α_2-adrenoceptor antagonist in the rat.

A decrease in the number of cortical β-adrenoceptors (down-regulation) has been associated with a number of chronic antidepressant therapies, including treatment with antidepressants of the tricyclic, monoamine oxidase inhibitor, and atypical classes, as well as with electroconvulsive therapy.[11] This β-adrenoceptor down-regulation can be ascribed to increased levels of norepinephrine in the brain. Since prejunctional α_2-adrenoceptors mediate norepinephrine levels through a negative-feedback mechanism,[23] antagonism of α_2-adrenoceptors should increase norepinephrine levels and also result in β-adrenoceptor down-regulation and possibly a concomitant antidepressant effect. It was therefore of interest that administration of **8b** at 0.5 mg/kg once daily for 14 days in the rat was found to significantly reduce the number of cortical β-adrenoceptors to a B_{max} of 57 ± 5 fmol/mg ($p < 0.05$) relative to a control B_{max} of 72 ± 3 fmol/mg.[24] On the basis of this finding and on its potency and selectivity as an α_2-adrenoceptor antagonist, **8b** should be an important agent for the pharmacological evaluation of the α_2-adrenoceptor and may have clinical application, e.g. for the treatment of depression.

Acknowledgment. We thank Dr. Henri Pinhas and Isabelle Jullien, Syntex Research France, for the chiral HPLC determinations and Dr. Frederick Hollander, University of California, Berkeley, for the X-ray crystallographic data. We are grateful to Lani Russell for preparing the manuscript.

(23) Gillespie, J. S. In *Adrenergic Activators and Inhibitors*, Part 1; Szekeres, L., Ed.; Springer-Verlag: Berlin, 1980.

(24) The B_{max} is the number of fmoles of [^{3}H]dihydroalprenolol which binds per mg of protein. A B_{max} of 50 was determined for desipramine after 14 days of once daily dosing at 7.5 mg/kg ip.

† Syntex Research, Palo Alto.
‡ Syntex Research, Edinburgh.

Robin D. Clark,*† David B. Repke†
Andrew T. Kilpatrick,‡ Christine M. Brown‡
Alison C. MacKinnon,‡ Ruth U. Clague‡
Michael Spedding*‡
Institute of Organic Chemistry
Syntex Research
Palo Alto, California 94304
and Department of Pharmacology
Syntex Research Center
Edinburgh, Scotland EH 14 4AP
Received February 13, 1989

LH-RH Antagonists: Design and Synthesis of a Novel Series of Peptidomimetics

Sir:

Luteinizing hormone releasing hormone (LH-RH) also known as gonadotropin releasing hormone (GnRH) was isolated and characterized by Schally[1] and co-workers in 1971. LH-RH is produced in the hypothalamus and controls secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the anterior pituitary. Various LH-RH agonists have been shown to produce an initial stimulation of gonadotropin release from the anterior pituitary, which results in ovulation in women with amenorrhea and testosterone secretion, together with spermatogenesis in hypogonadotropic men.[2] However, chronic administration of LH-RH agonists results in a paradoxical inhibition of the pituitary–gonadal axis characterized by a decrease of the levels of sex steroids and the atrophy of accessory sex organs.[3,4] This discovery of gonadal steroid suppression produced by an LH-RH agonist led to the successful development of clinical therapeutic agents such as Lupron (TAP Pharmaceuticals) for use in the treatment of hormone-dependent breast and prostatic carcinoma.[5]

Currently available LH-RH superagonists are polypeptides (containing 9–10 amino acid residues) and less than 1% bioavailable when administered orally. The very principle by which the superagonists induce the hypophysis to release large amount of LH during the first 2–3 weeks of desensitization period also produces undesirable side effects.[6] One attractive alternative to this problem has been to design and antagonist which, in principle, should attain the same goal through the direct blockade of LH release. It has been shown that rabbits which produced an antibody to LH-RH developed gonadal antropy[7] and that administration of rabbit anti-LH-RH serum to normally cycling rats prevented the preovulatory surge of LH and FSH as well as blocked ovulation.[8] We decided to try to develop an orally active, preferably nonpeptidic, antagonist of LH-RH, which we envisioned should competitively block the pituitary receptors and lead to the suppression of gonadal steroids.

It has been reported that an antifungal drug called ketoconazole (Nizoral, Janssen Pharmaceutica, Beerse, Belgium), when given orally to patients in a dosage of 200–1200 mg daily produces a dose-dependent suppression of serum testosterone and leads to the remission of prostate cancer.[9] The mechanism leading to the suppression of serum testosterone by ketoconazole was studied in great detail by Bhasin et al.[10] and it was concluded that the suppression of testosterone biosynthesis was primarily due to the inhibition of a number of enzymes in the biosynthetic pathway. During our search for a nonpeptidic lead in the LH-RH program, we became extremely interested in this antifungal compound and focused our attention primarily on its involvement at the pituitary level. To our surprise, we found that ketoconazole exhibits weak but competitive binding affinity for the pituitary LH-RH receptor. In this communication, we wish to report our

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(2) Hurley, D. M.; Brian, R.; Outch, K. *N. Engl. J. Med.* **1984**, *310*, 1069.

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(8) Arimura, A.; Debeljuk, L.; Schally, A. V. *Endocrinology* **1974**, *95*, 323.

(9) Trachtenberg, J.; Pont, A. *Lancet* **1984**, *2*, 433.

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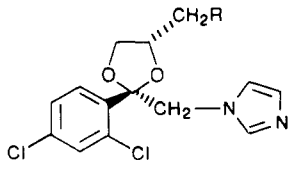
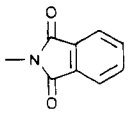
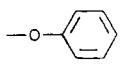
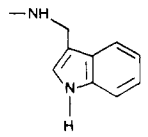
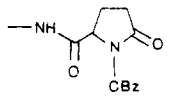
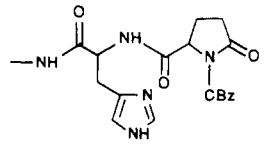
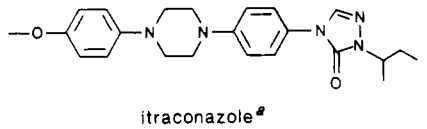
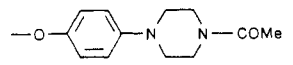
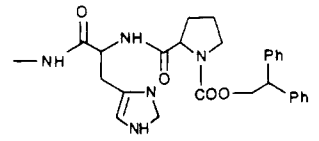
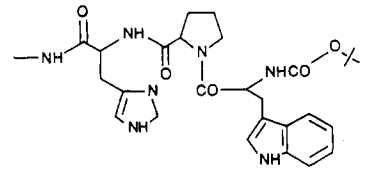
Table I. Data for Compounds 1-10



1-10

compds	R	IC_{50}, μM
1	-OH	>30
2		>30
3		>30
4		>30
5		>30
6		2.00
7	 itraconazole[a]	>30
8	 ketoconazole	2.00
9		0.48
10		0.50

1-10

[a] For Itraconazole, the imidazole ring is replaced by a triazole ring.

preliminary structure–activity relationship data (see Table I) obtained with ketoconazole and a few closely related analogues.

Chemistry

Racemic ketal alcohol **1**, which was synthesized following a published method,[11] was reacted with phthalimide (1 equiv) in the presence of triphenyl phosphine (1 equiv) and diethyl azodicarboxylate[12] (1.2 equiv) in THF at 0 °C for 30 min and at room temperature for 30 min, giving the desired imide **2** in 87% yield. Imide **2** was refluxed with hydrazine hydrate (1.5 equiv) in ethanol for 4 h, and a chromatographic purification of the crude product gave amine **11** in 76% yield. This amine was then coupled to a series of protected amino acids and dipeptides following either the mixed anhydride (1 equiv of *N*-methylmorpholine, 1 equiv of isobutyl chloroformate in either THF or CH_2Cl_2 at 0 °C to room temperature) or the EDAC coupling method.[13] Phenoxy ether **3** was made by a nucleophilic displacement of the mesylate (derived from **1**) with sodium phenoxide in DMF at 60 °C for 2 h (45% yield). Indole amine **4** was synthesized by reductive amination of **11** and indole-3-caroxaldehyde in 2-propanol with $NaCNBH_3$ at room temperature for 8 h (26% yield). The mixture of diastereomers (1:1) which resulted from the coupling of **11** and the peptides were tested in vitro

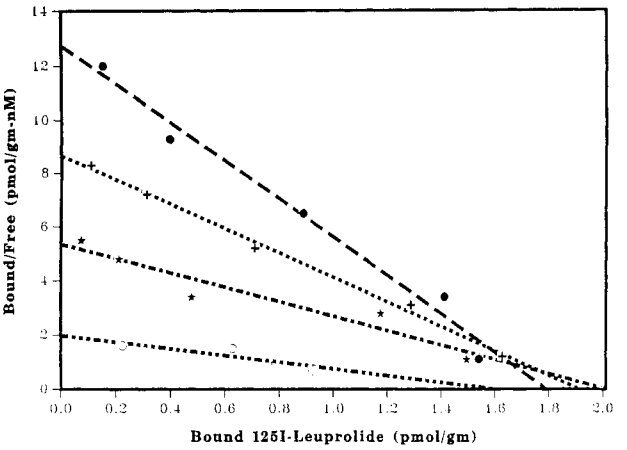


Figure 1. Rat pituitary membranes were coincubated with [^{125}I]leuprolide (varied from 15 to 1500 pM) in the absence (●) and in the presence of ketoconazole, at concentrations of 3.16×10^{-6} M (+), 1.0×10^{-5} M (*), and 3.16×10^{-5} M (○), for 120 min at 4 °C in 10 mM Tris-HCl buffer at pH 7.4. Nonspecific binding was determined at each [^{125}I]leuprolide concentration, in the presence of 1.0×10^{-5} M unlabelled leuprolide. Each point represents the mean of three replicate determinations. The data for each condition was fit to a one-site-binding model. The best fit parameter estimates for the control membrane preparations were $K_d = 140 \pm 11$ pM and $B_{max} = 1.79 \pm 0.09$ pmol/g of tissue. Increasing concentrations of ketoconazole were associated with a progressive and significant increase in K_d (at 3.16×10^{-6} M, $K_d = 221 \pm 9$ pM; at 1.0×10^{-5} M, $K_d = 375 \pm 65$ pM; at 3.16×10^{-5} M, $K_d = 811 \pm 479$ nM), evaluated by analysis of variance ($p < 0.01$). In contrast, the apparent receptor density was not significantly decreased ($B_{max} = 1.91 \pm 0.05$, 2.01 ± 0.25 and 1.60 ± 0.62 pmol/mg of tissue, respectively) relative to control.

(11) Heeres, J.; Backx, J. J.; Mostmans, J. H.; Van Cutsem, J. *J. Med. Chem.* **1979**, *22*, 1003. NMR (300 MHz, $CDCl_3$) δ: 7.60 (H, d, 8 Hz), 7.56 (H, s), 7.46 (H, d, 2.2 Hz), 7.27 (H, dd, 2.2 Hz, 8 Hz), 6.99 (2 H, s), 4.51 (H, d, 14.7 Hz), 4.39 (H, d, 14.7 Hz), 4.12 (H, m), 3.82 (H, dd, 6.8 Hz, 8 Hz), 3.64 (H, dd, 6.8 Hz, 8 Hz), and 3.34 (2 H, d, 5 Hz).

(12) Mitsunobu, O. *Synthesis* **1981**, 1.

(13) Synthesis of **10**: To a solution containing the amine **11** (dihydrochloride, 100 mg, 0.25 mmol), EDAC (1-ethyl-3-(3′,3′-dimethylamino)propylcarbodiimide, 50 mg, 0.26 mmol). HOBT (*N*-hydroxybenzotriazole, 101 mg, 0.748 mmol) and the tripeptide (^{t}Boc-NH-Trp-Pro-His-OH, 140 mg, 0.25 mmol) in dry DMF (50 mL) at −20 °C was added *N*-methylmorpholine (30 μL, 0.27 mmol). The reaction was stirred at −20 °C for 2 h and then at room temperature overnight. The contents were poured into a ethyl acetate/aqueous $NaHCO_3$ mixture. Extraction and purification by column chromatography gave **10** (152 mg, 72%). MS (POS FAB, MeOH) *m/z*: 849 (MH^+, 60%). Anal. ($C_{41}H_{47}N_9O_7Cl_2 \cdot 2H_2O$): C, H, N. Mp: 148–150 °C. Satisfactory MS (chemical ionization, NH_3), NMR (300 MHz, DMSO-d_6), and analysis data were obtained for all the entries in Table I.

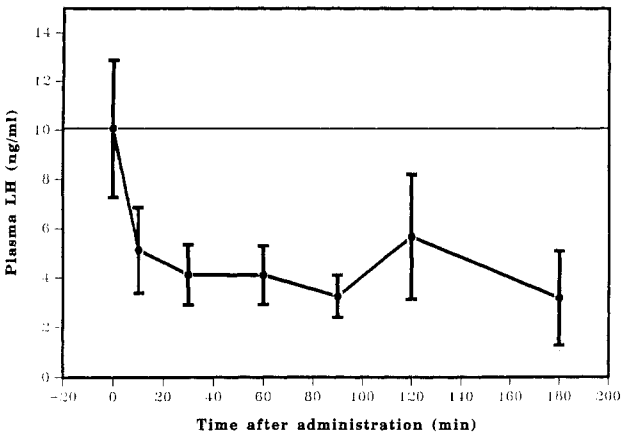
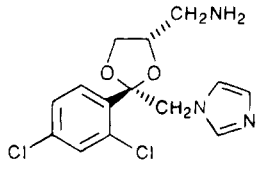


Figure 2. Three male Sprague–Dawley rats (approximately 300-g body weight) that were castrated 4 weeks before experimentation were treated with ketoconazole (100 mg/kg in 1 mL/kg 20% propylene glycol in 0.9% NaCl solution), administered as a single bolus dose by the intraduodenal route. Blood samples (0.5 mL) were obtained under light ether anesthesia by jugular venipuncture before dosing and at 10, 30, 60, 90, 120, and 180 min. Plasma luteinizing hormone (LH) concentration was determined by radioimmunoassay. Immunoaffinity-purified anti-rat-LH IgG was obtained from Dr. P. M. Conn of the University of Iowa. The standard curve was constructed with the national standard rat LH preparation (NIH-RP2) obtained from A. Parlow. 45 μL of rat plasma sample was diluted to 100 μL with 1% normal rabbit serum in Dulbeccos phosphate buffered saline (PBS; pH 7.4) and combined with 100 μL of [^{125}I]-rat-LH (Chemicon; 20 000 cpm) and 100 μL of antibody. The mixture was incubated for 24 h at 4 °C. Goat-antirabbit antibody (50 μL) was added to the mixture, and after 4 h at 4 °C, antibody-bound [^{125}I]LH was precipitated by centrifugation and counted by γ spectroscopy. Each sample was assayed in triplicate. The sensitivity of the assay was 50 pg per tube (1.1 ng/mL of plasma).

without further separation. In all cases, only L-amino acids were used.



11

Pharmacology

The affinities of **1–10** were tested in a rat pituitary LH-RH receptor competitive binding assay,[14] using [^{125}I]leuprolide as the radioligand. Ketoconazole (**8**) possessed micromolar affinity for LH-RH receptors. The interaction was competitive, because increasing concentrations of [^{125}I]leuprolide from 15 pM to 1.5 nM tested in the absence or presence of 3.16, 10, and 31.6 μM **8** demonstrated that only the affinity of radioligand but not the apparent binding site density was affected by **8** (Figure 1). Interstingly enough, itraconazole (**7**), a close relative of ketoconazole, failed to show any significant affinity even at a concentration as high as 30 μM. This may be due to the fact that the piperazine side chain of ketoconazole occupies a specific area with strict structural requirements while binding to the LH-RH receptor. A requirement for specific features of the ketoconazole side chain was further demonstrated by the lack of binding by analogues **1–5**. Compounds **6**, **9**, and **10**, which possess structural similarities to the amino terminal pyroGlu-His region of LH-RH, also interacted with the LH-RH receptor. Analogues **8** and **10** antagonized the leuprolide-induced release of LH from dispersed pituitary cells in culture,[15] with antagonist potencies (pA_2s; -log M) of 4.19 and 5.34, respectively. In castrated (30 days before the experiment began) male rats (n = 3, control LH 10.07 ng/mL, Figure 2), 100 mg/kg of **8** given intraduodenally produced a maximum 68% decrease in plasma LH concentration, sustained throughout a 6-h experimental period. Bhasin et al.[10] conducted a similar set of experiments where two groups of castrated male Sprague–Dawley rats were treated with 25 mg of ketoconazole in oil or oil alone by im injection every 8 h for 3 days. Plasma samples were collected at the end of a 3-day study and no significant change in either LH or FSH levels were seen between the groups. As stated earlier, our protocol was different in the sense that we used a 100 mg/kg bolus dose and sequentially sampled blood immediately after administration.

In summary, we found ketoconazole (**8**) to be an antagonist of LH-RH receptors both in vitro and in vivo, with micromolar affinity. We have also uncovered a novel series of peptidomimetics having submicromolar affinities.

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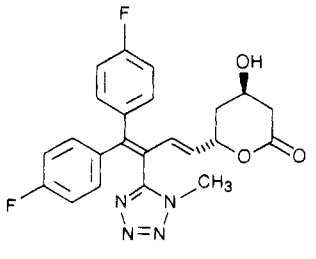
(15) Jinnah, H. A.; Conn, P. M. *Endocrinology* **1986**, *118*, 2599.

Biswanath De,* Jacob J. Plattner, Eugene N. Bush
Hwan-Soo Jae, Gilbert Diaz
Edwin S. Johnson, Thomas J. Perun
Abbott Laboratories, Pharmaceutical Research Division
Abbott Park, Illinois 60064
Received May 19, 1988

A Potent, Tissue-Selective, Synthetic Inhibitor of HMG-CoA Reductase

Sir:

Hypercholesterolemia (elevated levels of serum cholesterol) is a primary risk factor for coronary artery disease and atherosclerosis, which are major causes of death in western countries.[1] The enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) is the key regulatory enzyme in the biosynthesis of cholesterol and is, therefore, a prime target for therapeutic intervention in atherosclerosis.[2] Fungal metabolites, compactin and mevinolin, and synthetic analogues have been shown to be potent inhibitors of this enzyme.[3,4] Herein, we report a novel, tissue-selective, synthetic inhibitor of HMG-CoA reductase, **1** (BMY 22089).



1

As part of our synthetic effort, we have designed and prepared compounds having a substituted butadiene unit

(1) (a) Kannel, W. B.; Castelli, W. P.; Gordon, T.; McNamara, P. M. *Ann. Intern. Med.* **1971**, *74*, 1. (b) Endo, A. *J. Med. Chem.* **1985**, *28*, 401 and references therein.
(2) Brown, M. S.; Goldstein, J. L. *Science* **1986**, *232*, 34.

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