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Inhibition of Angiogenesis by the Antifungal Drug Itraconazole

Curtis R. Chong[†,‡,§], Jing Xu[†,‡], Jun Lu[†], Shridhar Bhat[†,‡], David J. Sullivan, Jr.[‡,¶], and Jun O. Liu[†,‡,∥,⊥,*]

[†]Department of Pharmacology and Molecular Sciences, [‡]The Johns Hopkins Clinical Compound Screening Initiative, [§]Medical Scientist Training Program, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, [¶]The Malaria Research Institute, W. Harry Feinstone Department of Molecular Microbiology and Immunology, The Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland 21205, [∥]The Solomon H. Snyder Department of Neuroscience, and [⊥]Department of Oncology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

ABSTRACT Angiogenesis, the formation of new blood vessels, is implicated in a number of important human diseases, including cancer, diabetic retinopathy, and rheumatoid arthritis. To identify clinically useful angiogenesis inhibitors, we assembled and screened a library of mostly Food and Drug Administration-approved drugs for inhibitors of human endothelial cell proliferation. One of the most promising and unexpected hits was itraconazole, a known antifungal drug. Itraconazole inhibits endothelial cell cycle progression at the G1 phase *in vitro* and blocks vascular endothelial growth factor/basic fibroblast growth factor-dependent angiogenesis *in vivo*. In attempts to delineate the mechanism of action of itraconazole, we found that human lanosterol 14α-demethylase (14DM) is essential for endothelial cell proliferation and may partially mediate the inhibition of endothelial cells by itraconazole. Together, these findings suggest that itraconazole has the potential to serve as an antiangiogenic drug and that lanosterol 14DM is a promising new target for discovering new angiogenesis inhibitors.

Since the angiogenesis hypothesis was first put forward in 1971, the physiologic and pathological roles of angiogenesis in various biological and disease processes have been subject to extensive scrutiny (*1*). The importance of angiogenesis in human diseases such as cancer is well established (*2, 3*). Significant progress in antiangiogenic drug discovery and development has also been made, culminating in the development of angiogenesis inhibitors as drugs for the treatment of cancer (*4*) and age-related macular degeneration (*5*). Angiogenesis inhibitors have been found to be particularly useful when used in conjunction with other chemotherapeutic drugs (*6*). Angiogenesis also contributes to the pathogenesis of a number of other diseases, including obesity, psoriasis, Kaposi's sarcoma, diabetic retinopathy, pulmonary hypertension, and arthritis (*7*). It is thus not surprising that ~500 million people worldwide may benefit from treatments that modulate angiogenesis.

A number of existing drugs have been found to possess antiangiogenic effects either serendipitously or by rational prediction. One of the first antiangiogenic drug candidates to enter clinical trials is TNP-470, a derivative of the antiamebic drug fumagillin (*8*), which was discovered in the late 1980s from a fungal contamination that inhibited endothelial cell culture growth (*9*). Other existing drugs such as thalidomide (*10*), nonsteroidal anti-inflammatory agents (*11*), and rapamycin (*12*) also inhibit angiogenesis and have shown promise in clinical trials for the treatment of cancer. Although new uses for several dozen existing drugs such as fumagillin have been found serendipitously or through knowledge of pharmaceutical side effects (*13, 14*), a systematic assembly and screening of libraries of existing drugs for

***Corresponding author,**
joliu@jhu.edu.

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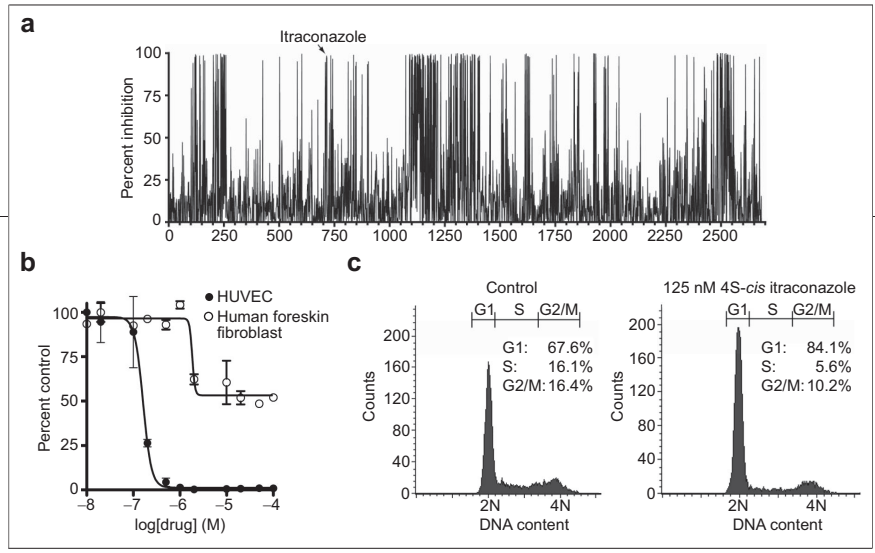


Figure 1. Itraconazole inhibits endothelial cell proliferation. a) Screening results for 2,604 existing drugs on HUVEC proliferation at 10 μM. b) Inhibition of proliferation by itraconazole. c) Cell cycle analysis of HUVEC showing G1/S arrest upon 4*S-cis* itraconazole treatment.

novel pharmacological activities did not begin until recently (*15*).

In an effort to uncover novel biological activity among existing drugs, we began to collect and assemble into a library drugs approved by the Food and Drug Administration and its foreign counterparts, as well as those that had entered phase 2 clinical trials. A preliminary screen of this library in a human umbilical vein endothelial cell (HUVEC) proliferation assay identified the immunosuppressive drug mycophenolic acid as a potent inhibitor of angiogenesis *in vitro* and *in vivo* (*16*). This led to the discovery of the type 1 inosine monophosphate dehydrogenase as a specific target for angiogenesis and the type 2 enzyme as a specific target for immunosuppression (*16*). Herein, we report that the known antifungal drug itraconazole also possesses potent antiangiogenic activity both *in vitro* and *in vivo*.

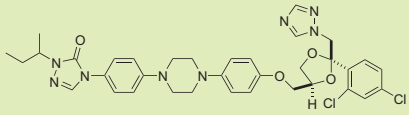
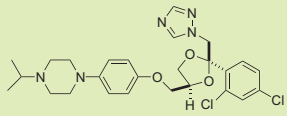
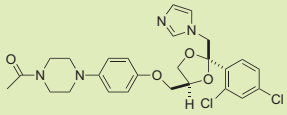
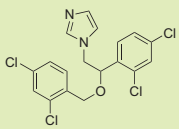
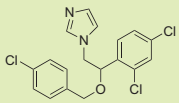
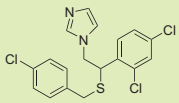
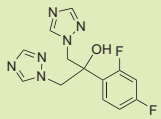
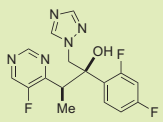
RESULTS AND DISCUSSION

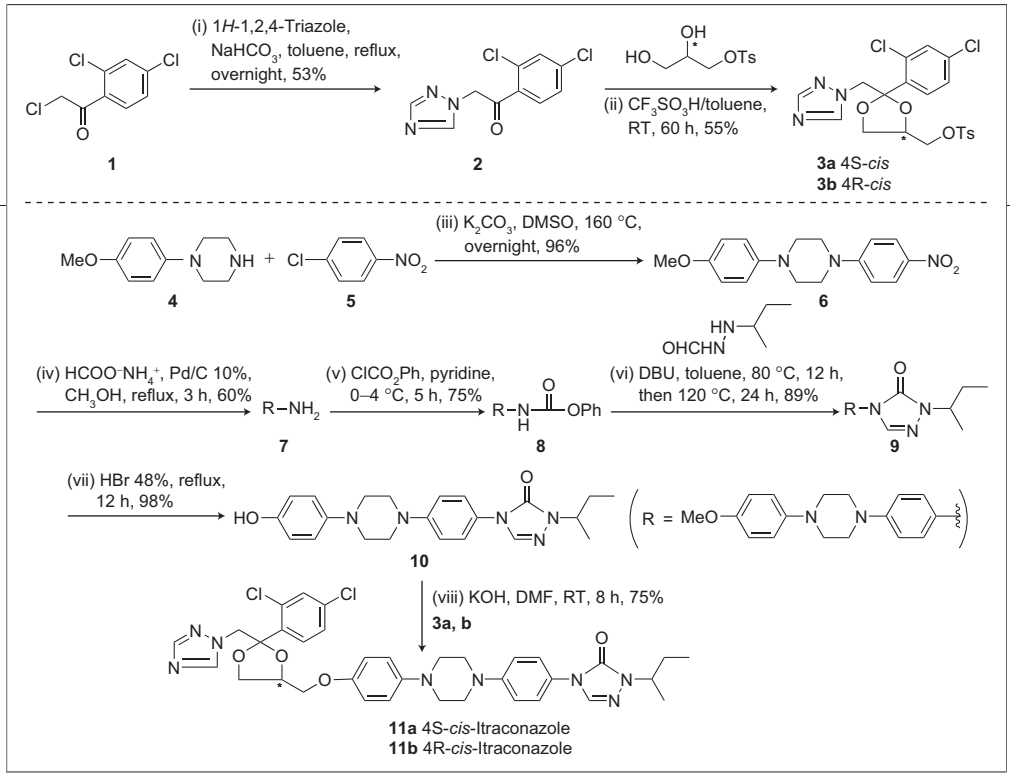
Endothelial cells form the inner lining of all blood vessels and constitute an essential part of new as well as pre-existing blood vessels. The proliferation, migration, and differentiation of endothelial cells are integral parts of angiogenesis. The majority of angiogenesis inhibitors discovered to date target endothelial cells. We thus employed an endothelial cell proliferation assay to screen our clinical drug library. The screen was carried out in 96-well plates with each drug at a final concentration of 10 μM. Thus, HUVEC were incubated with drugs for 36 h, and proliferation was measured by following incorporation of [^{3}H]-thymidine for the final 8 h. The preliminary screen identified 210 existing drugs with at least 50% inhibition at 10 μM, which belong to multiple drug classes (Figure 1, panel a). Some hits, such as antineoplastic and antiprotozoal drugs, are generally cytotoxic and are known to inhibit the proliferation of HUVEC and other cell types. These hits were excluded from further investigation. Other hits, including antacids, antibacterials, antiemetics, antihelminthics, antiobesity, and antiseptic drugs that are either for topical use or are not systemically absorbed, were also excluded from further studies. In addition, we put into lower priority those drugs whose IC_{50} values exceed peak plasma levels achieved with current clinical use, including antidepressants, antihyperlipidemics, antihypertensives, antipsychotics, cardiotonics, and steroids. However, it is worth pointing out that those drugs can still serve as promising leads for the development of antiangiogenic drugs. After the aforementioned filtration, several hits remained. One interesting hit was mycophenolic acid (*16*). Another hit, which was quite unexpected, was itraconazole (Table 1). Itraconazole has not been previously reported to inhibit endothelial cell proliferation or angiogenesis.

Itraconazole belongs to the family of azole antifungal drugs with several generations of structurally and mechanistically related analogues (*17*). Itraconazole displayed quite potent and selective inhibitory activity toward endothelial cells compared to other cell types tested. For example, itraconazole has little effect on the proliferation of human foreskin fibroblasts (HFF), with an $IC_{50} > 20$ μM in comparison to HUVEC ($IC_{50} = 0.16$ μM) (Figure 1, panel b). While it potently inhibited the proliferation of bovine aortic endothelial cells (BAEC), it is much less effective against Jurkat T cells or HeLa cells (Supplementary Figure 1). As shown in Table 1, itraconazole was unique among all azole antifungal drugs tested so far in its ability to selectively inhibit the proliferation of endothelial cells with high potency. In sharp contrast to itraconazole, the structurally related terconazole and ketoconazole were ~27- and 40-fold less potent against HUVEC, respectively. Intriguingly, whereas terconazole was inactive toward Jurkat T cells, like itraconazole, its analogue ketoconazole inhibited Jurkat T cell proliferation, albeit with low potency. Some of the most potent antifungal drugs of this superfamily, such as fluconazole and voriconazole, were inactive toward HUVEC, HFF, and Jurkat T cells (Table 1).

Itraconazole contains three stereocenters, which can yield a total of eight stereoisomers. The oral and intravenous formulations of itraconazole are supplied as a 1:1:1:1 mixture of four diastereomers (*18*). In an attempt to investigate whether inhibition of HUVEC by itraconazole

TABLE 1. IC_{50} values for the inhibition of HUVEC, HFF, and Jurkat T cell proliferation by itraconazole and other antifungal drugs

Drugs		IC_{50} (μM)		
		HUVEC	HFF	Jurkat
Itraconazole		0.16	>100	>100
Terconazole		7.1	ND	>100
Ketoconazole		10.4	19.6	24.8
Miconazole		2.47	28.2	10.8
Econazole		4.80	31.7	16.0
Sulconazole		>100	35.7	11.4
Fluconazole		>100	>100	>100
Voriconazole		>100	>100	>100



Scheme 1. Synthesis of itraconazole stereoisomers.

is stereoselective, we synthesized one pair of two diastereomers, the 4*S-cis* and 4*R-cis* itraconazole. The total synthesis of both isomers of itraconazole is shown in Scheme 1. Thus, a diastereoselective ketalization of **2** using a chirally pure glycerol monotosylate (step ii) afforded the intermediate **3a** and **3b**. Another intermediate, **10**, was synthesized *via* a five-step sequence starting from the piperazine precursor **4** and 4-chloronitrobenzene (**5**). The final coupling of tosylate **3a** or **3b** and phenol **10** was carried out under basic conditions to give either the 4*S-cis* or the 4*R-cis* itraconazole in good yield. The inhibitory activity of these diasteromers was then determined in the HUVEC proliferation assay. The 4*S-cis* diastereomer (IC_{50} = 0.056 ± 0.01 μM) was found to be ~20-fold more potent than the 4*R-cis* stereoisomer (IC_{50} = 1.1 ± 0.13 μM). In comparison, the racemic itraconazole has an IC_{50} of 0.16 μM. The significant influence of stereochemistry at one end of itraconazole on its activity suggests that this part of itraconazole may participate in a stereospecific interaction with target(s) in endothelial cells.

To further delineate the mechanism of inhibition of endothelial cell proliferation by itraconazole, we examined its effect on the cell cycle progression of HUVEC by fixing and staining cells with propidium iodide followed by fluoresence-activated cell sorting analysis. The 4*S-cis* diastereomer potently inhibits HUVEC cell cycle progression at the G1/S transition (Figure 1, panel c). Treatment of HUVEC with racemic itraconazole also led to an increase of cells in the G1 phase of the cell cycle and a corresponding decrease in cells in the S phase (data not shown). These results indicate that itraconazole inhibits HUVEC proliferation by blocking cell cycle progression in the G1 phase.

The molecular mechanism of action of itraconazole is well established for its antifungal activity: it inhibits lanosterol 14α-demethylase (14DM), which catalyzes an essential step in the biosynthesis of ergosterol required for the membrane integrity of fungal cells (*19*). The demethylation of lanosterol is a common step between fungi and humans in sterol biosynthesis prior to the divergence of the pathways leading to ergosterol in fungi and cholesterol in humans, respectively. Although itraconazole as well as other azole antifungal drugs preferably inhibit the fungal 14DM over its human counterparts, they do inhibit the human enzyme at higher concentrations. The IC_{50} values of itraconazole for human 14DM varied from 0.61 to 30 μM for unknown reasons (*20, 21*). Nevertheless, this raised the formal possibility that the inhibition of the endothelial cell cycle by itraconazole may be mediated at least in part through the inhibition of human 14DM.

Two complementary approaches were taken to assess the relevance of 14DM in the inhibition of endothelial cell proliferation by itraconazole. First, we synthesized a known potent inhibitor of human 14DM, azalanstat, and compared its effect on endothelial cells with that of itraconazole (*22, 23*). Similarly to itraconazole, azalanstat also blocked the cell cycle progression of HUVEC (Supplementary Figure 2) and BAEC in the G1 phase of the cell cycle (Supplementary Figure 3 and Table 2), suggesting that 14DM is required for endothelial cell proliferation. A hallmark of inhibitors of 14DM is that their potencies are dependent on the levels of cholesterol in cell culture medium (*24, 25*). We thus determined the potencies of both azalanstat and itraconazole in cell culture medium either containing or lacking cholesterol. As expected, azalanstat displayed higher potency toward endothelial cells in the absence of cholesterol (IC_{50} = 0.31 μM) than in its presence (IC_{50} = 1.2 μM) (Figure 2, panel a). Similarly, the inhibition of

TABLE 2. Effects of itraconazole and azalanstat on the cell cycle progression of BAEC[a]

	Control			Cholesterol		
	G1	S	G2/M	G1	S	G2/M
Control	66.4	18.7	14.2	65.5	22.1	11.8
Itraconazole	77.0	10.5	12.0	66.2	21.2	12.0
Azalanstat	74.5	16.8	8.8	70.2	19.4	10.0

[a]Values represent the percentage of cells in a given phase of the cell cycle.

endothelial cells by itraconazole was also sensitive to cholesterol, being less potent when cholesterol is present (IC_{50} = 0.044 *vs* 0.23 μM) (Figure 2, panel b). In contrast, an inhibitor of angiogenesis with unrelated mechanism of action, TNP-470, which works by inhibiting the type 2 methionine aminopeptidase (*26, 27*), inhibited endothelial cell proliferation with roughly equal potency in the absence and presence of cholesterol (Figure 2, panel c). Together, these observations suggest that itraconazole works at least in part by inhibiting cholesterol biosynthesis.

The second approach we took was to knock down the expression of human 14DM in HUVEC and determine the effect on cell proliferation. Thus, three different short hairpin RNAs (shRNAs) targeting the coding region of human 14DM messenger RNA were transiently expressed in 293T cells along with the expression plasmid for human 14DM with an C-terminal c-Myc tag. One of the constructs, pSSII-sih14DM, dramatically blocked the expression of ectopically expressed protein (Figure 3, panel a). The expression cassette for this shRNA was then moved to the lentiviral vector, pFUP2 (*28*), and the resulting lentiviruses were generated and used to transduce HUVEC. As shown in Figure 3, panel b, the human 14DM lentiviral shRNA blocked the expression of endogenous 14DM expression, as judged by reverse transcriptase polymerase chain reaction (RT-PCR) about 3 d after viral transduction. The transduced cells were allowed to grow, and their proliferation in the absence and presence of varying concentrations of itraconazole was determined at 7 d post-transduction. HUVEC transducted with human 14DM shRNA proliferate more slowly than those transduced with the control viruses, as judged by the amounts of [^{3}H]-thymidine incorporated at day 7 (Figure 3, panel c). Together, these results demonstrate that 14DM is essential for endothelial cell growth and suggest that human 14DM may serve as a novel target for developing angiogenesis inhibitors.

It has been reported that the statins, which inhibit 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, have biphasic effects on angiogenesis (*29*). At lower doses, statins stimulate angiogenesis, whereas at higher doses they inhibit angiogenesis. In agreement with previous reports, statins were also found to inhibit endothelial cell proliferation in our screen (Supplementary Table 1). The inhibition of angiogenesis by statins at higher doses has been attributed to the inhibition of prenylation of such signaling proteins as RhoA rather than sterol biosynthesis (*30*). Our demonstration that both

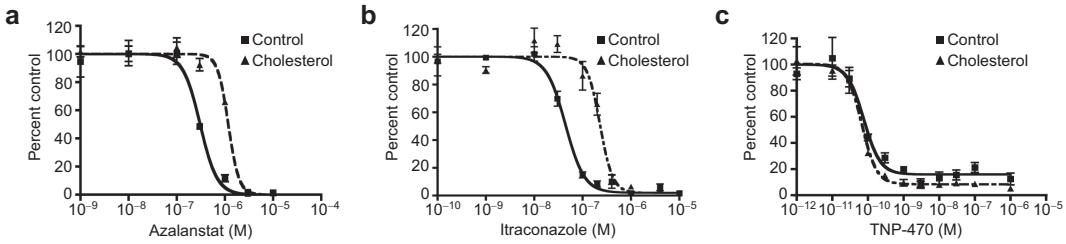


Figure 2. Inhibition of BAEC proliferation by itraconazole and azalanstat can be partially reversed by cholesterol. BAEC were incubated in DMEM, 10% LPDS with indicated concentrations of a) itraconazole, b) azalanstat, or c) TNP-470 either alone or in combination with 40 μg mL^{-1} free cholesterol for 36 h. Cells were then pulsed with 1 μCi [^{3}H]-thymidine for 8 h before they were harvested for scintillation counting.

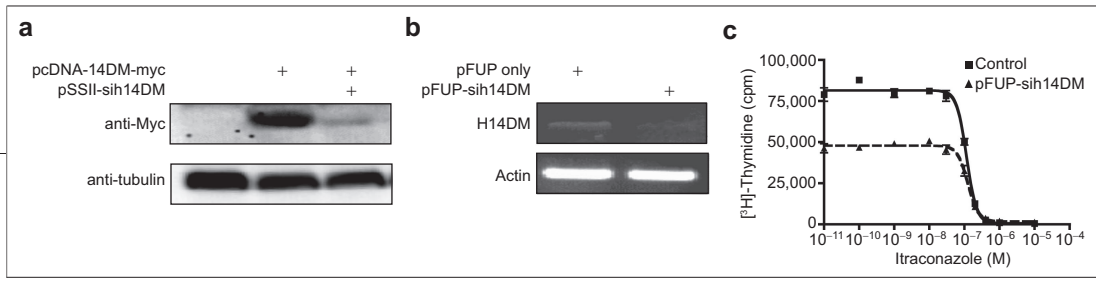


Figure 3. Knockdown of 14DM in HUVEC inhibits proliferation. a) Western blot of knockdown of transiently expressed 14DM protein. 293T cells were transfected with myc-tagged 14DM together with pSSII vector or pSSII-sih14DM. Cells were harvested 48 h after transfection, and cell lysate was subjected to SDS-PAGE followed by Western blot with antibodies as indicated. b) RT-PCR of 14DM knockdown HUVEC. HUVEC were transduced with pFUP or pFUP-sih14DM lentivirus. Cells were harvested 72 h after transduction, and total RNA was extracted and subjected to RT-PCR assay. c) Knockdown of human 14DM in HUVEC inhibits cell proliferation. HUVEC were transduced with lentiviruses containing 14DM knockdown shRNA, and the transduced cells were maintained in EGM-2 medium. Cell proliferation was determined in the absence and presence of varying concentrations of itraconazole at day 7 post-transduction.

chemical inhibition by azalanstat and RNA knockdown of 14DM caused inhibition of endothelial cell proliferation suggests that cholesterol biosynthesis is also essential for endothelial cell growth. It remains to be determined whether interference with cholesterol biosynthesis is partly responsible for the antiangiogenic effects of higher concentrations of statins.

It is apparent from the existing experimental evidence that 14DM is a potential target for itraconazole. However, it remains unclear whether 14DM is the only or major target of itraconazole for its antiangiogenic effects. Among the evidence in support of 14DM as a major target for itraconazole are the following: (i) Itraconazole does inhibit human 14DM *in vitro*. (ii) Similar profiles of effects on endothelial cells were observed for itraconazole and the known 14DM inhibitor azalanstat, including the common G1 cell cycle arrest and sensitivity to the presence of cholesterol in cell culture medium. (iii) 14DM appeared to be essential for HUVEC proliferation on the basis of RNA interference experiments. There are also several pieces of evidence that are inconsistent with 14DM being the target for itraconazole in endothelial cells: (i) Ketoconazole that is at least equally potent against 14DM *in vitro* is much less potent than itraconazole for inhibition of endothelial cells. (ii) Addition of cholesterol, although causing a 5-fold decrease in sensitivity of BAEC to itraconazole, did not completely reverse the inhibition by itraconzole, suggesting that inhibition of cholesterol biosynthesis is not sufficient to explain the effect of itraconazole in endothelial cells in the presence of normal concentrations of cholesterol in culture medium. The uncertainty on 14DM is further compounded by the discrepancy in the reported potency of itraconazole on human 14DM *in vitro*. It was reported earlier that the IC_{50} of itraconazole for recombinant human 14DM is 0.61 μM, similar to that of ketoconazole (*20*); however, in a more recent study, the IC_{50} was found to be much higher at 30 μM (*21*). Additional experiments will be required to provide an unambiguous answer to the question of whether 14DM is the antiangiogenic target of itraconazole.

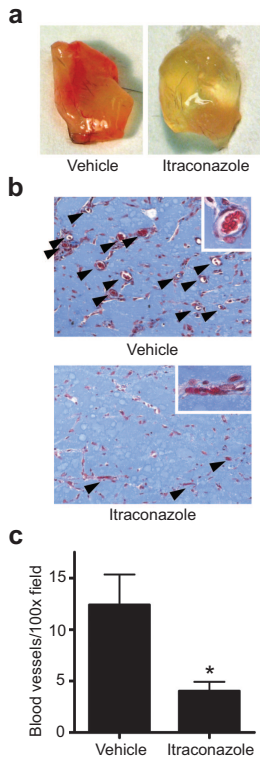


Figure 4. Intraperitoneal treatment of mice with itraconazole, 37.5 mg kg^{-1} d^{-1}, significantly inhibited angiogenesis as shown in a) representative Matrigel plugs and b) in 100× sections of plugs harvested from mice. c) Erythrocyte-filled blood vessels were counted per 100× field (*$p = 0.01$, $n = 6$ vehicle, $n = 8$ itraconazole).

To determine whether itraconazole inhibited angiogenesis *in vivo*, we tested itraconazole in a mouse Matrigel model (*31*). In humans, itraconazole is administered intravenously at a dose of 105 mg m^{-2} twice daily. Mice were thus treated with a comparable dose of itraconazole (112.5 mg m^{-2} or 37.5 mg kg^{-1}, intraperitoneally once daily). A significant decrease in angiogenesis was observed both macroscopically, as judged by the red color of the isolated Matrigel plugs (Figure 4, panel a), and microscopically upon staining thin sections for new blood vessels (Figure 4, panel b) in animals treated with itraconazole. Overall, there was a 67.5% decrease in new blood vessel formation in itraconazole-treated mice compared to vehicle-treated controls (Figure 3, panel c), indicating that itraconazole is capable of suppressing angiogenesis *in vivo*.

In order for itraconazole to have a clinically useful effect on angiogenesis in humans, its effective concentration for inhibiting endothelial cell proliferation has to be below the trough plasma level of the drug under existing dosing regimens. Fortunately, the IC_{50} value of itraconazole for HUVEC inhibition is well below the steady-state levels achieved with a standard oral 200 mg dose ($C_{min} = 2.6$ μM, $C_{max} = 3.2$ μM, $t_{1/2} = 64$ h) (www.ortho-mcneil.com/html/comi2/products_list.jsp), suggesting it is likely to exhibit antiangiogenic

effects *in vivo* under the current regimen of drug administration. Although itraconazole has been associated with rare cases of hepatotoxicity as its major side effect, it can be taken orally for up to 3 months to treat finger and toenail infections. Given the relatively safe history of itraconazole in the clinic and its well-established side effects, the gap between additional preclinical studies and human clinical trials can be easily narrowed, accelerating the development of itraconazole as a therapeutic antiangiogenic drug. Given that the 4*S-cis* diastereomer of itraconazole is significantly more potent than the other diastereomer, administration of the 4*S-cis* diastereomer alone could potentially further augment antiangiogenic activity. It is also worthwhile to screen itraconazole analogues that did not reach the clinic for even more potent antiangiogenic activity.

Although new uses for several dozen existing drugs such as thalidomide have been found serendipitously or through knowledge of pharmaceutical side effects, to date the largest systematic screen of existing drugs used <10% of the ~11,000 drugs known to man. Our results once again underscore the promise of systematically screening known drugs for new pharmacologic activities and suggest that itraconazole, a drug already widely used in the clinic, warrants further investigation as a treatment for angiogenesis-dependent diseases.

METHODS

Cell Culture. HUVEC were purchased from Cambrex Biosciences (Walkersville, MD) and maintained in EGM-2 medium (Cambrex), which contains vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and epidermal growth factor. Lipoprotein deficient serum (LPDS) was purchased from Intracell. BAEC and HeLa cells were maintained in Dulbecco's modified eagle's medium containing 10% fetal bovine serum (FBS). Jurkat cells were maintained in Roswell Park Memorial Institute (RPMI) medium containing 10% FBS. In a typical experiment, 5000–10,000 cells/well in 0.2 mL of EGM-2 medium were allowed to adhere for 8 h and then incubated with drug for 36 h. Cells were pulsed with 1 μCi [^{3}H]-thymidine for 8 h (MP Biomedicals, 6.7 Ci mmol^{-1}) and harvested using trypsin onto glass fiber filters (Wallac, Turku, Finland). The readout was performed on a Perkin Elmer MicroBeta plate reader. Cells used were under five passages.

HFF cells were the generous gift of Prof. Wade Gibson and were cultured at passage 2 in DMEM low glucose, 10% FBS, and 1% penicillin-streptomycin. Experiments were performed with 2500 cells/well in 0.2 mL and incubated for 96 h with drug. Plates containing cells were washed once with phosphate buffered saline (PBS), incubated with 1 μM Calcein-AM (Molecular Probes) in PBS for 4 h, and read in a fluorescent plate reader. IC_{50} values were determined using four-parameter logarithmic analysis with GraphPad Prism and are presented as mean ± SEM for triplicate experiments.

***In Vivo* Angiogenesis.** Female athymic nude 5-week-old, 25–30 g mice were purchased from NCI and treated in accordance with Johns Hopkins Animal Care and Use Committee procedures. In all animal experiments, the intravenous formulation of itraconazole was obtained from the Johns Hopkins Hospital Pharmacy. Control mice were treated with vehicle (40% hydroxypropyl-β-cyclodextrin, 2.5% propylene glycol, pH 4.5). Mice were pretreated for 3 d and then implanted subcutaneously with 0.5 mL of Matrigel (BD Biosciences) containing 100 ng mL^{-1} VEGF and 150 ng mL^{-1} bFGF. Drug treatment was continued daily for 10 d, mice were sacrificed, and plugs were harvested, fixed in neutral buffered formalin, and processed for histology using MAS-trichrome staining. A cross-section of the entire Matrigel plug was photographed at 100×, and erythrocyte-filled blood vessels were counted per field in a blinded manner. *P*-values comparing itraconazole *versus* vehicle treated mice were determined using the two-tailed Student's *t* test; the data are presented as mean ± SEM.

Synthesis of Stereoisomers of Itraconazole and Azalanstat. Details of the syntheses of the two stereoisomers of itraconazole and azalanstat can be found in Supporting Information.

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Supporting Information Available: This material is free of charge *via* the Internet.

REFERENCES

1. Folkman, J. (1971) Tumor angiogenesis: therapeutic implications, *N. Engl. J. Med. 285*, 1182–1186.
2. Hanahan, D., and Folkman, J. (1996) Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis, *Cell 86*, 353–364.
3. Folkman, J. (2006) Angiogenesis, *Annu. Rev. Med. 57*, 1–18.
4. Franson, P. J., and Lapka, D. V. (2005) Antivascular endothelial growth factor monoclonal antibody therapy: a promising paradigm in colorectal cancer, *Clin. J. Oncol. Nurs. 9*, 55–60.
5. Fine, S. L., Martin, D. F., and Kirkpatrick, P. (2005) Pegaptanib sodium, *Nat. Rev. Drug Discovery 4*, 187–188.
6. Kerbel, R. S. (2006) Antiangiogenic therapy: a universal chemosensitization strategy for cancer? *Science 312*, 1171–1175.
7. Carmeliet, P. (2003) Angiogenesis in health and disease, *Nat. Med. 9*, 653–660.
8. McCowen, M. C., Callender, M. E., and Lawlis, J. F., Jr. (1951) Fumagillin (H-3), a new antibiotic with amebicidal properties, *Science 113*, 202–203.
9. Ingber, D., Fujita, T., Kishmoto, S., Sudo, K., Kanamaru, T., Brem, H., and Folkman, J. (1990) Synthetic analogues of fumagillin that inhibit angiogenesis and suppress tumour growth, *Nature 348*, 555–557.
10. D'Amato, R. J., Loughnan, M. S., Flynn, E., and Folkman, J. (1994) Thalidomide is an inhibitor of angiogenesis, *Proc. Natl. Acad. Sci. U.S.A. 91*, 4082–4085.

11. Jones, M. K., Wang, H., Peskar, B. M., Levin, E., Itani, R. M., Sarfeh, I. J., and Tarnawski, A. S. (1999) Inhibition of angiogenesis by nonsteroidal anti-inflammatory drugs: insight into mechanisms and implications for cancer growth and ulcer healing, *Nat. Med. 5*, 1418–1423.
12. Guba, M., von Breitenbuch, P., Steinbauer, M., Koehl, G., Flegel, S., Hornung, M., Bruns, C. J., Zuelke, C., Farkas, S., Anthuber, M., Jauch, K. W., and Geissler, E. K. (2002) Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor, *Nat. Med. 8*, 128–135.
13. Ashburn, T. T., and Thor, K. B. (2004) Drug repositioning: identifying and developing new uses for existing drugs, *Nat. Rev. Drug Discovery 3*, 673–683.
14. O'Connor, K. A., and Roth, B. L. (2005) Finding new tricks for old drugs: an efficient route for public-sector drug discovery, *Nat. Rev. Drug Discovery 4*, 1005–1014.
15. Rothstein, J. D., Patel, S., Regan, M. R., Haenggeli, C., Huang, Y. H., Bergles, D. E., Jin, L., Dykes Hoberg, M., Vidensky, S., Chung, D. S., Toan, S. V., Bruijn, L. I., Su, Z. Z., Gupta, P., and Fisher, P. B. (2005) Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression, *Nature 433*, 73–77.
16. Chong, C. R., Qian, D. Z., Pan, F., Wei, Y., Pili, R., Sullivan, D. J., Jr., and Liu, J. O. (2006) Identification of type 1 inosine monophosphate dehydrogenase as an antiangiogenic drug target, *J. Med. Chem. 49*, 2677–2680.
17. Sheehan, D. J., Hitchcock, C. A., and Sibley, C. M. (1999) Current and emerging azole antifungal agents, *Clin. Microbiol. Rev. 12*, 40–79.
18. Kunze, K. L., Nelson, W. L., Kharasch, E. D., Thummel, K. E., and Isoherranen, N. (2006) Stereochemical aspects of itraconazole metabolism in vitro and in vivo, *Drug Metab. Dispos. 34*, 583–590.
19. Vanden Bossche, H., Marichal, P., Gorrens, J., and Coene, M. C. (1990) Biochemical basis for the activity and selectivity of oral antifungal drugs, *Br. J. Clin. Pract. Suppl. 71*, 41–46.
20. Lamb, D. C., Kelly, D. E., Waterman, M. R., Stromstedt, M., Rozman, D., and Kelly, S. L. (1999) Characteristics of the heterologously expressed human lanosterol 14alpha-demethylase (other names: P45014DM, CYP51, P45051) and inhibition of the purified human and Candida albicans CYP51 with azole antifungal agents, *Yeast 15*, 755–763.
21. Trosken, E. R., Adamska, M., Arand, M., Zarn, J. A., Patten, C., Volkel, W., and Lutz, W. K. (2006) Comparison of lanosterol-14 alpha-demethylase (CYP51) of human and Candida albicans for inhibition by different antifungal azoles, *Toxicology 228*, 24–32.
22. Swinney, D. C., So, O. Y., Watson, D. M., Berry, P. W., Webb, A. S., Kertesz, D. J., Shelton, E. J., Burton, P. M., and Walker, K. A. (1994) Selective inhibition of mammalian lanosterol 14 alpha-demethylase by RS-21607 in vitro and in vivo, *Biochemistry 33*, 4702–4713.
23. Burton, P. M., Swinney, D. C., Heller, R., Dunlap, B., Chiou, M., Malonzo, E., Haller, J., Walker, K. A., Salari, A., Murakami, S., Mendizabal, G., and Tokes, L. (1995) Azalanstat (RS-21607), a lanosterol 14 alpha-demethylase inhibitor with cholesterol-lowering activity, *Biochem. Pharmacol. 50*, 529–544.
24. Martinez-Botas, J., Suarez, Y., Ferruelo, A. J., Gomez-Coronado, D., and Lasuncion, M. A. (1999) Cholesterol starvation decreases p34(cdc2) kinase activity and arrests the cell cycle at G2, *FASEB J. 13*, 1359–1370.
25. Fernandez, C., Lobo Md Mdel, V., Gomez-Coronado, D., and Lasuncion, M. A. (2004) Cholesterol is essential for mitosis progression and its deficiency induces polyploid cell formation, *Exp. Cell Res. 300*, 109–120.
26. Griffith, E. C., Su, Z., Turk, B. E., Chen, S., Chang, Y.-W., Wu, Z., Biemann, K., and Liu, J. O. (1997) Methionine aminopeptidase (type 2) is the common target for angiogenesis inhibitors AGM-1470 and ovalicin, *Chem. Biol. 4*, 461–471.
27. Sin, N., Meng, L., Wang, M. Q. W., Wen, J. J., Bornmann, W. G., and Crews, C. M. (1997) The anti-angiogenic agent fumagillin covalently binds and inhibits the methionine aminopeptidase, MetAP-2, *Proc. Natl. Acad. Sci. U.S.A. 94*, 6099–6103.
28. Pan, F., Means, A. R., and Liu, J. O. (2005) Calmodulin-dependent protein kinase IV regulates nuclear export of Cabin1 during T-cell activation, *EMBO J. 24*, 2104–2113.
29. Weis, M., Heeschen, C., Glassford, A. J., and Cooke, J. P. (2002) Statins have biphasic effects on angiogenesis, *Circulation 105*, 739–745.
30. Vincent, L., Soria, C., Mirshahi, F., Opolon, P., Mishal, Z., Vannier, J. P., Soria, J., and Hong, L. (2002) Cerivastatin, an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme a reductase, inhibits endothelial cell proliferation induced by angiogenic factors in vitro and angiogenesis in in vivo models, *Arterioscler., Thromb., Vasc. Biol. 22*, 623–629.
31. Passaniti, A., Taylor, R. M., Pili, R., Guo, Y., Long, P. V., Haney, J. A., Pauly, R. R., Grant, D. S., and Martin, G. R. (1992) A simple, quantitative method for assessing angiogenesis and antiangiogenic agents using reconstituted basement membrane, heparin, and fibroblast growth factor, *Lab Invest. 67*, 519–528.