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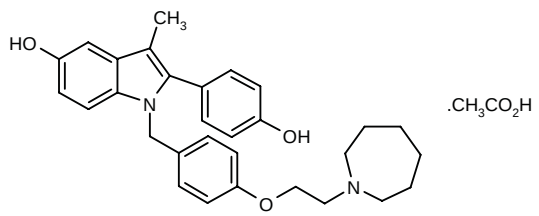
Bazedoxifene Acetate

Selective Estrogen Receptor Modulator

Treatment and Prevention of Osteoporosis

TSE-424
WAY-140424

2-(4-Hydroxyphenyl)-3-methyl-1-[4-[2-(perhydroazepin-1-yl)ethoxy]benzyl]-1H-indol-5-ol acetate



$C_{30}H_{34}N_2O_3.C_2H4O_2$

Mol wt: 530.6612

CAS: 198481-33-3

CAS: 198481-32-2 (as free base)

EN: 257955

Abstract

In order to develop a new tissue selective estrogen with superior preclinical pharmacology, a multifaceted approach to screening/characterization was employed utilizing a series of *in vitro* and *in vivo* models. *In vitro* transcription data demonstrate that all SERMs are not equivalent. This was especially evident when assessing hepatic lipase promoter activation where bazedoxifene was a relatively potent agonist and raloxifene was inactive. When bazedoxifene and raloxifene uterine responses are compared they are different enough that when codosed, bazedoxifene inhibits the stimulation of the rodent uterus attributable to raloxifene. There are several preclinical endpoints where the two drugs differ, some subtler than others. The structural differences between bazedoxifene and raloxifene are apparently sufficient to confer differences in their pharmacology, which also supports the potential for development of more refined molecules that could achieve the optimal therapeutic target profile for a SERM. Bazedoxifene acetate's preclinical profile suggests that it is closer to this profile than other currently disclosed SERMs and is "best in class" to date.

Synthesis

The discovery synthetic route leading to bazedoxifene has been described in the chemical literature (1) and is shown in Scheme 1. Briefly, a Bischler indole synthesis between α-bromopropiophenone (I) and 4-(benzyloxy)aniline hydrochloride (II) yielded the 3-methyl indole core (III). The side chain precursor was prepared by alkylation of 4-(hydroxybenzyl alcohol (IV) with ethyl bromoacetate followed by conversion of the benzyl alcohol to the benzyl chloride with $SOCl_2$. The side chain precursor (V) was appended to the core indole in the presence of NaH in DMF. The indole (VI) was subsequently and sequentially treated with $LiAlH_4$, triphenylphosphine/ CBr_4 and hexamethyleneimine (homopiperidine) to render the protected precursor of bazedoxifene free base (VII). Lastly, hydrogenolysis of the benzyl protecting groups followed by treatment of the free base with AcOH in acetone delivered bazedoxifene as its acetic acid salt (1). An alternative, more convergent synthesis for this class of compounds has been described in the patent literature (2).

Description

White crystalline solid, m.p. 170.5-2.5 °C.

Introduction

Classically, estrogens are known as sex steroids affecting the reproductive tract that are required for the development of secondary sexual characteristics. However, estrogens are no longer regarded as strictly reproductive hormones and the appreciation of their effect on many organ systems has grown considerably in recent years. For example, although hormone replacement therapy was first developed to relieve menopausal

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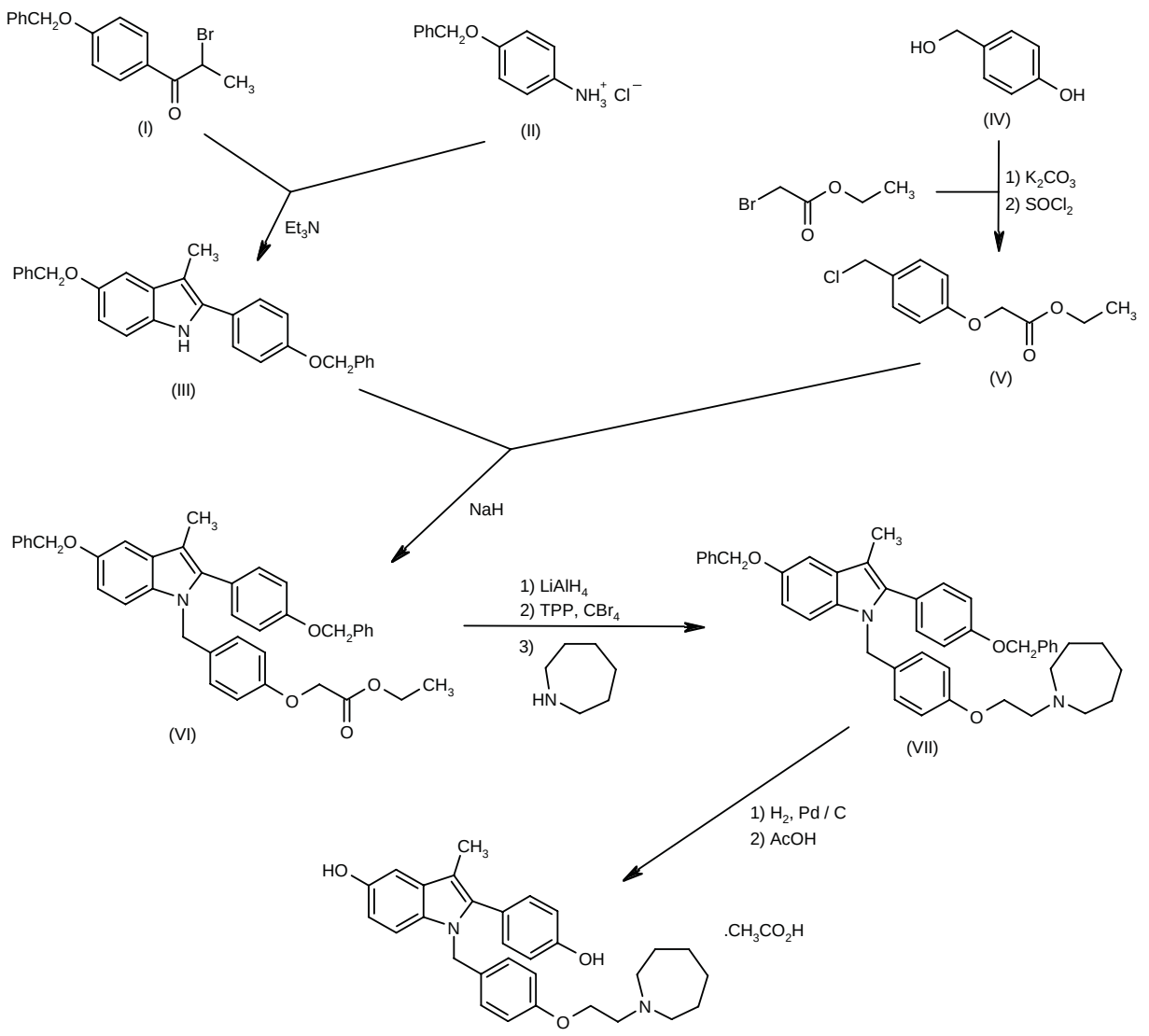
Scheme 1: Synthesis of Bazedoxifene Acetate



hot flushes, its indications have expanded to include the treatment of vaginal atrophy and prevention of osteoporosis. Partly because such large numbers of women have used replacement therapy, a sizable body of evidence has accumulated strongly suggesting that estrogens have beneficial effects on many other organs including the bladder (improving tone and reducing incontinence), colon (reduction of cancer risk), brain (improved cognition) and cardiovascular system (improved lipid profile, reduction of risk of disease).

Estrogens can exert their effects on cells in several ways, and the most well-characterized mechanism of action is via their interaction with estrogen receptors (ERs), leading to alterations in gene transcription. To date, two ERs have been discovered: ERα and ERβ. ERs are ligand-activated transcription factors and belong to the nuclear hormone receptor superfamily. Other members of this family include the progesterone, androgen, glucocorticoid and mineralocorticoid receptors. Upon binding ligand, ERs dimerize and can activate gene transcription either by directly binding to specific sequences on DNA (known as response elements) or by interacting with other transcription factors (such as AP1), which in turn bind directly to specific DNA sequences (3-5). A variety of coregulatory proteins can also interact with the ligand-bound receptor and further modulate its transcriptional activity (6). It has also been shown that estrogen receptors can suppress NFκB-mediated transcription in a ligand-dependent manner (7-9). Thus, ER signaling is a complex process with many opportunities for intervention.

The development of therapeutically useful and selective ER ligands has become possible, not only because of our increased understanding of the complexities of estrogen biology, but also because a wide variety of ER ligands have been developed over the years, some of which exhibit unexpected activities. ERs have a relatively large and flexible binding pocket (10) which can accommodate structurally diverse ligands. These ligands include steroids (e.g., 17β-estradiol, estrone), phytoestrogens (*e.g.*, genistein, coumestrol) and xenobiotics (*e.g.*, polychlorinated biphenols). Traditionally, compounds having roughly the same biological effects as 17β-estradiol, the most potent endogenous estrogen, are referred to as ER agonists. Those which, when given in combination with 17β-estradiol, block its effects are called ER antagonists.

It has been known for some time that estrogen receptors adopt different conformations when binding ligands. However, the consequence and subtlety of these changes has only recently been revealed. The three-dimensional structures of ERα and ERβ have been solved by co-crystallization with various ligands and clearly show the repositioning of helix 12 in the presence of an estrogen receptor antagonist which sterically hinders the protein sequences required for receptor-coregulatory protein interaction (11, 12). In addition, the technique of phage display has been used to identify peptides that interact with estrogen receptors in the presence of different ligands (13). For example, a peptide was identified that distinguished between ERα bound to the full estrogen receptor agonists 17β-estradiol and diethylstilbesterol. A different peptide was shown to distinguish between clomiphene bound to ERα and ERβ. These data indicate that each ligand potentially places the receptor in a unique and unpredictable conformation that is likely to have distinct biological activities.

As mentioned above, ER ligands have been historically classified as ER agonists or antagonists. In reality, there is a continuum between these activities and indeed some compounds behave as estrogen receptor agonists in some tissues and estrogen receptor antagonists in others. The precise reason why the same compound can have cell-specific effects has not been elucidated, but the differences in receptor conformation and/or in the milieu of coregulatory proteins have been suggested.

Tamoxifen, a case in point, was initially developed as an ER antagonist for breast cancer treatment. It was subsequently discovered that, while an ER antagonist in the breast, it had ER agonist activity in the bone and uterus (14). This unexpected finding of mixed ER agonist and antagonist activity within a single compound spawned efforts to develop other selective compounds with improved profiles, and now tamoxifen is referred to as a first-generation SERM or tissue-selective estrogen (15, 16).

The development of raloxifene (a second-generation SERM), originally aimed at the breast cancer treatment market to compete with tamoxifen, was redirected toward the treatment and prevention of osteoporosis in postmenopausal women. Preclinical data revealed that it spared bone and lowered LDL cholesterol, while demonstrating minimal estrogenic impact on the uterus and mammary gland. Other SERMs including levormeloxifene, idoxifene and droloxifene were also placed into clinical trials for different indications but were dropped from development as all three compounds displayed uterine stimulatory effects preclinically, clinically or both (17-19). In fact, raloxifene is not without side effects. While the effects on the uterine endometrium are currently reported as not clinically relevant, raloxifene has been associated with an increased incidence of hot flushes (20, 21). This effect is problematic given that women experience accelerated bone loss during the first 5-7 years of menopause, making raloxifene unsuitable for use in this population. Thus, there is apparently room for improvement in the SERM pharmacological profile.

Two tissue-selective estrogens, lasofoxifene and bazedoxifene, are in development for the primary indication of prevention and treatment of osteoporosis. Lasofoxifene (Pfizer) preclinical data have been described in the literature (22). Bazedoxifene acetate's (Wyeth-Ayerst) preclinical characterization has shown it to be competitive within the class with improved uterine characteristics compared to raloxifene and lasofoxifene. Additionally, the CNS effects of bazedoxifene are predicted to be minimal based upon preclinical models of vasomotor instability (23).

Pharmacological Actions

In vitro *activity*

Bazedoxifene competitively inhibits 17β-estradiol binding to both ERα ($K_i \cong 0.1$ nM) and ERβ ($K_i \cong 0.3$ nM). In the MCF-7 cell line (human breast cancer cell line) transfected with an ERE-tk luciferase reporter, bazedoxifene showed no agonist activity when dosed alone, but inhibited the 17β-estradiol transactivation (EC_{50} = 0.03 nM) with an IC_{50} = 3.7 nM (1). Bazedoxifene inhibited the 17β-estradiol stimulated (EC_{50} = 0.01 nM) proliferation of MCF-7 cells with an IC_{50} = 0.19 nM. In HEPG-2 cells (human liver cells) transfected with promoters associated with estrogen action in the cardiovascular system such as Apo (a) and hepatic lipase, bazedoxifene displayed estrogen-like action by inhibiting activity on these promoter systems (EC_{50} = 130 nM and 215 nM, respectively). This was in contrast to either tamoxifen or raloxifene, which were either inactive or only weakly active on these promoters (24). The ability of bazedoxifene to bind competitvely to ERs while exhibiting estrogen-like activity in a promoter and cell-type selective manner is the hallmark of SERM-type action and the prominent characteristic of this drug.

In vivo *activity*

Bazedoxifene's primary indication is the treatment and prevention of postmenopausal osteoporosis and its

preclinical activity for this indication was assessed in the 6-week ovariectomized rat model of osteopenia. In this model, sexually mature female rats were ovariectomized and treated with a subcutaneous dose of 17β-estradiol (2 μg/rat) or an oral dose of ethinyl estradiol (0.3 mg/kg), orally administered bazedoxifene at various doses or with vehicle. Bone mineral density (BMD) was analyzed by peripheral quantitative computerized tomography (pQCT) or by dual energy x-ray absorptiometry (pDEXA). Vehicle-treated, ovariectomized animals lost significant BMD at the proximal tibia and lumbar vertebrae relative to sham operated animals, while administration of 17β-estradiol or bazedoxifene (doses of 0.3 mg/kg and above) maintained BMD (1). Additionally, the maintenance of BMD by bazedoxifene was evaluated in compressive force testing analysis on bone cores taken from the L4 vertebra. While ovariectomy results in a loss of resistance to compressive force, bazedoxifene at doses of more than or equal to 0.3 mg/kg demonstrated compressive force resistance equivalent to sham operated and estradiol-treated animals (24). In addition to displaying estrogen-like agonist activity on preventing bone loss in this model, bazedoxifene-treated animals significantly reduced total cholesterol levels with doses as low as 0.1 mg/kg.

In contrast to bazedoxifene's beneficial estrogenic action on bone and cholesterol, there is no evidence from preclinical models that it shows an estrogenic, stimulatory effect on the uterus. For example, bazedoxifene was dosed subcutaneously (0.2 and 2.0 mg/kg) in immature female rats without eliciting an increase in uterine weight or endometrial epithelial cell hypertrophy/hyperplasia. This was in contrast to what was observed with ethinyl estradiol or raloxifene. Both of these compounds caused significant increases in uterine wet weight, albeit to different degrees (1). Interestingly, when a 0.2 mg/kg dose of raloxifene was combined with increasing doses of bazedoxifene, the uterine stimulation caused by raloxifene was reversed. The combination treated animals' uterine wet weights remained at control levels and histological changes associated with raloxifene were normalized (24). These uterine data suggest that bazedoxifene is a more selective SERM than raloxifene and, combined with the lack of an estrogenic agonist effect on MCF-7 cell proliferation, support the contention that bazedoxifene will not stimulate the uterus and breast which are the primary tissues of concern when contemplating the risks associated with postmenopausal treatment with hormonal therapies.

Clinical Studies

The phase I and II data reveal bazedoxifene to be safe, very well tolerated and efficacious on biochemical markers of bone metabolism and lipid metabolism, correlating well with the preclinical pharmacology (25). The phase III trials for bazedoxifene with comparisons to raloxifene are currently in progress.

Source

Wyeth Research (US).

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