stimulation of prostate cancer cells. Indeed, initial studies have shown that known ES inhibitors reduce ES activity within prostate cancer cells (17).

Targeting ES in the treatment of hormone-dependent breast cancer is still in its early stages, with no compound having entered the market that specifically targets ES, and only a limited number of compounds having entered clinical trials. ES activity has been shown to be higher in estrogen-dependent breast cancer tissue, resulting in the local production of E1; as such, ES is an important target, since the reduction of E1 biosynthesis would lead to a decrease in subsequent E2 biosynthesis and therefore a reduction in the stimulation of estrogen-dependent breast cancer cells (18, 19). Currently, only AR inhibitors have been shown to be highly useful in the clinic in the treatment of hormone-dependent breast cancer, and ES inhibitors would aid in total estrogen ablation. Thus, there is a great deal of interest in this field and a number of steroidal and nonsteroidal compounds are currently (or have been) under investigation, with recent compounds showing some promising results, suggesting they may be close to entering the market. The two major classes of inhibitors will now be considered, namely steroidal and nonsteroidal.

STEROIDAL INHIBITORS OF ESTRONE SULFATASE

The first steroidal inhibitor of ES discovered was danazol, a synthetic isoxazole derivative of 17α-ethynyltestosterone (Fig. 3) (20, 21), which was found to provide ~60% inhibition of ES at 10 μM in human breast adenocarcinoma MCF7 cells (22).

The level of biological activity observed with danazol led researchers to evaluate one of the natural substrates for ES, namely ADIOLS, which was found to be a reversible inhibitor, giving a K_i value of 2.0 μM against microsomal placental ES (23). Derivatization of the sulfate moiety was undertaken, leading to the discovery of estrone-3-*O*-methylphosphonothionate (E1-MTP) (Fig. 3), which was found to inhibit ES in a reversible, competitive and time-dependent manner; as such, E1-MTP was the first compound to be specifically designed for the inhibition of ES, showing an IC_{50} value of 90 nM against ES in intact MCF7 cells (14 times more potent than danazol). More importantly, E1-MTP was found to be more stable than ADIOLS, which suggested that a stable sulfonate-based compound could be synthesized against this biochemical target.

Further derivatization of the sulfonate moiety led to a series of promising but reversible inhibitors, eventually leading to the discovery of estrone-3-*O*-sulfamate (EMATE) (Fig. 3), which was found to be a highly potent inhibitor, displaying up to 95% inhibition at 2 nM (24, 25). More importantly, EMATE was found to irreversibly inhibit ES in a time- and concentration-dependent manner. However, clinical trials suggested that there were significant problems with the use of EMATE in terms of its chemical stability and the high level of estrogenic properties of both the initial sulfamated compound, as well as E1 resulting from the hydrolysis of the sulfamate moiety. As a result, it was withdrawn from clinical trials and further derivatization of EMATE was undertaken in an effort to reduce its estrogenicity (Tables II and III).

The derivatives of EMATE synthesized and evaluated against ES were, in general, found to possess weaker biological activity compared to EMATE, without a major decrease in estrogenic properties. However, four compounds were discovered to possess excellent inhibitory activity with decreased estrogenicity (Table III). Compounds **11** and **12** (STX-213) gave IC_{50} values of 12 and 1 nM, respectively (under similar conditions, EMATE gave an IC_{50} value of 8 nM), and STX-213 was therefore eight times more potent than EMATE. As a result of its potency, lack of estrogenicity and improved stability with respect to EMATE, STX-213 recently entered phase I clinical trials (26). Potent inhibitory activity was also observed for the benzyl derivative **15**, which showed an IC_{50} value of 3 nM, while the (3-pyridyl)methyl derivative **17** (IC_{50} = 1 nM) was found to be equipotent to STX-213. Furthermore, recent studies with STX-213 in rats have shown that this compound possesses an excellent pharmacokinetic profile (27), and as such could be the first ES inhibitor to enter the clinic.

Further derivatization of the C(17)=O functionality of EMATE was undertaken, resulting in a number of compounds (**19**-**38**) possessing potent inhibitory activity (Table IV) (28-30). However, only compound **38** was found to have extremely potent inhibitory activity in comparison to EMATE, with an IC_{50} value of 0.45 nM. An initial consideration of the structure–activity relationships (SAR) of compounds **27-38** suggested a strong correlation between the overall

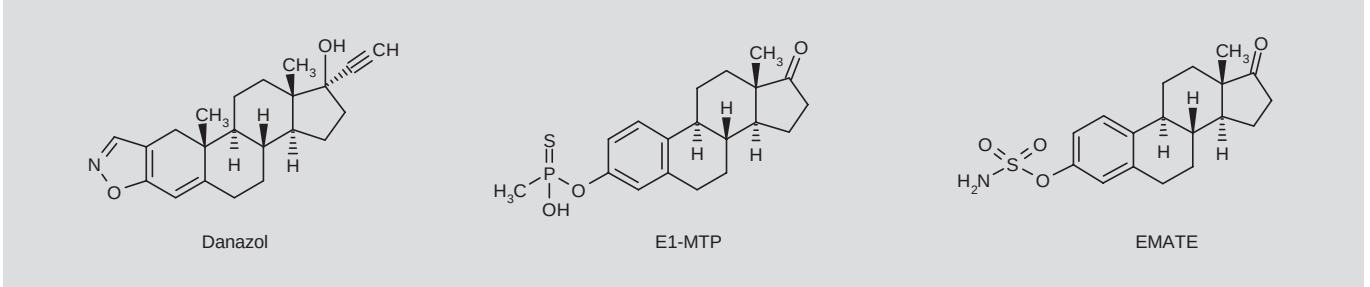
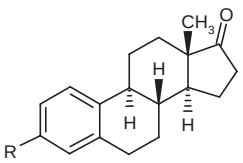


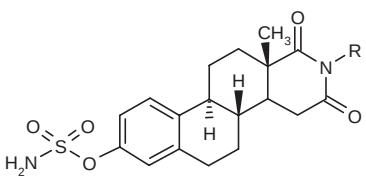
Figure 3. Initial steroidal inhibitors of estrone sulfatase.

Table II. *Estrone-derived estrone sulfatase inihibitors (32, 65-67).*



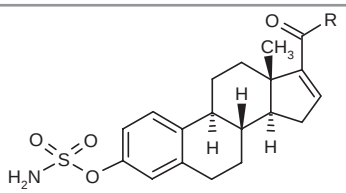
Compound	R	% Activity at concentration	Cell line
EMATE	OSO_2NH_2	99% at 0.1 μM	Intact MCF7 cells
1	$-OSO_2NHCH_3$	80% at 0.1 μM	Intact MCF7 cells
2	$-OSO_2N(CH_3)_2$	50% at 0.1 μM	Intact MCF7 cells
3	$-OSO_2C_6H_4CH_3$	30% at 0.1 μM	Intact MCF7 cells
4	$-OSO_2CH_3$	28% at 10 μM	Intact MCF7 cells
5	$-OSO_2C_4H_9$	17% at 10 μM	Intact MCF7 cells
6	$-OPO_2H$	80% at 10 μM	Intact MCF7 cells
7	$-OPO_2CH_3$	41% at 10 μM	Intact MCF7 cells
8	$-NHSO_2NH_2$	53% at 50 μM	Placental microsomes
9	$-SSO_2NH_2$	12% at 50 μM	Placental microsomes

Table III. *D-ring derivatives of EMATE evaluated against placental microsomes (68, 69).*



Compound	R	IC_{50} (nM)
EMATE	–	8
10	H	20
11	Me	12
12	Pr	1
13	C_5H_{11}	150
14	Cyclopropyl-CH_2	74
15	CH_2Ph	3
16	Vinyl	80
17	3-Pyr-CH_2	1
18	4-*t*-Bu-$PhCH_2$	> 100

Table IV. *C(17) derivatization of EMATE evaluated against purified estrone sulfatase (28, 30, 70).*

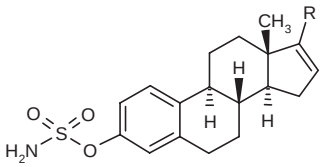


Compound	R	IC_{50} (nM)
19	NH-Cyclopentyl	1.0
20	$NH(CH_2)_2OMe$	2.8-15.5
21	NH-*i*-Pr	3.2-17.0
22	$N(Et)_2$	3.6
23	1-Pyrrolidinyl	4.4-6.0
24	$N(i\text{-}Pr)_2$	4.8
25	NHPr	5.0-19.0
26	NH-Allyl	5.6
27	(2-Pyridinyl)-NHNH	6.0
28	NH(4-COOH-Benzyl)	6.0
29	NH-2-Pyridazinyl	9.4
30	$NH(CH_2)_4OH$	9.8
31	$NH(CH_2)_5COOCH_3$	10.0
32	NHPh	10.0
33	OEt	12.0
34	NHMe	12.0
35	OPr	13.0
36	$NHC(CH_3)_2CH_2OH$	26.0
37	OH	50.0
38	$NH(CH_2)_6Me$	0.45

inhibitory activity and alkyl chain length of the substituent at the C(17) position of the steroid backbone.

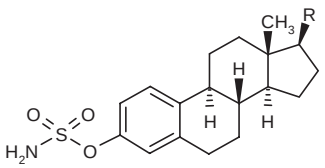
Further derivatization of the D-ring within EMATE involving the insertion of a C=C at the C(16) position of the steroidal backbone resulted in a range of highly potent inhibitors of ES (Table VA/VB). Indeed, compound **39** (IC_{50} = 0.02 nM) was found to be 400 times more potent than EMATE. The reduction of the C(16)-C(17) double bond, however, led to a derivative of **39** (compound **42**) which was found to be a weaker inhibitor than the parent C=C-containing com-

Table VA. *C(17) modifications of estrone derivatives evaluated against purified estrone sulfatase (29, 70).*



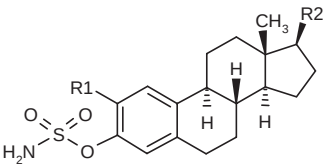
Compound	R	IC_{50} (nM)	Estrogenicity
39	Ac	0.02	Y
40	3-Pyridyl	12.0	N/A
41	$C(CH_3)_2OH$	13.0	N/A

Table VB. *C(17) modification of estrone derivatives evaluated against purified estrone sulfatase (29, 70).*



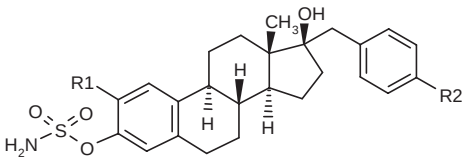
Compound	R	IC_{50} (nM)	Estrogenicity
42	Ac	2.0	N
43	Et	0.020	Y
44	Pr	0.034	N
45	Bu	0.096	N
46	(*E*)-Propylidene	0.027	Y
EMATE	–	8	Y

Table VI. *A-ring and C-(17) modifications of estrone derivatives evaluated against purified estrone sulfatase (29, 71-73).*



Compound	R_1	R_2	IC_{50} (nM)
47	CN	OAc	0.31
48	OMe	Et	1.5
49	OMe	OSO_2NH_2	32
50	Et	OSO_2NH_2	33
51	OEt	OSO_2NH_2	100

Table VII. *A-ring and C-(17) modifications of estrone-derived inhibitors evaluated against purified estrone sulfatase (71-73).*



Compound	R_1	R_2	IC_{50} (nM)
52	SMe	H	44
53	SMe	*t*-Bu	80
54	OMe	H	430
55	H	*t*-Bu	4300
EMATE	–	–	18

pound. However, the derivatization of the C(17) moiety within **42** led to a range of highly potent inhibitors, in particular compounds **43**, **44** and **46**, which were found to be equipotent to **39**. However, while **39** was found to possess estrogenic properties, compounds **44** and **45** did not. As such, the double bond appears to play an important role in determining the inhibitory activity and the estrogenic property of the inhibitor (29).

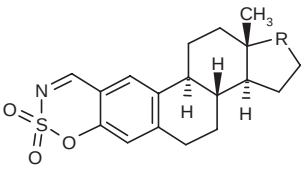
Derivatization of both the A- and D-ring was also investigated (Tables VI and VII); however, the modifications did not, in general, improve inhibitory activity significantly in comparison to EMATE. Compound **47** (IC_{50} = 0.31 nM) was found to be more potent than EMATE (Table VI) and contained small functionalities in both the A- and D-rings. The SAR consideration of these compounds suggested that the use of large bulky groups resulted in a reduction in the overall inhibitory activity (Table VII) (30).

The oxathiazine-based compounds involved major derivatization of the A-ring of the steroid backbone; however, the most potent compound was **56**, which was found to have an IC_{50} value of 9 nM in intact MCF7 cells and was therefore equipotent to EMATE (Table VIII). Continued derivatization of both ring A and ring D of the steroid backbone led to a range of irreversible inhibitors which possessed greatly reduced estrogenic properties in comparison to EMATE (31).

Derivatives of EMATE containing substituent(s) on the A-ring have also been studied, resulting in a number of inhibitors that were found to possess inhibitory activity equipotent to EMATE (Table IX). 4-Nitro-EMATE (**72**) (32) and 2-chloro-EMATE (**75**) (33) were found to have IC_{50} values of 0.8 nM against ES in human placental microsomes. The synthesis and subsequent evaluation of other substituted (nonhalogenated) compounds resulted in weak inhibitors in comparison to EMATE (34).

Researchers in the field have considered the use of nonsulfamated derivatives of E1, resulting in the synthesis of a small range of potent and irreversible inhibitors of ES. Compound **85** (the 3-formyl derivative of E1) (Fig. 4) was found to be the most potent inhibitor, with an IC_{50} value of 0.42 µM; for comparison, EMATE was found to have an IC_{50} value of 0.056 µM under the same conditions. However, the remaining compounds were shown to possess poor inhibitory activity; indeed, compounds **81**-**84** gave IC_{50} values of > 50 µM (35).

Table VIII. *Inhibition data for steroidal oxathiazine derivatives evaluated against intact MCF7 cells (31).*



Compound	R	IC_{50} (nM)
56	C=O	9
57	CH-OAc	12
58	CH-OH	20
59	$C=CHCH_3$ (Z)	63
60	$C=CHCH_2CH_3$ (Z)	58
61	$C=CHCH_2CH_3$ (E)	< 1000
62	$C=C=CH_2$	74
63	$CH-CH_3$	45
64	$CH-CH_2CH_3$	50
65	$CH-(CH_2)_2CH_3$	120
66	$C=CHCO_2Et$	36
67	$C=C(CN)_2$	22
68	$CH-OCH_3$	35
69	CH-αCl	62
EMATE	–	8

NONSTEROIDAL INHIBITORS OF ESTRONE SULFATASE

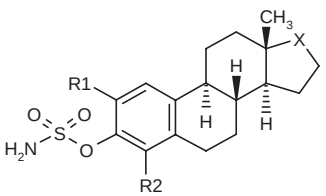
In an effort to overcome the inherent problems with steroidal inhibitors (e.g., estrogenic properties), the synthesis of nonsteroidal inhibitors was considered, since these compounds (lacking the steroidal backbone) were expected to possess greatly reduced estrogenic properties. One of the first series of nonsteroidal inhibitors considered was a substituted indole-based compound; the most potent was found to be 3-methyl-1-pentafluorophenylmethyl-6-sulfooxy-2-(4-sulfooxyphenyl)-4-trifluoromethyl indole (**86**) (Fig. 5), which proved to be a reversible, competitive inhibitor, with an IC_{50} value of 80 μM using partially purified enzyme from calf uterus (36).

The development of the nonsteroidal inhibitors mimicked that of steroidal inhibitors in that the majority of compounds incorporated the sulfamate moiety. Furthermore, the compounds were assumed to mimic aspects of the steroid backbone, in particular the four rings of the steroid backbone, which presumably conferred a degree of hydrophobicity to the inhibitor similar to that of the steroidal backbone.

A-ring mimetics

A series of compounds based on the 4-*O*-sulfamoyl-*N*-alkanoyltyramine backbone (Table X) were initially studied, with the most potent inhibitor being **94**, which gave an IC_{50} value of 56 nM (37). It was hypothesized that the phenylsulfamate moiety mimicked the A-ring of EMATE, while the alkyl moiety contributed to the overall hydrophobicity of the inhibitor. The most potent tyramine-based compound (**96**) (Fig. 6) showed an IC_{50} value of 0.4 nM (in

Table IX. *Examples of EMATE-derived irreversible estrone sulfatase inhibitors (32-34, 70, 71, 74, 75).*



Compound	X	R_1	R_2	Activity	Cell line	Inhibitory concentration (μM)
70	CH_2	H	H	97%	Intact MCF7 cells	0.01
71	C=O	NO_2	H	0.07 μM	PM	–
72	C=O	H	NO_2	0.8 nM	PM	–
73	C=NOH(E)	H	H	> 99%	Intact MCF7 cells	0.1
74	C=O	F	H	5.6 nM	PM	–
75	C=O	Cl	H	0.8 nM	PM	–
76	C=O	Br	H	1.7 nM	PM	–
77	C=O	I	H	6.1 nM	PM	–
78	C=O	OCH_2CH_3	H	2 nM	Intact MCF7 cells	–
79	C=O	OCH_3	H	30 nM	PM	–
80	$CH(\beta\text{-}SO_2NH_2)$	OCH_3	H	39 nM	PM	–
EMATE	–	–	–	8 nM	–	–

PM, placental microsomes.

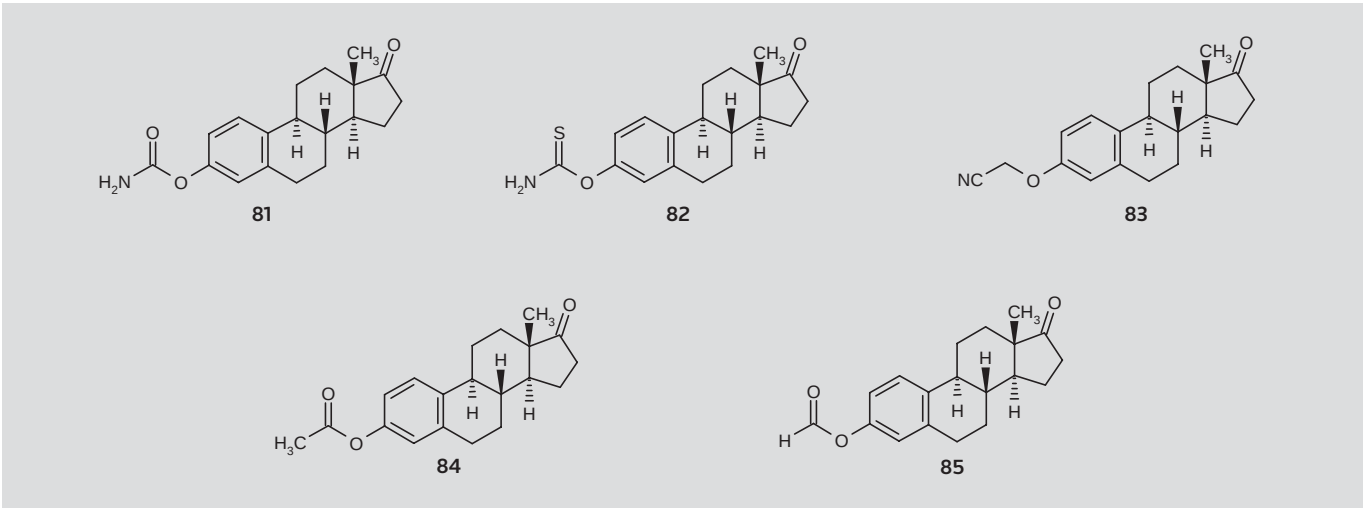


Figure 4. Non-sulfamoyl-based irreversible inhibitors of estrone sulfatase.

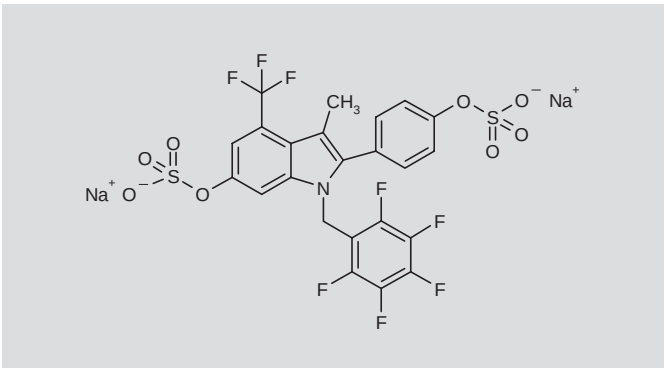


Figure 5. 3-Methyl-1-pentafluorophenylmethyl-6-sulfooxy-2-(4-sulfooxyphenyl)-4-trifluoromethylindole (**86**).

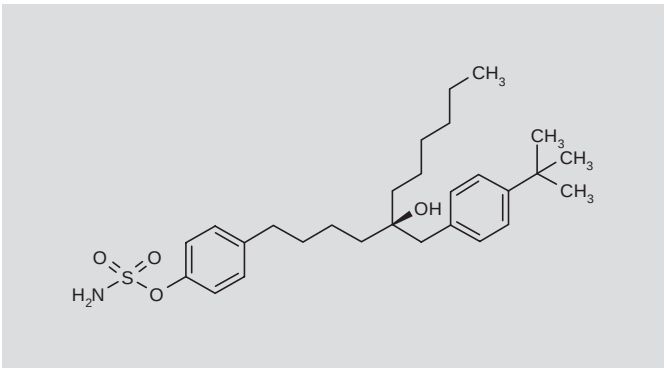
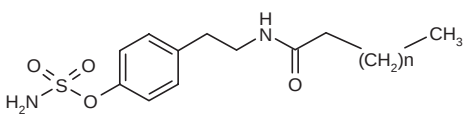


Figure 6. Potent inhibitor of estrone sulfatase (**96**) (38).

Table X. *First monoaryltyramine-based estrone sulfatase inhibitors (37).*

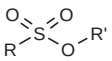


Compound	n	IC_{50} (nM)
87	4	14,300
88	5	1880
89	6	600
90	7	253
91	8	180
92	9	74
93	10	61
94	11	56
95	12	158

homogenates of HEK-293 cells transfected with ES), compared to 0.9 nM for EMATE (38). However, derivatization of the aliphatic chain (so as to increase hydrophobicity) did not improve the potency of these compounds; indeed, the inhibitory activity decreased with any change in the alkyl moiety.

In our group, we considered a series of sulfamated derivatives of phenol and straight-chain alkyl alcohol in an effort to determine the pharmacophore and in an attempt to discover highly potent inhibitors of ES. Our studies have shown that the aminosulfonated straight-chain compounds (Table XI), in particular the sulfamate derivatives of the unsubstituted straight-chain alcohol-based compounds **97**-**99**, were devoid of any inhibitory activity when evaluated up to an inhibitor concentration of 10 mM. However, the methanesulfonate derivatives of the unsubstituted straight-chain alcohol-based compounds **100** and **101** were found to possess weak inhibitory activity, e.g., **100** gave 28% inhibition at a concentration of 1 mM using human placental microsomes. Furthermore, β-halogenated derivatives of the straight-chain alcohol-based compounds were found to possess weak inhibitory activity, with **102** showing 60%

Table XI. *Sulfamated straight-chain alcohol inhibitors of estrone sulfatase using a placental microsome assay (41).*

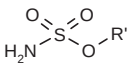


Compound	R	R′	Percent inhibition	Inhibitor concentration (mM)
97	NH_2	$CH_3(CH_2)_5CH_2$-	0	10
98	NH_2	$CH_3(CH_2)_6CH_2$-	0	10
99	NH_2	$CH_3(CH_2)_7CH_2$-	0	10
100	CH_3	$CH_3(CH_2)_7CH_2$-	28.0	1
101	CH_3	$CH_3(CH_2)_{10}CH_2$-	17.0	1
102	NH_2	Cl_3CCH_2-	60.0	1
103	NH_2	Cl_2CHCH_2	30.0	1
104	NH_2	$ClCH_2CH_2$	15.0	1
Coumate	–	–	99.5	1
EMATE	–	–	99.8	1

inhibition at 1 mM (39-42). While these compounds were weak inhibitors of ES, the latter series of compounds showed potential physicochemical properties that may play a part in the inhibition of ES. That is, the observation that β-substitution with halogen resulted in an increase in inhibitory activity suggested the involvement of the acid dissociation constant (pK_a).

As previously mentioned, we also considered the sulfamate derivatives of phenol in an attempt to develop extensive SAR, in particular, our hypothesis regarding the involvement of pK_a (Table XII). The results of the biochemical evaluation suggested that, in general, most of the sulfamate derivatives of phenol (and its substituted derivatives) possessed weak inhibitory activity in comparison to EMATE. The SAR analysis did, however, suggest a strong relationship between biological activity and pK_a. For example, compounds **107** and **119** were found to have IC_{50} values of 2089 and 120 μM (43), respectively, whereas they showed pK_a values of 10.0 and 8.28,

Table XII. *Sulfamated substituted phenolic inhibitors of estrone sulfatase using the placental microsome assay at an inhibitor concentration of 1 mM (41).*



Compound	R'	Percent inhibition	IC_{50} (μM)	pK_a
105	Phenyl	29.7	> 10,000	–
106	4-Me-Ph	27.4	> 10,000	10.2
107	3-Me-Ph	39.5	2089 ± 50	10.0
108	4-F-Ph	37.0	537 ± 21.2	9.8
109	3-F-Ph	79.6	2089 ± 50.0	9.16
110	4-Cl-Ph	37.6	1585 ± 66.1	9.5
111	3-Cl-Ph	62.0	537 ± 21.2	9.0
112	4-Br-Ph	58.8	912 ± 12.4	9.29
113	3-Br-Ph	75.1	257 ± 6.3	8.95
114	4-I-Ph	66.0	560 ± 16.2	–
115	3-I-Ph	89.4	120 ± 1.2	–
116	4-CN-Ph	74.4	300 ± 3.3	8.02
117	3-CN-Ph	84.3	191 ± 4.3	8.54
118	4-NO_2-Ph	82.5	330 ± 10.3	7.15
119	3-NO_2-Ph	90.4	120 ± 3.9	8.28
Coumate	–	99.5	12 ± 0.16	–
EMATE	–	99.8	0.5 ± 0.01	–

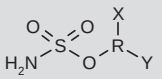
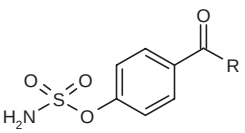


Figure 7. New pharmacophore for the inhibition of estrone sulfatase (R = aliphatic or aromatic carbon backbone; X = electron-withdrawing groups; Y = additional functionality including fused or adjacent/remote ring structures) (42).

respectively; that is, an increase in potency was observed with a decrease in pK_a. The study also suggested that an optimum pK_a of 8.3 was required for potent inhibitory activity and that the parent nonsulfamated compound possessing a strong acidic character would undergo self-hydrolysis during biochemical evaluation, leading to weak inhibitory activity. The two series of compounds developed allowed us to propose a pharmacophore for the development of new inhibitors of ES (Fig. 7).

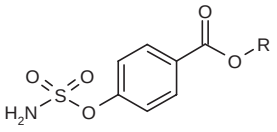
Based on the pharmacophore, a number of sulfamated derivatives of benzoic acid ester- and phenyl ketone-based compounds (Tables XIII and XIV) were synthesized and evaluated (44-47). The results showed that the compounds based on the 4-*O*-sulfamoylated derivatives of 4-hydroxyphenyl ketone-based inhibitors (**120**-**128**) were good inhibitors of ES. For example, **127** gave an IC_{50} value of 3.4 µM and was therefore less potent than EMATE; however, all the sulfamated compounds were found to be irreversible inhibitors, inhibiting ES in a time- and concentration-dependent manner (48). The 4-sulfamoylated derivative of the *n*-alkyl-based esters of 4-hydroxybenzoic acid (**129**-**138**) were found to be comparable in activity to the phenylketone-based compounds (Table XIV), e.g., compounds **134**, **135** and **136** showed IC_{50} values of 3.8, 3.4 and 5.0 µM, respectively.

Table XIII. *Sulfamated phenyl ketone inhibitors of estrone sulfatase using the placental microsome tissue assay (40).*



Compound	R	IC_{50} (µM)
120	H	254
121	CH_3	302
122	C_2H_5	116.4
123	C_3H_7	39.8
124	C_4H_9	20.9
125	C_6H_{13}	5.0
126	C_7H_{15}	5.6
127	C_8H_{17}	3.4
128	C_9H_{19}	13
EMATE	–	0.5

Table XIV. *Sulfamated phenyl ester inhibitors of estrone sulfatase evaluated in placental microsomes (48).*



Compound	R	IC_{50} (µM)
129	CH_3	31.6
130	C_2H_5	31.6
131	C_3H_7	13.2
132	C_4H_9	10.5
133	C_5H_{11}	5.9
134	C_6H_{13}	3.8
135	C_7H_{15}	3.4
136	C_8H_{17}	5.0
137	C_9H_{19}	4.8
138	$C_{10}H_{21}$	22.4
139	*c*-C_5H_9	9.3
140	*c*-C_6H_{11}	1.7
141	*c*-C_7H_{13}	0.5
142	*c*-C_8H_{15}	0.17
EMATE	–	0.5
Coumate	–	13.8
667-Coumate	–	0.21

The compounds were therefore, in general, found to be weaker than EMATE; however, compound **141** was equipotent, with an IC_{50} of 0.5 µM (48-50). Derivatization of the straight alkyl chain to a cycloalkyl moiety led to the development of compound **142** (IC_{50} = 0.17 µM using human placental microsomes), which was found to be an extremely potent inhibitor of ES in comparison to EMATE and 667-coumate (49).

AB-ring mimetics

Tetrahydronaphthol (THN) sulfamate derivatives were the first A- and B-ring mimetics to be synthesized (**143**-**148**; Fig. 8). Derivatization of the "B-ring" within the THN backbone led to a number of alternative series of inhibitors (**149**-**154**; Fig. 9), with **149** being the most potent irreversible inhibitor, with an IC_{50} of 1 mM in human placental microsomes (51).

The most significant series of AB-ring mimetics were based on the coumarin backbone and a number of coumarin-based sulfamate derivatives were synthesized (**155**-**160**; Table XV) with the parent inhibitor coumate. In general, the derivatization of the coumate backbone led to a potent series of inhibitors that were found to possess nonestrogenic activity and were found to inhibit ES in a time- and concentration-dependent manner, with compounds **157**, **159** and **160** showing 99% inhibition at 10 µM; indeed, **160** was found to exert 97% inhibitory activity at a concentration of 1 µM against human placental microsome-based ES. However, all of these compounds were found to be, in general, weaker than EMATE (52).

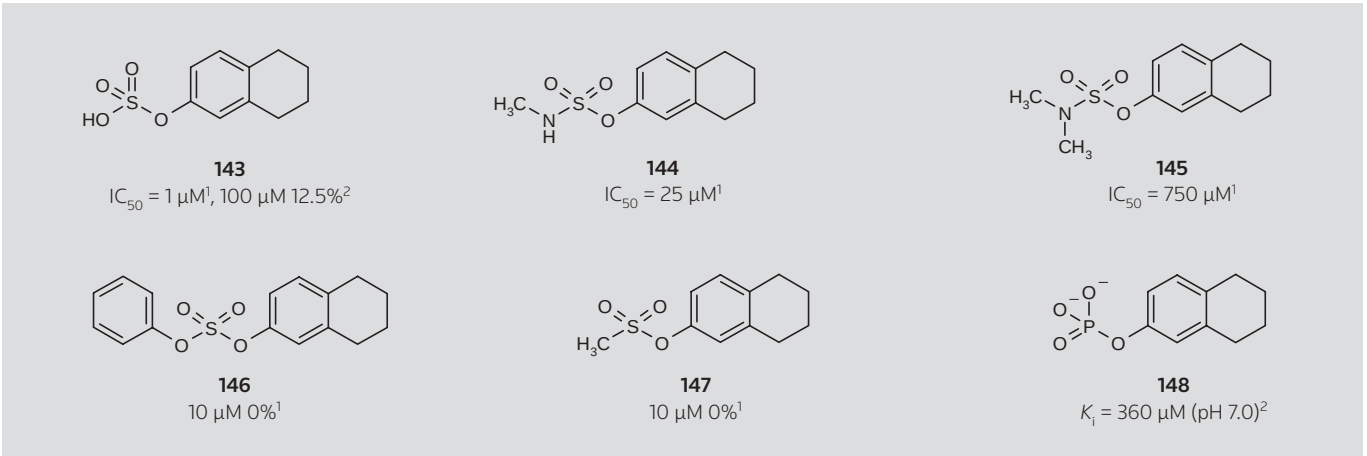


Figure 8. Tetrahydronaphthol sulfamate derivatives and activity. [1]Intact MCF7 breast cancer cell line assay; [2]human placental microsome assay (51).

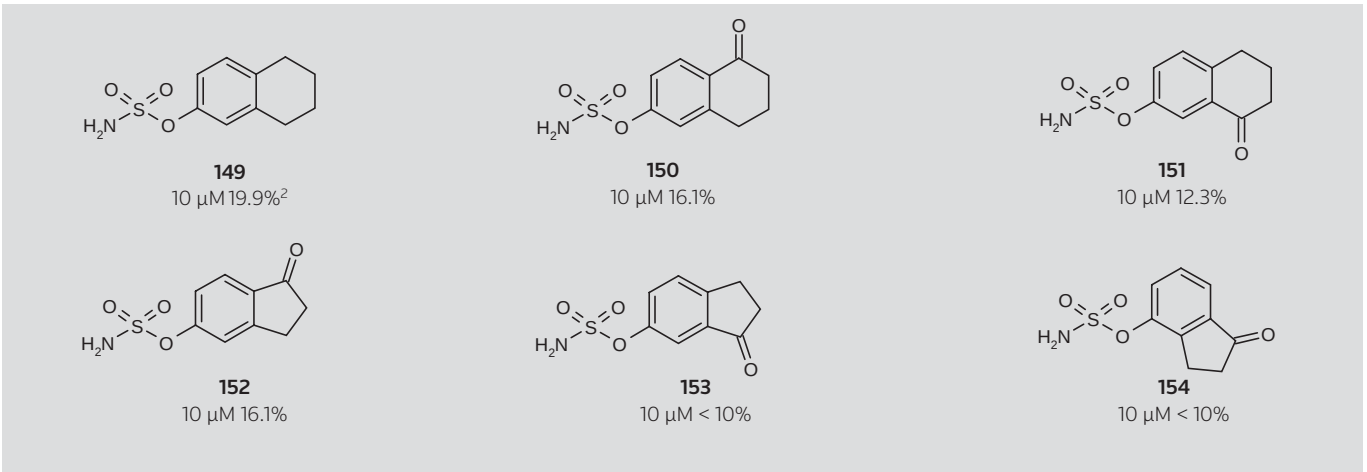
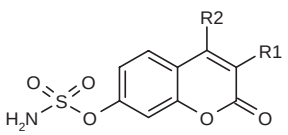


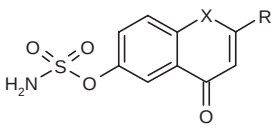
Figure 9. Modified tetrahydronaphthol sulfamate derivatives and their activity in the human placental microsome assay (51).

Table XV. *Coumate and derivatives and their respective activity (52).*



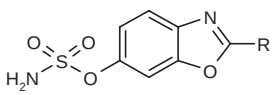
Compound	R_1	R_2	% Inhibition of estrone sulfatase in placental microsomes	
			1 μM	10 μM
Coumate	H	CH_3	63	93
155	H	H	–	78
156	CH_3	CH_3	88	97
157	H	CH_2CH_3	88	> 99
158	H	$CH_3CH_2CH_2$	94	96
159	CH_2CH_3	CH_3	96	> 99
160	$CH_3CH_2CH_2$	CH_3	97	> 99

Table XVI. *Modified aminosulfonated isocoumarin derivatives (76, 77).*



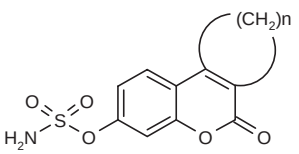
Compound	X	R	IC_{50} (nM)
161	O	*n*-Propyl	722
162	O	*n*-Nonyl	403
163	O	1,1-Dimethylnonyl	78
164	O	*t*-Butyl	22
165	O	4-Pentylbicyclo[2.2.2]oct-1-yl	11
166	O	1-Adamantyl	5.6
167	S	1-Adamantyl	0.34

Table XVII. *Modified aminosulfonated oxazolidine derivatives (78, 79).*



Compound	R	IC_{50} (nM)
168	1-Adamantyl	2800
169	(1-Adamantyl)methyl	1792
170	(2-Adamantylidene)methyl	196
171	Cyclohexylidenemethyl	319

Table XVIII. *Tricyclic 667-coumate derivatives and their respective inhibitory activity (52).*



		% Inhibition of estrone sulfatase in placental microsomes	
Compound	n	0.1 μM	1 μM
667-Coumate	5	91	> 99
172	3	37	91
173	4	63	93
174	6	89	> 99

Derivatization of the coumarin backbone led to further inhibitors that were found to possess potent inhibitory activity. Indeed, **166** and **167** showed IC_{50} values of 5.6 and 0.34 nM, respectively (Table XVI). Further derivatization of the coumarin backbone led to oxazolidine derivatives (Table XVII); however, these compounds were found to be weak inhibitors in comparison to coumate, e.g., **168** gave an IC_{50} value of 2800 nM and was therefore 500 times weaker than the corresponding coumate derivative.

ABC-ring mimetics

Currently, only the series of tricyclic derivatives of coumate exist which can be considered as ABC-ring mimics. These compounds (**172**-**175**; Table XVIII and Fig. 10) have proved to be extremely potent nonsteroidal inhibitors of ES. Although a number of these tricyclic compounds have been synthesized, only 667-coumate (or STX-64; IC_{50} = 8 nM in placental microsomes) has entered phase I clinical trials for the treatment of advanced metastatic estrogen-dependent breast cancer in postmenopausal women, since it was shown to be more potent than EMATE (IC_{50} = 25 nM) (52). This compound completed phase I clinical trials in 2005 and demonstrated acceptable pharmacokinetics when administered daily, with minor side effects. The compound has therefore been progressed to phase II clinical trials (53). In a recent study, 667-coumate was evaluated in parallel to STX-213 with regard to endometrial cancer (conducted in a mouse model) and STX-213 was shown to be the more effective in reducing tumor proliferation at 1 mg/kg (27).

ABD-ring mimetics

A number of different types of ABD-ring mimetics have been synthesized, although only the isoflavone sulfamate-based compounds (**176**-**180**) (Table XIX) have shown any promise as irreversible inhibitors (the parent compound **178** was shown to be highly estrogenic) of ES and were synthesized as the monosulfamate derivative (e.g., **179** was found to possess 83% inhibitory activity in intact MCF7 cells at 1 μM) or the bis-sulfamate derivative (e.g., **180** was found to be the most potent inhibitor, showing 90% inhibitory activity under similar conditions); however, the ABD-ring mimetics were found to be weaker inhibitors of ES in comparison to EMATE. A small number of sulfate derivatives were also synthesized, e.g., daidzein 4′,7-di-*O*-sulfate (**176**) and daidzein 4′-*O*-sulfate (**177**), which were found to be potent competitive inhibitors, giving IC_{50} values of 6 and 1.5 μM, respectively, and K_i values of 1 and 5.91 μM, respectively (54).

AC-ring mimetics

A series of AC-ring mimetics based on the biphenyl backbone were synthesized and biochemically evaluated (41, 42, 46). A series of 4-alkyl ester derivatives were also investigated (**181**-**184**; Table XX),

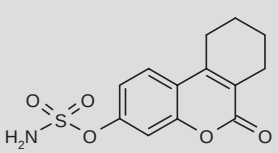
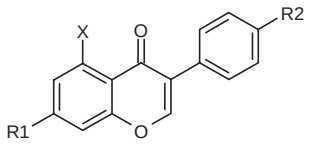


Figure 10. Tricyclic oxepin sulfamate **175** (52).

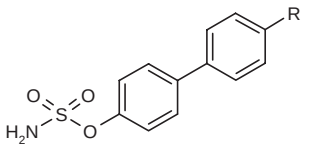
Table XIX. *Natural flavonoid-based inhibitors of estrone sulfatase (54).*



Compound	X	R_1	R_2	Activity
176	H	OSO_3^-	OSO_3^-	1 μM[a]
177	H	OH	OSO_3^-	5.9 μM[a]
178	H	OH	OH	0[b]
179	OH	OSO_2NH_2	H	83%[b]
180	OH	OSO_2NH_2	OSO_2NH_2	90%[b]

[a]K_i in μM; [b]percent inhibition of intact MCF7 cells at 1 μM.

Table XX. *Biphenyl-based inhibitors of estrone sulfatase (41-43).*



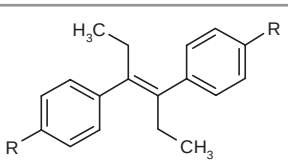
Compound	R	IC_{50} (μM)
EMATE	–	0.1
181	4-CN	6.7
182	4-COOMe	5.2
183	4-COOEt	4.2
184	4-COOPr	3.5
185	H	76

and proved to be weak inhibitors of ES when compared to EMATE but were found to be 10-20 times more potent than the unsubstituted biphenyl sulfamate **185**, which showed an IC_{50} value of 76 μM while EMATE under similar conditions gave an IC_{50} value of 0.1 μM.

AD-ring mimetics

AD-ring mimetics have thus far been based on the stilbene backbone (Table XXI) and, similar to the flavonoid-based inhibitors, include both the mono- and bis-sulfamated derivatives, with the latter showing greater inhibitory activity, e.g., compound **186** gave an IC_{50} value of 10 nM against ES in intact MCF7 cells. Derivatization of the sulfamate group to the dimethylsulfamate moiety (e.g., **187**) decreased inhibitory activity, presumably due to steric interactions; the compound showed an IC_{50} of 10 μM and also acted as a reversible inhibitor (55). However, due to the highly estrogenic nature of the parent backbone, in particular for (*E*)-stilbene, the use of these compounds has been more investigational (56).

Table XXI. *Examples of AD-ring mimetics as inhibitors evaluated in intact MCF7 cells (55).*



Compound	R	IC_{50}
186	OSO_2NH_2	10 nM
187	$OSO_2N(CH_3)_2$	10 μM

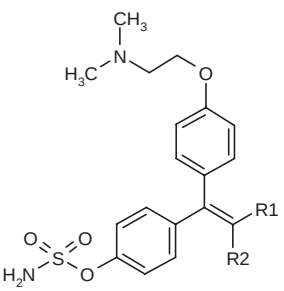
Hydroxytamoxifen sulfamate derivatives have also been investigated (Table XXII), in particular the (*E*)-isomer (**188**) and (*Z*)-isomer (**189**) of hydroxytamoxifen, and whereas previous sulfamate-based inhibitors were found to act as irreversible inhibitors, these two compounds proved to be reversible inhibitors (K_i = 35.9 and 500 μM, respectively); indeed, these compounds are the only sulfamate-based compounds to date that do not possess irreversible inhibition against ES (57).

Alternative inhibitors of ES

A novel series of compounds were prepared by Jütten et al. (59), who initially evaluated a series of derivatives of the natural product madurahydroxylactone (MHL) (Fig. 11), a secondary metabolite produced by the soil bacterium *Nonomuria rubra,* which were shown to possess potent inhibitory activity against ES. A number of thiosemicarbazone derivatives of MHL have been synthesized and evaluated; for example, **190** (Fig. 11) was found to have an IC_{50} of 0.46 μM compared to 0.08 μM for EMATE and 2.6 μM for coumate. Compound **190** was also shown to be a noncompetitive inhibitor, with a K_i value of 0.35 μM, and was devoid of estrogenic activity; these compounds also showed low acute toxicity. The active pharmacophore of **190** was considered and led to structure **191** (Fig. 11); derivatization of **191** led to the development of a series of compounds (**192-200**; Table XXIII) with inhibitory activity comparable to EMATE and 667-coumate (58, 59).

6-Adamantan-2-ylidene-hydroxybenzoxazole (**201**; Fig. 12) was derivatized to the formate derivative and evaluated against ES in comparison to the sulfamate derivative of **201**. It was observed that the formate derivative had an IC_{50} value of 1.5 μM compared to 0.26

Table XXII. *Hydroxytamoxifen sulfamate estrone sulfatase inhibitors in rat liver microsomes (57).*



Compound	R_1	R_2	K_{iapp}
188	Ph	Et	35.9 μM
189	Et	Ph	> 500 μM

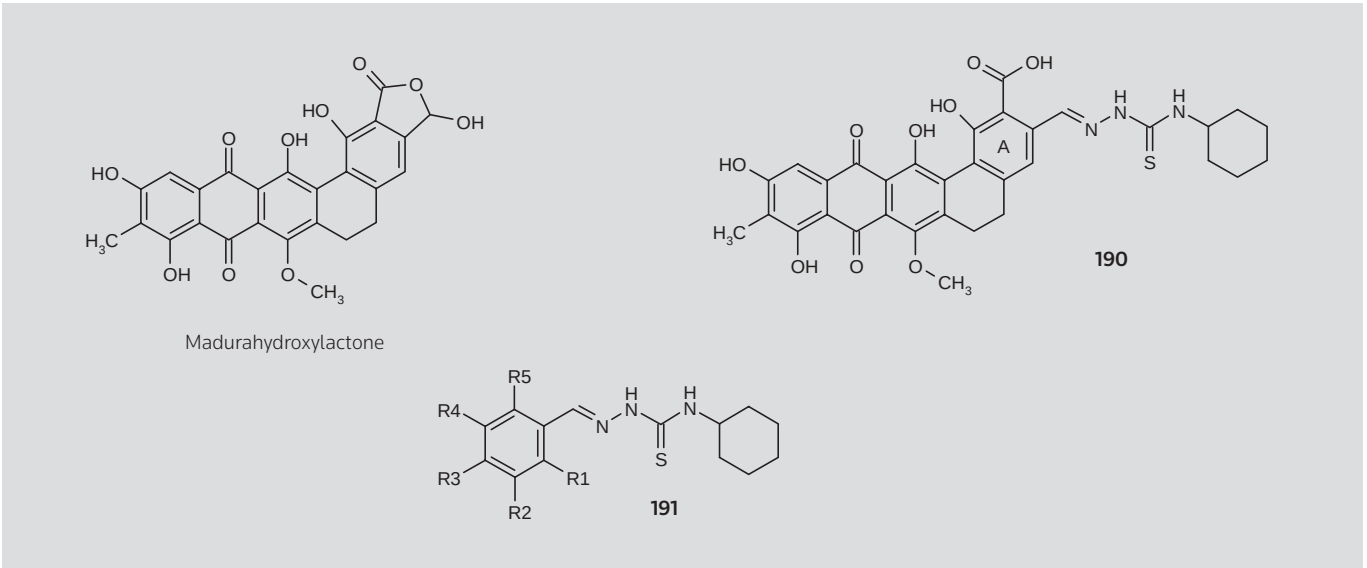
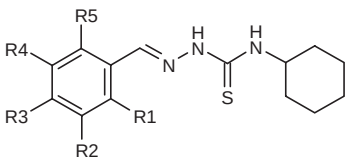


Figure 11. Madurahydroxylactone, madurahydroxylactone cyclohexylthiosemicarbazone (**190**) and the proposed active pharmacophore (**191**) (59).

Table XXIII. *Derivatives of **191** and their inhibitory activities in comparison to EMATE and 667-coumate (58, 59).*



Compound	R_1	R_2	R_3	R_4	R_5	IC_{50} (μM)
192	COOH	OH	H	H	H	4.2
193	COOH	OH	H	H	CH_3	2.5
194	COOH	OH	CH_3	H	H	0.73
195	OH	H	H	COOH	H	0.25
196	OH	COOH	H	H	H	0.15
197	H	COOH	OH	H	H	0.05
198	COOH	OH	OH	H	H	4.5
199	OH	H	H	SO_3Na	H	4.6
200	OH	H	H	COOH	H	5.0
EMATE	–	–	–	–	–	0.08
667-Coumate	–	–	–	–	–	2.6

μM for the sulfamate derivative of **201**, indicating that the formate derivative was less potent than the sulfamate derivative (35).

Nussbaumer et al. (60) reported a novel series of reversible inhibitors that inhibited ES in a non-time-dependent manner (**202**) and which included derivatives of the sulfamate moiety. Derivatization of **202** resulted in a series of potent inhibitors (Fig. 13, Table XXIV), of which compound **213** was found to be the most potent inhibitor, with an IC_{50} value of 1.89 μM; however, this compound was found to be 60 times weaker than EMATE.

Dual inhibitors

As previously mentioned, estrogens are also biosynthesized via the AR pathway from androgen precursors within breast cancer tissue, and as such, the inhibition of AR together with ES would be expect-

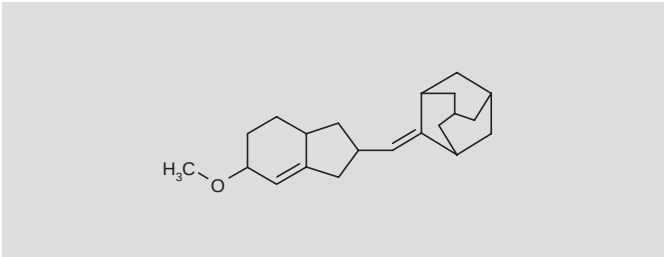
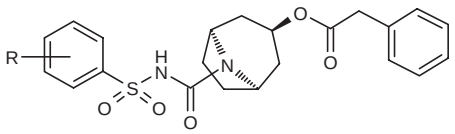


Figure 12. 6-Adamantan-2-ylidene hydroxybenzoxazole (**201**).

ed to provide an alternative mode of total inhibition of E1 biosynthesis. Dual inhibitors were initially introduced by Ahmed et al. (61) based on the phenyl azole backbone and have more recently been derivatized to the benzyl azole backbone in a similar manner to the potent AR inhibitors anastrozole and letrozole (Fig. 14). From this latter series of compounds, **214** (Fig. 15) was identified as the most potent, giving 83% and 70% inhibition, respectively, of ES and AR in JEG-3 cells; however, the compound was found to be a weak inhibitor in comparison to anastrozole and 667-coumate (62, 63). Additionally, Foster et al. (64) found that **STX-681** (Fig. 16) inhibited both AR- and ES-overexpressing MCF7 cells; indeed, this compound proved to be a better dual inhibitor in comparison to STX-64.

Table XXIV. *Novel nortropinyl-arylsulfonylureas as reversible inhibitors of estrone sulfatase (60).*



Compound	R	IC_{50} (μM)
207	4-Cl	6.72
208	4-F	39.2
209	4-Br	6.15
210	4-H	> 30
211	4-Me	37.1
212	4-CF_3	7.47
213	3,5-diCF_3	1.89

CONCLUSIONS

In conclusion, ES has proven to be an important enzyme in estrogen ablation, since potent estrogens have been shown to play a major role in the initiation and progression of hormone-dependent breast cancer. Thus far, however, only a limited number of compounds have

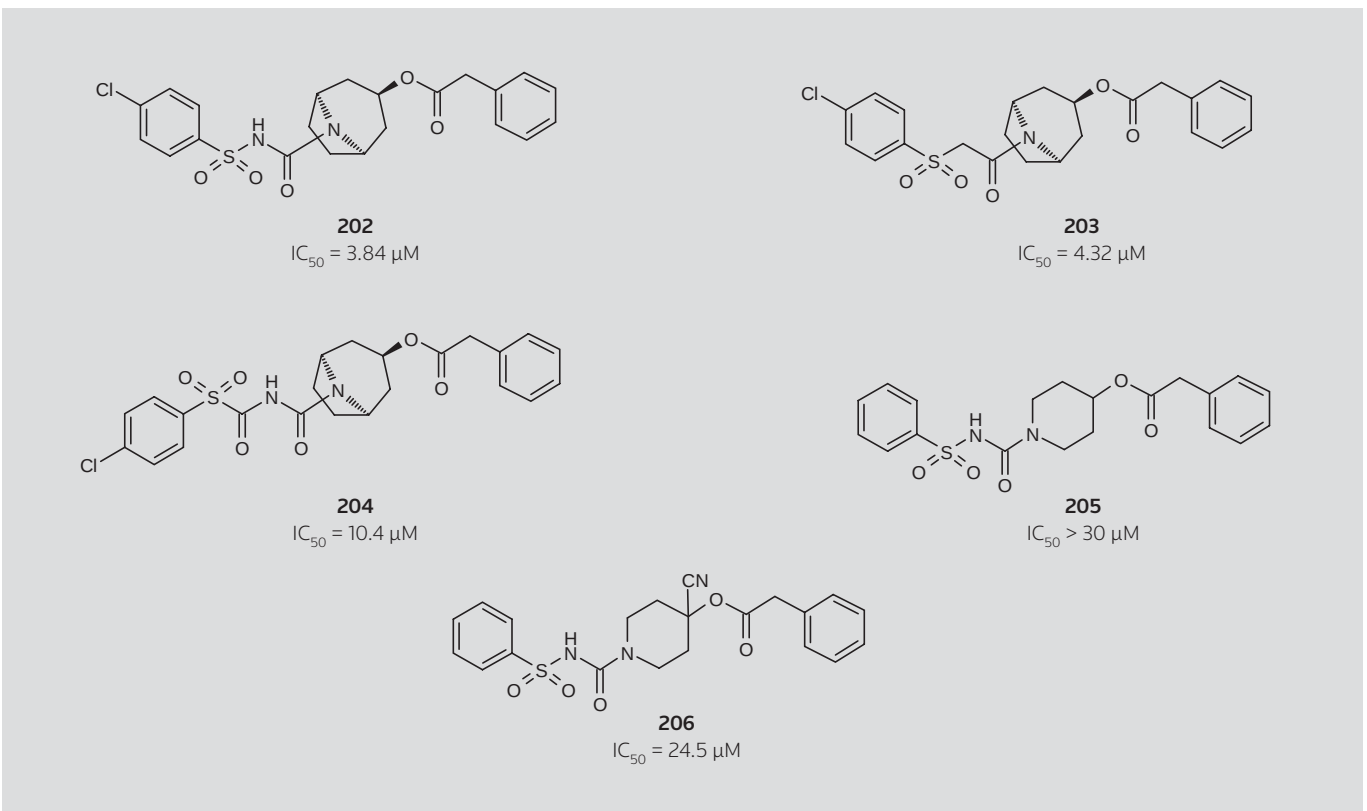


Figure 13. Novel nortropinyl-arylsulfonylureas as reversible inhibitors of estrone sulfatase (60).

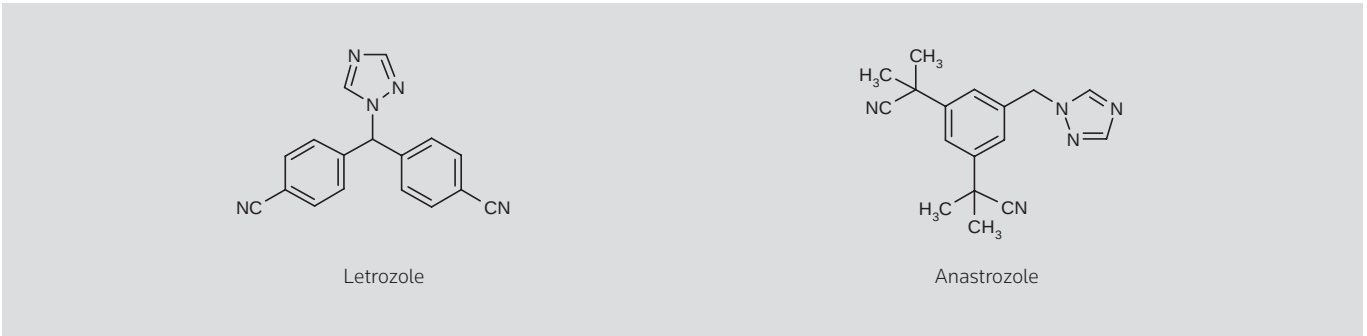


Figure 14. Aromatase inhibitors which have been used as templates for the development of "dual" inhibitors.

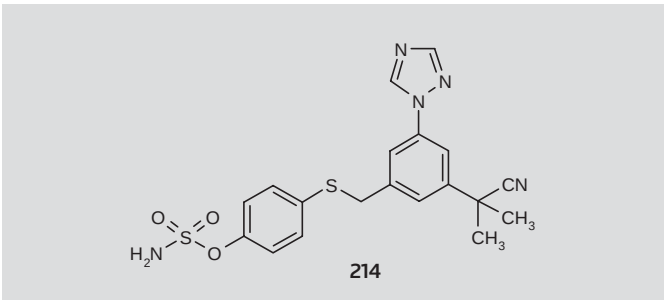


Figure 15. Dual inhibitor of estrone sulfatase and aromatase (63).

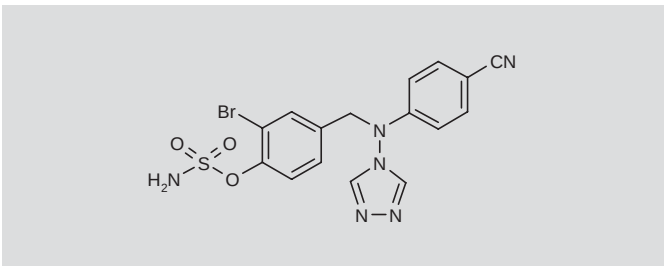


Figure 16. STX-681 (64).

entered clinical trials, with ST-213 (a steroidal inhibitor) proving to possess some potential as a drug substance for the treatment of hormone-dependent breast cancer. Additionally, STX-64 (a non-steroidal inhibitor) has now progressed into phase II clinical trials and STX-681 (an inhibitor of both AR and ES) appears to be a potential candidate in total estrogen ablation via inhibition of the two most important enzymes involved in estrogen biosynthesis.

DISCLOSURES

The authors state no conflicts of interest.

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