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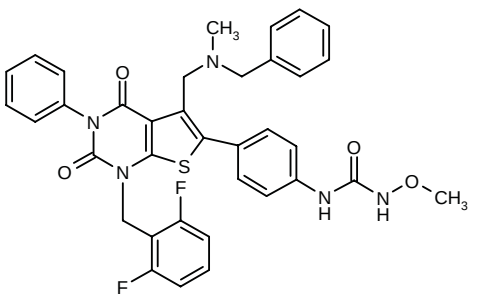
TAK-013

Treatment of Endometriosis
Treatment of Uterine Fibrosis
GnRH Antagonist

N-[4-[5-(*N*-Benzyl-*N*-methylaminomethyl)-1-(2,6-difluorobenzyl)-2,4-dioxo-3-phenyl-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidin-6-yl]phenyl]-*N*'-methoxyurea



$C_{36}H_{31}F_2N_5O_4S$

Mol wt: 667.7339

CAS: 308831-61-0

CAS: 308831-62-1 (as monohydrochloride)

EN: 295240

Abstract

Treatment of sex hormone-dependent disorders (*e.g.*, endometriosis, uterine leiomyoma, uterine fibrosis, breast and prostate cancer) first consisted of therapy with gonadotropin-releasing hormone (GnRH) receptor agonists to suppress gonadal steroidogenesis via downregulation of receptors. However, GnRH agonists have the disadvantage of being associated with an initial gonadal hormonal flare effect which can worsen symptoms. Peptidic GnRH receptor antagonists were next considered an option for treatment of hormone-dependent disorders. These agents successfully suppressed gonadotropin release with the first dose and were not accompanied by any adverse flare effects. However, peptide GnRH receptor antagonists exhibit poor oral bioavailability. Research, therefore, has focused on the discovery of potent orally active, nonpeptide small-molecule GnRH receptor antagonists. TAK-013 is one such compound that exhibited potent activity *in vitro* and *in vivo* and was chosen for further development.

Synthesis

Cyclization of 1-(4-nitrophenyl)acetone (I) with ethyl 2-cyanoacetate (II) by means of NH_4OAc, AcOH, S and diethylamine gives 2-amino-4-methyl-5-(4-nitrophenyl)-thiophene-3-carboxylic acid ethyl ester (III), which is cyclized with phenyl isocyanate (IV) in pyridine to yield the thieno[2,3-*d*]pyrimidinedione derivative (V). Alkylation of compound (V) with 2,6-difluorobenzyl chloride (VI) by means of K_2CO_3 and KI in DMF affords the adduct (VII), which is brominated with NBS and AIBN in chlorobenzene to provide the bromomethyl derivative (VIII). Reaction of compound (VIII) with *N*-benzyl-*N*-methylamine (IX) by means of DIEA in DMF gives the tertiary amine (X), which by reduction of the nitro group with H_2 over Pd/C in ethyl ether/formic acid yields the primary amine (XI). Finally, this compound is treated with CDI, *O*-methylhydroxylamine (XII) and TEA in dichloromethane (1-5). Scheme 1.

Introduction

Gonadotropin-releasing hormone (GnRH; or luteinizing hormone-releasing hormone [LHRH]) is a hypothalamic decapeptide amide secreted in a pulsatile manner that plays a crucial role in the regulation of reproductive processes. The hormone binds to the GnRH G-protein-coupled receptor on the anterior pituitary where is induces the synthesis and secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), both mediators of gonadal steroidogenesis and gametogenesis (6).

Several sex hormone-dependent disorders such as prostate cancer, breast cancer, endometriosis, uterine leiomyoma, uterine fibrosis and precocious puberty improve with peptidic GnRH agonist therapy. GnRH agonists cause the suppression of gonadal steroidogenesis via downregulation of the GnRH receptor which is known as biochemical castration. However, treatment with GnRH agonists has the disadvantage that it is associated

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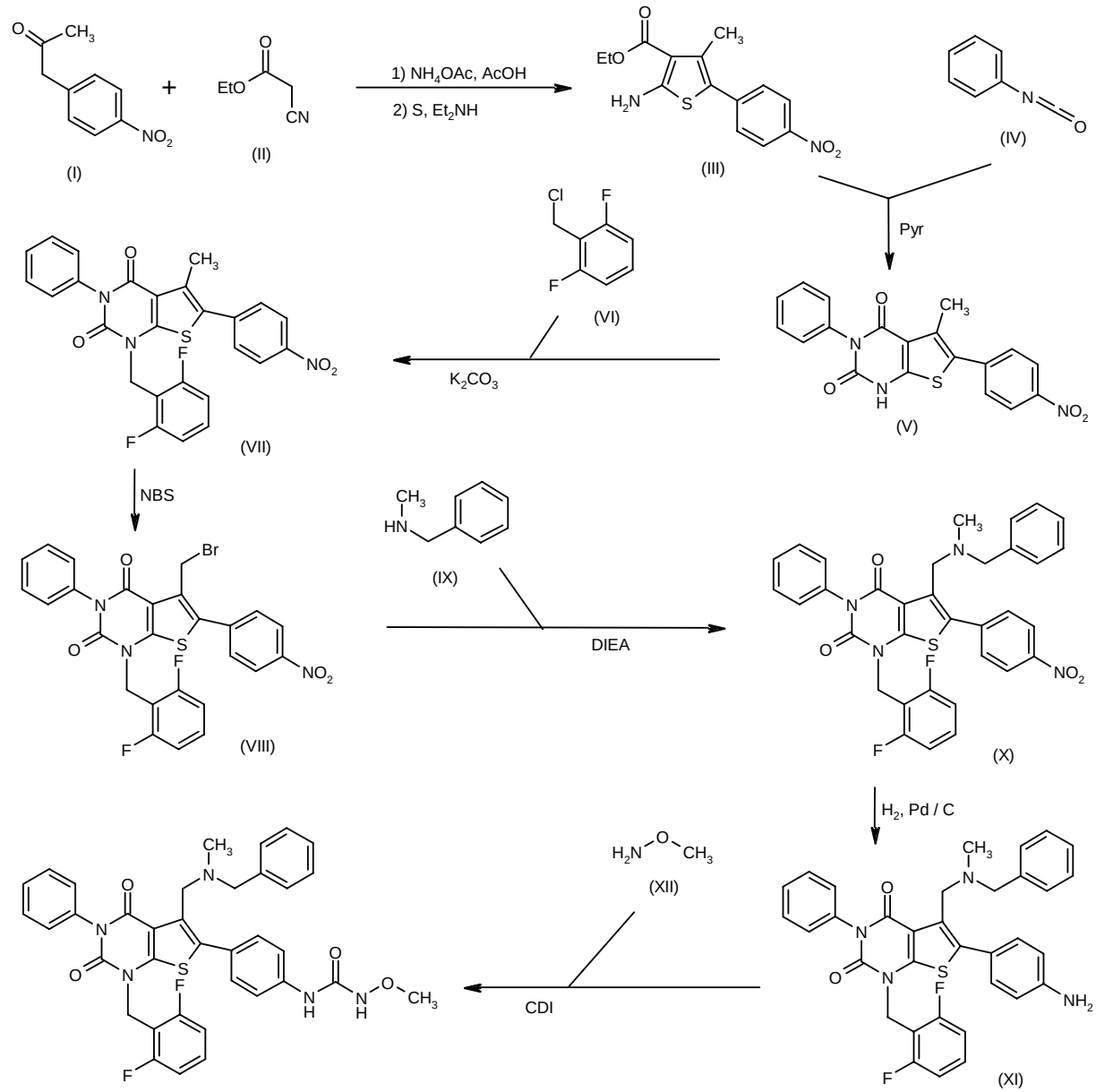
Scheme 1: Synthesis of TAK-013



with an initial hormonal flare effect (*i.e.*, gonadal hormone surge) which can worsen symptoms (7-11).

Peptidic GnRH receptor antagonists, on the other hand, have been shown to suppress gonadotropin release starting with the initial dose without any flare effects (8, 12). However, because these agents are peptides, they exhibit poor oral bioavailability. Research, therefore, has been initiated to find potent orally active, nonpeptide small-molecule GnRH receptor antagonists for the treatment of sex hormone-dependent disorders.

The first potent and orally active nonpeptide GnRH receptor antagonist described was T-98475, a thieno-[2,3-*b*]pyridin-4-one derivative (13). Although this agent possesses excellent GnRH antagonist activity *in vitro*, its bioavailability *in vivo* was not sufficient. Attempts to improve on this compound led to the discovery of TAK-013, a thieno[2,3-*b*]pyrimidine-2,4-dione bearing a unique 4-(3-methoxyureido)phenyl group at position 6. TAK-013 has exhibited potent activity *in vitro*, possessing highly specific antagonist activity for the human GnRH receptor as compared to other species. Moreover, in monkeys

potent inhibitory activity was demonstrated along with good oral absorption *in vivo*. TAK-013 was therefore chosen for further development as a treatment for sex hormone-dependent diseases (1, 2, 14, 15).

Pharmacological Actions

Binding studies using the human GnRH receptor expressed in CHO cells and [^{125}I]-leuprolin showed that the affinity of TAK-013 for the receptor was 40 times higher than that of the endogenous ligand; the IC_{50} value for inhibiting [^{125}I]-leuprolin binding to the receptor was 0.1 nM. TAK-013 displayed selectivity for the human GnRH receptor over the monkey (IC_{50} = 0.6 nM) and rat receptor (IC_{50} > 1000 nM). TAK-013 was highly specific for the GnRH receptor; IC_{50} values for TAK-013 obtained in binding assays for other systems (*e.g.*, 120 other receptors, 9 ion channels and 7 transporters) were not less than 0.5 μM. TAK-013 also showed efficacy and species specificity in an *in vitro* functional assay examining inhibition of GnRH-stimulated arachidonic acid release from CHO cells expressing human (IC_{50} = 0.06 nM) and monkey (IC_{50} = 10 nM) GnRH receptors. TAK-013 was 220-fold more potent in cells expressing the human receptor (IC_{50} = 0.06 *vs.* 10 nM) (2, 5).

TAK-013 (10 and 30 mg/kg p.o. suspended in methylcellulose) exhibited potent activity and excellent oral absorption *in vivo* in castrated cynomolgus monkeys. While the 10 mg/kg dose resulted in effective suppression of plasma LH levels (20% of baseline values at 8-24 h postdosing), the higher dose caused almost complete suppression of plasma LH levels (11% of baseline levels at 24 h postdosing). C_{max}, t_{max} and $AUC_{0\text{-}6\ h}$ values obtained at a dose of 10 mg/kg were 0.21 μM, 6 h and 0.85 μM·h, respectively.

Clinical Studies

A randomized, double-blind, placebo-controlled, ascending dose, parallel-group phase I trial conducted in 50 healthy premenopausal women (who discontinued oral contraceptives 1 week prior to the study onset), examined the safety, tolerability, efficacy, pharmacokinetics and metabolism of multiple (5, 10, 25, 50 or 100 mg once daily for 14 days) oral doses of TAK-013. The compound was well tolerated with no adverse events or significant changes in vital signs or ECG parameters observed in any treatment group. Plasma LH and estradiol levels were rapidly, effectively and dose-dependently reduced on day 1 and levels remained suppressed on day 14; recovery was observed beginning at 48 h after the last dose. No significant effects were observed on FSH levels. C_{max} and AUC values on day 1 were linearly related to dose. Median t_{max} and mean $t_{1/2}$ values were 2 h and 8-12 h, respectively. When the pharmacokinetic parameters from day 14 were compared to those obtained on day 1, there was a significant reduction in C_{max} and AUC (56%) values and the mean $t_{1/2}$ was longer (22-53 h) and inversely related to dose (53 and 22 h after 5 and 100 mg, respectively). Dose-dependent increases in the urinary 6-hydroxycortisol:cortisol ratio (consistent with cytochrome P450 3A4 [CYP3A4] induction) were observed, with a maximum enhancement seen on day 6. Induction of CYP3A4 could be responsible for the reduction in AUC and the inverse relationship between $t_{1/2}$ and dose observed after multiple dosing (16).

A randomized, double-blind, placebo-controlled, ascending dose, parallel-group, phase I trial conducted in 36 healthy postmenopausal women examined the safety, tolerability, efficacy, pharmacokinetics and metabolism of single (50, 100 or 200 mg) and multiple (25, 50 or 100 mg b.i.d. for 14 days starting 1 week after single dosing) oral doses of TAK-013. TAK-013 was well tolerated with no adverse events or significant changes in vital signs or ECG parameters observed in any treatment group. Single dosing rapidly reduced plasma LH and FSH levels for at least 48 h postdosing; no difference in median AUC or C_{min} values were noted between doses. C_{max} and AUC values were dose-proportional and the median t_{max} and mean $t_{1/2}$ values obtained for single dosing were 2-4 h and about 24 h, respectively. Multiple dosing resulted in dose-dependent reductions in plasma LH and FSH levels which were sustained for at least 36 h after the first dose. Steady state was achieved by day 7. C_{max} and AUC values were also dose-proportional. Changes in the pharmacokinetics were noted with multiple dosing, although the pharmacodynamics induced by TAK-013 were not altered. Significant reductions in mean $t_{1/2}$ (5.3 h) and AUC (76%) values were observed between the single dose and the last dose of the repeated-dose schedule. It was concluded that TAK-013 administration induced CYP3A4 since a dose-dependent increase in the urinary 6-hydroxycortisol:cortisol ratio was observed after multiple dosing. This increase could also be responsible for the decrease in $t_{1/2}$ and AUC values seen after multiple dosing (17).

TAK-013 is currently undergoing phase I clinical trials in Japan and phase II trials in Europe and the U.S. involving patients with endometriosis and uterine fibrosis (18).

Manufacturer

Takeda Chemical Industries, Ltd. (JP).

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