small increase in $(Na)_c$ from 12 m*M* to 14 m*M* is sufficient to account for a fourfold increase in pump activity, can be dismissed on the grounds that it is quantitatively inconsistent with any of the known kinetic properties of the basolateral Na^+ pump (*13*). Alternative possibilities are that some signal other than $(Na)_c$ (i) increases the turnover rate or stoichiometry (Na ions pumped per pump cycle) of a fixed number of already operating pumps or (ii) that there is an increase in the number of operating pumps resulting from the activation of pumps already in the membrane that were previously quiescent, or that there is a recruitment of new pumps into the membrane.

We reported that after the addition of galactose to the solution bathing the mucosal surface of *Necturus* small intestine there is a slow increase in the K^+ conductance of the basolateral membrane that parallels the increase in pump activity. This increase is blocked by Ba^{2+} and by prior treatment of the tissue with metabolic inhibitors (*14*). We further reported that all of these effects can be mimicked by exposure of the tissue to a 12 percent hypotonic solution, which presumably results in cell swelling. The increase in K^+ conductance could be caused either by an increase in the conductance of a fixed number of already operating channels or an increase in the number of conductive channels; the latter could result from the activation of quiescent channels already present in the membrane or from the recruitment of new channels into that barrier.

Regardless of underlying mechanism, these two sets of findings clearly indicate that in response to an increase in Na^+-coupled solute entry into the cell across the apical membrane there is an increase in the ability of the cell to actively extrude Na^+ across the basolateral membrane, with no increase in $(Na)_c$, and simultaneously to recycle K^+ across that barrier. Both processes act in concert to prevent potentially large increases in cell Na^+ and K^+ activities in response to large increases in the rate of Na^+ entry and in Na^+ and K^+ pump activity (*15*). It is possible that these responses are "triggered" by cell swelling and serve to prevent inordinate increases in cell volume that would otherwise ensue. The immediate signal (or signals) for these responses and their underlying mechanisms remain to be elucidated.

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$$\frac{I'_{sc}}{I_{sc}} = \frac{1 + [K_{Na}/(Na)_c]^n}{1 + [K_{Na}/(Na)'_c]^n}$$

where I_{sc} and $(Na)_c$ are the control values, and the primes designate the values in the presence of the sugar. Assuming $n = 3$, and substituting the data given in Table 1 into the above equation, we obtain a negative value for K_{Na}, an excluded solution; the situation is even worse if we chose a noncooperative interaction between Na and the pump! Further, it can be readily shown that it is impossible to reconcile the above equation with the experimental data with reasonable values of K_{Na} or n unless one assumes that K_{Na}, $(I_{sc})_m$, or n, or a combination of these, are affected by the Na-sugar cotransport process.
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27 January 1984; accepted 6 March 1984

Allylamine Derivatives: New Class of Synthetic Antifungal Agents Inhibiting Fungal Squalene Epoxidase

Abstract. *A new class of synthetic antifungal agents, the allylamines, has been developed by modification of naftifine, a topical antimycotic. SF 86-327, the most effective of these compounds so far, is highly active in vitro against a wide range of fungi and exceeds clinical standards in the oral and topical treatment of guinea pig dermatophytoses. SF 86-327 is a powerful specific inhibitor of fungal squalene epoxidase, a key enzyme in sterol biosynthesis.*

The search for new antimycotics is currently focused on structures related to the azole-based antimycotics (clotrimazole, miconazole, ketoconazole, and others) (*1*). There have been comparatively few reports in the scientific and patent literature of new structural classes.

Naftifine (*2–4*), a new topical antimycotic, is structurally distinct from all other antifungals (Fig. 1). It was first obtained after acid hydrolysis of heterocyclic spiro-naphthalenones (*5*), and its antifungal activity was discovered during routine screening. Naftifine provided the starting point for synthetic modifications aimed at the development of more potent, orally active compounds. Many related compounds synthesized were found to have considerable antifungal

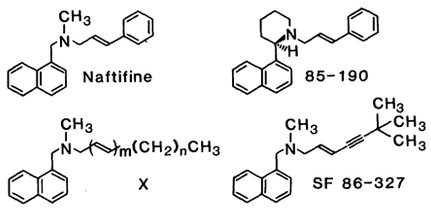

Fig. 1. Chemical structure of naftifine, piperidine derivative 85-190, compound X, and SF 86-327.

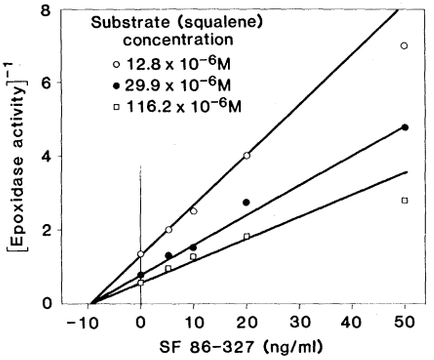

Fig. 2. Inhibition of *C. albicans* squalene epoxidase by SF 86-327 (Dixon plot at three substrate concentrations). Squalene epoxidase was assayed under optimum conditions with microsomal preparations from *C. albicans* (*14*). Microsomes (0.4 mg of protein) were incubated with soluble cytoplasm (2.5 mg of protein from supernatant obtained at 200,000*g*), 1m*M* reduced nicotinamide adenine dinucleotide, 0.1 m*M* flavin-adenine dinucleotide, Tween 80 (0.2 mg/ml), and [^{3}H]squalene (New England Nuclear) in a total volume of 0.5 ml of 0.1*M* phosphate buffer (*p*H 7.4) containing 0.5 m*M* dithiothreitol. SF 86-327 was added in ethanol (final concentration, 1 percent). After 60 minutes at 30°C, the mixtures were saponified and lipids extracted in petrol. The labeled products (squalene-2,3-epoxide and sterols) were isolated by thin-layer chromatography, counted for radioactivity, and expressed as nanomoles of squalene incorporated per milligram of protein per hour. Results are derived from two separate experiments, each with triplicate incubations.

Table 1. Spectrum and MIC's of the antifungal activity of SF 86-327. MIC's were determined by serial dilution with Sabouraud's dextrose broth (*p*H 6.5) in test tubes. The test compound was dissolved in dimethyl sulfoxide and serially diluted with the growth medium. The growth control for yeasts was read after 48 hours of incubation (30°C), for molds after 72 hours, and for dimorphic fungi and all dermatophytes after 7 days. MIC was defined as that concentration at which there were no macroscopic signs of fungal growth.

Species	Number of strains tested	MIC (μg/ml)	
		Range	Mean
Trichophyton spp.	64	0.0015 to 0.006	0.004
Epidermophyton floccosum	24	0.0015 to 0.006	0.004
Microsporum canis	24	0.006 to 0.01	0.007
Aspergillus spp.	7	0.02 to 1.56	0.63
Sporothrix schenckii	4	0.1 to 0.4	0.2
Candida albicans	78	6.25 to 100	29.3
Candida spp.	42	0.1 to > 100	33.0

Table 2. Efficacy of SF 86-327 against *Trichophyton* and *Candida* skin infections in guinea pigs. In *Trichophyton* infections, male albino guinea pigs weighing 260 to 340 g were used (eight or ten animals per dose group). An open glass cylinder 2 cm high and 3.5 cm in diameter was laid on the lumbar region of the back and 0.1 ml of the inoculum, containing 10^6 infective particles of *T. mentagrophytes* (animal isolate), was abraded into the skin encircled by the cylinder with a roughened glass pestle. After this the control animals developed typical mycotic foci. Lesions reached a climax on the 11th to 15th day, with marked redness, incrustation, scaling, and (in some cases) ulceration, followed by a healing phase that extended from the climax to the 30th day after inoculation. For oral treatment the test compound was suspended in water containing 2 percent methyl cellulose and 0.5 percent Tween 80, and 0.5 ml of each of the various doses was administered by stomach tube. Treatment was once daily for nine consecutive days starting on the day of inoculation. Control animals received the suspension only. For topical treatment, 0.4 ml of the test solution (polyethylene glycol 400 and ethanol, 75:25 by volume) was spread on the infected area. Treatment was once daily for seven consecutive days starting 48 hours after inoculation. Control animals received solvent only. Mycological status was assessed on either the first or third day after the last treatment, depending on whether the compound was administered orally or topically, by culturing hairs from the infected lesions. After incubation, cultures were evaluated microscopically for fungal growth in the region of hair roots (*13*). In *Candida* infections, an inoculum of 0.1 ml containing 3×10^7 colony-forming units of *C. albicans* was spread on a shaved circular area (diameter, 4 cm) on the backs of the animals with a roughened glass pestle. The infected area was kept occluded for 3 days until treatment began (twice daily for five consecutive days). The test compound was suspended in a carrier containing Tween 80, Aqualose L75, and distilled water (1:3:6 by volume). The mycological status of the infected skin was assessed 10 days after inoculation by incubating scaly skin material from the infected area on Sabouraud's 4 percent dextrose agar plates. Control animals received suspension medium only.

Fungus	Dose or concentration	Number of animals mycologically cured
	Oral administration	
T. mentagrophytes	2 mg/kg	1 of 10
	4 mg/kg	7 of 10
	6 mg/kg	10 of 10
	Control	0 of 10
	Topical administration	
T. mentagrophytes	0.015 percent	1 of 8
	0.03 percent	7 of 8
	0.06 percent	8 of 8
	Control	0 of 8
C. albicans	0.5 percent	6 of 10
	1.0 percent	9 of 9
	Control	1 of 10

activity. They are collectively termed allylamine derivatives, reflecting the fundamental importance of this structural feature for biological activity.

Some of the derivatives studied have been previously reported, as in a presentation describing structure-activity correlations in a series of compounds of general type *X* (*6*). Another compound whose activity in vitro and in vivo was studied in detail was the piperidine derivative 85-190. It has been observed that antifungal activity is restricted to the (*R*)-enantiomer (proved by x-ray), the (*S*)-enantiomer being significantly less active in vitro and devoid of activity in vivo (*7*). This indicates the high degree of structural specificity required for the activity of these compounds.

We report here a new allylamine derivative, SF 86-327 (*8*), with remarkable antimycotic properties. The side chain of this compound features an acetylene group conjugated with the allylamine function, a structural element hitherto unknown in medicinal chemistry. SF 86-327 is active in vitro against all fungi tested so far (Table 1). The most striking activity is displayed against dermatophytes of the genera *Trichophyton, Microsporum*, and *Epidermophyton* [112 strains; minimum inhibitory concentration (MIC), 0.0015 to 0.01 μg/ml]; *Aspergillus* (seven strains; MIC, 0.02 to 1.56 μg/ml); and *Sporothrix schenckii* (four strains; MIC, 0.1 to 0.4 μg/ml). The susceptibility of various yeast species and strains in vitro is subject to a much wider variation, the MIC for the 120 strains tested ranging from 0.1 to > 100 μg/ml.

The uniformly high activity of SF 86-327 against dermatophytes indicates a high specificity, underlined by the primarily fungicidal action against these organisms. The type of activity against yeasts was found to depend on the species—being, for example, primarily fungicidal against *Candida parapsilosis* and primarily fungistatic against *C. albicans*.

SF 86-327 was active in various guinea pig dermatomycosis infections after topical or oral application (Table 2). Excellent oral activity against trichophytosis was achieved with a dose of 4 to 6 mg/kg given daily for 9 days. In parallel experiments, 40 to 80 mg of griseofulvin or ketoconazole per kilogram were necessary to achieve comparable activity, whereas naftifine was not active below 150 mg/kg. Similar results have been obtained in guinea pigs infected with *Microsporum canis*.

The outstanding efficacy of SF 86-327 as a topical antifungal agent can be shown in the trichophytosis model by

applying a 0.03 to 0.06 percent solution of the drug to the infected skin for 7 days. In direct comparison, a 2 percent solution of econazole or tolnaftate led to a cure rate of 0 and 50 percent, respectively [(number of animals mycologically cured/number of animals tested) × 100]. Infection of guinea pigs with *C. albicans* also responded to daily topical application of SF 86-327 for 5 days at a concentration of 1 percent.

The significance of SF 86-327 and other allylamines as a new class of compounds is underlined by their novel mode of action. This involves the inhibition of ergosterol biosynthesis at the point of squalene epoxidation (*8*). The fungicidal action is thought to result from a combination of sterol deficiency and heavy intracellular accumulation of squalene (*9*). SF 86-327 is a powerful inhibitor of squalene epoxidase preparations from *C. albicans* (Fig. 2), with apparently noncompetitive kinetics and an inhibition constant of $3 \times 10^{-8} M$. Squalene epoxidase is a key enzyme in sterol biosynthesis, being the first in the pathway to require molecular oxygen, a fact of considerable evolutionary significance (*10*). To our knowledge, the allylamines are the first specific inhibitors of this enzyme to be reported. Squalene epoxidase is also involved in mammalian cholesterol biosynthesis (*11*), but preliminary studies (*12*) indicate that the mammalian epoxidase is three to four orders of magnitude less sensitive to SF 86-327 than is the fungal enzyme. Further investigation of the molecular basis for the inhibition and its high specificity may enable even more potent inhibitors to be designed and certainly should increase our insight into the biosynthesis of sterols in general.

SF 86-327 was selected for preclinical development and clinical trials. The results of preliminary clinical investigations are encouraging (*8*). However, the full clinical spectrum of SF 86-327 is not yet known. In view of the novel mode of action of the allylamines, they might prove useful against fungal infections that are resistant to currently available antimycotics and in the treatment of other diseases caused by pathogens dependent on sterol biosynthesis.

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31 January 1984; accepted 20 March 1984

Action of the *e* Locus of Mice in the Response of Phaeomelanic Hair Follicles to α-Melanocyte–Stimulating Hormone in Vitro

Abstract. *Dibutyryl adenosine 3',5'-monophosphate (dibutyryl cyclic AMP) induced eumelanin synthesis in hair bulb melanocytes of recessive yellow* (e/e) *mice in vitro, whereas α-melanocyte–stimulating hormone (α-MSH) did not. In contrast, the melanocytes of lethal yellow* (A^y/a) *mice produced eumelanin in response to both dibutyryl cyclic AMP and α-MSH. These results suggest that the* e *locus controls a mechanism that determines the function of an α-MSH receptor.*

Mammalian melanocytes are capable of producing two types of pigment: black or brown melanin (eumelanin) and yellow melanin (phaeomelanin). These melanins differ in their chemical and physical properties (*1*, *2*). In the house mouse, the type of melanin produced in hair bulb melanocytes is determined by the agouti (*a*) and extension (*e*) loci. Coat color of the mutants at the *a* locus ranges from extreme black (*a/a*), in which only eumelanin is deposited, to yellow (A^y/a), which is composed mainly of phaeomelanin. In contrast, the recessive yellow (*e*) gene, which is an allele at the *e* locus, is epistatic to the alleles at the *a* locus. Therefore, mice of genotype *e/e* exhibit hairs pigmented mainly with phaeomelanin regardless of their *a*-locus genotype.

Earlier studies have shown that the alleles at the *a* locus exert their effect on melanocytes indirectly by modifying the local tissue environment (*3*–*5*), whereas the *e* locus is considered to function within melanocytes (*6*, *7*). Geschwind *et al.* found that lethal yellow (A^y/a) mice that had been producing phaeomelanin were induced to produce eumelanin when treated with α-melanocyte–stimu-

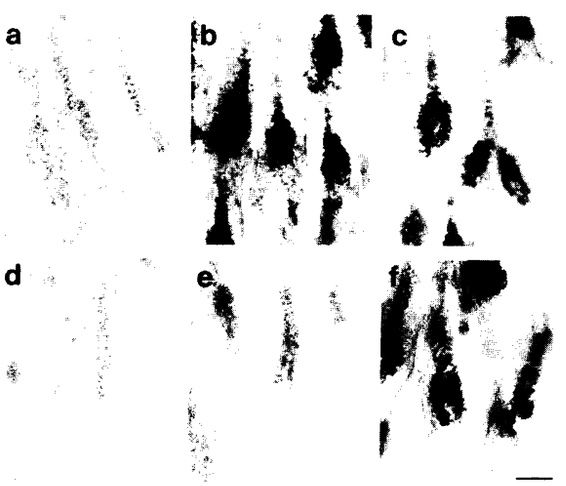

Fig. 1. Induction of eumelanin synthesis in genotypically yellow hair bulbs in vitro. Each explant was cultured in Ham's F-12 medium supplemented with 10 percent fetal bovine serum for 48 hours. Top: Hair bulbs of lethal yellow (A^y/a) explants. Only phaeomelanin was found in the explants cultured in the control medium (a). Eumelanin appeared when explants were cultured in the presence of 1 μg/ml α-MSH (b) or 1 m*M* dibutyryl cyclic AMP (c). Bottom: Hair bulbs of recessive yellow (*e/e*) explants. Only phaeomelanin was found in the explants cultured in the control medium (d) and in the explants cultured in the medium containing 1 μg/ml α-MSH (e). Treatment with 1 m*M* dibutyryl cyclic AMP gave rise to eumelanin synthesis in hair bulb melanocytes (f). Scale bar represents 100 μm.