Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 8, No. 14, pp. 1891–1896, 1998

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

17α-ALKYL- OR 17α-SUBSTITUTED BENZYL-17β-ESTRADIOLS: A NEW FAMILY OF ESTRONE-SULFATASE INHIBITORS

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Received 8 April 1998; accepted 9 June 1998

Abstract: A series of 17α-derivatives of 17β-estradiol was synthesized and tested for their ability to inhibit the estrone-sulfatase activity transforming estrone sulfate to estrone. A strong inhibitory activity was obtained when an alkyl side chain or a substituted benzyl was introduced at position 17α of estradiol. The 17α-(3'-bromobenzyl)-estradiol (**26**) and 17α-(4'-*t*-butylbenzyl)-estradiol (**30**) were the most potent estrone-sulfatase inhibitors obtained in our study with IC_{50} values of 24 and 28 nM, respectively. They also represent a new family of estrone-sulfatase inhibitors. These compounds are about 300-fold more effective in interacting with the enzyme than the substrate estrone sulfate itself.

Introduction

It is well established that estrogens act as important endocrine growth factors for at least a third of breast cancers,[1] and that breast tumors are able to produce estrogen from circulating inactive precursors.[2-4] Among the enzymes involved in local biosynthesis of steroids, estrone sulfatase [E.C. 3.1.6.2], the enzyme that catalyzes the hydrolysis of estrone sulfate (E_1S) to the active hormone estrone (E_1), plays a key role in regulating estrogen formation in breast tumors.[4,5] Since estrone sulfate is the most abundant circulating C18-steroid in women,[6,7] the inhibition of estrone sulfatase is attractive to reduce the level of mitogenic estrone and estradiol (after reduction by 17β-HSD type 1). Thus, inhibitors of estrone sulfatase are potential agents for the treatment of estrogen-dependent diseases such as breast cancer (Fig. 1).

Over the past few years, several steroidal and nonsteroidal inhibitors of estrone sulfatase have been developed.[5] Most of these inhibitors have the common characteristic of an aromatic ring substituted at C3 (or pseudo C3 for nonsteroids) that mimicks the phenolic A-ring of the enzyme substrate, estrone sulfate. With the steroidal estrone nucleus, a wide variety of chemical groups was introduced at C3 to induce an inhibitory effect (**4-11**, Fig. 2).[5,8-20] The most potent inhibition was obtained with the sulfamate group, and estrone sulfamate (**4**, $R = OSO_2NH_2$) was found to inhibit estrone sulfatase in a time-dependent manner.[11] This inactivating group was thereafter added to a nonsteroidal nucleus such as tetrahydronaphthol (**12**),[21] coumarin (**13**),[22] diethylstilbestrol (**14**),[5] or N-alkanoyl tyramine (**15**).[23,24]

0960-894X/98/$19.00
PII: S0960-894X(98)00330-8

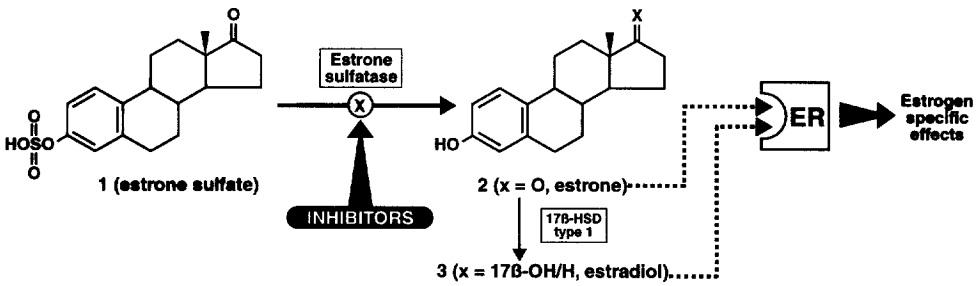


Figure 1. Biosynthesis of the active estrogens, estrone and estradiol, showing the key role of estrone sulfatase and its corresponding inhibitors; ER: estrogen receptor.

In the course of our studies on the development of 17β-hydroxysteroid dehydrogenase (17β-HSD) type 1 inhibitors,[25,26] we synthesized a series of 17α-substituted estradiols. These compounds were also tested for their ability to inhibit estrone-sulfatase activity. The results of this preliminary SAR-study were the starting point of our work on the development of estrone-sulfatase inhibitors.[27] In this communication, we report a new family of estrone-sulfatase inhibitors (**16-30**, Fig. 2). In contrast to the already known inhibitors of estrone sulfatase that contain a pharmacophore at position C3 of steroidal A-ring, the reported inhibitors have novel substituents located at another position (i.e., C17α of steroidal D-ring).

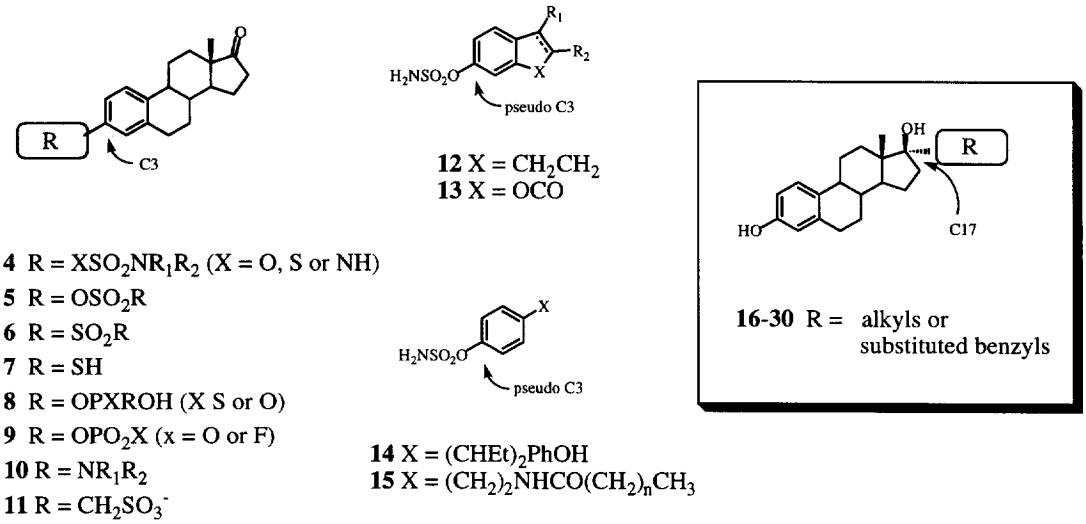


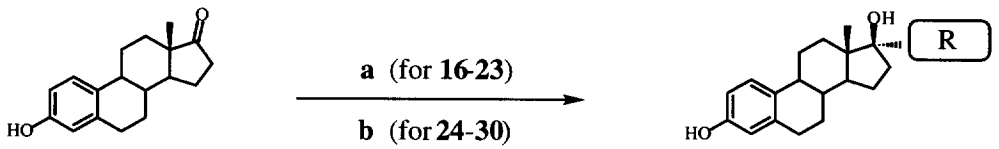
Figure 2. Chemical structures of known (**4-15**) and new (**16-30**) inhibitors of estrone sulfatase.

Chemistry

17α-(Alkyl or substituted benzyl)-estradiols (**16-30**) were directly synthesized by a stereoselective addition of an organolithium or organomagnesium reagent to the C17-ketone of estrone (Scheme 1). Since the methyl-18 on the β-face of steroid is known to direct the nucleophile attack on the less hindered α-face,[28] we obtained only the 17α-isomer. We found the use of lithium reagents suitable for alkyl addition, while magnesium reagents were suitable for benzyl (and substituted benzyls) addition. Indeed, the reaction of a hindered ketone, such as the C17-ketosteroid, with a Grignard reagent having a β-hydrogen (our alkyl series) gave mainly the product of reduction with a low yield of alkylated product. Although a new methodology ($Ce(III)Cl_3$ and RMgX) has been described recently by Li *et al.*,[28] we alternatively used an alkyllithium reagent for the introduction of alkyl groups (**16-23**). Alkyllithium was then prepared by the lithium-iodine exchange method (*t*-BuLi, *n*-pentane/ethyl ether) described by Bailey and Punzalan,[29] and added dropwise into a 0 °C-solution of estrone dissolved in dry THF. Compounds **16-23** were thus obtained in yields ranging from 30% to 63% with starting estrone as the other detectable product. The yields were not, however, corrected for the estrone recovered. Contrary to the alkyl series, it was possible to introduce benzyl or a substituted benzyl group to a C17-ketosteroid by a Grignard reaction. Appropriate Grignard reagents were first generated at 0 °C from substituted benzyl bromide or chloride in dry ethyl ether rather than in dry THF. A solution of estrone in dry THF was then added to a Grignard-reagent preparation giving 17α-benzylated derivatives **24-30** in yields 25% to 49%. As above, these yields were not optimized and corrected for the recovery of starting estrone, the other material observed. All compounds reported in this study (**16-30**) were characterized by FTIR, ^1H NMR, ^{13}C NMR, and MS analysis. In addition, purity was verified by HPLC and combustion analysis.

Enzymatic assay

The enzymatic reaction was carried out at 37 °C in 300 μL of 0.1 M Tris-acetate buffer (pH 7.0) containing 5 mM of ethylenediaminetetraacetate (EDTA), 10% glycerol, 7 nM of labeled estrone sulfate ($[^3H]$-E_1S), and tested compounds dissolved in ethanol or only ethanol for control (10 μL). After 1 h of incubation with homogenized JEG-3 cells, as the source of estrone-sulfatase activity, the reaction was stopped with an excess of unlabeled E_1S (225 μM) and the addition of xylene (1.25 mL). The tubes were then shaken and centrifuged at 2500 RPM for 10 min to separate organic and aqueous phases. Radioactivity in 750 μL of organic phase ($[^3H]$-E_1) was determined by liquid scintillating counting with a Beckman LS3801 (Irvine, CA). Data were expressed as % of E_1 produced (100% for control without inhibitor) versus the concentrations of the tested compound. The IC_{50} was calculated using an unweighted iterative least-squares method for 4-parameters logistic curve fitting (DE_{50} program, CHUL Research Center, Qc).



2 (estrone)

16-23 R = alkyls
24-30 R = substituted benzyls

Scheme 1. Synthesis of inhibitors **16-30**. (**a**) R(alkyl)-I, *t*-BuLi, *n*-pentane/Et_2O, THF; (**b**) R(substituted benzyl)-Br (or Cl), Mg, Et_2O, THF.

Table 1. Inhibition of estrone-sulfatase activity by 17α-(alkyl or substituted benzyl)-estradiols

Compounds	R	Substituent name	IC_{50} (nM) [a]
		ALKYL SERIES	
3	H	Hydride	84000
16	$(CH_2)_2CH_3$	propyl	5640
17	$(CH_2)_3CH_3$	butyl	3490
18	$(CH_2)_4CH_3$	pentyl	1980
19	$(CH_2)_5CH_3$	hexyl	930
20	$(CH_2)_6CH_3$	heptyl	780
21	$(CH_2)_7CH_3$	octyl	440
22	$(CH_2)_9CH_3$	decyl	~1000
23	$(CH_2)_{11}CH_3$	dodecyl	~6000
		BENZYL SERIES	
24	CH_2Ph	benzyl	310
25	CH_2Ph-2'-Br	2'-bromobenzyl	840
26	CH_2Ph-3'-Br	3'-bromobenzyl	24
27	CH_2Ph-3'-Cl	3'-chlorobenzyl	110
28	CH_2Ph-3',4'-Cl_2	3',4'-dichlorobenzyl	80
29	CH_2Ph-4'-OCH_3	4'-methoxybenzyl	110
30	CH_2Ph-4'-*t*-Bu	4'-*t*-butylbenzyl	28
E_1S (enzyme substrate)			7600

[a] Transformation of [^{3}H]-E_1S to [^{3}H]-E_1; error ± 10 %.

Inhibition of estrone sulfatase

The estrone sulfatase activity was assayed with homogenized JEG-3 cells by measuring the [^{3}H]-E_1 obtained from [^{3}H]-E_1S. The results were expressed as IC_{50} values. From a preliminary screening study (data not shown) with a series of 17α- or 16α-(propyl derivative)-estradiols initially synthesized as inhibitors of 17β-HSD type 1,[25,26] we observed that some compounds were more potent inhibitors of estrone-sulfatase activity than danazol (17β-hydroxy-17α-pregna-2,4-dien-20-ynol-[2,3-d]-isoxazol), the first reported inhibitor.[30] Indeed, compounds with allyl or a propyl group were better inhibitors than analogs with polar groups (alcohol or epoxide). In addition, a 17α-positioning of the novel substituents was found suitable to a 16α-positioning. From this preliminary SAR-study, it was decided to synthesize a series of estradiol derivatives bearing an alkyl, a benzyl, or a substituted benzyl group at position 17α (Table 1).[27]

For linear alkyl derivatives of estradiol (**16-23**), the inhibition of estrone sulfatase increased with the side-chain length and reached a maximum with the octyl group (**21**, IC_{50} = 440 nM). The high hydrophobicity induced by a longer alkyl side chain seems to be an important factor for inhibition of estrone sulfatase. However, a side chain that is too long, such as decyl (**22**) or dodecyl (**23**), provoked the opposite effect, probably by involving a steric hindrance. The 17α-benzyl-estradiol (**24**) was thereafter tested and its IC_{50} value (310 nM) was found slightly better than the IC_{50} value of the optimized alkyl derivative **21**. Preliminary substitution of a benzyl group led to further valuable information. Thus, a bromine or a chlorine atom in the *meta* (C3') position as well as a dihalogenated (C3' and C4') increased the potency of the inhibitor at the nano-molar level (24 nM for **26**). On the other hand, a halogen in *ortho* (C2') decreased the inhibitory activity. Interestingly, the two *para* (C4') alkyl substitutions depicted in Table 1 increased markedly the potency of this new family of inhibitors. The less polar 4'-*t*-butylbenzyl group provided a better enzyme inhibition than the more polar 4'-methoxy group (28 and 110 nM, respectively, for **30** and **29**). Both compounds are, however, more potent inhibitors than 17α-octyl-estradiol (**21**).

In summary, we have demonstrated the importance of a substituent at position 17α of 17β-estradiol for the inhibition of estrone-sulfatase activity. Until now, the development of inhibitors was exclusively based on a modification of the sulfate group at position 3 (A-ring) of the enzyme substrate. Herein we report for the first time that novel substituents located at steroidal position 17α (D-ring) inhibit strongly the estrone-sulfatase activity. Among the chemical groups studied, the substituted benzyls gave higher inhibition than alkyls. To date, the 17α-(3'-bromobenzyl)-estradiol (**26**) and 17α-(4'-*t*-butyl)-estradiol (**30**) were the most potent estrone-sulfatase inhibitors obtained in our study with IC_{50} values of 24 and 28 nM, respectively. These compounds are approximately 300-fold more effective in interacting with estrone sulfatase than the substrate E_1S itself. When compared to estrone sulfamate, the most potent known inhibitor of estrone sulfatase, compound **30** is only 7-fold less potent. Studies are now in progress to optimize this new family of inhibitors and to determine the mechanism of inhibition. These additional results will be reported as a full paper in due time.

Acknowledgements: We gratefully acknowledge the financial support of the Medical Research Council of Canada (MRC) and the Fonds de la Recherche en Santé du Québec (FRSQ). We are grateful to the Laboratory of Molecular Endocrinology (Dr. Fernand Labrie, Director) for providing chemical and biological facilities. We also thank Mr. Roger Lachance for performing the enzymatic assay.

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