

## YM116, 2-(1*H*-Imidazol-4-ylmethyl)-9*H*-carbazole, Decreases Adrenal Androgen Synthesis by Inhibiting C17-20 Lyase Activity in NCI-H295 Human Adrenocortical Carcinoma Cells

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Received September 16, 1998    Accepted November 18, 1998

**ABSTRACT**—The concentrations of androstenedione and dehydroepiandrosterone, products of C17-20 lyase, in the medium after a 6-hr incubation of NCI-H295 cells were decreased by YM116 (2-(1*H*-imidazol-4-ylmethyl)-9*H*-carbazole) (IC<sub>50</sub>: 3.6 and 2.1 nM) and ketoconazole (IC<sub>50</sub>: 54.9 and 54.2 nM). 17 $\alpha$ -Hydroxyprogesterone, a product of 17 $\alpha$ -hydroxylase, was increased by YM116 (1–30 nM) and by ketoconazole (10–300 nM) and then was decreased at higher concentrations of both agents (IC<sub>50</sub>: 180 nM for YM116, 906 nM for ketoconazole), indicating that YM116 and ketoconazole were 50- and 16.5-fold more specific inhibitors of C17-20 lyase, respectively, than 17 $\alpha$ -hydroxylase. Compatible with these findings, progesterone, a substrate of 17 $\alpha$ -hydroxylase, was increased by these agents. Cortisol production was inhibited by YM116 and ketoconazole (IC<sub>50</sub>: 50.4 and 80.9 nM, respectively). YM116 was a 14-fold more potent inhibitor of androstenedione production than cortisol production, whereas ketoconazole was a nonselective inhibitor of the production of both steroids. YM116 and ketoconazole inhibited the C17-20 lyase activity in human testicular microsomes (IC<sub>50</sub>: 4.2 and 17 nM, respectively). These results demonstrate that YM116 reduces the synthesis of adrenal androgens by preferentially inhibiting C17-20 lyase activity.

**Keywords:** CYP17, Adrenal androgen, Ketoconazole, Adrenocortical cell, YM116

The adrenal cortex synthesizes the C21 steroids (glucocorticoids and mineralcorticoids) and the C19 steroids (androgens) from cholesterol (1). During this steroidogenic process, C17-20 lyase plays a key role in synthesizing adrenal androgens such as dehydroepiandrosterone and androstenedione (2). This enzyme catalyzes the cleavage of the C17-C20 carbon-carbon bond of the C21 hydroxylated intermediates, 17 $\alpha$ -hydroxypregnenolone and 17 $\alpha$ -hydroxyprogesterone, to form dehydroepiandrosterone and androstenedione, respectively. Adrenal androgens are released from the adrenals and converted to testosterone or dihydrotestosterone in the peripheral tissues. Therefore, the specific inhibition of C17-20 lyase could result in a decrease in not only the production of androgenic hormones in the adrenal cortex, but also the synthesis of testosterone in the testis, suggesting that inhibitors of this enzyme may be useful

for the treatment of androgen-dependent diseases such as prostate cancer (3, 4).

It was found in clinical practice that ketoconazole, an antifungal agent, produced a drop in the level of circulating androgens and a corresponding increase in circulating progesterone and 17 $\alpha$ -hydroxyprogesterone levels (5–8). These findings suggest that this agent may be able to inhibit C17-20 lyase activity preferentially. Since it is reported that 17 $\alpha$ -hydroxylase and C17-20 lyase activities reside in a single enzyme (CYP17 or P450 17 $\alpha$ ) (9), there is a possibility that ketoconazole inhibits 17 $\alpha$ -hydroxylase as well. In vitro studies have shown that the potency of ketoconazole in inhibiting 17 $\alpha$ -hydroxylase activity was about 1/10 or comparable to that of C17-20 lyase (10–12). However, the basal cortisol levels in the patients who underwent a long-term administration of ketoconazole were not affected or were only slightly lowered (8, 10). These clinical findings are rather unexpected, because ketoconazole is a potent inhibitor of 11 $\beta$ -hydroxylase and

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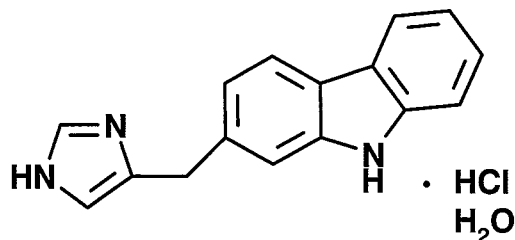


Fig. 1. Chemical structure of YM116.

cholesterol side-chain cleavage in vitro (13, 14). The clinical findings of ketoconazole-treated patients with respect to androgens and cortisol synthesis may be complicated by several actions induced by this agent. Ketoconazole has been used clinically in the treatment of advanced prostate cancers. Although this agent has received a favorable evaluation in some studies, it has proved less promising in other studies (15, 16). However, ketoconazole was withdrawn from clinical use because it caused gastrointestinal intolerance (nausea and vomiting) and/or hepatic lesions. These combined findings stimulated a search for a more specific and safer inhibitor of C17-20 lyase that could block adrenal androgens and testosterone (3, 17).

We have synthesized 2-(1*H*-imidazol-4-ylmethyl)-9*H*-carbazole, YM116 (Fig. 1), which is a novel non-steroidal imidazole derivative and inhibits C17-20 lyase activity in rat testicular microsomes. YM116 reduced the prostatic weight to castration levels by decreasing the serum concentrations of testosterone and dehydroepiandrosterone sulfate in rats (18). In this study, to clarify the site(s) of action of YM116 in its inhibition of the biosynthetic pathways of adrenal androgens and cortisol, we determined the concentrations of various steroid hormones that were released from human adrenocortical carcinoma cells during a 6-hr incubation, and we compared the hormone levels in the presence and absence of YM116 or ketoconazole. In addition, we have examined the inhibitory effect of YM116 on C17-20 lyase activity in human testicular microsomes and compared its potency with those of inhibitors of this enzyme reported recently (19–21).

## MATERIALS AND METHODS

### Materials

Ketoconazole was obtained from Paesel+Lorei GMBH & Co. (Frankfurt, Germany) and 17 $\alpha$ -[7-<sup>3</sup>H]-hydroxypregnenolone (748 GBq/mmol) was from DuPont NEN (Boston, MA, USA). The radioimmunoassays (RIA) used were obtained from the following sources: progesterone, 17 $\alpha$ -hydroxyprogesterone, de-

hydroepiandrosterone (Diagnostic Products Co., Los Angeles, CA, USA); androstenedione, 11-desoxycortisol (ICN Biomedicals, Inc., Costa Mesa, CA, USA); cortisol (Incstar Co., Stillwater, MN, USA). 2-(1*H*-Imidazol-4-ylmethyl)-9*H*-carbazole monohydrochloride monohydrate (YM116), CB7630 (19) and liarozole (20) were synthesized at Yamanouchi Pharmaceutical Co., Ltd. (Tokyo). All other reagents were of the analytical grade commercially available.

### Production of steroid hormones by human adrenocortical carcinoma cells

Human adrenocortical carcinoma cells, NCI-H295 cells, were obtained from the American Type Culture Collection (Rockville, MD, USA). These cells were maintained in RPMI medium 1640 (Life Technologies, Gaithersburg, MD, USA) with 1% ITS plus (6.25  $\mu$ g/ml insulin, 6.25  $\mu$ g/ml transferrin, 6.25 ng/ml selenous acid, 1.25 mg/ml bovine serum albumin (BSA), and 5.35  $\mu$ g/ml linoleic acid) (22), 10 nM hydrocortisone, 10 nM estradiol, 10 mM HEPES, 2% fetal bovine serum (FBS) and antibiotics. For the experiments, the cells were subcultured at a density of  $1 \times 10^5$  cells/well in a 12-well culture plate and incubated for 5 days. The 1 ml medium was replaced by serum-free RPMI 1640 medium containing 10 mM HEPES and 0.01% BSA, and the test compound in ethanol was added to the medium. After incubation in a CO<sub>2</sub> incubator at 37°C for 6 hr, the cells and medium were separated by centrifugation at  $500 \times g$  for 5 min, and an aliquot of the supernatant was obtained to measure the concentration of steroid hormones. The inhibitory activity of the test compound was evaluated by the difference in the amounts of steroid hormones released from the cells during a 6-hr incubation in the presence or absence of the test compound. The concentrations of 17 $\alpha$ -hydroxyprogesterone, androstenedione, dehydroepiandrosterone, progesterone, cortisol and 11-desoxycortisol were each measured by a specific RIA. The precision of the RIAs used in this study was determined by evaluating replicates of various concentrations of steroid hormones. The assay precisions for 17 $\alpha$ -hydroxyprogesterone, androstenedione, dehydroepiandrosterone, progesterone, cortisol and 11-desoxycortisol were checked; and under these assay conditions, the intraassay variations were 4.7%, 6.5%, 5.6%, 3.8%, 4.3% and 4.1%, respectively, while the interassay variations were 5.1%, 11.3%, 4.0%, 5.1%, 6.5% and 12.7%, respectively, for these steroids.

### Preparation of human testicular microsomes

Human testes were obtained from a male Japanese patient, aged 81 years, with informed consent during orchiectomy for prostate cancer. Testicular microsomes were prepared according to the method described by

Schatzman et al. (23). Briefly, the testes were minced with scissors and homogenized in 4 vol. of 50 mM phosphate buffer with 0.25 M sucrose and 1 mM EDTA, pH 7.4, with a polytron homogenizer (Kinematica GMBH, Lucerne, Switzerland). The homogenate was centrifuged at  $800 \times g$  for 10 min and then at  $9,000 \times g$  for 20 min. The resulting supernatant was again centrifuged at  $140,000 \times g$  for 1 hr. After decantation of the supernatant, the microsomal pellets were washed and resuspended in a solution of 50 mM phosphate buffer (pH 7.4) and glycerol (3:1). All of the above procedures were performed at 4°C. The final protein concentration was 50 µg/ml. The protein concentration was determined by a BioRad protein assay kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

#### Measurement of C17-20 lyase activity

The C17-20 lyase activity was determined by incubating 5 µg of human testicular microsomes in an incubation mixture containing (in a final volume of 200 µl) 50 mM phosphate buffer (pH 7.4), 118 nM  $17\alpha$ -[ $^3\text{H}$ ]-hydroxy-pregnenolone (4.63 kBq), the NADPH generating system (1 mM NADPH, 5 mM G6P, 2 IU/ml G6P dehydrogenase) and 2 µl of a test compound and/or solvent at 37°C for 1 hr. Following the quenching of incubates by 500 µl ethyl acetate, the mixture was centrifuged at  $2,000 \times g$  for 5 min and 400 µl of the upper layer was evaporated under decompression. The pellet was redissolved in 200 µl of 9% tetrahydrofuran/hexane and filtered using Samprep C02-LH (Millipore Co., Bedford, MA, USA).

Dehydroepiandrosterone was separated and quantified from its peak area relative to the peak of the internal standard using high-performance liquid chromatography (HPLC), with a Shimadzu LC-10A system equipped with an autosampler (Shimadzu, Kyoto) and a flowscintillation analyzer (Series A-500, Flow-One/beta; Packard Instrument Co., Meriden, CT, USA), according to the method described by Schatzman et al. (23). The radioactivity in the fraction of dehydroepiandrosterone was defined as the activity of C17-20 lyase.

#### Analytical procedures

To determine the  $\text{IC}_{50}$  value, i.e., the concentration required to decrease the concentration of hormone in the incubation medium of untreated control cells by 50%, the logarithm of the concentration of a test compound vs the concentration of each hormone was linearized by least-squares fitting (SAS Software; SAS Institute Japan, Tokyo). The regression equation was used to determine the  $\text{IC}_{50}$  values.

#### Statistical analyses

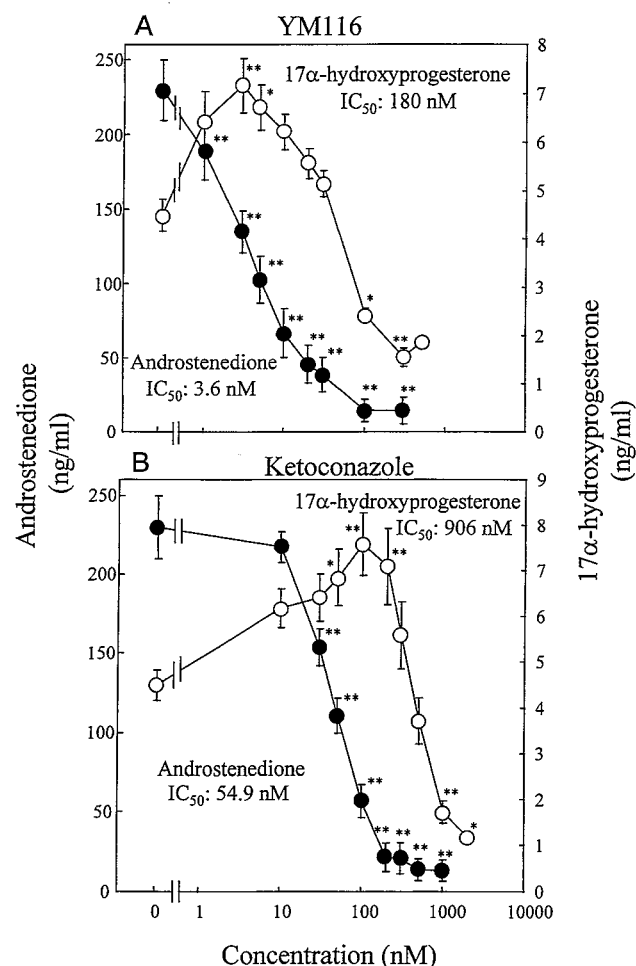
Comparisons between experimental groups were made

using the one-way ANOVA test, followed by the Dunnett's multiple range test. Differences were accepted as significant at the  $P < 0.05$  level.

## RESULTS

### *Amounts of $17\alpha$ -hydroxyprogesterone, androstenedione and dehydroepiandrosterone released from human adrenocortical carcinoma cells*

$17\alpha$ -Hydroxyprogesterone, androstenedione and de-



**Fig. 2.** Effects of YM116 and ketoconazole on the amounts of  $17\alpha$ -hydroxyprogesterone and androstenedione released from human adrenocortical carcinoma cells. NCI-H295 cells were incubated for 6 hr with various concentrations of YM116 (A) or ketoconazole (B). The concentrations of  $17\alpha$ -hydroxyprogesterone (○) and androstenedione (●) in the incubation medium were determined by its specific RIAs. The results are the mean  $\pm$  S.E.M. of 8 wells in 3 separate experiments. The  $\text{IC}_{50}$  value is the concentration required to decrease the concentration of hormone in the incubation medium of untreated control cells by 50%. Statistical significance was analyzed by one-way ANOVA, followed by the Dunnett's multiple range test. \* $P < 0.05$ , \*\* $P < 0.01$ , significantly different from the control value in the absence of YM116 or ketoconazole.

hydroepiandrosterone are the main products of  $17\alpha$ -hydroxylase/C17-20 lyase (24). The concentration of  $17\alpha$ -hydroxyprogesterone in the incubation medium was significantly increased, by up to 60% of the control value during the 6-hr incubation in the presence of 1–30 nM YM116 (Fig. 2A). Higher concentrations of YM116 in the medium resulted in a decrease in the  $17\alpha$ -hydroxyprogesterone concentration. Concentration-related changes in  $17\alpha$ -hydroxyprogesterone were also observed in ketoconazole-treated cells, but a higher concentration of this agent was needed compared to YM116 (Fig. 2B). The  $IC_{50}$  values of YM116 and ketoconazole were 180 and 906 nM, respectively. Both YM116 and ketoconazole decreased the androstenedione concentration in a concentration-dependent manner, with  $IC_{50}$  values of 3.6 and 54.9 nM, respectively. These findings indicated that YM116 was a 50-fold more potent inhibitor for C17-20 lyase than for  $17\alpha$ -hydroxylase. Ketoconazole was a less selective inhibitor of  $17\alpha$ -hydroxylase/C17-20 lyase. The dehydroepiandrosterone concentration in the medium was also decreased by YM116 and ketoconazole, with  $IC_{50}$  values of 2.1 and 54.2 nM, respectively (Fig. 3).

#### Amount of progesterone released from human adrenocortical carcinoma cells

The concentration of progesterone, one of the sub-

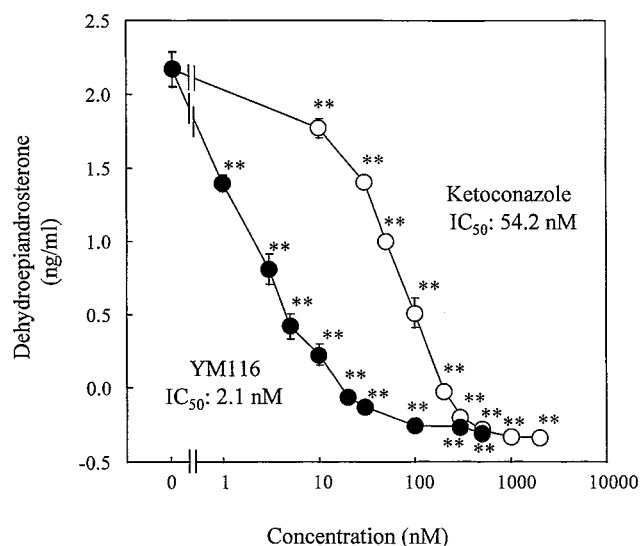
strates of  $17\alpha$ -hydroxylase, was significantly increased by YM116 in a concentration-dependent manner, consistent with the hypothesis that this agent inhibited CYP17 activity (Fig. 4). In contrast, ketoconazole decreased elevated levels of progesterone at higher concentrations.

#### Effects on cortisol and 11-desoxycortisol synthesis in human adrenocortical carcinoma cells

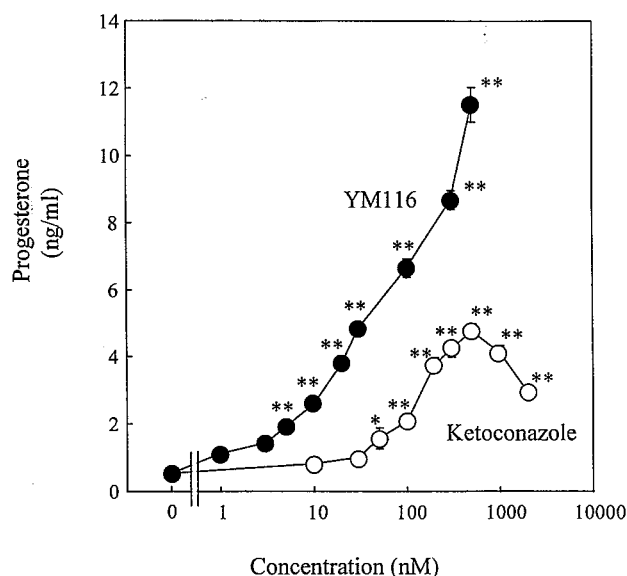
YM116 and ketoconazole inhibited cortisol production with  $IC_{50}$  values of 50.4 and 80.9 nM, respectively (Fig. 5), suggesting that the inhibitory effects of YM116 and ketoconazole on cortisol production were 14 times and 1.5 times less potent than their inhibitory effects on androstenedione production, respectively. YM116 was a more specific inhibitor for C17-20 lyase compared to ketoconazole. The concentration of 11-desoxycortisol was increased by YM116 at a concentration of 1–30 nM, but decreased at higher concentrations of this agent (Fig. 6).

#### Inhibition of human testicular C17-20 lyase activity

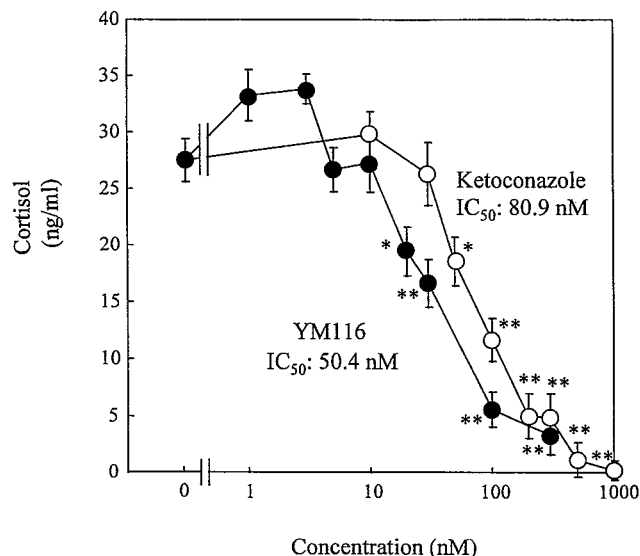
YM116 inhibited the human testicular C17-20 lyase activity in a concentration-dependent manner, with an  $IC_{50}$  value of 4.2 nM (Table 1). The  $IC_{50}$  values of ketoconazole, CB7630 and liarozole were 17, 4.3 and 68



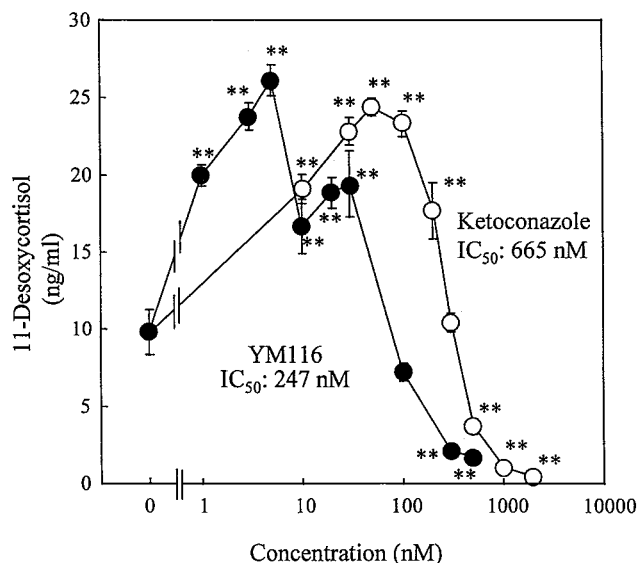
**Fig. 3.** Inhibitory effect of YM116 and ketoconazole on dehydroepiandrosterone synthesis in human adrenocortical carcinoma cells. NCI-H295 cells were incubated for 6 hr with various concentrations of YM116 (●) or ketoconazole (○). The concentration of dehydroepiandrosterone was determined by its specific RIA. The results are the mean  $\pm$  S.E.M. of 9 wells in 3 separate experiments. \*\* $P < 0.01$ , significantly different from the control value in the absence of YM116 or ketoconazole (one-way ANOVA, Dunnett's multiple range test).



**Fig. 4.** Increase in progesterone synthesis by YM116 and ketoconazole in human adrenocortical carcinoma cells. NCI-H295 cells were incubated for 6 hr with various concentrations of YM116 (●) or ketoconazole (○). The concentration of progesterone in the incubation medium was determined by its specific RIA. The results are the mean  $\pm$  S.E.M. of 9 wells in 3 separate experiments. \* $P < 0.05$ , \*\* $P < 0.01$ , significantly different from the control value in the absence of YM116 or ketoconazole (one-way ANOVA, Dunnett's multiple range test).



**Fig. 5.** Effects of YM116 and ketoconazole on cortisol synthesis in human adrenocortical carcinoma cells. NCI-H295 cells were incubated for 6 hr with various concentrations of YM116 (●) or ketoconazole (○). The concentration of cortisol in the incubation medium was determined by its specific RIA. The results are the mean  $\pm$  S.E.M. of 9 wells in 3 separate experiments. \* $P < 0.05$ , \*\* $P < 0.01$ , significantly different from the control value in the absence of YM116 or ketoconazole (one-way ANOVA, Dunnett's multiple range test).



**Fig. 6.** Effects of YM116 and ketoconazole on 11-desoxycortisol synthesis in human adrenocortical carcinoma cells. NCI-H295 cells were incubated for 6 hr with various concentrations of YM116 (●) or ketoconazole (○). The amount of 11-desoxycortisol released from the cells was determined by its specific RIA. The results are the mean  $\pm$  S.E.M. of 9 wells in 3 separate experiments. \*\* $P < 0.01$ , significantly different from the control value in the absence of YM116 or ketoconazole (one-way ANOVA, Dunnett's multiple range test).

**Table 1.** Inhibitory effects of YM116, ketoconazole, CB7630 and liarozole on human testicular C17-20 lyase activities

Inhibition of human C17-20 lyase activity*	
	IC <sub>50</sub> (nM)
YM116	4.2
Ketoconazole	17
CB7630	4.3
Liarozole	68

\*Results expressed as the concentration required to inhibit the enzyme activity by 50% (IC<sub>50</sub>).

nM, respectively. YM116 was about 4 and 16 times more potent than ketoconazole and liarozole, respectively, and equipotent to CB7630 in inhibiting C17-20 lyase activity.

## DISCUSSION

The NCI-H295 cell line used in this study was established from an invasive primary human adrenocortical carcinoma (25). These cells express most of the enzymes needed for adrenal steroidogenesis (26) and actually form glucocorticoids, mineralcorticoids and androgens. The ability of these cells to produce cortisol and adrenal androgens (such as androstenedione, dehydroepiandrosterone and its sulfate) suggests that these cells could act as a model system for the identification of the factors regulating the relative production of cortisol and adrenal androgens (27). One of the main purposes in this study was to examine the inhibitory effect of YM116 on 17 $\alpha$ -hydroxylase/C17-20 lyase activity, which synthesizes dehydroepiandrosterone and androstenedione from pregnenolone and progesterone, respectively, in NCI-H295 cells. This process is catalyzed by a single enzyme known as CYP17 (28, 29).

A low concentration (1–30 nM) of YM116 induced a 60% increase in the 17 $\alpha$ -hydroxyprogesterone level during a 6-hr incubation of NCI-H295 cells, whereas further increased concentrations of this agent (more than 100 nM) resulted in a decrease in the 17 $\alpha$ -hydroxyprogesterone level, with an IC<sub>50</sub> value of 180 nM (Fig. 2). In contrast, YM116 concentration-dependently decreased the concentrations of dehydroepiandrosterone and androstenedione, with IC<sub>50</sub> values of 2.1 and 3.6 nM, respectively. YM116 inhibited the production of androstenedione at a 50-fold lower concentration than that which inhibited the production of its precursor, 17 $\alpha$ -hydroxyprogesterone, suggesting a preferential inhibition of the C17-20 lyase activity. Ketoconazole also decreased the productions of 17 $\alpha$ -hydroxyprogesterone and androstenedione, with IC<sub>50</sub> values of 906 and 54.9 nM, respectively, showing a preferential inhibition of the

C17-20 lyase activity but at a lesser extent compared to YM116. YM116 and ketoconazole increased concentrations of progesterone, but at higher concentrations, ketoconazole decreased them (Fig. 4). The reason for this difference remains unknown, but ketoconazole is reported to reduce the conversion of cholesterol to pregnenolone by inhibiting CYP11A1 (P450<sub>scc</sub>) activity (13, 14). It is likely that the decrease in progesterone concentration induced by ketoconazole at high concentrations is due to a decreased supply of pregnenolone, a precursor of progesterone, although the effects of YM116 and ketoconazole on CYP11A1 was not examined in this study. These findings were compatible with observations in clinical studies (5–8) that a low dose of ketoconazole reduced the serum testosterone concentration with a concomitant increase in the  $17\alpha$ -hydroxyprogesterone concentration. Thus, it is concluded that YM116 is a more potent and selective inhibitor of C17-20 lyase than ketoconazole in inhibiting adrenal androgen production in human adrenocortical carcinoma cells.

Gazdar et al. (25) showed that in NCI-H295, very high concentrations of pregnenolone,  $17\alpha$ -hydroxypregnenolone and dehydroepiandrosterone were present, along with more modest concentrations of progesterone,  $17\alpha$ -hydroxyprogesterone and androstenedione, indicating a significant lyase pathway from  $17\alpha$ -hydroxypregnenolone. However, the amount of androstenedione secreted from NCI-H295 used in this study was about 100-fold more than that of dehydroepiandrosterone, indicating a significant lyase pathway from  $17\alpha$ -hydroxyprogesterone. The reason for the difference between the original findings and our observations remains unknown. However, there is a possibility that a subpopulation of these cells can be selected that use preferentially  $17\alpha$ -hydroxyprogesterone or culture conditions might alter the transcriptional expression of steroidogenic enzymes (30).

The direct inhibition of C17-20 lyase activity YM116 and ketoconazole was demonstrated in microsomes obtained from human testes, with an  $IC_{50}$  value of 4.2 nM for YM116 and 17 nM for ketoconazole, indicating that YM116 was a fourfold more potent inhibitor than ketoconazole (Table 1). Other inhibitors reported recently, such as CB7630 and liarozole, also inhibited C17-20 lyase activity. As mentioned above, YM116 was a 15-fold more potent inhibitor of androstenedione production than ketoconazole in NCI-H295 cells ( $IC_{50}$ : 3.6 nM for YM116 and 54.9 nM for ketoconazole). These results clearly showed that there was a significant difference in the relative potency of these two agents between microsomes and cells, although there was no significant change in the range of the potency of the two agents. Albertson et al. (11) reported that there was no difference in the concentration of ketoconazole required to inhibit the

enzymatic activities of  $17\alpha$ -hydroxylase and C17-20 lyase. In addition, Engelhardt et al. (31) showed using human adrenal tissue slices that ketoconazole inhibited C17-20 lyase activity at a concentration 9 times lower than that which inhibited  $17\alpha$ -hydroxylase activity. Although the reason(s) for this difference remains unknown, the activity of  $17\alpha$ -hydroxylase/C17-20 lyase is reported to be dependent on numerous factors, such as its lipid environment and the presence of electron transport components (32); namely,  $17\alpha$ -hydroxylase/C17-20 lyase requires the presence of NADPH and NADPH cytochrome P450 reductase for activity. The amount of reductase as well as cytochrome b5 changed the relative activities of  $17\alpha$ -hydroxylase/C17-20 lyase (33, 34). In addition, the treatment of microsomes by detergent also affected the activities of the enzyme because of the disruption of the microsomal structure (32). Although YM116 inhibited rat C17-20 activity competitively (18), the inhibitory effects of this agent may be modified by several factors in microsomal preparations. Thus, NCI-H295 cells may act as an ideal model system for the determination of the ability of agents to affect adrenal steroidogenesis, eliminating the involvement of numerous factors associated with in vitro assay conditions.

YM116 and ketoconazole each inhibited cortisol production, with  $IC_{50}$  values of 50.4 and 80.9 nM (Fig. 5), suggesting that the inhibitory effect of each agent on cortisol production was 14 or 1.5 times less potent than its corresponding inhibitory effect on androstenedione production. The concentration of 11-desoxycortisol was increased by YM116 at a concentration of 1–30 nM and was then decreased at higher concentrations of this agent (Fig. 6). These changes were very similar to those in the  $17\alpha$ -hydroxyprogesterone concentration, indicating the existence of a possible inhibitory site between 11-desoxycortisol and cortisol. Detailed studies with  $11\beta$ -hydroxylase showed an additional blockage of this enzyme by ketoconazole (35). However, ketoconazole has had little effect on cortisol levels in clinical practice, although cortisol production in response to ACTH stimulation was blunted (36). Further study is necessary to determine the in vivo effect of YM116 on serum cortisol concentrations in humans.

Finally, it is reported that ketoconazole is metabolized by the cytochrome P450 enzyme CYP3A4 and that this agent inhibits biotransformation of other drugs (37). Because YM116 and ketoconazole have an imidazole ring in their structures and imidazoles inhibit oxidative drug metabolism by forming a tight complex with the heme of P450, we have examined the inhibitory effects of these agents on CYP3A4 activity using testosterone as a substrate. Our preliminary results showed that ketoconazole was about a 30 times more potent inhibitor of CYP3A4

than YM116 (H. Miura et al., unpublished data).

In conclusion, the results of this study demonstrated that YM116 is a potent and selective inhibitor of C17-20 lyase in human adrenocortical carcinoma cells, leading to a significant reduction in adrenal androgen production. Further studies are required to determine the efficacy of this agent for the treatment of hormone-dependent prostate cancer in clinical studies.

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