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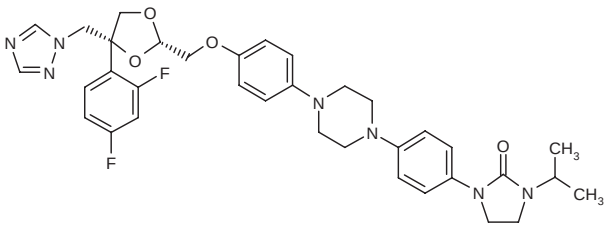
R-126638

Antifungal Agent

Azoline

(+)-1-[4-[4-[4-[4(*R*)-(2,4-Difluorophenyl)-4-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2(*S*)-ylmethoxy]phenyl]piperazin-1-yl]phenyl]-3-isopropylimidazolidin-2-one



$C_{35}H_{39}F_2N_7O_4$
Mol wt: 659.7257

CAS: 219923-85-0

CAS: 219923-86-1 (as hydrochloride)

EN: 272538

Abstract

Dermatomycoses are among the most common cutaneous fungal infections in humans. The agents most frequently used to treat dermatomycoses are the ergosterol biosynthesis inhibitors, which include a subclass known as the azoles. R-126638 is a novel azole currently under investigation as a potential oral therapy for a number of fungal infections of the skin, including tinea pedis, tinea corporis, tinea cruris, tinea versicolor and seborrheic dermatitis. This compound has shown potent antifungal activity against a number of dermatophytes, *Candida* spp. and *Malassezia* spp. both *in vitro* and *in vivo*. Comparison of the antifungal activities of R-126638 with those of itraconazole, terbinafine and ketoconazole has yielded promising results.

Synthesis

R-126638 can be prepared by condensation of bromomethyl derivative (I) with phenol intermediate (II) by means of either NaOH in DMF at 50 °C (1) or NaH in DMF/toluene at 60 °C (2). Scheme 1.

Synthesis of Intermediate (I)

Intermediate (I) can be prepared by three different ways:

a) Friedel-Crafts' acylation of 1,3-difluorobenzene (III) with chloroacetyl chloride (IV) by means of $AlCl_3$ yields α-chloro-2,4-difluoroacetophenone (V), which by reaction with 1,2,4-triazole (VI) and triethylamine in refluxing ethyl acetate gives the α-(1*H*-1,2,4-triazol-1-yl)-2,4-difluoroacetophenone (VII) (3). Reaction of acetophenone (VII) with 2-hydroxy-2-methylpropionitrile (VIII) and NH_4OH as catalyst at room temperature, provides a cyanohydrine intermediate, which after hydrolysis with refluxing HCl yields the acid intermediate (IX). Reduction of compound (IX) with $LiAlH_4$ in THF affords diol (X), which is cyclized with 2-bromoacetaldehyde diethyl acetal (XI) by means of MsOH in CH_2Cl_2 to yield a racemic mixture of 2-(bromomethyl)-1,3-dioxolanes. Finally this mixture is resolved by chiral chromatography, to provide the desired *cis*-enantiomer 1-[2(*S*)-(bromomethyl)-4(*R*)-(2,4-difluorophenyl)-1,3-dioxolan-4-ylmethyl]-1*H*-1,2,4-triazole (I) (1, 2). Scheme 2.

b) Reaction of acetophenone (VII) with trimethylsulfoxonium iodide (XII) by means of NaOH in toluene affords the epoxide derivative (XIII), which is hydrolyzed by means of aqueous H_2SO_4 at 60 °C to afford the already described intermediate (X) (2). Scheme 2.

c) Reaction of 2-chloro-1-(2,4-difluorophenyl)-1-ethanone (V) with chloro iodomethane (XIV) by means of MeLi/LiBr complex in THF affords the oxirane (XV), which after treatment with an excess of acetone (XVI) and a boron catalyst yields dioxolane (XVII). Hydrolysis of compound (XVII) with HCl in refluxing methanol/water gives the diol derivative (XVIII), which is condensed with 1,2,4-triazole (VI) by means of NaH in DMF to provide 2-(2,4-difluorophenyl)-3-(1,2,4-triazol-1-yl)propane-1,2-diol (X) (1). Scheme 2.

Synthesis of Intermediate (II)

Arylation of 1-(4-methoxyphenyl)piperazine (XIX) with 1-chloro-4-nitrobenzene (XX) by means of K_2CO_3 in DMSO gives compound (XXI), which after catalytic

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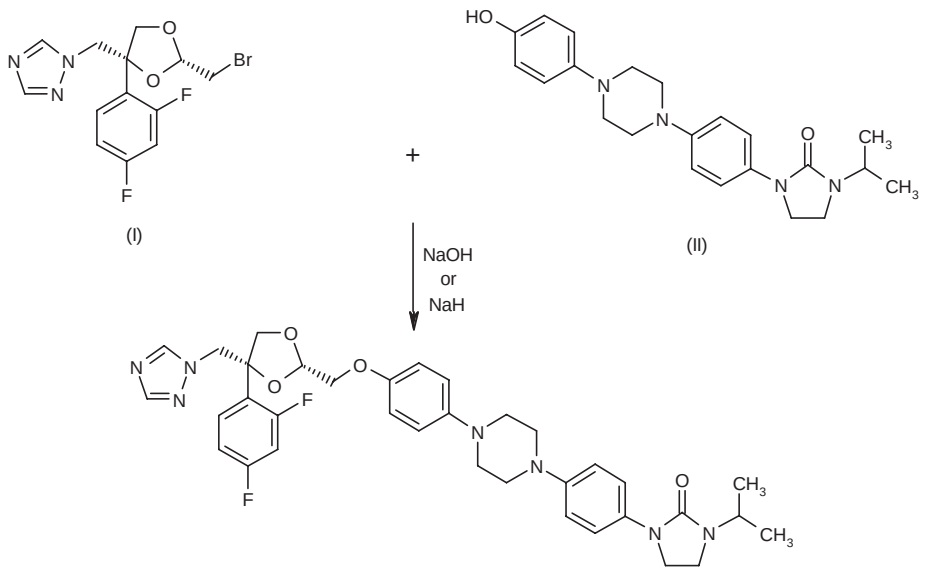
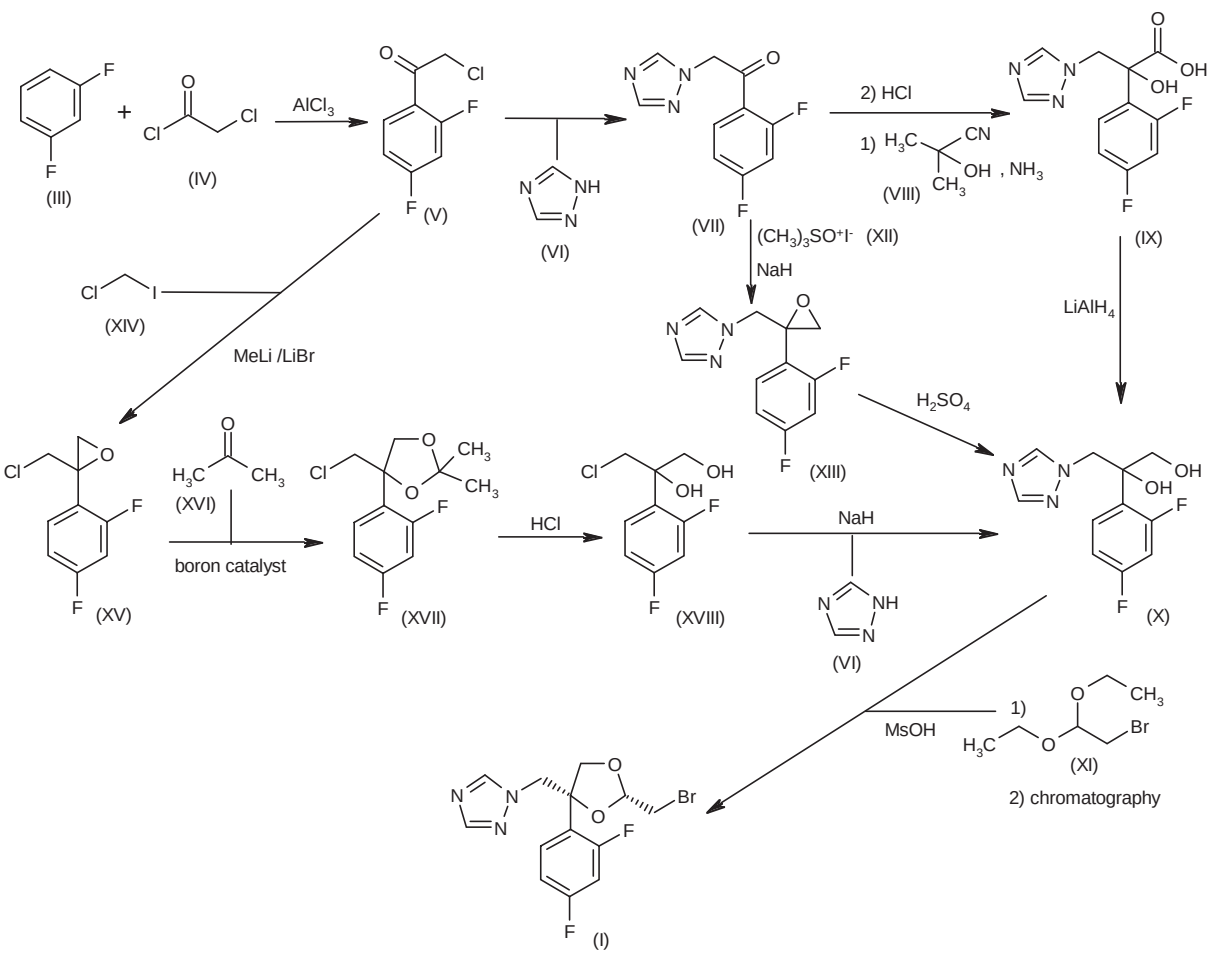
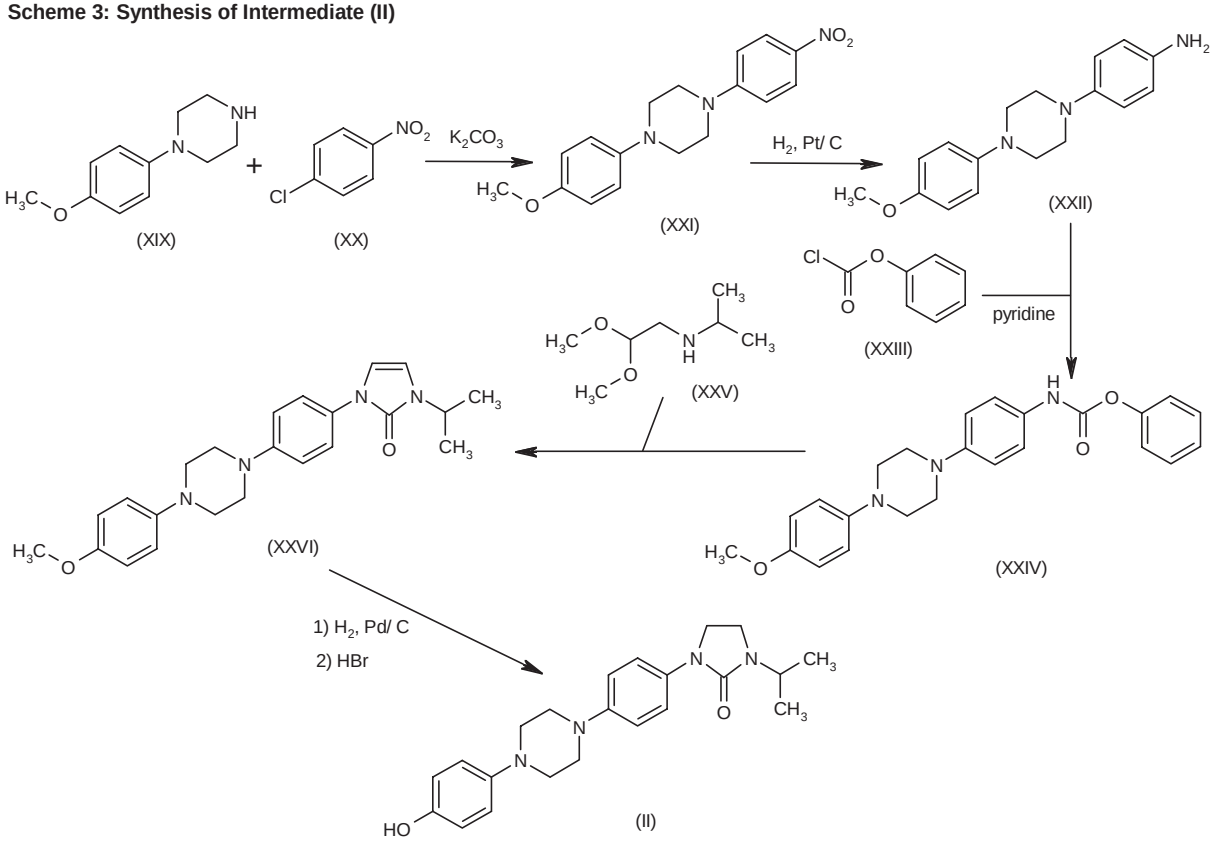
Scheme 1: Synthesis of R-126638



Scheme 2: Synthesis of Intermediate (I)



Scheme 3: Synthesis of Intermediate (II)



hydrogenation with Pt/C affords the aniline (XXII). Acylation of intermediate (XXII) with phenyl chloroformate (XXIII) in $CHCl_3$/pyridine yields carbamate (XXIV) (4), which by reaction with *N*-(2,2-dimethoxyethyl)-2-propaneamine (XXV) in dioxane at reflux, in the presence of DMAP and triethylamine, provides the imidazolone (XXVI). Finally, hydrogenation of compound (XXVI) in AcOH and Pd/C followed by deprotection with HBr/AcOH gives phenol intermediate (II) (2). Scheme 3.

Background

Dermatomycoses are widespread, common human superficial and cutaneous fungal infections. Caused by filamentous fungi such as *Trichophyton, Microsporum* or *Epidermophyton* spp., dermatomycoses are often difficult to treat (5, 6).

Ergosterol biosynthesis inhibitors account for most of the antifungal agents currently used for the treatment of dermatomycoses and can be classified into three groups: azoles, allylamines and morpholines. First- and second-generation azoles, including ketoconazole, itraconazole, fluconazole and voriconazole, are currently on the market for the treatment of life-threatening systemic fungal infections, but pharmacological investigation continues to identify new azole compounds with applications in dermatology (2, 7, 8).

One such novel azole, R-126638, is currently being evaluated in clinical trials as a potential antifungal agent for the oral treatment of dermatophytosis (2).

Preclinical Pharmacology

R-126638 and itraconazole exhibited similar inhibitory effects on ergosterol synthesis in an *in vitro* study; the respective IC_{50} values for inhibition of ergosterol synthesis were 2.2 and 2.8 nM in *Candida albicans*, 280 and 310 nM in *Microsporum canis*, 22 and 82 nM in *Trichophyton mentagrophytes*, and 33 and 18.5 nM in *Trichophyton rubrum*. Judging from its inhibition of ergosterol synthesis, together with the observed accumulation of 14α-methylsterols, R-126638 appears to inhibit cytochrome P-450 CYP51 activity. In human hepatoma cells, R-126638 and itraconazole inhibited cholesterol synthesis only at much higher concentrations (IC_{50} = 3.1 and 1.4 μM, respectively) (9).

The *in vitro* and *in vivo* antifungal activities of R-126638 were studied in comparison with those of itraconazole and terbinafine. The *in vitro* antifungal potencies (MIC_{50} and MIC_{90}, respectively) against a panel of 24 *Candida* isolates were 0.1 and > 8 μg/ml for R-126638, 0.1-0.32 and > 8 μg/ml for itraconazole and > 20 and > 20 μg/ml for terbinafine. With respect to *in vitro* activity against dermatophyte isolates, R-126638 showed com-

parable or somewhat lower potency than the reference compounds. The MIC_{50} and MIC_{90} values for R-126638 were, respectively, 0.016-0.032 and 0.093-0.13 μg/ml for *Epidermophyton floccosum*, 0.032 and 0.524 μg/ml for *Microsporum* spp., 0.01-0.063 and 0.032-0.80 μg/ml for *T. mentagrophytes*, 0.25-0.32 and 0.5-0.796 μg/ml for *T. rubrum*, 0.13-0.32 and 0.5-1.0 μg/ml for *Trichophyton tonsurans*, and 0.1 and 1.22 μg/ml for other *Trichophyton* spp. Excellent activity was also observed against *Malassezia* spp. (MIC_{50} = 0.010 μg/ml *vs*. 0.10 μg/ml for ketoconazole). The compound was rarely active against the other pathogenic fungi examined, including *Aspergillus* spp. (MIC_{50} > 20 μg/ml). *In vivo*, R-126638 showed antifungal activity 3-fold greater than that of itraconazole and markedly greater than that of terbinafine in guinea pigs infected with *M. canis*. The ED_{50} values (prophylactic treatment from day 0 to day 12) assessed on day 7 in this model were < 0.31, 0.84 and > 10 mg/kg/day p.o. for R-126638, itraconazole and terbinafine, respectively. The compound was also 5-fold more potent than itraconazole and terbinafine in mice infected with *T. mentagrophytes*, with ED_{50} values on day 7 of < 0.63, 2.60 and > 5.00 mg/kg/day p.o. for R-126638, itraconazole and terbinafine, respectively (2, 9, 10). Superior therapeutic efficacy (treatment from day 3 for 3 days) compared to itraconazole was also observed in guinea pigs with *M. canis* and *T. mentagrophytes* infections (10).

The *in vitro* activity of R-126638 against *Malassezia* spp., the species involved in pityriasis versicolor and seborrheic dermatitis, was compared with that of ketoconazole using two techniques. Results from the agar diffusion technique showed lower MICs for R-126638 as compared to ketoconazole against *Malassezia globosa, Malassezia obtuse, Malassezia slooffiae, Malassezia restricta* and 2 isolates of *Malassezia sympodialis*. The second technique examined the *in vitro* production of hyphae on human stratum corneum; results showed a reduction in the production of hyphae from 12% to 2% and 3% with R-126638 and ketoconazole, respectively. Exposure to 0.01-1.0 μg/ml R-126638 or ketoconazole did not produce obvious surface differences relative to untreated cell cultures, as determined by scanning electron microscopy (SEM). Transmission electron microscopy (TEM), however, did reveal partial to complete necrosis of cytoplasmic organelles. Concentrations of 0, 0.01, 0.1 and 1.0 μg/ml caused complete necrosis of 6%, 60%, 76% and 100%, respectively, of cells exposed to R-126638 and of 6%, 41%, 62% and 97%, respectively, of cells exposed to ketoconazole (11).

Pharmacokinetics and Metabolism

The tolerability, safety and pharmacokinetics of oral R-126638 were assessed in a randomized, double-blind, placebo-controlled, multiple-dose trial in healthy volunteers. The 12 study participants received 1 week of treatment with 100 mg/day R-126638 or placebo, followed by 1 week of treatment with 200 mg/day R-126638 or placebo. The drug was generally well tolerated. No serious adverse events were reported and none of the participants discontinued treatment. Most subjects exhibited peak plasma concentrations 1-2 h after R-126638 administration. C_{max} and $AUC_{0\text{-}24h}$ appeared to increase in a dose-proportional manner after both the first and last dose of R-126638. The terminal elimination half-life estimated after the last administration of R-126638 on day 7 was 56 h for the lower dose and 81 h for the higher dose, with an average accumulation factor of 4. Only negligible urinary excretion was observed (12). The results of this study and some that follow are summarized in Table I.

Clinical Studies

Using a corneofungimetry bioassay method, the antifungal activity of R-126638 was determined *ex vivo* in skin strips taken from volunteers after oral administration of the drug. Cyanoacrylate skin surface strippings (CSSSs) were obtained at different time points from the forearms of 16 healthy subjects receiving R-126638 (100 or 200 mg once daily for 1 week) and used to culture 5 fungal strains. R-126638 began to show effects on day 4, when the fungi tested showed reductions of 24.2% for *M. globosa*, 31.2% for *C. albicans*, 14.1% for *T. mentagrophytes*, 11.1% for *M. canis* and 35.9% for *T. rubrum* on the higher dose. On day 7, the reductions observed were 31.3%, 57.9%, 39.6%, 26.0% and 45.3%, respectively. A significant posttreatment effect (reflecting the retention time of the active drug in the skin) was noted against 4 of 5 strains on day 10 (reductions ranging from 31.3% to 44.2% on the higher dose) and against 4 of 5 strains on day 14 (reductions ranging from 16.3% to 33.9%) (13, 14).

The efficacy of R-126638 against *Malassezia* yeasts on the forehead of patients with seborrheic dermatitis was assessed in a clinical trial. Ten patients received R-126638 (200 mg) and mycological and clinical evaluations were performed before and after 3, 7 and 28 days of treatment. As compared to baseline, R-126638 significantly reduced the number of living yeasts and the number of yeasts/mm^2 of scales at all time points. Clinical parameters such as desquamation, erythema, itching and global clinical evaluation (as determined by the patient and the investigator) showed significant improvement at days 7 and 28, but not at day 3. Scaliness severity did not show significant improvement and no effect was observed on sebum secretion (15).

A pilot trial assessed the effects of R-126638 200 mg once daily for 3 or 5 days in 20 patients with tinea pedis. A negative KOH microscopic evaluation of scales taken at the border of an active lesion at day 28 was found in 60% of the 10 patients receiving 3 days of treatment and in 70% of the 10 patients receiving 5 days of treatment. Results from the culture assay at day 28 revealed negative results for 40% of patients receiving 3 days of treatment and for 70% of patients receiving 5 days of treatment. At day 28, all patients receiving 3 days of treatment and 90% of patients receiving 5 days of treatment exhibited marked improvement or cure according to global

Table I: Clinical studies of R-126638 (from Prous Science Integrity®).

Indication	Design	Treatments	n	Conclusions	Ref.
Healthy volunteers	Randomized Double-blind	R126638, 100 mg p.o. o.d. x 1 wk → [if safe & tolerable] 200 mg p.o. o.d. x 1 wk (n=8) Placebo (n=4)	12	R-126638 100-200 mg was safe and well tolerated in healthy volunteers	12
Healthy volunteers	Open	R-126638, 100 mg o.d. x 1 wk (n=8) R-126638, 200 mg o.d. x 1 wk (n=8)	16	After 4 days of treatment, antifungal effects of both doses of R-126638 were observed in the stratum corneum of skin. These effects persisted for several days after stopping treatment	13
Infection, fungal, Tinea pedis	Open	R-126638, 200 mg p.o. o.d. x 3 d (n=10) R-126638, 200 mg p.o. o.d. x 5 d (n=10)	20	Oral R-126638 given for 3 or 5 days was effective in improving signs and symptoms of tinea pedis	16
Infection, fungal, Pityriasis versicolor	Open	R-126638, 200 mg p.o. o.d. x 3 d	19	R-126638 given for 3 days significantly reduced signs and symptoms (erythema, itching, desquamation) in patients with pityriasis versicolor. All patients achieved mycological cure at 30 days after onset of treatment	17

clinical evaluation. The median value of the sum of different signs and symptoms assessed using a 4-point scale was 10 before inclusion in both treatment groups. At day 14, this value was about 3.0 with 3 days of therapy and 3.5 with 5 days of therapy. At day 28, the corresponding values were 1.0 and 2.0, respectively (16).

An open-label, proof-of-concept trial examined the effects of 3-day oral therapy with R-126638 at a dose of 200 mg/day in 19 patients with pityriasis versicolor. At day 10, 42% of patients exhibited negative KOH microscopic results, and by day 30 all patients showed mycological cure. All signs and symptoms except hypopigmentation were significantly reduced at days 10 and 30. The median value of the sum of signs and symptoms was 8.0 at inclusion, 5.0 at day 4, 3.0 at day 10 and 2.0 at day 30. Global clinical evaluation showed improvement at all follow-up visits and all patients experienced cure at day 30. No serious adverse events were observed and all patients completed the study. Nine patients reported minor adverse events such as cold, headache and muscle pain (17).

Drug Interactions

The effects of R-126638 on mammalian cytochrome P-450-dependent reactions were investigated as an indication of its potential for drug-drug interactions. R-126638 inhibited the 1α-hydroxylation of 25-hydroxyvitamin D_3 with an IC_{50} of > 10 μM, compared to a value of 3.5 μM for itraconazole. Both the 24-hydroxylation of 25-hydroxyvitamin D_3 and the conversion of 1,25-dihydroxyvitamin D_3 to polar metabolites were inhibited by R-126638 and itraconazole at micromolar concentrations. R-126638 had minimal effects on CYP11A1, CYP11B1, CYP17, CYP19 or CYP26 at concentrations up to 10 μM. Furthermore, at a concentration of 10 μM, R-126638 did not clearly inhibit CYP1A2, CYP2A6, CYP2D6, CYP2C8, CYP2C9, CYP2C10, CYP2C19 or CYP2E1. The potential for interaction with testosterone 6β-hydroxylation and ciclosporin hydroxylation was lower for R-126638 than for itraconazole, although both drugs produced a similar inhibition of midazolam hydroxylation (9).

Source

Barrier Therapeutics, Inc. (US).

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