Chapter 12. Antifungal Agents

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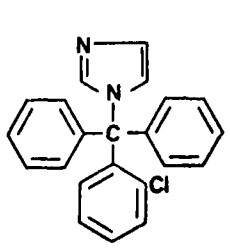
Introduction - Since the last review two years ago there have been no new major antifungal drugs introduced into clinical practice. Clotrimazole, 1, and miconazole, 2, are regarded as promising advances in two editorials.[1,2] The possibilities of miconazole for the treatment of systemic mycoses including reports on its experimental and clinical evaluation was the subject of a symposium.[3] The use of and the mode of action of 5 fluorocytosine (5FC), 3, was comprehensively reviewed.[4]

It was suggested that the synthesis of chitin might be a suitable site for a future antifungal drug. Chitin is specific to fungal cells, is essential to their structure, is produced precisely at the site of invasion of tissue - the hyphal apex and its synthesis is accomplished by one enzyme - chitin synthase.[5]

New Antifungal Agents - A series of 1 - (2-alkyl-2-phenylethyl)-1H-imidazole derivatives were synthesized from corresponding phenylacetonitriles, 4,[6].

Most activity was found in analogues with ortho-para distribution on the phenyl ring. Significant broad-spectrum activity was associated with at least four carbon atoms in the alkyl chain. Trichostatin, 5, an analogue of a primary hydroxamic acid was isolated from Streptomyces hygroscopicus. It was active in vitro against dermatophytes but not yeasts.[7] A non-polyene antibiotic (H537 SY2) isolated from Streptomyces yokosukanensis was active against Candida spp.[8] The structure has not yet been elucidated but appears to contain three amino sugars and a carbonyl group. A group of quinolizidine-derived hemiaminals and beta-tert-amino



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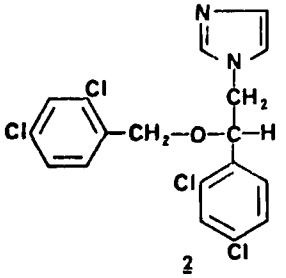

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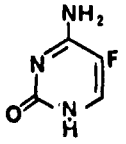

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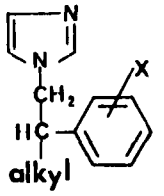

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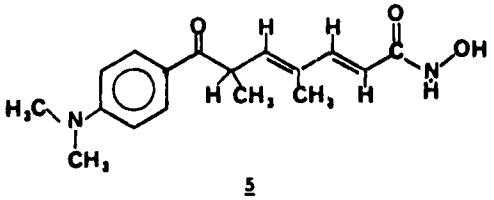

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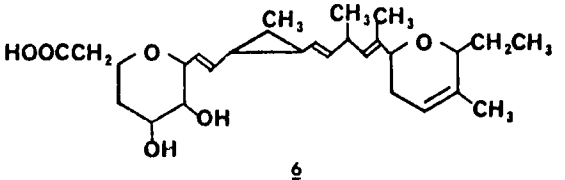

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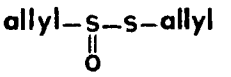

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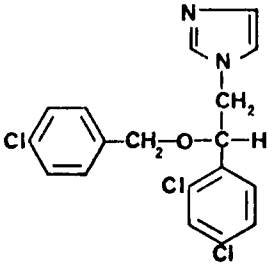

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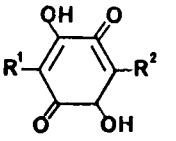

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sulfides were prepared.[9] Only those α-thiohemiaminals possessing the C-1 equatorial methyl and the C-4 equatorial 3-furyl groups were active antifungal agents and their in vitro activity was greater than amphotericin B. Four thiocyanatopyrazole derivatives were synthesized and in vitro activity was demonstrated against a number of dermatophytic fungi. Electron microscopic studies on Trichophyton mentagrophytes exposed to 1, 3-dimethyl -4-thiocyanato-5-amino-pyrazole showed an increase of the plasma membrane with intra and extra cytoplasmic processes. The nuclear and mitochondrial membranes deteriorated and there was final plasmolysis.[10] Inhibition of T.mentagrophytes was inhibited by some 7-alkyl amino pyrazolo (1, 5-a) pyrimidines. The degree of inhibition increased with the length of 7-alkylamino chain up to C_8 units then decreased. Unsaturated chains had greater activity than saturated chains.[11] Aculeacin, the structure of which has not yet been elucidated, is a peptide antibiotic containing palmitic acid, isolated from Aspergillus aculeatus. It was active against yeasts, dermatophytes and phytopathogenic fungi, and although not strongly fungicidal, inhibited filamentous fungi. Toxicity for mice was low.[12]

A metabolite of Aspergillus tamarii A3064[1] was active against candida, cryptococci and gram-positive bacteria.[13] Preliminary studies suggest that the structure includes a phenolic hydroxyl and the presence of the epidithiodiketopiperazine ring system. Ambruticin (W7783), 6, was isolated from Polyangium cellulosum var fulvum. A cyclopropyl-polyene-pyran acid, it was highly active against Coccidioides immitis, Histoplasma capsulatum and Blastomyces dermatitidis.[14] Allicin, 7, an extract from Allium sativum, was active in vitro against yeasts and dermatophytes but not against aspergilli.[15] The observed effect was dependent on the inoculum size.

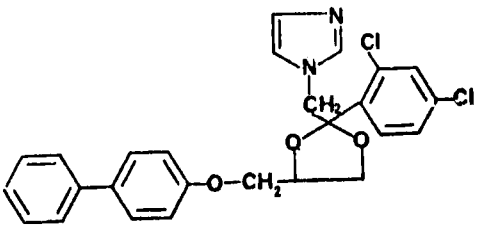
Laboratory Methods - A potential bioassay for nystatin depending on nystatin-induced potassium efflux from Saccharomyces cerevisiae, measured

by flame photometry, was described. [16] Polyenes were assayed by two methods involving the replacement of intracellular potassium by rubidium and measuring its subsequent release from cells exposed to a polyene antibiotic. Using S. cerevisiae as the test organism, the release rubidium was measured by atomic absorption spectrophotometry. [17] A radiometric approach used ^{86}Rb to replace the potassium in a strain of Candida parapsilosis. This method enabled amphotericin B to be assayed within the range 0.06 - 2.9 μg/ml.[18] The rapid assay of amphotericin B, 8, in less than thirty minutes was achieved with the use of high-pressure liquid chromatography.[19] Absorption was at 405 nm and the lower level of sensitivity was 0.02 μg/ml. There was no interference from 5FC. 5FC was assayed in the presence of amphotericin B after heating the serum sample at 100°C for forty-five minutes which inactivated the polyene. [20] C.stellatoidea was used in the bioassay of miconazole by radial diffusion.[21] Difficulties in assessing the susceptibility testing of yeasts to imidazoles were recognized and a turbimetric technique independent of inoculum size was developed. [22] A Coulter counter was used to assess the ED_{50} and the MIC of a wide range of antifungal agents against strains of S.cerevisiae and C. albicans. The degree of inhibition of cell division and of protein synthesis could be compared.[23] Econazole, 9, was compared to other agents by the Warburg assay. [24] The antifungal activity of 5FC was measured by disc diffusion susceptibility testing against 216 yeasts using 1 μg and 10 μg discs. [25] The 10 μg disc was preferred. Disc diffusion susceptibility was used to determine the MIC of yeasts against amphotericin B, nystatin and 5FC. [26] It was demonstrated that the antifungal activity of imidazoles was reduced by complex media - Sabouraud's glucose and brain heart infusion - but not by a synthetic amino acid medium or modified yeast nitrogen broth. No inhibition was shown with any of the media by amphotericin B methyl ester. [27]

In vitro and animal studies - 15-Aza-24-methylene-D-homocholestadiene, a naturally occurring azasterol with antimycotic properties was shown to competitively inhibit sterol 24 (28) methylene reductase in S.cerevisiae.[28] 2, 5-Dihydroxy-1, 4 benzoquinones were shown to decrease the vegetative growth and inhibit spore germination in twelve fungal species. Substitution at 3 and 6 in the quinone ring affected these properties; the most active compounds generally had a low polarity. [29] The antibiotic phacidin was inhibitory against T.mentagrophytes, T. rubrum and Epidermophyton floccosum.[30] 2, 4-Diamino-6- {2-(3,4-dichlorophenyl) acetamido} quinazoline inhibited the incorporation of labelled precursors into the RNA and protein of C. neoformans. Experimental infections in mice responded to intraperitoneal therapy. [31] In vitro, tolciclate was more active against dermatophytes than clotrimazole or miconazole.[32] Versicolin was active against T. rubrum both in vitro and in experimental infections of mice and guinea pigs. [33] The activity of amphotericin B was prolonged using the antioxidant propyl gallate. [34] The inhibitory effect of amphotericin B methyl ester on protoplasts of C. albicans was inhibited by the presence of 85 mM KCl or 45 mM $MgCl_2$.[35] Amphotericin B inhibited phosphate uptake in C. albicans, especially in young (2h) cells and this effect was stronger than growth inhibition. [36] The sterol structure of two polyene-resistant mutants of C. albicans showed that one accumulated

lichesterol and fecosterol, and the other eburicol and lanosterol.[37] In polyene-resistant strains of C. krusei, C. parakrusei and C. tropicalis isolated from patients, the sterols were the same as in the wild types but present in lower concentrations.[38] In an auxotroph of C. albicans, the resistance to polyenes was related to fatty acid composition rather than sterols. Greatest resistance was shown when the strain was grown in the presence of oleic or linoleic acid.[39] The action of candicidin on C. albicans at various stages of the growth cycle was related to differing fatty acid composition of the cells.[40] It was suggested that the divalent ions Ca++ and Mg++ can interact with membrane sterols in C. albicans creating steric hinderance to the absorption of candicidin. K+ and NH_{4+} reversed the inhibition of glycolysis by candicidin but did prevent antibiotic absorption.[41] Ultrastructural changes were observed in the cells of A. fumigatus from infected mice treated with clotrimazole.[42] Miconazole was highly active against Histoplasma capsulatum and Blastomyces dermatitidis and serum levels in man could be expected to exceed the MIC. C. immitis, Sp.schenckii, C. neoformans, C. albicans and A. fumigatus were less sensitive and Fusarium spp. were resistant.[43]

The mode of action of miconazole against C. albicans was studied by biochemical, cytochemical and ultrastructural techniques.[44, 45, 46] Changes were observed in oxidative and peroxidative enzymes and it was suggested that fungistatic levels of miconazole produce an increase in the production of intracellular hydrogen peroxide which is broken down by catalase. At fungicidal levels, the catalase was inhibited leading to lethal levels of hydrogen peroxide. Miconazole is also a potent inhibitor of demethylation at the C-14 position in ergosterol biosynthesis. The emergence of miconazole-resistant candida during treatment was reported.[46] Econazole inhibited fungal growth by its damaging effects on the various membrane systems of fungal cells.[48] Ultrastructural changes in the cell wall followed the exposure of C. albicans to econazole.[49] Econazole was more active than miconazole in vitro against filamentous fungi.



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R34,000, 10, was effective orally in controlling murine coccidioidomycosis.[51] 5FC sensitive cells of candida possess an electron dense layer dividing the inner layer of the cell wall. The dense layer is absent in resistant strains.[52] Following exposure to 5FC, yeast cells enlarge. Ultrastructure studies of treated cells show an enlarged nucleus and a thin cell comparative to the changes caused by DNA synthesis inhibitors in mammalian cells.[53] Studies on the influence of 5FC on nucleic and synthesis in yeasts suggested that it may act by the formation of 5-fluorodeoxyuridine monophosphate which inhibits thymidylate synthetase and also by the incorporation of fluorouridylic acid into RNA.[54] A wide range of purines and pyrimidines except thymidine inhibited the action of 5FC.[55]

Griseofulvin was shown to interfere with the normal polymerization of microtubule protein; it also acted directly on tubulin in common with other mitotic drugs. [56]

Drug Combinations:- Minocyline at 10 µg/ml or less was synergistic with amphotericin B. Doxycycline had a reduced effect but demeclocycline and tetracycline were ineffective. [57] In vitro synergism between amphotericin B and rifampin was demonstrated against Candida spp.[58] and C.immitis.[59] The combination however, was not superior to amphotericin B alone in murine coccidioidomycosis. Therapy of murine aspergillosis with amphotericin B in combination with rifampin or 5FC was more effective than amphotericin B alone but did not result in complete cure.[60] Amphotericin B and rifampin were used successfully for pulmonary aspergillosis in a leukemic patient. [61] In vitro inhibition of aspergilli was increased by the combination of amphotericin B with 5FC and rifampin.[62] Five patients with systemic infection due to candida or Torulopsis glabrata were successfully treated by amphotericin B in combination with 5FC. [63, 64] The effect of the combination of amphotericin B and 5FC on C. albicans appeared to be sequential, amphotericin B acting first. [65]

The combination of amphotericin B and griseofulvin was used with success in a diabetic with pulmonary and rhinocerebral mucormycosis. [66] Sulfamethoxazole acted synergistically with both clotrimazole [67] and miconazole[68] against Candida spp. In vitro synergism was shown between clotrimazole and both amphotericin B and 5FC [69] but there was antagonism between miconazole and amphotericin B.

Therapeutic and Pharmacologic Studies:- In a double blind study, a 1% solution of ciclopirox was effective against T. rubrum, T.violaceum, T. mentagrophytes and Malassezia furfur. [71] Tolciclate treatment produced a 78% cure rate in a multicentre trial against dermatophytes.[72] Three patients with indwelling catheters and a candidal urinary tract infection were successfully treated with oral nifuratel.[73] The value of intravenous 5FC was illustrated by the case report of a patient with candida septicemia following severe trauma. [74] Four out of fifteen patients receiving 5FC had evidence of bone marrow toxicity which was related to serum levels above 125 µg/ml.[75] The nephrotoxicity of amphotericin B was reduced by the infusion of 25 gram of mannitol with each dose. [76] In a double blind study involving eleven patients, no difference was observed whether or not mannitol was given with the amphotericin B. Renal biopsies showed a hitherto undescribed vacuolization of smooth muscle cells in the media of arterioles and arteries associated with amphotericin B. [77] Miconazole and clotrimazole were both effective topically against dermatophytes, pityriasis, candida and erythrasma.[78]

The topical pharmacology of chlormidazole, clotrimazole, miconazole and econazole and the relative value of topical antifungal drugs was discussed. [79] The general pharmacology of imidazoles was reviewed. [80] Pharmacologic studies of intravenous miconazole in patients with coccidioidomycosis revealed a biphasic clearance from the blood. Passage into cerebrospinal fluid did not occur in therapeutic amounts.[81] A more

detailed study in normal subjects and patients with renal failure showed an initial serum half life of 0.38 h., then 2.08 h and finally a prolonged phase of 24.1 h. Renal insufficiency did not affect the rate of elimination from the body. [82] Five of the patients with coccidioidal meningitis responded to miconazole intravenously and four more intrathecally. [83] No response was recorded in two patients with cryptococcal meningitis. A leukemic patient with disseminated candidiasis was successfully treated with miconazole. [84] Intravenous miconazole was also used with success in a patient with chronic esophageal candidiasis [85] and a patient with an extensive cavitatory infection with Sporotrichum schenckii. [86] The latter patient had received amphotericin B alone and in combination with 5FC without effect. The failure of oral polyenes in the management of candidiasis of the digestive tract was followed successfully by oral miconazole. [87] Preliminary studies indicate the value of long term miconazole for patients with paracoccidioidomycosis. [88] The use of intravenous miconazole should be accompanied by careful hematologic studies as six consecutive patients showed an anemia and thrombocytosis. The effect was dose related and reversible. There were no hemorrhagic or thrombotic episodes. [89]

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