

Expert Opinion

1. Introduction
2. Description of onychomycosis
3. Challenges to the therapy of onychomycosis
4. Drugs currently approved for onychomycosis in the main markets
5. New antifungal compounds in development for onychomycosis
6. Discovery and characterization of AN-2690
7. Clinical summary
8. Expert opinion

Anti-Infectives

Recent progress on the topical therapy of onychomycosis

Michael RK Alley, Stephen J Baker, Karl R Beutner & Jacob Plattner[†]

[†]Anacor Pharmaceuticals, 1060 East Meadow Circle, Palo Alto, CA 94303, USA

Onychomycosis is a fungal infection of the fingernails and toenails that results in thickening, discoloration, splitting of the nails and lifting of the nail from the nail bed. The disease is caused by dermatophytes and has a high incidence within the general population, especially among older individuals. Present treatment options include both oral and topical drugs, with oral therapies giving better outcomes; however, neither of these treatment options provides high cure rates that are durable. The difficulty in treating onychomycosis results from the deep-seated nature of the infection within the nail unit (nail plate, nail bed and surrounding tissue) and the inability of drugs to effectively reach all sites. Ongoing drug development activities have focused on novel delivery technologies to facilitate penetration of existing antifungal drugs through the nail plate and on the discovery of inherently penetrable antifungals. AN-2690 represents an oxaborole antifungal that is designed to penetrate the nail plate and is showing promising results in clinical trials.

Keywords: antifungal agent, dermatophyte, fungal infection, leucyl-tRNA synthetase, nail penetration, onychomycosis, oxaborole, tinea unguium

Expert Opin. Investig. Drugs (2007) 16(2):157-167

1. Introduction

Onychomycosis is a progressive fungal infection of the nail unit that leads to the destruction and deformity of toenails and (less frequently) fingernails. This condition is common and represents ~ 50% of all nail disorders. Onychomycosis has a high occurrence throughout the world, with recent epidemiological data indicating a prevalence of 6.5 – 13.8% in North America [1]. The infection shows an increasing incidence in older individuals and 1 study reported that 48% of people aged 70 years are infected with onychomycosis [2]. The susceptibility to onychomycosis is higher in men than in women, although women seek medical treatment more frequently.

The infection is caused by fungi that infect the nail unit (the nail bed, the nail plate and surrounding tissue) and these include yeasts, dermatophytes and other molds. By far the most common fungi that cause onychomycosis are the dermatophytes, which account for ~ 90% of all cases. Dermatophytes are also the cause of skin fungal infections [3] and many patients with a nail infection also have a co-existing skin infection. The *Trichophyton* spp., *Microsporum* spp. and *Epidermophyton* spp. are the main causative dermatophytes, with *Trichophyton rubrum* and *T. mentagrophytes* representing the two most common isolates [3-5].

2. Description of onychomycosis

Tinea unguium is the medical term that is used to describe a nail infection caused by a dermatophyte, whereas onychomycosis is used more broadly to characterize all fungal nail infections. Distal subungual onychomycosis represents the most

informa
healthcare

common presentation of tinea unguium [6]. Distal subungual onychomycosis starts by the microorganism (usually a dermatophyte) invading the stratum corneum of the hyponychium and distal nail bed. Subsequently, the infection moves proximally in the nail bed and invades the ventral nail surface of the nail plate. The infection is characterized by discoloration, separation of the nail plate from the nail bed (onycholysis), accumulation of subungual debris and nail plate dystrophy. Proximal subungual onychomycosis is the least common variant of onychomycosis. This condition starts by fungal invasion of the stratum corneum of the proximal nail fold and subsequently of the nail plate. White superficial onychomycosis occurs when the nail plate is invaded directly by the causative organism and is characterized by the presence of white, chalky patches on the nail plate. The patches may coalesce to cover the whole nail plate. The potential end point of all forms of onychomycosis is total dystrophic onychomycosis and occurs when the entire nail plate and nail bed are invaded by the fungus.

Although onychomycosis is generally not life threatening, the disease adversely affects the quality of life of its victims. In those cases in which the nail unit is seriously compromised, patients can experience pain and discomfort at the site of infection. Because of the high incidence of onychomycosis and the inadequacy of present treatment modalities, considerable research efforts have been directed to finding improved therapeutic options. This review summarizes the recent developments for new treatments of onychomycosis.

3. Challenges to the therapy of onychomycosis

Onychomycosis has proven to be a challenging infection to treat, with treatment failures and relapses being common occurrences [7-9]. In a study reported in 1998, it was found that 22.2% of patients whose toenail onychomycosis had been cured by oral therapy experienced a relapse during a 3-year follow-up period [10]. For an antifungal drug to be effective, it must disseminate throughout the nail unit and kill the pathogen. When a sample taken from the nail bed of an infected patient shows a negative culture and negative microscopy, this is termed a mycological cure; however, a clinical cure includes not only elimination of the fungi from the nail unit but also the formation of clear, new nail growth that is absent of dystrophic characteristics. Because of the slow rate of growth of toenails (~ 1 mm/month), evidence of a clinical cure can take 9 – 12 months. For this reason, drug treatment periods for onychomycosis are lengthy and require 3 – 12 months. During this treatment period, the infected nails can be monitored for growth of new clear nail and for the presence of viable dermatophytes.

The current treatment modalities for onychomycosis include mechanical procedures, systemically administered antifungals and topical drug therapy. Although infected nails can be surgically removed, recurrence of the onychomycosis can occur as the new nail grows back and there is no evidence

that the surgical approach is effective in producing a sustained disease-free period. In addition, because surgical avulsion is quite traumatic, this procedure is rarely used today. An alternative procedure of chemically removing the nail using a urea ointment is also not widely used by current practitioners. In preference, podiatrists perform periodic trimming and debridement of affected nails as a means of reducing symptoms, with the added hope of minimizing progression by stabilizing the disease.

Whereas mechanical procedures to treat onychomycosis only offer marginal benefit to the patient, drug therapies have provided some advancement in the attempt to effectively treat this disease; however, the overall success in treating onychomycosis is still far from optimal (as mentioned in Section 3) and this almost certainly results from the unique anatomical features of the nail unit and its pervasive colonization by dermatophytes during infection. The varied anatomy of the nail unit provides an opportunity for the fungal pathogen to establish a deep-seated infection by invading and proliferating into the nail plate, the nail bed and the surrounding tissue. Systemically administered drugs must not only reach the nail bed but must also achieve sufficient concentration in the nail plate to eliminate dermatophytes at this location. It is more likely that systemically administered drugs reach the nail plate via the nail matrix, which is a continually proliferating epidermal tissue that serves as the origin of new nail growth. Access to the nail plate from the newly formed cells in the matrix is a slow process due to the very slow growth rate of toenails. As a consequence of this slow growth rate, oral therapy must be continued for some time and this may have the inherent disadvantage of causing systemic adverse effects.

On the other hand, topically administered drugs face even more challenges to reach all of the required sites of these deep-seated infections. This difficulty in achieving relevant fungicidal concentrations throughout the nail unit directly relates to the nail plate's unique properties, its thickness and relatively compact structure. The nail plate is a hard yet slightly elastic convex structure that consists of ~ 25 layers of dead, keratinized, flattened cells that are tightly bound together. The nail plate itself consists of three layers: the dorsal and intermediate layers derived from the matrix, and the ventral layer derived from the nail bed [11]. The upper (dorsal) layer is a few cell layers thick and consists of hard keratin. It constitutes the main barrier to drug diffusion into and through the nail plate. The intermediate layer constitutes 75% of the whole nail thickness and consists of soft keratin. Below the intermediate layer is the ventral layer of soft keratin, a layer that is a few cells thick, that connects to the underlying nail bed in which many pathological changes can occur. Thus achieving an effective drug concentration in the ventral nail plate and the nail bed is of great importance in the treatment of nail diseases.

Chemically, the nail plate mainly consists of fibrous proteins – keratins – which are highly cross-linked with disulfide bonds. Coupled with the highly compact structure

of the keratinized cells in the nail plate, these highly cross-linked proteins within the cells present a formidable barrier to the entry of topically applied agents [12]. In 1 study, the concentration of an applied drug across the nail dropped ~ 1000 times from the outer surface to the inner surface [13]. As a result, the drug concentration had presumably not reached a therapeutically effective level in the inner ventral layer.

Another factor contributing to the difficulty of topically applied antifungal drugs to penetrate the nail plate is the apparent mismatch of drug physicochemical properties with the biophysical properties of the nail plate [14]. Most existing antifungal drugs were originally designed for oral and/or skin applications and are consequently fairly lipophilic molecules, only sparingly soluble in water and have molecular weights of ≥ 300 Da. On the other hand, with its dense keratin fabric network and a high capacity for flux of water, the nail plate has been described as a hydrophilic gel [15-17]. Thus most of the known antifungal agents will not find a compatible environment in traversing the nail plate.

4. Drugs currently approved for onychomycosis in the main markets

4.1 Oral agents

Systemic drug treatment is currently the most effective method of treating onychomycosis. Even so, 20 – 25% of the patients fail to respond [18] and recurrence of disease after successful treatment is common. Terbinafine and itraconazole (Figure 1) are the two systemic treatments of choice, with terbinafine showing greater efficacy and lower rates of recurrence than itraconazole [19].

Terbinafine, a member of the allylamine family of antifungals, has a broad spectrum of activity and exerts fungicidal activity against most fungal pathogens. This compound also inhibits squalene epoxidase, an important enzyme in the biosynthesis of the essential membrane component ergosterol [20]. Terbinafine is active against dermatophytes, *Malassezia furfur*, *Aspergillus* spp. and some *Candida* spp. (including *Candida parapsilosis*). A single dose of terbinafine 250 mg p.o. administered to humans produces peak plasma concentrations of 1 $\mu\text{g/ml}$ within 2 h. It is > 99% protein bound and has a half-life of ~ 36 h. It is administered at a dose of 250 mg q.d. with treatment duration of 6 and 12 weeks for fungal infection of the fingernail and toenail, respectively. Using this regimen, 38.2% of toenails showed a clinical cure when examined at 48 weeks [21]. Therapeutic levels of drug persist in the nail for 3 – 6 months after therapy is discontinued. Liver toxicity has been reported for terbinafine and hepatic function tests are recommended for patients who use terbinafine continuously for > 6 weeks. Terbinafine is metabolized by CYP enzymes and has been noted to have a number of drug interactions [22].

Itraconazole, which is from the azole class of antifungal agents, inhibits lanosterol 14 α -demethylase and thus stops

the biosynthesis of ergosterol. It has a broad spectrum of activity against species including dermatophytes, *Candida* spp., *Aspergillus* spp. and *M. furfur* [23]. Blood levels of itraconazole after a single dose of 200 mg administered to humans reached a peak level of 0.2 – 0.3 $\mu\text{g/ml}$ after 4 – 5 h. It is 99.8% protein bound and has a half-life of 21 h. It is administered as either 200 mg q.d. for 12 weeks or 200 mg b.i.d. for 7 days, followed by 3 weeks with no treatment and repeated for 3 months [22]. Therapeutic levels of itraconazole persist in the nail for 3 – 6 weeks after therapy is discontinued. Itraconazole has also been associated with liver damage and liver function tests are required if continuous treatment exceeds 1 month. This agent specifically inhibits the CYP3A4 isoenzyme system and may consequently increase plasma concentrations of drugs metabolized by this pathway [22].

Griseofulvin (Figure 1) is a natural product that was isolated from *Penicillium griseofulvin* in 1939 [24] and has been used for treating dermatophytosis since its introduction in 1958. Griseofulvin acts by binding to microtubular proteins, which results in the inhibition of cell mitosis and the formation of multinucleated fungal cells [25]. The drug is administered orally and is effective against a wide variety of dermatophytes. Griseofulvin has been used in complicated, difficult to treat tinea capitis and onychomycosis.

Fluconazole is an orally active, synthetic, bis-triazole antifungal agent with activity against a wide range of fungi, including most *Candida* spp. and (as with other azoles) inhibits lanosterol 14 α -demethylase. Although fluconazole is not approved in the US or Europe for the treatment of onychomycosis, it is used off-label for this indication. A number of clinical trials studying the kinetics and efficacy of fluconazole have been reported [22].

4.2 Topical agents

Treatment of onychomycosis by topical methods has been met with limited success for the reasons described in Section 3. The two main topical treatments that are used today are ciclopirox and amorolfine (Figure 2), both of which are formulated in lacquers that are painted onto the infected nails. The lacquer dries to leave a water-insoluble film on top of the infected nail, which subsequently acts like a drug depot releasing the drug into the nail plate. Ciclopirox is a synthetic compound and is a member of the hydroxypyridone family of antifungal agents. The hydroxypyridone antifungals are active against many pathogenic fungi such as dermatophytes, *M. furfur* and *Candida* spp. Ciclopirox is believed to work by inhibiting metal-dependant enzymes by chelating the polyvalent cations (Fe³⁺ or Al³⁺) [26,27]. Ciclopirox has antifungal, antibacterial and anti-inflammatory activities. It is administered to the infected nails daily and this treatment regimen continues for ≥ 6 months. Ciclopirox persists in the nail for 14 days after therapy is completed. Clinical response rates for the treatment of onychomycosis are in the range of 7 – 10%.

Amorolfine belongs to the morpholine group of synthetic antifungal agents. Its mechanism of action involves the

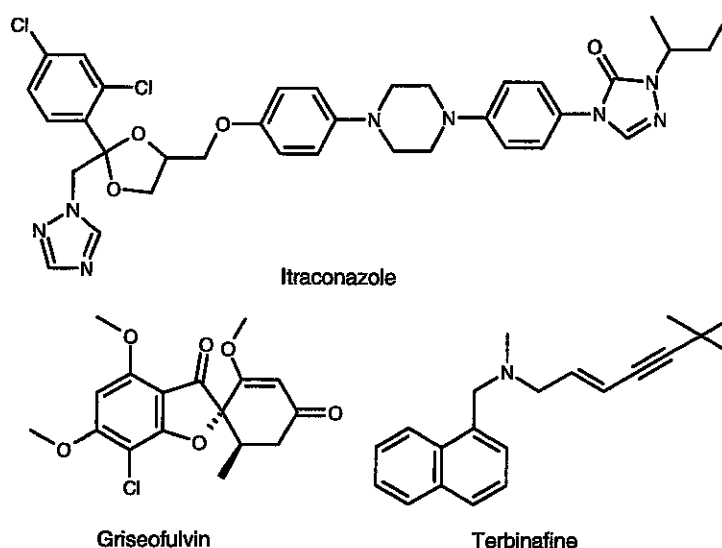


Figure 1. Chemical structures of itraconazole, griseofulvin and terbinafine.

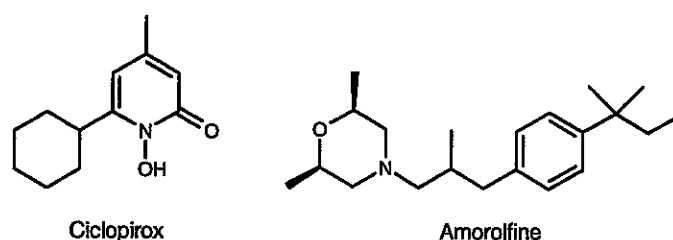


Figure 2. Chemical structures of ciclopirox and amorolfine.

inhibition of two important steps in the ergosterol pathway [28]. Amorolfine is fungicidal against *C. albicans* and *T. mentagrophytes*. It is administered once or twice weekly to the infected nails for 6 – 12 months and (as with ciclopirox) persists in the nail for 14 days after therapy is completed.

5. New antifungal compounds in development for onychomycosis

Due to the treatment limitations described in Section 3 for onychomycosis, it is not surprising that significant R&D efforts have focused on the discovery of improved therapies. In general, these efforts have emphasized eliminating the deficiencies of currently approved drugs, which include long treatment times, concurrent side effects of oral drugs and poor efficacy of topical agents. The new treatment modalities that are presently in clinical development for onychomycosis are listed in Table 1.

As noted from the entries in Table 1, most of the approaches represent re-formulations of known antifungal compounds with penetration enhancers in an effort to

increase penetration of the active agent into and through the nail plate. The only systemic drug in development is itraconazole (Hyphanox™), which is being developed by Barrier Therapeutics as a once-daily oral formulation. With respect to topical treatments for onychomycosis, the use of novel delivery vehicles and permeation enhancers applied to existing classes of antifungals (e.g., azoles or allyl amines) represents the main R&D approach that has been pursued over the past 10 years. Very little work has been reported on approaches focusing on discovering antifungal compounds that have intrinsic nail-penetrating properties. S-291-ND is one such compound and involves the use of a topical NO-generating formulation that can be applied directly to the toenail. NO is a highly reactive molecule that has antimicrobial activity [29] and may also have intrinsic nail-penetrating capabilities due to its low molecular weight. The other novel approach is represented by the boron-containing antifungal compound, AN-2690. Now in clinical trials, this agent was specifically designed to overcome the nail barrier when applied topically so that it can achieve high concentrations in the nail bed [30].

Table 1. Onychomycosis treatments in clinical development.

Compound	Company	Stage	Description
Abafungin (Abasol™, York Pharma)	York Pharma	Pre-registration (in UK)	A topical 2-aminothiazole membrane intergity antagonist
Topical butenafine gel	Mylan	Phase III	Topical formulation of ergosterol synthesis inhibitor butenafine
AN-2690	Anacor Pharmaceuticals	Phase II	Topical boron-containing antifungal with inherent nail-penetrating characteristics
NM-100060	NexMed/Novartis	Phase II	Topical formulation of terbinafine with penetration enhancer
Itraconazole (Hyphanox™, Barrier Therapeutics)	Barrier Therapeutics	Phase II	Once-daily oral formulation of itraconazole in 200-mg tablets
Organo-gel delivery of allylamine antifungals	MediQuest Therapeutics	Phase II	Proprietary organo-gel delivery of naftifine or terbinafine
Topical NO (S-291-ND)	ProStrakan	Phase II (in UK)	Cream formulation of NO precursors that liberate NO on the nail
Onycofitex	Bentley Pharma	Phase VII	Antifungal nail lacquer formulation of clotrimazole
Transdermal SEPA®	MacroChem	Phase I	Econazole in a nail lacquer with SEPA, a transdermal absorption enhancer

6. Discovery and characterization of AN-2690

6.1 Designing a compound to penetrate the nail plate

Given the many failures and poor efficacy of topical treatments using known antifungal agents [31], a completely new approach was contemplated. Topical antifungal agents are applied to the outer surface (dorsal layer) of the nail plate and treatment is then dependent on the passive diffusion of the drug through this thick, keratin-rich, lipid-containing barrier. Several reviews on onychomycosis therapy suggest that topical antifungal drugs fail in clinical trials because of the lack of penetration of the drug through the nail plate [11,32,33]. To the authors' knowledge, there has been no reported chemical optimization effort to identify an antifungal agent for delivery through a nail plate to treat onychomycosis topically.

Studies have shown that nail penetration is dependent on molecular weight, octanol:water partition coefficient and water solubility, with smaller, more polar, water-soluble molecules capable of penetrating to a greater extent than larger lipophilic molecules [15,34]. In addition to keratin, the nail plate contains lipids but (unlike the skin) these are not at such high concentrations; furthermore, they seem to be localized in the thin upper dorsal and lower ventral layers with a much lower concentration throughout the remaining nail plate [17]. Another consideration was the suggestion in the literature that the antifungal potency of certain drugs may be reduced in the presence of keratin [35]. This is believed to be due to the drug binding to keratin, thus requiring a higher drug concentration to compensate for this loss.

With this knowledge, a medicinal chemistry-based project was initiated to develop a small-molecule antifungal agent with appropriate physicochemical properties to treat onychomycosis topically. The criteria that was used for such a molecule included low molecular weight (< 250 Da), polar (cLogP < 2.5), good water solubility (> 0.1 mg/ml) and

retention of antifungal activity in the presence of 5% keratin powder.

6.2 Structure-activity relationship and chemical properties of AN-2690

Initially, 1-phenyl-substituted benzoxaboroles (1; Figure 3) were identified as having broad-spectrum antifungal activity with reasonable minimum inhibitory concentration (MIC) values [30]. These had a molecular weight of ~ 200 Da; however, these 1-phenylbenzoxaboroles had a high cLogP (> 3.5), and were not very water soluble. Initially, 1-phenylbenzoxaboroles were synthesized with hydrophilic substituents appended to the 1-phenyl group. Although these modifications improved water solubility, they did not reduce the cLogP by any significant margin. Furthermore, all of these substitutions led to an undesirable gain in molecular weight.

To lower molecular weight further, the 1-phenyl group was replaced with a 1-hydroxyl group to give compound 2. This immediately lowered molecular weight to ~ 130 – 180 Da and retained equivalent broad-spectrum antifungal activity to the 1-phenyl derivatives. In addition to the large reduction in molecular weight, these compounds also had lower cLogP values as an aromatic ring had been replaced by a hydroxyl group. Furthermore, this modification increased water solubility. Several of these derivatives were synthesized and the most potent compounds were found to contain a halogen *para* to the boron. Activity was lost by replacing the halogen or by moving the fluoro group from the 5-position to another position on the aromatic ring. This study resulted in the identification of AN-2690 as having the best combination of potency and optimum physicochemical properties.

AN-2690 has a low molecular weight (152 Da), good solubility in water (~ 1 mg/ml) and a cLogP of 1.24 (cLogP calculated using CS ChemDraw Ultra 10). It has

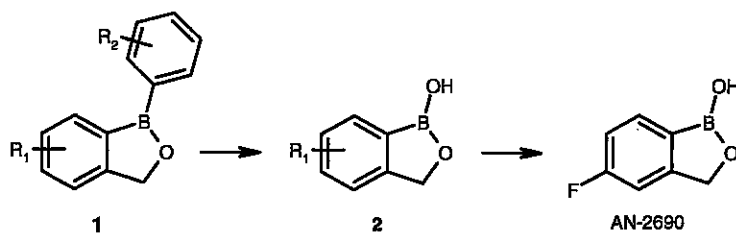


Figure 3. Chemical structures of 1-phenyl substituted benzoxaboroles.

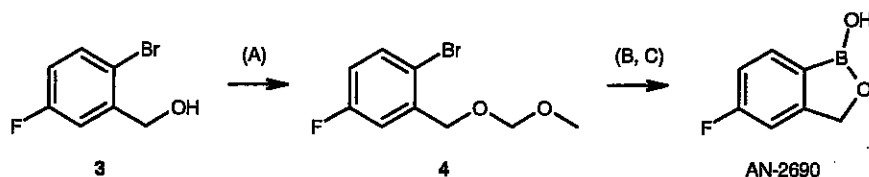


Figure 4. The typical synthesis of AN-2690. Conditions: (A) $\text{CH}_3\text{OCH}_2\text{Cl}$, $i\text{-Pr}_2\text{NEt}$ and CH_2Cl_2 at room temperature; (B) $n\text{-BuLi}$, $(i\text{-PrO})_3\text{B}$ and THF at -78°C to room temperature; and (C) 6N HCl and THF at room temperature [30].

broad-spectrum antifungal activity against dermatophytes, yeast, molds and other filamentous fungi with MIC values in the low microgram per milliliter range. AN-2690 is fungicidal against *T. rubrum* at 4 to 8 times its MIC.

AN-2690 is synthesized using the method shown in Figure 4. The scale-up development of this compound has been relatively simple and it has been synthesized at the 30-kg scale using a modification of this scheme.

The stability of these boron-containing oxaboroles is good and comparable with other small non-boron-containing organic molecules. They are easy to handle and require no specialized equipment. They are purified and analyzed using standard methods including recrystallization, column chromatography, HPLC and LCMS. They are stable when stored at room temperature for prolonged periods and are also stable under acidic or basic conditions as well as at elevated temperatures.

To determine whether the activity of AN-2690 was affected by the presence of keratin, a MIC study was performed against *T. rubrum* in the presence and absence of 5% powdered keratin. This showed that the MIC of AN-2690 was essentially the same, leading to the conclusion that the activity of this compound is not affected by keratin binding.

6.3 Nail-penetration studies

Nail penetration of AN-2690 and ciclopirox was performed by the group of Maibach using their published methodology [36]. In this study, human cadaver finger nails were mounted in a one-cell diffusion chamber on top of a cotton ball wetted with saline to act as the nail bed. AN-2690 was formulated at 10% w/v in ethanol:propylene glycol (4:1)

as a vehicle and compared with ciclopirox (8% w/w in commercial lacquer) [37]. These test articles were applied at a dose of 10 μl to the top of the nail plates once daily for 14 days. At the study end, the top and bottom sections of the dosed area of the nail plate were micro-dissected and analyzed for test compound. The result of the cotton ball analysis (i.e., the amount of radiolabeled material to penetrate through the nail plate and deposit into the cotton ball as determined by scintillation counting) is shown in Figure 5. AN-2690 penetrated through the nail plate in remarkably high concentrations and the degree of penetration through the nail plate was far superior to ciclopirox. A total of 3 other AN-2690 derivative compounds were also studied for nail penetration, 1 analog whereby the fluoro group was replaced by chloro and 2 1-arylbenzoxaborole derivatives. The chloro analog showed almost equal penetration to AN-2690, whereas the 1-arylbenzoxaboroles did not show such good penetration as could be expected as these congeners had a higher molecular weight and were more lipophilic. This result showed that the small, polar, water-soluble nature of AN-2690 matched the requirements to penetrate the nail plate providing an antifungal agent with an unprecedented ability to penetrate human nails.

In a final *in vitro* demonstration of activity, AN-2690, ciclopirox and amorolfine were studied in a model of infection using the TurChub[®] cells (MedPharm Ltd; Figure 6) [101,102]. In this study, the antifungal agent must first pass through full thickness human nail plates to show a zone-of-inhibition against the dermatophyte cultured in the flask below the nail plate. AN-2690 (10% w/v in ethyl acetate:propylene glycol 1:1) was compared with ciclopirox

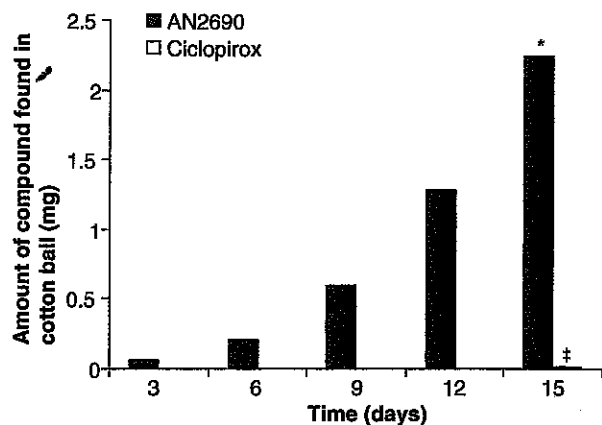


Figure 5. Cumulative total amount of AN-2690 and ciclopirox found under the nail plate in cotton ball supports at each time point following a 14-day, multiple-dose nail-penetration study.

*2.24 mg. †0.009 mg.

(8% w/w in commercial lacquer) and amorolfine (5% w/w in commercial lacquer). Using 5 – 6 replicates, test articles were dosed daily for 5 days at a concentration of 40 $\mu\text{l}/\text{cm}^2$. The vehicle showed no zone-of-inhibition nor did ciclopirox or amorolfine in their commercial lacquers. By contrast, AN-2690 showed a significant zone-of-inhibition, demonstrating its ability to penetrate full thickness nail plates and disseminate through a large area of the receiver cells at concentrations above its MIC value to prevent the growth of the dermatophyte.

These *in vitro* results have proven that this focused medicinal chemistry approach had successfully resulted in the development of a polar, low molecular weight, water-soluble antifungal compound that penetrates full thickness nails and should be suitable for the topical treatment of onychomycosis.

6.4 Mechanism of action of AN-2690

The oxaborole AN-2690 represents a new class of antifungal protein synthesis inhibitors, which specifically inhibits the cytoplasmic leucyl-tRNA synthetase, an aminoacyl-tRNA synthetase (AARS). Although eukaryotic protein synthesis inhibitors are common (e.g., cycloheximide [38] and anisomycin [39]), specific fungal inhibitors are less common because of the similarity between the fungal and human enzymes involved in protein synthesis. Despite this close homology, it is still possible to get specific antifungal protein synthesis inhibitors as demonstrated by the sordarins, which target the highly conserved fungal ribosomal protein elongation factor 2 [40]. Although there is ~ 63% homology between the human and yeast cytoplasmic leucyl-tRNA synthetases, AN-2690 has > 1000-fold selectivity for fungi with MIC values ranging from 0.125 to 8 $\mu\text{g}/\text{ml}$, whereas the LC_{50} for

human hepatocytes (HepG2) is > 1000 $\mu\text{g}/\text{ml}$ as measured in an MTS cell viability assay. As expected, the difference between the bacterial and human AARS enzymes is much larger so it is not surprising that the archetypal AARS inhibitor, mupirocin, is an antibacterial [41]. However, the relatively close homology between the fungal and human AARS enzymes has not prevented antifungal AARS inhibitors being found; for example, even mupirocin, which is used solely as an antibacterial topical drug, does have some antifungal activity as demonstrated by its efficacy in a guinea-pig *T. mentagrophytes* ringworm model [42]. Other examples of more well-known specific antifungal AARS inhibitors are icofungipen and cispentacin [43–46].

The AARS enzymes perform the first step in protein synthesis by attaching their cognate amino acid to either the 2'- or 3'-hydroxyl group of the terminal adenosine of tRNA. This is performed in a 2-step process: first, the amino acid is activated by the attachment of the α -carboxyl group to the 5'-phosphate of AMP; and second, this activated amino acid is transferred to either the 2'- or 3'-hydroxyl group of the adenosine on the 3'-terminus of the tRNA with the corresponding anticodon. The accuracy of this reaction in protein synthesis is essential as it is the linchpin in the translation of the genetic code into protein, linking the triplet codon to the amino acid. Leucyl-tRNA synthetase, as for the AARS for isoleucine, valine, methionine, alanine, lysine, proline and phenylalanine, possess additional proofreading mechanisms, which can edit either the incorrect amino acid-AMP intermediate or the incorrect aminoacylated tRNA product, to improve the fidelity of tRNA aminoacylation [47]. The homologous AARS enzymes for leucine, isoleucine and valine possess two structurally distinct domains: one domain contains the site of aminoacylation and the other domain bears an editing site that proofreads the aminoacylated tRNA [48–50]. The archetypal AARS inhibitor mupirocin inhibits isoleucyl-tRNA synthetase [51] by binding to the aminoacylation active site as an isoleucine adenylate analog [52]. The AARS inhibitors cispentacin and icofungipen are thought to act as amino acid analogs and (as with mupirocin) probably bind to the aminoacylation site. Although AN-2690 inhibits an AARS, its mechanism of action is very different to these known AARS inhibitors. This was made apparent early in genetic studies in *Saccharomyces cerevisiae* and *C. albicans* when all of the AN-2690 resistance mutations were mapped to the editing domain of leucyl-tRNA synthetase. As would be expected for a drug that bound to the editing site, AN-2690 is a non-competitive inhibitor with respect to leucine, ATP or tRNA. The oxaborole AN-2690 represents a new class of inhibitors that exerts their action on AARS via the editing domain. As the editing active site has evolved to accommodate a larger repertoire of compounds than the aminoacylation active site, the editing site may prove to be a better target for designing compounds that inhibit this validated drug target.

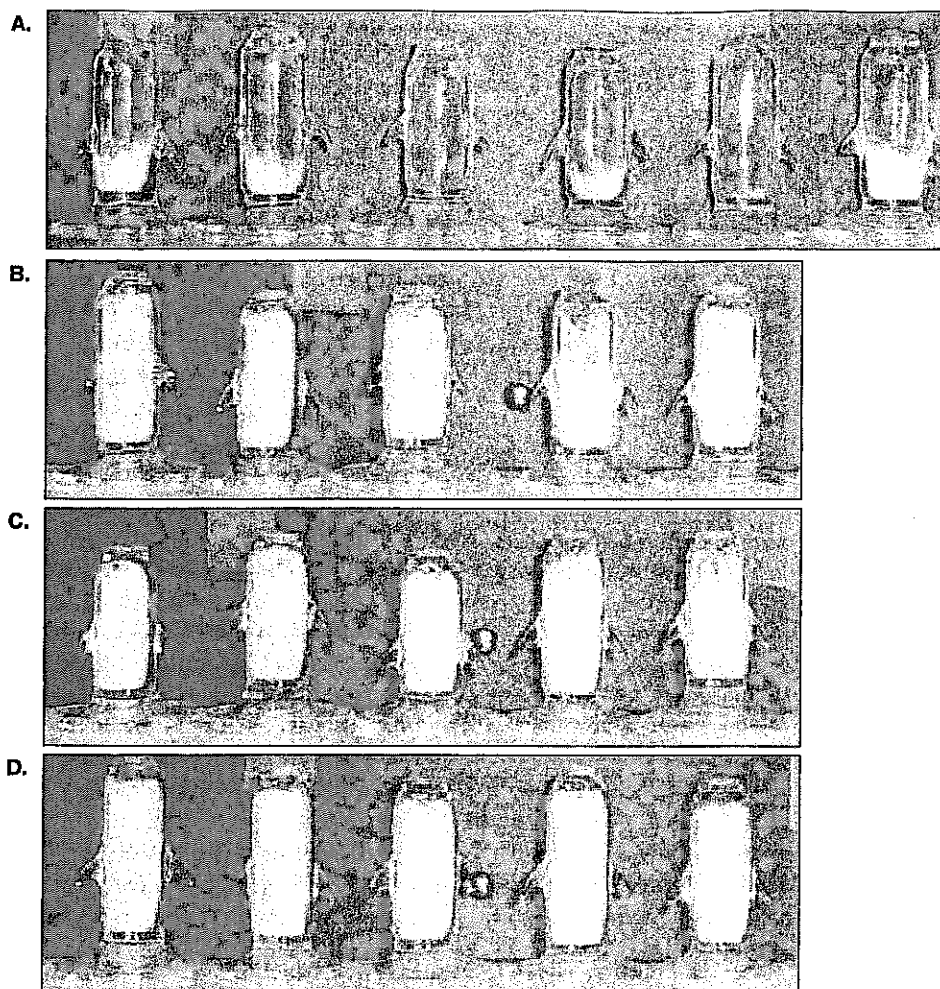


Figure 6. *In vitro* demonstration of activity in a model of infection using the TurChub® cells holding full thickness nails. **A.** TurChub result of AN-2690: 10% w/v in ethyl acetate:propylene glycol 1:1. **B.** TurChub result of placebo: ethyl acetate:propylene glycol 1:1. **C.** TurChub result of ciclopirox: 8% w/w in commercial lacquer. **D.** TurChub result of amorolfine: 5% w/w in commercial lacquer.

7. Clinical summary

The initial clinical development of AN-2690 focused on establishing proof-of-principle clinical efficacy and the *in vivo* confirmation of the bioavailability of AN-2690 noted in the nail-penetration studies. To accomplish the latter, the infected nails of 15 subjects were treated daily with 7.5% w/v AN-2690. Whereas multiple plasma samples of all patients at all timepoints demonstrated no systemic exposure, analysis of the nail plates demonstrated high levels of AN-2690 that were sustained even weeks after treatment was stopped. At the end of the treatment, the mean nail plate level of AN-2690 was 28.8 ± 22.0 $\mu\text{g}/\text{mg}$ of nail plate (range: 13.4 – 91 $\mu\text{g}/\text{mg}$). These levels are ~ 5000 times the MIC_{90} and 600 times the minimum fungicidal concentration (MFC) against *T. rubrum*. AN-2690 was present at levels of 20-fold above the MFC for *T. rubrum* [103] on examination of the nail

clippings 13 weeks after dosing was stopped. All fungal cultures were negative at the end of the 28-day treatment period. A total of 8 weeks after treatment, the mean level of AN-2690 in the treated nail plates was 2.5 ± 2.5 $\mu\text{g}/\text{mg}$ (range: 0 – 8.4 $\mu\text{g}/\text{mg}$). These levels are ~ 400-fold the MIC_{90} and 50-fold the MFC against *T. rubrum*.

To establish clinical proof-of-principle efficacy, an open-label trial was performed. The open design was deemed appropriate because spontaneous improvement of the disease is not part of the known natural history of onychomycosis and there have been no published trials demonstrating any significant placebo or vehicle effect in onychomycosis. This trial enrolled 2 sequential cohorts of 30 subject. All subjects had mild-to-moderate, KOH-positive (direct microscopic examination of a sample from the nail bed for fungal forms), distal subungual onychomycosis. Subjects were treated with AN-2690 solution 5% or 7.5% w/v once daily for a

treatment period of 6 months. Efficacy was measured by evaluating KOH, fungal culture and length of new clear nail growth as measured from serial digital images. The primary end point for this trial was the proportion of subjects who had ≥ 2 mm of new clear nail growth and a negative culture at the end of treatment.

At the end of the 6-month treatment period, the primary end point was reached by 45% (13 out of 29) of the subjects treated with 5% AN-2690 and 50% (15 out of 30) of those treated with 7.5% AN-2690. Also of note, 33 of the subjects were culture positive for dermatophytes at baseline, only 1 was culture positive at 2 weeks, and all were culture negative after 4 weeks of treatment with only rare sporadic positive cultures thereafter. Combining the two cohorts, the average new clear nail growth at the end of treatment was 3.1 mm with 25% (15 out of 60) of the subjects having ≥ 5 mm of new clear nail growth and negative fungal cultures. These results not only establish the clinical proof-of-principle for this product, but in the context of published trials in onychomycosis would appear to demonstrate a level of efficacy not previously seen with other topical treatments and possibly comparable with results reported with oral therapies [102,103]. It should be noted that due to the slow growth of nails, even after the elimination of the fungi, the final determination of efficacy is usually made 48 – 52 weeks after the initiation of treatment in most onychomycosis trials. Although these initial results are very promising, the definitive answer as to the efficacy of this new drug awaits the 6-month follow up of these subjects as well as the results of an ongoing vehicle-controlled dose-ranging trial.

8. Expert opinion

Onychomycosis is a challenging disease to treat. At present, oral therapy is most effective as it attacks the disease from the nail bed and is also integrated into the nail plate at the point of the nail matrix. As nails are at the extremities of our body, good circulation is also required for oral drugs to be effective. New oral therapies will need to be very potent to overcome poor circulation in older patients and either show

an extremely high level of safety over a prolonged dosing period or be very effective under a shorter dosing paradigm, both of which are very challenging problems. Current topical therapy has mostly failed to treat the disease, presumably because of the inability of antifungal agents to penetrate the nail plate and kill the infecting pathogen in the nail bed; however, a new topical antifungal in clinical development, AN-2690, has now shown that this problem may be solved. By matching the physicochemical properties of the antifungal with those of the nail plate, good nail penetration with an antifungal has been achieved and early clinical studies using AN-2690 are validating this approach; therefore, new topical treatments for onychomycosis that show efficacy competitive to oral therapy are most likely to come from a new generation of antifungal agents that are specifically designed to penetrate the nail plate. This new generation promises to dramatically raise the bar for topical and maybe even oral therapy.

To conclude, fungal infection of the nail unit (onychomycosis) represents a therapeutic challenge because of the deep-seated nature of the infection and the difficulty of both oral and topical drugs to reach all sites of infection within the nail unit and to eliminate the pathogenic organism. Ongoing research and development activities are focusing on novel delivery technologies to facilitate nail penetration of antifungal agents and on the discovery of new antifungal compounds with intrinsic nail-penetrating characteristics. Several of these new approaches are now undergoing clinical studies. The outcome of these trials is expected over the next several years and may lead to new therapeutic options for patients with this difficult to treat disease.

Acknowledgements

The authors thank A You, V Sanders and L Hogan for critically reading and editing of the manuscript. The authors are grateful to Y Freund and B Pinto for obtaining the MTS data on the HepG2 cells.

J Plattner, S Baker, K Beutner and MR Alley are employed and supported by Anacor Pharmaceuticals.

Bibliography

Papers of special note have been highlighted as either of interest (*) or of considerable interest (**) to readers.

- GHANNOUM MA, HAJJEH RA, SCHER R *et al.*: A large-scale North American study of fungal isolates from nails: the frequency of onychomycosis, fungal distribution, and antifungal susceptibility patterns. *J. Am. Acad. Dermatol.* (2000) 43(4):641-648.
- ELEWSKI B, CHARIF MA: Prevalence of onychomycosis in patients attending a dermatology clinic in northeastern Ohio for other conditions. *Arch. Dermatol.* (1997) 133:1172-1173.
- HOSSAIN MA, GHANNOUM MA: New developments in chemotherapy for non-invasive fungal infections. *Expert Opin. Investig. Drugs* (2001) 10(8):1501-1511.
- GUPTA AK, JAIN HC, LYNDE CW *et al.*: Prevalence and epidemiology of onychomycosis in patients visiting physicians' offices: a multicenter Canadian survey of 15,000 patients. *J. Am. Acad. Dermatol.* (2000) 43:244-248.
- SHEAR NH, GUPTA AK: Terbinafine for the treatment of pedal onychomycosis. A foot closer to the promised land of cured nails. *Arch. Dermatol.* (1995) 131:937-942.
- HABIF TB: Nail diseases. In: *Clinical dermatology*. Chapter 25, Habif TB (Ed.), Mosby, Inc., Philadelphia, USA (2004) 4:875.
- SCHER RK, BARAN R: Onychomycosis in clinical practice: factors contributing to

Recent progress on the topical therapy of onychomycosis

- recurrence. *Br. J. Dermatol.* (2003) 149(Suppl. 65):5-9.
8. ARRESE JE, PIÉRARD GE: Treatment failures and relapses in onychomycosis: a stubborn clinical problem. *Dermatology* (2003) 207:255-260.
 9. GUPTA AK, LYNCH LE: Onychomycosis: review of recurrence rates, poor prognostic factors, and strategies to prevent disease recurrence. *Cutis* (2004) 74(Suppl. 1):10-15.
 - Review of challenges to treatment and causes of treatment failures to onychomycosis.
 10. TOSTI A, PIRACCINI BM, STINCHI C *et al.*: Relapses of onychomycosis after successful treatment with systemic antifungals: a three-year follow-up. *Dermatology* (1998) 197:162-166.
 11. MURDAN S: Drug delivery to the nail following topical application. *Int. J. Pharm.* (2002) 236:1-26.
 - Comprehensive review of nail unit anatomy and factors that govern the penetration of drugs into the nail plate.
 12. GUPCHUP GV, ZATZ JL: Structural characteristics and permeability properties of the human nail: a review. *J. Cosmet. Sci.* (1999) 50:363-385.
 13. STÜTTGEN G, BAUER E: Bioavailability, skin- and nail penetration of topically applied antimycotics. *Mykosen* (1982) 25:74-80.
 14. HANEKE E: Fungal infections of the nail. *Semin. Dermatol.* (1991) 10:41-53.
 15. MERTIN D, LIPPOLD BC: *In-vitro* permeability of the human nail and of a keratin membrane from bovine hooves: influence of the partition coefficient octanol/water and the water solubility of drugs on their permeability and maximum flux. *J. Pharm. Pharmacol.* (1997) 49:30-34.
 16. WALTERS KA, FLYNN GL, MARVEL JR: Physicochemical chemical characterization of the human nail: permeation pattern for water and the homologous alcohols and the difference with respect to stratum corneum. *J. Pharm. Pharmacol.* (1983) 35:28-33.
 17. KOBAYASHI Y, MIYAMOTO M, SUGIBAYASHI K *et al.*: Drug permeation through the three layers of the human nail plate. *J. Pharm. Pharmacol.* (1999) 51:271-278.
 18. SIGURGEIRSSON B, BILLSTEIN S, RANTANEN T *et al.*: L.I.O.N. study: efficacy and tolerability of continuous terbinafine (Lamisil®) compared to intermittent itraconazole in the treatment of toenail onychomycosis. *Br. J. Dermatol.* (1999) 141(Suppl. 56):5-14.
 19. DE CUYPER C, HINDRYCKX PH: Long-term outcomes in the treatment of toenail onychomycosis. *Br. J. Dermatol.* (1999) 141(Suppl. 56):15-20.
 20. GHANNOUM MA, RICE LB: Antifungal agents: mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance. *Clin. Microbiol. Rev.* (1999) 12:501-517.
 - Review of the mechanism of action for common antifungal agents.
 21. DE BACKER M, DE KEYSER P, DE VROEY C *et al.*: A 12-week treatment for dermatophyte toe onychomycosis: terbinafine 250 mg/day versus itraconazole 200 mg/day – a double blind comparative trial. *Br. J. Dermatol.* (1996) 134(Suppl. 46):16-17.
 22. BARAN R, GUPTA AK, PIÉRARD GE: Pharmacology of onychomycosis. *Expert Opin. Pharmacother.* (2005) 6(4):609-624.
 23. RICHARDSON MD, WARNOCK DW: In: *Fungal infections, diagnosis and management*. Third Edition, Blackwell Publishing, Malden, USA (2003).
 24. WEINBERG ED: Antifungal agents. In: *Principles of medicinal chemistry*. Chapter 35, Foye WO (Ed.), Leo & Febiger, Malvern, USA (1990) 3:731.
 25. GULL K, TRINCI AP: Griseofulvin inhibits fungal mitosis. *Nature* (1973) 244:292-294.
 26. GUPTA AK: Ciclopirox: an overview. *Int. J. Dermatol.* (2001) 40:305-310.
 27. GUPTA AK, PLOTT T: Ciclopirox: a broad-spectrum antifungal with antibacterial and anti-inflammatory properties. *Int. J. Dermatol.* (2004) 43(Suppl. 1):3-8.
 28. POLAK A: Mode of action of morpholine derivatives. *Ann. NY Acad. Sci.* (1988) 544:221-228.
 29. FANG FC: Mechanisms of nitric oxide-related antimicrobial activity. *J. Clin. Invest.* (1997) 99(12):2818-2825.
 30. BAKER SJ, ZHANG YK, AKAMA T *et al.*: Discovery of a new boron-containing anti-fungal agent, 5-fluoro-1,3-dihydro-1-hydroxy-2,1-benzoxaborole (AN2690), for the potential treatment of onychomycosis. *J. Med. Chem.* (2006) 49:4447-4450.
 - Description of the discovery and antifungal properties of the oxaborole family of antifungals.
 31. GUPTA AK, RYDER JE, BARAN R: The use of topical therapies to treat onychomycosis. *Dermatol. Clin.* (2003) 21:481-489.
 32. ROBERTS DT: Onychomycosis: current treatment and future challenges. *Br. J. Dermatol.* (1999) 141(Suppl. 56):1-4.
 33. BAKER SJ, HUI X, MAIBACH HI: Progress on new therapeutics for fungal nail infections. *Ann. Rep. Med. Chem.* (2005) 40:323-334.
 34. KOBAYASHI Y, KOMATSU T, SUMI M *et al.*: *In vitro* permeation of several drugs through the human nail plate: relationship between physicochemical properties and nail permeability of drugs. *Eur. J. Pharm. Sci.* (2004) 21:471-477.
 - Delineation of the physicochemical properties of organic compounds that enhance penetration into the nail plate.
 35. TATSUMI Y, YOKOO M, SENDA H *et al.*: Therapeutic efficacy of topically applied KP-103 against experimental tinea unguium in guinea pigs in comparison with amorolfine and terbinafine. *Antimicrob. Agents Chemother.* (2002) 46:3797-3801.
 36. HUI X, CHAN TCK, BARBADILLO S *et al.*: Enhanced econazole penetration into human nail by 2-n-nonyl-1,3-dioxolane. *J. Pharm. Sci.* (2003) 92:142-148.
 37. HUI X, BAKER SJ, WESTER RC *et al.*: *In vitro* penetration of a novel oxaborole antifungal (AN2690) into the human nail plate. *J. Pharm. Sci.* (2006) (In Press).
 38. COLOMBO B, FELICETTI L, BAGLIONI C: Inhibition of protein synthesis by cycloheximide in rabbit reticulocytes. *Biochem. Biophys. Res. Commun.* (1965) 18:389-395.
 39. GROLLMAN AP: Inhibitors of protein biosynthesis. II. Mode of action of anisomycin. *J. Biol. Chem.* (1967) 242(13):3226-3233.
 40. JUSTICE MC, HSU MJ, TSE B *et al.*: Elongation factor 2 as a novel target for selective inhibition of fungal protein synthesis. *J. Biol. Chem.* (1998) 273(6):3148-3151.
 41. FULLER AT, MELLOWS G, WOOLFORD M *et al.*: Pseudomonica

- acid: an antibiotic produced by *Pseudomonas fluorescens*. *Nature* (1971) 234(5329):416-417.
42. NICHOLAS RO, BERRY V, HUNTER PA *et al.*: The antifungal activity of mupirocin. *J. Antimicrob. Chemother.* (1999) 43(4):579-582.
 43. PETRAITIS V, PETRAITIENE R, KELAHER AM *et al.*: Efficacy of PLD-118, a novel inhibitor of candida isoleucyl-tRNA synthetase, against experimental oropharyngeal and esophageal candidiasis caused by fluconazole-resistant *C. albicans*. *Antimicrob. Agents Chemother.* (2004) 48(10):3959-3967.
 44. ZIEGELBAUER K: Decreased accumulation or increased isoleucyl-tRNA synthetase activity confers resistance to the cyclic β -amino acid BAY 10-8888 in *Candida albicans* and *Candida tropicalis*. *Antimicrob. Agents Chemother.* (1998) 42(7):1581-1586.
 45. ZIEGELBAUER K, BABCZINSKI P, SCHONFELD W: Molecular mode of action of the antifungal β -amino acid BAY 10-8888. *Antimicrob. Agents Chemother.* (1998) 42(9):2197-2205.
 46. CAPOBIANCO JO, ZAKULA D, COEN ML *et al.*: Anti-Candida activity of cispentacin: the active transport by amino acid permeases and possible mechanisms of action. *Biochem. Biophys. Res. Commun.* (1993) 190(3):1037-1044.
 47. HENDRICKSON TL, SCHIMMEL P: Transfer RNA-dependent amino acid discrimination by aminoacyl-tRNA synthetase. In: *Translation mechanisms*. Lapointe JPD, Brakier-Gingras L (Eds), Landes Bioscience, Georgetown, USA (2003):34-64.
 - A review of the well-integrated steps that occur in protein synthesis including the crucial step accomplished by the aminoacyl-tRNA synthetases.
 48. CUSACK S, YAREMCHUK A, TUKALO M: The 2 Å crystal structure of leucyl-tRNA synthetase and its complex with a leucyl-adenylate analogue. *EMBO J.* (2000) 19(10):2351-2361.
 49. FUKAI S, NUREKI O, SEKINE S *et al.*: Structural basis for double-sieve discrimination of L-valine from L-isoleucine and L-threonine by the complex of tRNA(Val) and valyl-tRNA synthetase. *Cell* (2000) 103(5):793-803.
 50. NUREKI O, VASSYLYEV DG, TATENO M *et al.*: Enzyme structure with two catalytic sites for double-sieve selection of substrate. *Science* (1998) 280(5363):578-582.
 51. HUGHES J, MELLOWS G: Interaction of pseudomonic acid A with *Escherichia coli* B isoleucyl-tRNA synthetase. *Biochem. J.* (1980) 191(1):209-219.
 52. SILVIAN LE, WANG J, STEITZ TA: Insights into editing from an ile-tRNA synthetase structure with tRNA^{ile} and mupirocin. *Science* (1999) 285(5430):1074-1077.

Websites

101. <http://www.medpharm.co.uk/downloads/Skin%20and%20nail%20dec%202003.pdf> MedPharm website (Accessed 3 January 2007).
102. http://www.anacor.com/news/posters/PPP_AN2690.pdf Anacor website (Accessed 3 January 2007).
103. http://www.anacor.com/news/posters/aaps_2006_final.pdf Anacor website (Accessed 3 January 2007).

Affiliation

Michael RK Alley,
 Stephen J Baker,
 Karl R Beutner &
 Jacob Plattner[†], Senior Vice President R&D
[†] Author for correspondence
 Anacor Pharmaceuticals, 1060 East Meadow
 Circle, Palo Alto, CA 94303, USA
 Tel: +1 650 739 0719;
 Fax: +1 650 730 0139;
 E-mail: jplattner@anacor.com