Charles University in Prague

Faculty of Pharmacy in Hradec Králové

Department of Pharmaceutical Chemistry and Drug Control

Derivatives of Rhodanine as Potential Antifungal and Antimycobacterial Drugs

Diploma Thesis

Hradec Králové 2007 Morid Mahmoudi Majd

I would like to say thank you to RNDr. V. Opletalová, Ph.D. for helping me in writing the thesis. And I should thank my parents for their support during my study. I would also like to thank to Ms. V. Hronová for performing the elemental analyses, to Ms. I. Vencovská for recording the IR spectra, to Assoc. Prof. PharmDr. J. Kuneš, CSc. for recording and interpreting the NMR spectra, to PharmDr. J. Jampílek, Ph.D. for HPLC analyses, and to Ms. Ida Dufková and Mgr. M. Vejsová for performing antifungal tests.

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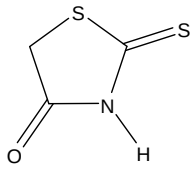
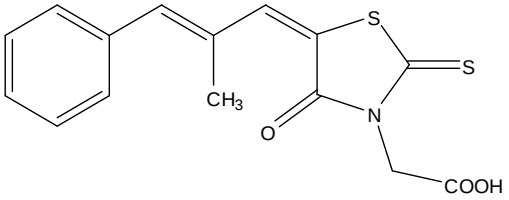
1. INTRODUCTION AND THE AIM OF THE WORK

Rhodanine (2-thioxo-1,3-thiazolidin-4-one) was first prepared by Nencki in 1877 [1]. Since that time various derivatives of rhodanine and *N*-substituted rhodanines were prepared. Some of them have also been screened for their biological activities [2 – 4].

Boyd [5] searched various databases to obtain an indication of how common the rhodanine ring is. From the database search results that the prevalence of rhodanine-containing compounds of pharmaceutical interest is very small despite the fact that the compounds exhibit a wide variety of bioactivity. Epalrestat is the only derivative of rhodanine that has been used clinically. It is an aldose reductase inhibitor and is used to slow eye damage associated with diabetes and to prevent diabetic peripheral neuropathy [5].



rhodanine

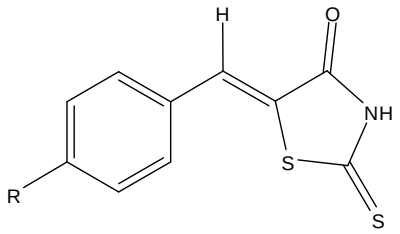


epalrestat

The rarity of clinically effective compounds with a rhodanine ring can be viewed in two ways. An optimistic believer in drug discovery by serendipity might see that the rhodanines are a relatively unexploited structural motif and therefore have the potential to lead to novel, patentable compounds. On the other hand, a pragmatist looking rationally at the track record might see the rhodanines as a motif that has yielded little useful information to date and is therefore unlikely to do so in future [5, 6].

Recent studies performed at the Department of Pharmaceutical Chemistry and Drug Control of the Faculty of Pharmacy in Hradec Králové showed that condensation product of rhodanine with acetylpyrazines were practically

ineffective in antifungal, antimycobacterial, antiproliferative assays [7], but benzylidenerhodanines derived from ring substituted benzaldehydes exhibited antifungal properties (MIC = 15.62 – 250 μmol.l^{-1}). 5-Benzylidene-2-thioxo-1,3-thiazolidine-4-one and 5-(4-chlorobenzylidene)-2-thioxo-1,3-thiazolidine-4-one inhibited the growth of a highly resistant pathogen *Absidia corymbifera* [8].



5-benzylidene-2-thioxo-1,3-thiazolidine-4-one: R = H

5-(4-chlorobenzylidene)-2-thioxo-1,3-thiazolidine-4-one: R = Cl

The aim of my diploma thesis is to prepare additional condensation products of rhodanine with aldehydes. The compounds will then be submitted to testing of their antifungal and antimycobacterial activities.

2. THEORETICAL PART

2.1. Mycobacterial diseases

There are more than 70 species of mycobacteria. Of these, two are major pathogens – *Mycobacterium tuberculosis* (Koch, 1882) and *Mycobacterium leprae* (Hansen, 1874). The remaining mycobacteria are environmental organisms – collectively known as MOTTS (**M**ycobacteria **O**ther **T**han **T**uberculosis), NTM (**N**on-**T**uberculous **M**ycobacteria) or atypical mycobacteria. NMT, especially *Mycobacterium avium* complex, are facultative human pathogens that are responsible not only for disseminated disease in patients with AIDS but also for various opportunistic infections affecting organs (e.g. lung, skin, eyes) of people without AIDS [9 – 13].

2.2. Tuberculosis (TB) and possibilities manage it

2.2.1. The disease tuberculosis

Tuberculosis is an infectious disease caused by the microorganism *Mycobacterium tuberculosis*. It can affect several organs of the human body, including the brain, the kidneys and the bones; but most commonly it affects the lungs (Pulmonary Tuberculosis). The first stage of the infection usually lasts for several months. During this period, the body´s natural defenses (immune system) resist the disease, and most or all of the bacteria are walled in by a fibrous capsule that develops around the area. Before the initial attack is over, a few bacteria may escape into the bloodstream and be carried elsewhere in the body, where they are again walled in. In many cases, the disease never develops beyond this stage – and is referred to as TB infection. If the immune system fails to stop the infection and it is left untreated, the disease progresses to the second stage, active disease. There, the germ multiplies rapidly and destroys the tissues of the lungs (or the other affected organ). In some cases, the disease, although halted at first, flares up after a latent period. Sometimes, the latent period is many years, and the bacteria become active when the opportunity presents itself, especially when immunity is low.

The second stage of the disease is manifested by destruction or "consumption" of the tissues of the affected organ. When the lung is affected, it results in diminished respiratory capacity, associated with other symptoms; when other organs are affected, even if treated adequately, it may leave permanent, disabling scar tissue [14].

2.2.2. *Mycobacterium tuberculosis*

Mycobacterium tuberculosis is a fairly large nonmotile rod-shaped bacterium distantly related to the Actinomycetes. The rods are 2 – 4 μm in length and 0.2 – 0.5 μm in width. Chains of cells in smears made from in vitro-grown colonies often form distinctive serpentine cords. This observation was first made by Robert Koch who associated cord factor with virulent strains of the bacterium.
It is an obligate aerobe. For this reason, in the classic case of tuberculosis, the *M. tuberculosis* complexes are always found in the well-aerated upper lobes of the lungs. The bacterium is a facultative intracellular parasite, usually of macrophages, and has a slow generation time, 15 – 20 hours, a physiological characteristic that may contribute to its virulence.

Two media are used to grow *M. tuberculosis*. Middlebrook´s medium which is an agar based medium and Lowenstein-Jensen medium which is an egg based medium. *M. tuberculosis* colonies are small and buff colored when grown on either medium (Figure 1). Both types of media contain inhibitors to keep contaminants from out-growing *M. tuberculosis*. It takes 4 – 6 weeks to get visual colonies on either type of media.

Figure 1. Colonies of *M. tuberculosis* on Lowenstein-Jensen medium [15]

The cell wall structure of *M. tuberculosis* deserves special attention because it is unique among procaryotes and it is a major determinant of virulence for the bacterium. The cell wall complex contains peptidoglycan, but otherwise it is composed of complex lipids. Over 60% of the mycobacterial cell wall is lipid. The lipid fraction of *M. tuberculosis* cell wall consists of three major components:

- **Mycolic acids** are unique α-branched lipids found in cell walls of *Mycobacterium* and *Corynebacterium.* They make up 50% of the dry weight of the mycobacterial cell envelope. Mycolic acids are strong hydrophobic molecules that form a lipid shell around the organism and affect permeability properties at the cell surface. Mycolic acids are thought to be a significant determinant of virulence in *M. tuberculosis*. Probably, they prevent attack of the mycobacteria by cationic proteins, lysozyme and oxygen radicals in the phagocytic granule. They also protect extracellular mycobacteria from complement deposition in serum.
- **Cord factor** is responsible for the serpentine cording mentioned above. Cord factor is toxic to mammalian cells and is also an inhibitor of polymorphonuclear migration. Cord factor is most abundantly produced in virulent strains of *M. tuberculosis*.

- **Wax-D** in the cell envelope is the major component of Freund´s complete adjuvant.

The high concentration of lipids in the cell wall of *M. tuberculosis* has been associated with these properties of the bacterium:

- Impermeability to stains and dyes
- Resistance to many antibiotics
- Resistance to killing by acidic and alkaline compounds
- Resistance to osmotic lysis via complement deposition
- Resistance to lethal oxidations and survival inside of macrophages [15].

2.2.3. Management of tuberculosis

The following approaches have been in practice or studied as potential measures in attempting to control tuberculosis:

2.2.3.1. The sanatorium with fresh air, cleanliness, and a nutritious diet

2.2.3.2. Vaccination

2.2.3.3. Genomics

2.2.3.4. Immunotherapy

2.2.3.5. Chemotherapy [16].

2.2.3.1. The sanatorium with fresh air, cleanliness, and a nutritious diet

The introduction of the sanatorium cure provided the first real step against TB. Hermann Brehmer, a Silesian botany student suffering from TB, was instructed by his doctor to seek out a healthier climate. He traveled to the Himalayan mountains where he could pursue his botanical studies while trying to rid himself of the disease. He returned home cured and began to study medicine. In 1854, he presented his doctoral dissertation bearing the auspicious title, Tuberculosis is a Curable Disease. In the same year, he built an institution in Gorbersdorf where, in the midst of fir trees, and with good nutrition, patients were exposed on their balconies to continuous fresh air. This setup became the blueprint for the subsequent development of sanatoria, a powerful weapon in the battle against an insidious opponent [17].

2.2.3.2. Vaccination

Active immunization is one of the essential components to control TB, although vaccination is ineffective nowadays. Until now one billion people have been vaccinated with BCG (**B**acilli **C**almette-**G**uerin). In general, the efforts to make new four classes of vaccines candidates are being worked on: the rationally attenuated strains of *M. tuberculosis*, BCG vaccines, protein subunit vaccines, and nucleic acid vaccines [16].

- *The rationally attenuated strains of M. tuberculosis*

  The manipulation of *M. tuberculosis* chromosomes generates bacterial strains, which lack the pathogenicity but elicit a protective immune response.

- *BCG vaccines*

  Advances in the discovery and characterization of genes and antigens of *M. tuberculosis* had led to substantial progress toward the development of improved vaccines since BCG vaccines were first used successfully. The cheap BCG vaccines with minor side effects can safely be given to children. It is proven now that BCG does have some protective effects in children and is effective against meningitis TB, but it does not prevent the emergence of pulmonary TB, particularly in the adulthood.

  Protein subunit vaccines

  Purified proteins as vaccine candidates have several advantages over attenuated organisms. They are inherently safe and have no propensity to cause disease, which is an important consideration when vaccinated individuals have been exposed to HIV. The efficacy of such vaccines has been demonstrated in mice and guinea pigs.

- *Nucleic acid vaccines*

  The hypothesis that naked DNA vaccines and DNA encoding influenza nucleoprotein lend immunity to influenza in mice has been applied to M. tuberculosis also. Many nucleic acid vaccines have shown efficacy in experimental TB.

2.2.3.3. Genomics

The most significant developments in the area of TB are perhaps the sequencing of the mycobacterial genome. Among the estimated 4500 genes, every drug target and every antigen or protein elicits an immune response. Among them 40% of is known, another 44% have some sequence homology, whereas function of 16% are completely unknown, and they may account for specific mycobacterial functions.

2.2.3.4. Immunotherapy

Immunotherapy is a therapeutic means whereby the immune system is stimulated by the injection of inactivated mycobacteria, resulting in the activation of Th1 cells and inactivation of Th2 cells, the two kinds of helper cells. Cell mediated immune (CMI) and delayed type of hypersensitive (DTH) responses play very important roles in host during *M. tuberculosis* infection. Several studies on the effectiveness of *Mycobacterium vaccae*, as an immunotherapeutic agent for TB control have recently been carried out. It is thought to be a powerful Th1 adjuvant and has a beneficial effect with enough evidence of its use as an immunotherapeutic agent [16].

2.2.3.5. Chemotherapy

Chemoterapy of TB started in 1940´s. In 1943, anti-TB research resulted in discovery of the active anti-TB agents, and strategies have been devised to treat TB from time to time. A number of agents have been discovered since then, including *para*-aminosalicylic acid (PAS), isoniazid (INH), pyrazinamide (PZA), cycloserine, ethionamide, rifampicin (RMP), and ethambutol. The majority of these drugs were discovered through broad random screening. Very little optimization was undertaken. As no biochemical tools to study biochemical reactions were known at that time, the modifications were made without any regard to drug targets.. This lack of understanding of drug action because of ignorance in the biochemistry of mycobacterium and urgency to develop drugs against this devastating disease led to random screening of compound libraries in

fact, among other reasons, the difficulty in manipulating *M. tuberculosis* has hindered efforts to delineate the mode of the action of these agents.

Recent improvements in biological techniques have allowed mechanism of action of many of these agents to be uncovered and more carefully studied. Current treatment involve multiple drug regimens that extend for months at the time, and pharmacology of these treatments regimens can be complex, specially for the treatment of multidrug resistant tuberculosis (MDR TB) [16].

Generally the targets, for an anti-TB drug, involve the biosynthetic pathways which are involved in the production of macromolecules (the proteins, the nucleic acids, or cell wall polymers). Many well known anti-TB drugs target the biosynthesis of these macromolecules. The recent development in genetic engineering of *M. tuberculosis* have now offered many targets to be validated and screen libraries of compounds against them to develop new anti-TB agents. In selecting targets for anti-TB agents, it is advantageous to avoid targets that are close to counterparts in mammalian cells. It is also desirable that new targets should be specific to mycobacteria to limit the transfer of resistant factors from other bacteria. Further, new drugs must act on a target that is essential for bacterial survival, and ideally they should be active against mycobacterium throughout their growth cycle both inside and outside mammalian cells during infection.

The following targets for anti-TB drugs have been studied:

- *Protein Synthesis*

  Streptomycin, an aminoglycoside for widespread use in the treatment of TB, disrupts the protein synthesis in bacteria. Streptomycin resistance in M. tuberculosis is because of mutation altering the 16s ribosomal subunit in the RNA molecule. Most of aminoglycosides act through this mechanism.

- *Nucleic Acids*

  Sulphonamides, the structural analogs of *p*-aminobenzoic acid, inhibit biosynthesis of tetrahydrofolic acid, and thereby block the production of purine and pyrimidine bases required for nucleic acids synthesis in

microbes. A detailed study of enzymes involved in tetrahydrofolate biosynthesis may lead to a rational design of new and novel anti-TB drugs.

- *DNA Topoisomerases*

  Another promising target is DNA topoisomerase particularly DNA gyrase, a type 2 topoisomerase. Biosynthesis of nucleotides has recently been reported to be a good target particularly for TB in HIV-cases. Very recently, thymididne monophosphate kinase (dTMKase) has been suggested as a validated target to develop new anti-TB agents, particularly for the treatment of MDR TB and TB in HIV infected patients.

- *Cell Wall Macromolecules Biosynthesis*

  Based upon most recent developments in the ultra structure and biochemistry of *M. tuberculosis*, its cell envelope, consisting of three structural components, the plasma membrane, the cell wall, and the capsule, has been identified as the most important target to develop new drugs. Plasma membrane appears to be a typical bacterial membrane contributing very little towards the pathological processes [16].

- *Mycolic Acids and Other Lipids*

  The cell envelope of *M. tuberculosis* is based on unusual lipid molecules, ranging from inert waxes to biologically-active glycolipids. The mycolic acids are high-molecular weight (70 – 90 carbon), 3-hydroxy fatty acids covalently bound to arabinogalactan, as the dominant part of the cell envelope. An initial key mycolic acid biosynthetic step is a *delta*-5 desaturation of tetracosanoate, followed by elongation. It was shown that the cyclopropene analogue of *Z*-tetracos-5-enoate is a specific inhibitor of mycolic acid biosynthesis, establishing the principal that it was possible to target key lipid biosynthetic targets. Current studies are aimed at the detailed elucidation of the role of the antimycobacterial agent isoxyl in this key step. Isoniazid (isonicotinic acid hydrazide) and thiolactomycin are

involved in the elongation cycle of mycolic acids and the detailed mechanisms of action of these drugs are being studied. The translocation of mycolic acids into the cell walls of mycobacteria is being studied by designing inhibitors and substrates of the key "antigen-85" tranferase [16, 18].

- *Isocitrate Lyase as a Target for Combating Latent Infection*

  An enzyme isocitrate lyase (absent in mammals) responsible for the conversion of isocitrate to glyoxylate is a very hot molecule and is considered to be a promising target for new drug development for TB. It has very attractive feature of being bacterium-specific [16].

2.2.3.5.1. Current therapy

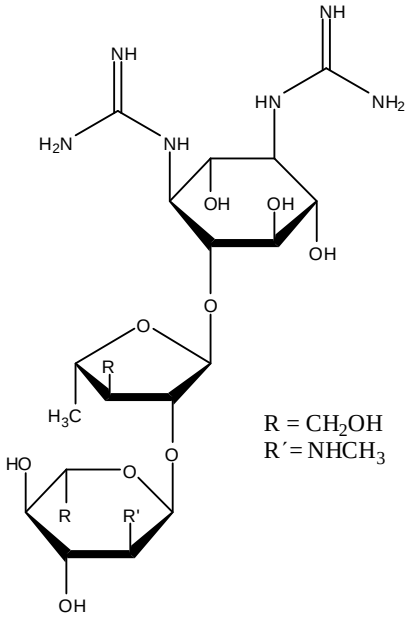
Drugs used to treat TB include broad spectrum and narrow spectrum agents, and different drug combinations are used in different types of TB discussed briefly below. The aims of the current chemotherapy are:

- conversion of the sputum cultures to negative in shortest time
- prevention of emergence of drug resistance
- asurance of a complete cure without relapse [16].

The DOT (**D**irectly **O**bserved **T**herapy) strategy is actively promoted by the World Health Organization (WHO) for TB patients in an effort to control the global emergency of TB. DOT means that a supervisor watches the client swallowing the medication for all doses over the course of treatment. This ensures that a TB client takes the correct drugs, the correct dose, and at the correct times. DOT may happen on an inpatient or outpatient basis The DOT supervisor may be a health worker or a trained and supervised community member. There must be a clearly defined line of accountability between the TB control staff and the person administering DOT. It is important to ensure confidentiality and that DOT is acceptable to the patient [19].

The first drug shown to be effective was **streptomycin**, in the late 1940´s. Streptomycin is an aminoglycoside antibiotic isolated from S*treptomyces griseus.* It consists of three components, streptidine streptose, and *N*-methyl-L-glucosamine. Because of its poor absorbance from the gastrointestinal tract, it is

administered intramuscularly and very occasionally by interthecal route. It penetrates the inner membrane of *M. tuberculosis* and binds to the 30S subunit of the ribosome. Because of many toxic manifestations on the peripheral and central nervous system at higher doses, and hypersensitivity reactions, it is not a drug of popular choice. In addition, it was soon found that an initial response was often followed by treatment failure, and that drug resistance developed in a high proportion of patients within a few months [16].



streptomycin

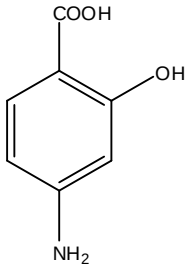
A further advance occurred with the addition of ***p*-aminosalicylic acid** (PAS), which markedly reduced the development of resistance to streptomycin. For many years PAS was considered a first line drug for the chemotherapy of tuberculosis and was generally included in combination regimes. However, the introduction of more effective and generally better tolerated agents have relegated to an alternative drug status [20].

The chemotherapy era, with consistent achievement of high cure rates, really began in 1952 with the discovery of **isoniazid** and its use in combination regimens. Isoniazid, a hydrazide of isonicotinic acid, has been one of the most effective and most commonly used antituberculous agents. It has potent bactericidal activity against all populations of organisms in patient with

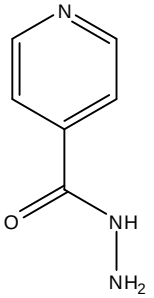
tuberculosis. It is very inexpensive and relatively safe. Isoniazid continues to be 1 of 2 drugs, along with rifampicin (rifampin), that are central to treatment of tuberculosis. It is the only drug with a well established role in chemoprophylaxis. Inhibition of mycolic acid biosynthesis may be a primary action. Isoniazid may prevent elongation of the "very-long-chain fatty acid precursors" of mycolic acid. Isoniazid also inhibits the first step that is specific for mycolic acid synthesis, a desaturase enzyme [16, 20].

While searching for new isoniazide analogs, the antimycobacterial activity of **pyrazinamide** was revealed. No other changes on its molecule lead to improving of the antituberculous activity. Pyrazinamide has potent sterilizing activity in the acid pH of the intracellular environment and extracellulary in highly inflamed tissue. It is well absorbed after peroral administration and hydrolyzed by liver to pyrazinoic acid, an active metabolite. Its addition for the first 2 months to regimens containing isoniazid and rifampicin appears to be essential to achieving very high rates of relapse-free cure.

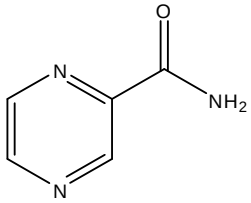
The main adverse effect is hepatotoxicity. It is relatively costly in comparison with other older antituberculous agents [16, 20].



***p*-aminosalicylic acid**



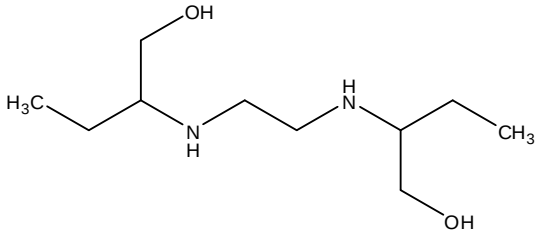
isoniazid



pyrazinamide

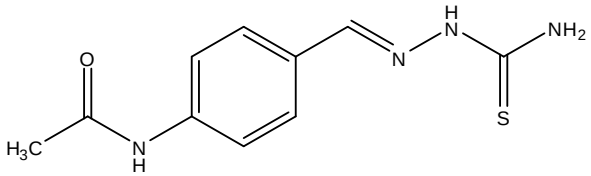
Ethambutol was introduced in 1962 [17]. It is a synthetic agent, active only against dividing mycobacteria. It is the most active member of the group of ethylendiamine derivatives, but also compounds with 2-aminopropanol, 2-aminoisopentanol or 3-amino-2-butanol in their molecules are of some activity. Ethambutol posseses optical activity with only *R*-(+) isomer being of a high antituberculous activity. Mechanism of action includes interference with the synthesis and stabilization of RNA. Its main use is as a companion drug, to

prevent the development of resistance to more potent agents. It is increasingly replacing thioacetazone in countries with high HIV prevalence. The main toxicity of ethambutol is visual loss, which is usually reversible [16].

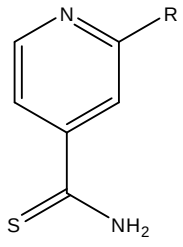


ethambutol

Another class of antituberculous drugs includes -CSNH-moiety containing compounds. To this group belong thiosemicarbazones (=N-NH-CS-NH_2) and thioamides (-CS-NH_2). Thiosemicarbazones of aliphatic aldehydes or ketones are highly toxic compounds, therefore only some thiosemicarbazones of aromatic or heterocyclic aldehydes found their place in therapy. Until today, only **thioacetazone (**thiosemicarbazone of 4-acetamidobenzaldehyde) is in practice. It is a drug of modest efficacy, but with a role in preventing development of resistance to a more potent antituberculous drug. The advantage of this drug is a low cost, so it is widely used particularly in countries with very limited resources. Among thioamides two therapeutically used drugs – **ethionamide** and **prothionamide –** belong**.** They are synthetic derivatives, thioamides of 2-ethyl and 2-propylisonicotinic acids. They are considered to be interchangeable. Their mechanism of action is similar to that of isoniazid, inhibition of mycolic acid biosynthesis [16, 20].



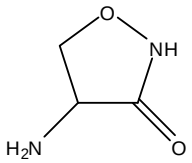
thioacetazone



ethionamide: R = C_2H_5

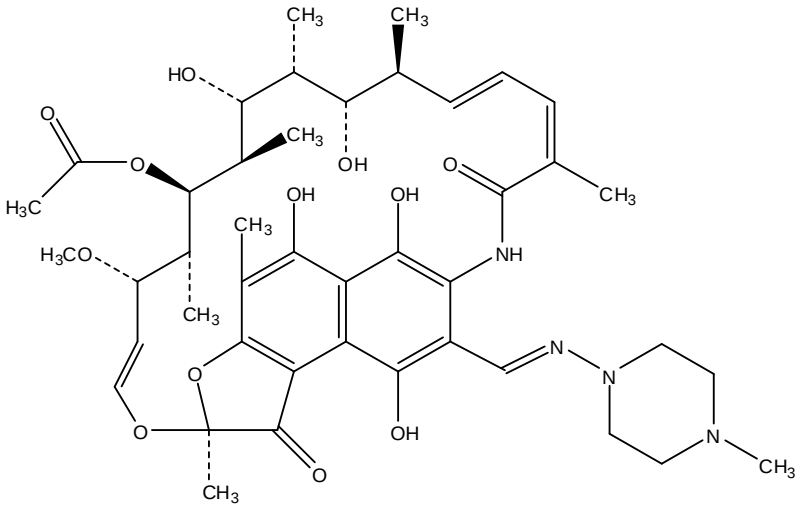
prothionamide: R = C_3H_7

Besides streptomycine, other antibiotics have been found effective against mycobateria. **Cycloserine** is used sometimes as a second-line drug for treatment of tuberculosis, usually against organisms resistant to other drugs [16, 20].

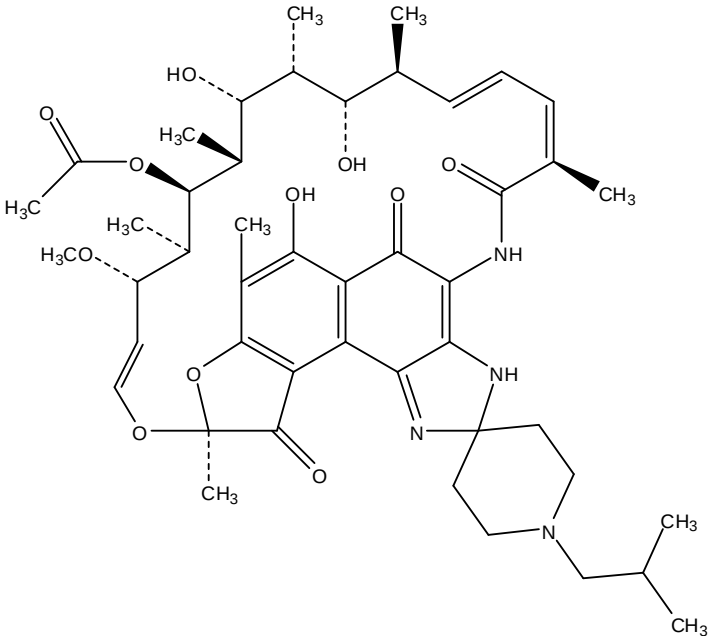


cycloserine

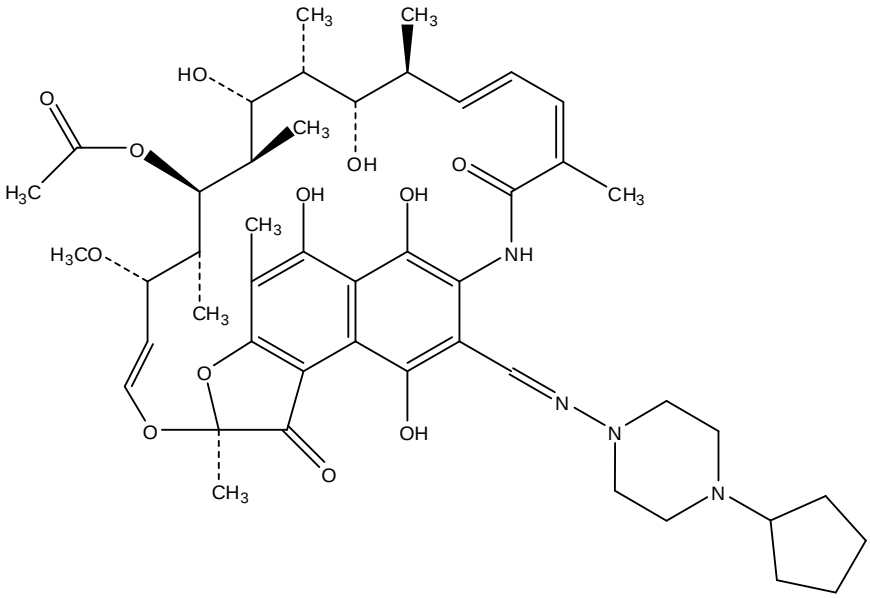
Nowadays**, rifampicin** is an essential element of modern short course treatment regimens. Because of its activity against non-mycobacterial organisms, it has a high potential of misuse in the community, which increase prevalence of rifampicin-resistant tuberculosis. Rifamycins bind to the β-subunit of bacterial DNA-dependent RNA polymerases to prevent chain initiation. Like isoniazid, rifampicin can cause hepatotoxicity, but it is usually well tolerated. The success of rifampicin in the treatment of tuberculosis stimulated researchers to manipulate the rifamycin molecule to produce other, and possibly better, antibiotics. There are two newer ansamycins with antimycobacterial activity. **Rifabutin** is a spiropiperidyl rifamycin derivative that is more active against *M. avium* complex, with a lower MIC and lower natural resistance rate. **Rifapentine** is generally more active against *M. tuberculosis* than rifampicin although strains resistant to rifampicin are usually cross-resistant to rifapentine [21, 22].



rifampicin

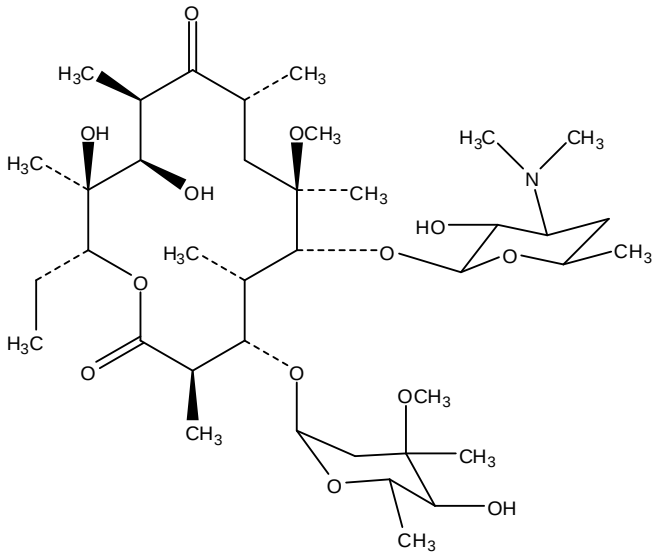


rifabutin

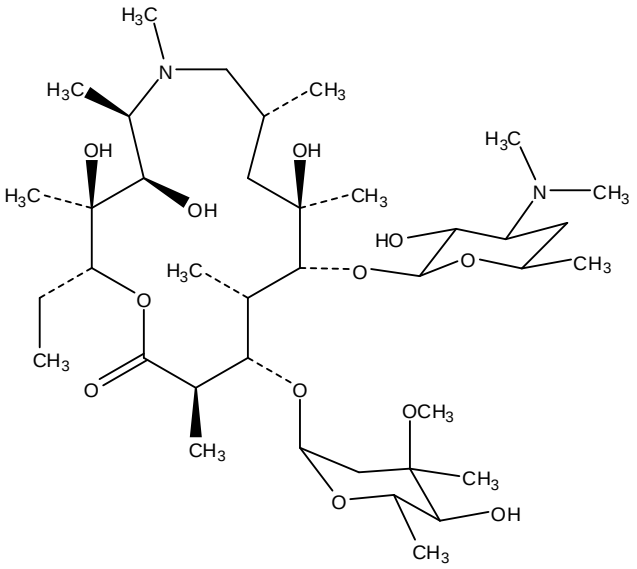


rifapentine

New semisynthetic macrolides, e.g. **clarithromycin** and **azithromycin**, also exert antimycobacterial activity. These antibiotics bind to high-affinity site in the peptidyl t-RNA binding region of the bacterial 50S ribosome subunit, causing dissociation of peptidyl-tRNA from ribosomes and inhibition of bacterial protein synthesis. They appear to be promising in the treatment of mycobacterial infections, particularly those caused by non-TB species [16].

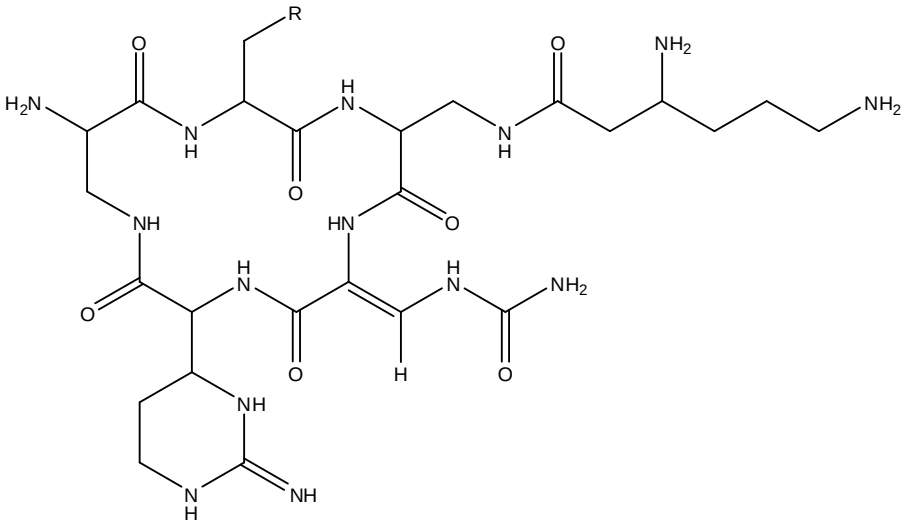


clarithromycin



azithromycin

Capreomycin is strongly basic peptide isolated from *S. capreolus*. Four capreomycin designated 1A, 1B, 2A, 2B have been isolated. The clinical agent contains primarly 1A and 1B. It was released in the early 1970´s exclusively as a tuberculostatic drug. It is a second line agent employed in combination with other antituberculous drugs [20].

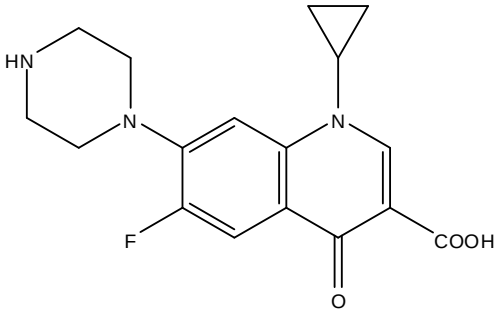


capreomycin 1A: R = OH

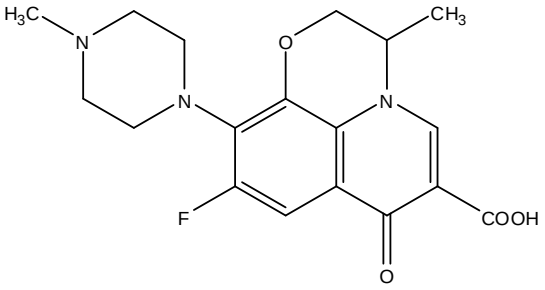
capreomycin 1B: R = H

.

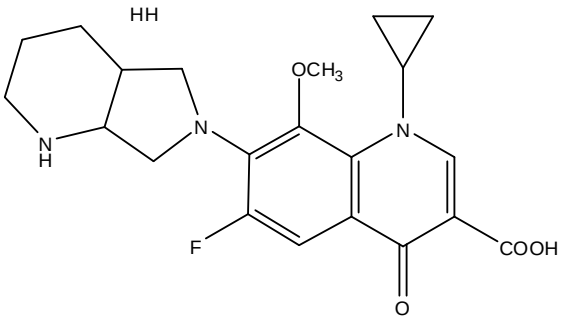
Quinolinones exhibit bactericidal effects that involve an interaction of the drug with DNA-gyrase and DNA-topoisomerase IV. The new members of this group with a heterocyclic ring in position 7 and fluoro-substitution in position 6 and/or 8 are very active against mycobacteria with low MIC values. **Ciprofloxacin**, **ofloxacin** and **sparfloxacin** and **moxifloxacin** have been used as part of multi drug regimes to cure patients infected with *M. tuberculosis* and *M. avium* [16, 23].



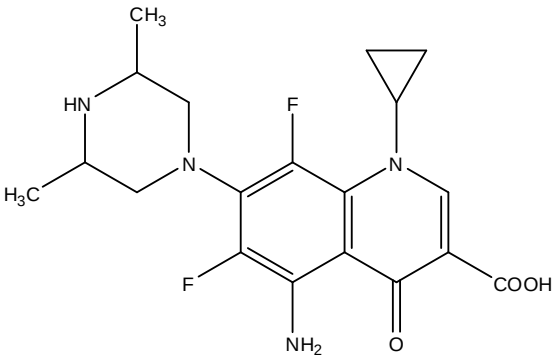
ciprofloxacin



ofloxacin



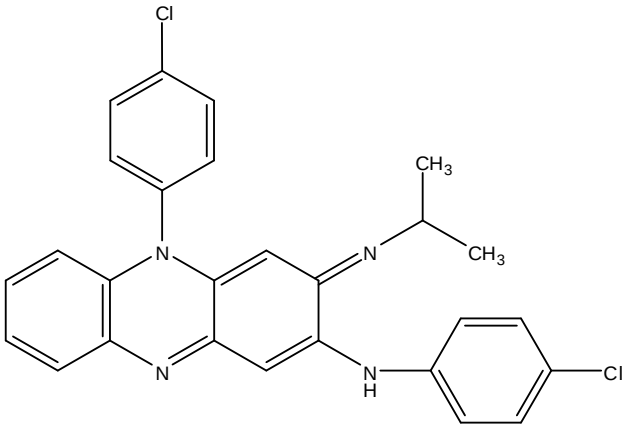
moxifloxacin



sparfloxacin

Clofazimine is a phenazine dye used for the treatment of leprosy. It has been shown to be active in vitro against *M. tuberculosis* the drug is only indicated as an addition to two or more reliable agents in regimens for multidrug-resistant disease. Liposomized, encapsulated clofazimine has been reported to be more effective then clofazimine alone in experimental studied in mice [16, 20, 24].

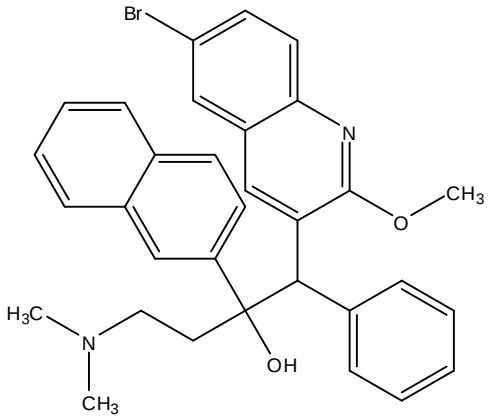
Some clofazimine analogues are currently under study as new potential antituberculous drugs [16].



clofazimine

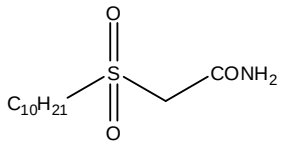
2.2.3.5.2. New structural classes of anti-tuberculosis agents

The diarylquinoline R207910 (also known as **TMC207**) is a promising member of a new class of anti-mycobacterial agents. TMC207 was demonstrated to have high bactericidal activity when combined with first- or second-line antituberculous drugs [25]. The target and mechanism of action is different from those of other anti-TB agents implying low probability of cross-resistance with existing TB drugs. TMC207 is postulated to inhibit the proton pump of the *M. tuberculosis* adenosine triphosphate (ATP) synthase that is the main source of fuel for *M. tuberculosis* [26].



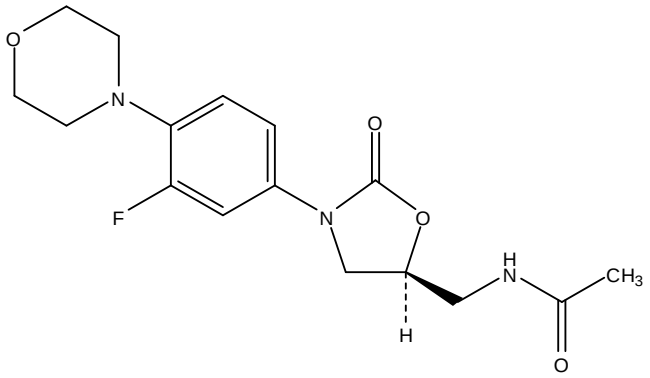
TMC207

Mycobacteria produce a wide array of complex fatty acids, such as mycocerosic acid and mycolic acids, not found in mammalian cells. While the synthesis of fatty cids occurs in all living organisms, mycobacteria posses accessory fatty acid synthase (FAS) enzymes with specialized substrate and product specificities and are considered as attractive target for TB drug development. β-Ketoacyl synthase (KAS) is responsible for the fatty acids synthesis, and catalyzes the most mechanistically complex of the sequences of FAS reactions. A new class of compounds designed to inhibit the β -ketoacyl synthase reaction of fatty acid synthesis have been developed. Acetamides containing alkyl sulfonyl substituents were most effective of all tested compounds [27].



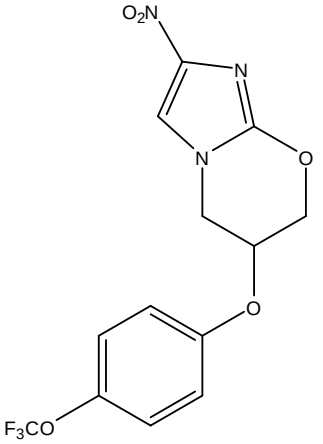
2-(decylsulfonyl)acetamide

Oxazolidinones are a new class of antibacterial protein synthesis inhibitors with inhibition uniquely in the initiation phase of protein synthesis. **Linezolid** is the first oxazolidinone to be developed and approved to treat single or multiple resistant grampositive bacterial infections. In vitro studies have shown good activity of linezolid against *M. tuberculosis*, including MDR strains, but prolonged use of linezolid in the treatment of MDR TB frequently caused significant toxicity including anemia and peripheral neuropathy. Although oxazolidinones have promising potential for the treatment of MDR TB, more extensive clinical studies are needed to evaluate their efficacy and toxicity, as well aw the degree of resistance development by mycobacteria [28, 29, 30].



linezolid

A particularly promising candidate for TB treatment is nitroimidazopyran **PA824**, derived from 5-nitroimidazole. PA824 is a prodrug that requires activation by a bacterial F420-dependent glucose-6-phosphate dehydrogenase and nitroreductase to activate components that then inhibit bacterial mycolic acid and protein synthesis. Additional studies are needed to evaluate PA824 in terms of its ability to shorten TB therapy in combination with other drugs without significant relapse, drug resistance or toxixity. PA824 is currently being evaluated in clinical trials by the Global Aliance for TB Drug Development [28].



PA824

Other recently discovered chemical structures with the antituberculous potential include thiolactomycin, trypanthrin [16], 5-arylidene-2-thiohydantoins, benzoxazoles, benzothiazoles, benzoic acid hydrazones, chalcones, coumarins, purines [27], to name at least some. Carbohydrates and various marine

compounds have also been tested for antituberculous activity [16, 27]. Drugs used for treatment of other diseases, such as antifungal azoles and phenothiazines have good anti-tuberculosis activity and could be candidates for further evaluation for the treatment of TB [28].

2.3. Mycoses and possibilities to manage them

2.3.1. Mycoses

Most fungal infections (mycoses) involve superficial invasion of the skin or the mucous membranes of body orifices. These infections are divided into two etiologic groups:

- the dermatophytoses (tinea infections), which are contagious superficial epidermal infections caused by various *Epidermophyton*, *Microsporum* and *Trichophyton* species
- mycoses caused by pathogenic saprophytic yeasts, which are contagious and usually superficial infections involving the skin and mucous membranes.

Some species of saprophytic fungi (e.g. *Aspergilllus, Candida* and *Cryptococcus* species) under certain conditions are capable of invading deeper body cavities and causing systemic mycoses. Such infections may become serious and occasionally life-threatening, and they are frequently difficult to treat [20].

2.3.2. Fungi

All living things can be classified into one of five fundamental kingdoms of life, and the term fungus refers generically to all members of the kingdom Fungi. There are more than a million species of fungi, but only about 400 cause diseases relevant to man, animals, or plants. Fungi, including those pathogenic to humans and animals, are eukaryotic microorganisms. Classically, there are two broad groups of fungi: yeasts and moulds. Yeasts reproduce principally by budding (or fission) and moulds reproduce principally by elongation at the tips of filamentous growth forms. All fungal genera of medical importance can be placed into one of five sexual groups, even if sexual reproduction has not been observed. These groups correspond to the five phyla of the kingdom Fungi and are:

- the ascomycetes (Phylum Ascomycota)
- basidiomycetes (Phylum Basidiomycota)
- zygomycetes (Phylum Zygomycota)
- chytridiomycetes or chytrids (Phylum Chytridiomycota)

- Fungi Imperfecti

The first four groups are the true sexual groups (or phyla) because they are characterized by the production of sexual spores known as ascospores, basidiospores, zygospores, and oospores, respectively. Fungi that infect people come from all the groups except the chytridiomycetes. The chytrids are important as causes of diseases in agriculture and in lower cold-blooded animals. The asexual genera of the Fungi Imperfecti (also sometimes known as the phylum Deuteromycota) are different from the known sexual genera in that a sexual form is not known. Some genera are classified as *dematiaceous*, meaning that melanin in the cell walls of its conidia, hyphae, or both results in a darkly colored fungus [31].

2.3.3. Management of mycoses

2.3.3.1 Chemotherapy

So far, chemotherapy has been the only possibility to manage mycoses. The selection of a suitable drug is dependent on the type of the disease. Superficial mycoses have been most often treated the non-specific antifungal agents derived from antiseptics. Fatty acids in perspiration have been found to be fungistatic, and this discovery has led to the introduction of fatty acids in therapy. The use of copper and zinc salts provides the added antifungal activity of the metal ion. Aromatic acids, especially salicylic acid, which also has a useful keratolytic action, and its derivatives are employed for their topical fungistatic effect. A variety of alkylated or halogenated phenols and their derivatives are useful for the treatment of local fungal infections. The antifungal activity of the aforementioned compounds is largely confined to local dermatophytic infections [20].

The treatment of systemic mycoses has acquired increased importance in recent years as a result of the increased incidence of opportunistic yeast infections in immunocompromised patients. The widespread use of immunosuppressants following organ transplant operations and the AIDS epidemic have been major contributions to this situation [20, 31]. Clinical needs for novel antifungal agents have altered steadily with the rise and fall of AIDS-related mycoses, and the

change in spectrum of fatal disseminated fungal infections that has accompanied changes in therapeutic immunosuppressive therapies [32].

The targets of all antifungal agents used in the clinic (and of some agents that entered or approached clinical development but have not been marketed) are summarized in Figure 2. This figure shows that, contrary to occasional pessimistic comments, a considerable diversity of antifungal targets already exists. Nevertheless, in terms of numbers of classes of agents that can be used to treat life-threatening mycoses, the targets are heavily focused, directly or indirectly, on the cell envelope (wall and plasma membrane), and particularly on the fungal membrane sterol, ergosterol, and its biosynthesis. Targets elsewhere in the cell would therefore be a welcome innovation for systemically bioavailable antifungal agents. The search for new molecular targets for antifungals has generated considerable research using modern genomic approaches, so far without generating new agents for clinical use [32].

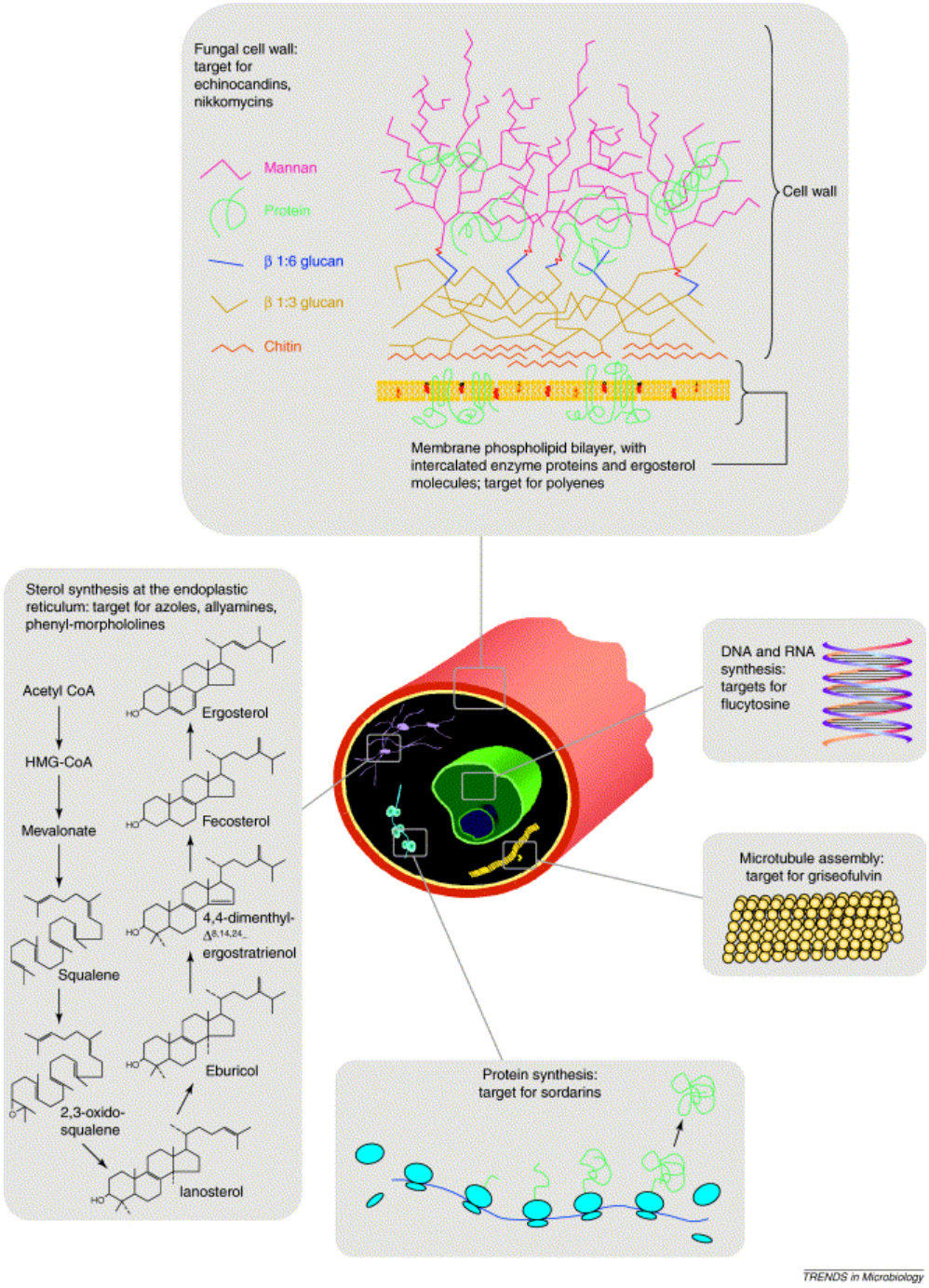


Figure 2. Target areas for antifungal agents [32].

2.3.3.1. Current therapy

Examples of non specific antifungal agents used for local treatment are given in Figure 3.

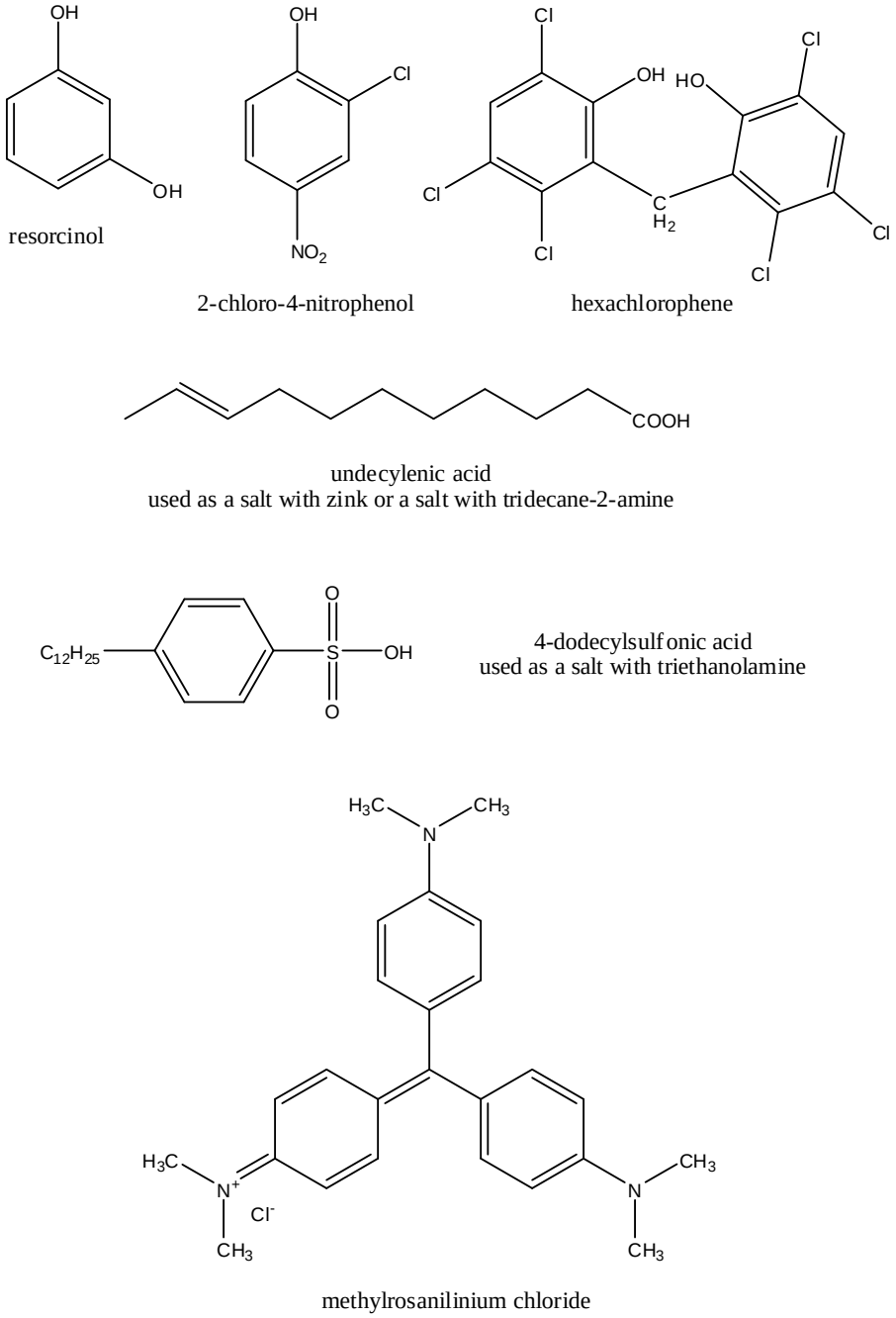
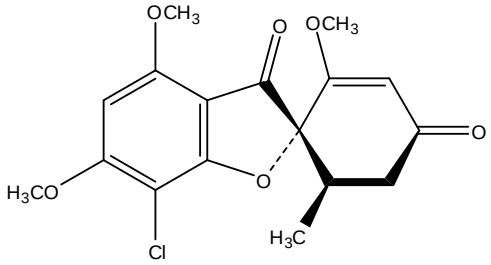


Figure 3. Examples of non specific antifungal agents used for local treatment.

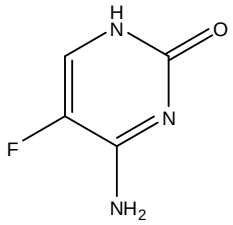
Deep dermatophytic infections, resistant to topical therapy, maybe treated systematically. The earliest inhibitory agent specified to fungal species was

griseofulvin. The precise mechanism of this compound is still unknown, but the favoured explanation is that it interferes with microtubule assembly. The selective toxicity of griseofulvin for fungi is only moderate (liver toxicity is recognized as an occasional hazard), and its spectrum of action is mainly restricted to the dermatophyte fungi – causes of ringworm and athletes foot.



griseofulvin

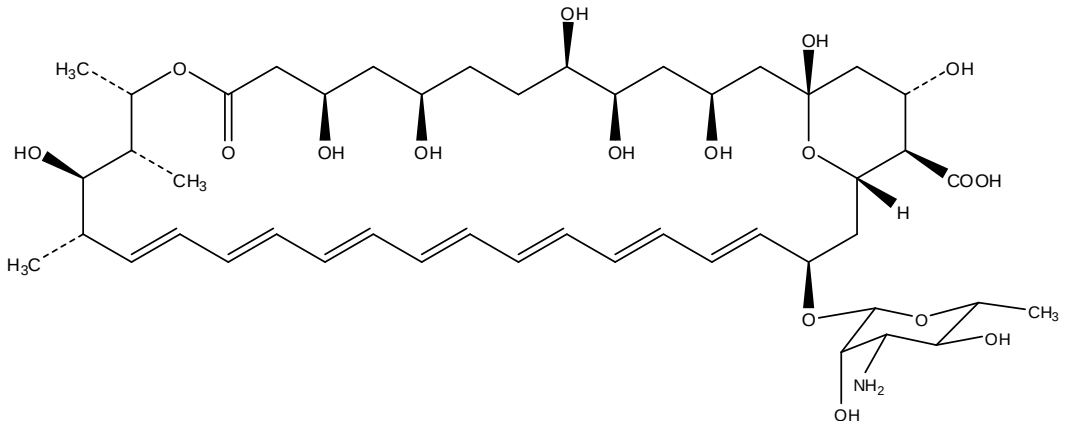
Flucytosine (5-fluorocytosine, 5-FC) is an antimetabolite type of antifungal drug. It is chemically 4-amino-5-fluoropyrimidin-2-one It is activated by deamination within the fungal cells to 5-fluorouracil. It inhibits fungal protein synthesis by replacing uracil in fungal RNA. Flucytosine also inhibits thymidylate synthetase via 5-fluorodeoxyuridine monophosphate and thus interferes with fungal DNA synthesis [31, 32].



flucytosine

Polyene antifungal agents include amphotericin B, natamycin and nystatin. **Amphotericin B** was isolated by Gold et al. from *Streptomyces nodosus* in 1955. It is an amphoteric compound. It composed of a hydrophilic polyhydroxyl chain along one side and a lipophilic polyene hydrocarbon chain on the other. It is poorly soluble in water. Amphotericin B is now available in four formulations. The classic amphotericin B deoxycholate formulation has been available since

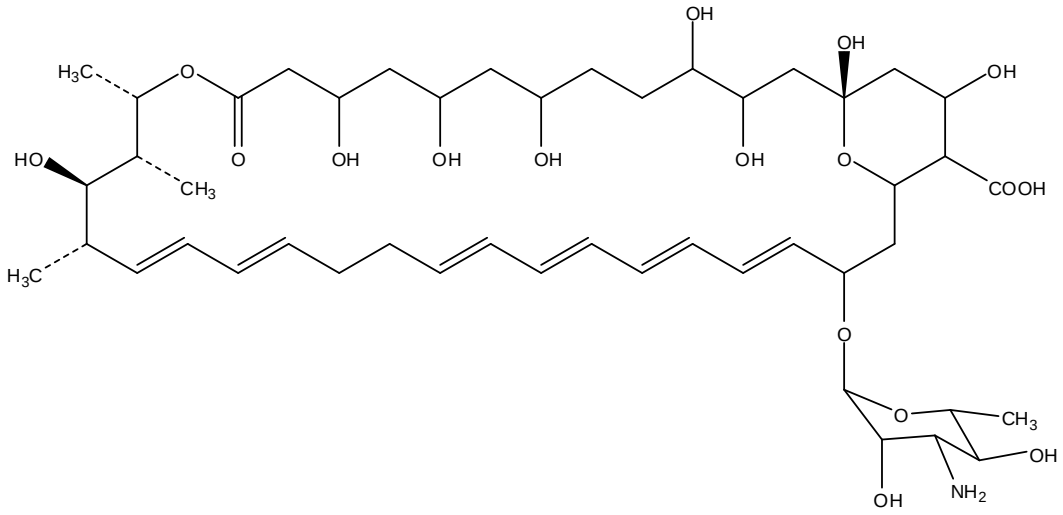
1960 and is a colloidal suspension of amphotericin B. A bile salt, deoxycholate, is used as the solubilizing agent. This preparation has a number of toxicities that are partially ameliorated when a lipid carrier is used. Nonconventional formulations of amphotericin B for injection include liposomal amphotericin B, a colloidal dispersion of an amphotericin B and sodium cholesteryl sulfate complex, and a phospholipid complex. Amphotericin B binds to sterols, preferentially ergosterol. This binding disrupts osmotic integrity of the fungal membrane, resulting in leakage of intracellular potassium, magnesium, sugars, and metabolites and then cellular death [31, 32].



amphotericin B

Natamycin is a polyene antifungal antibiotic produced by the growth of *Streptomyces natalensis*. It is used for the local treatment of oral candidiasis (lozenges) and fungal keratitis (ophthalmic suspension and ointment). Tablets have been given orally for the treatment of intestinal candidiasis. Natamycin has also been used topically for fungal skin infections and for candidal and trichomonal infections of the vagina [33].

Nystatin is a polyene antifungal antibiotic used for the prophylaxis and treatment of candidiasis of the skin and mucous membranes. It interferes with the permeability of the cell membrane of sensitive fungi by binding to sterols, chiefly ergosterol. Its main action is against *Candida* spp. It has been used with anti-bacterials in various regimens to suppress the overgrowth of gastrointestinal flora and as part of selective decontamination regimens [33].

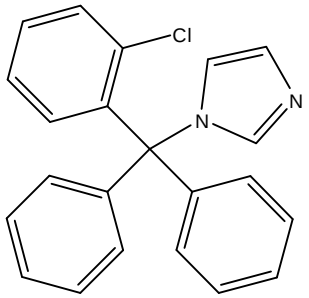


nystatin

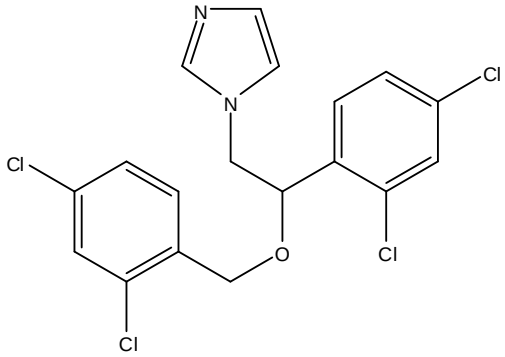
Imidazoles and triazoles are the largest class of antifungal agents in clinical use. Their main effect is to inhibit 14α-demethylation of lanosterol in the ergosterol biosynthetic pathway, but in some fungal species, they can also inhibit the subsequent 22-desaturase step. With ergosterol depleted and replaced with unusual sterols, the normal permeability and fluidity of the fungal membrane is altered, with secondary consequences for membrane-bound enzymes, such as those involved in cell wall synthesis.

The principal molecular target of azole antifungals is a cytochrome P450 – Erg11p or cyp51p, according to different gene based nomenclatures, which catalyses the oxidative removal of the 14α-methyl group of lanosterol and eburicol in fungi by a typical P450 mono-oxygenase activity [32].

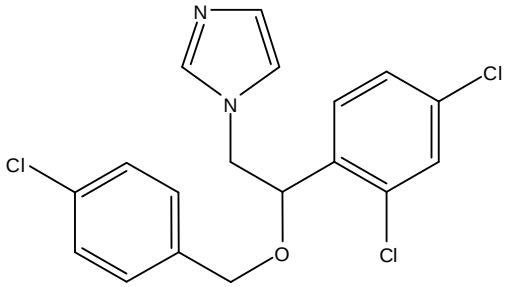
Although the first report of antifungal activity of an azole compound, benzimidazole, was already described in 1944, it was not until after the introduction of topical chlormidazole in 1958 that researchers became interested in the antifungal activity of azole compounds. In the late 1960´s, three new topical compounds were introduced – **clotrimazole**, **miconazole** and **econazole**.



clotrimazole



miconazole



econazole

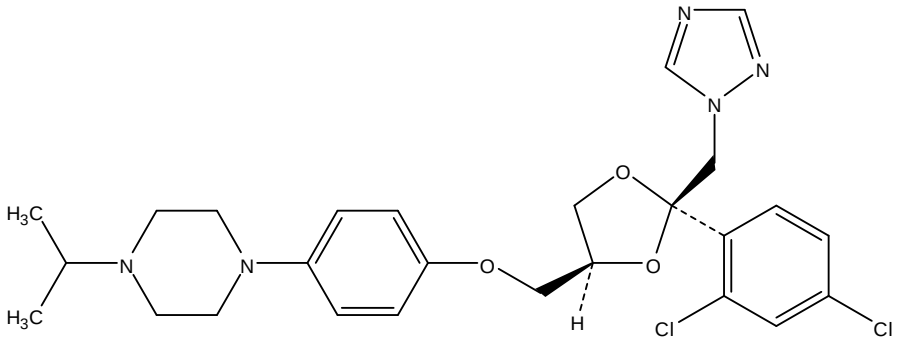
Miconazole, a phenethyl imidazole synthesized in 1969, was the first azole available for parenteral administration (although not before 1978). The drug has a limited spectrum of activity including dermatophytes, *Candida* spp., dimorphic fungi. The agent has proven to be an effective topical antifungal agent, but toxicity associated with the vehicle used for intravenous administration has limited its parenteral use. Miconazole has recently been withdrawn from the market.

In 1981, the Food and Drug Administration (FDA) approved the systemic use of **ketoconazole**. For almost a decade it would be regarded as the standard and was the only available oral agent for the treatment of systemic fungal infections. Until the introduction of the triazoles, ketoconazole was indicated as the drug of choice in chronic mucocutaneous candidiasis and as an effective alternative to amphotericin B in less severe (non-immunocompromised) cases of blastomycosis, histoplasmosis, coccidioidomycosis, and paracoccidioidomycosis. Ketoconazole has not been adequately evaluated in deep-seated candida infections or cryptococcosis and was ineffective in aspergillosis and mucormycosis. Over

the years, a number of clinically relevant shortcomings of this compound became evident. Ketoconazole was largely fungistatic and proved to be less effective in immunocompromised patients. Thus, the poor response rates and frequent recurrences of major fungal infections, as well as the toxicity associated with ketoconazole therapy, led to the search for a second chemical group of azole derivatives, namely the triazoles [34].

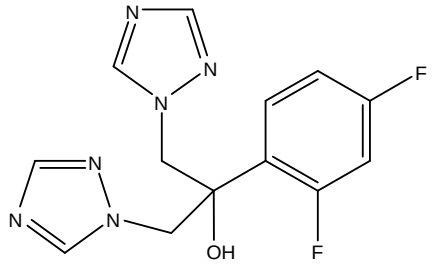
In general, the triazoles demonstrate a broader spectrum of antifungal activity and reduced toxicity when compared with the imidazole antifungals.

Terconazole, the first triazole marketed for human use, was active in the topical treatment of vaginal candidiasis and dermatomycoses [33, 34].



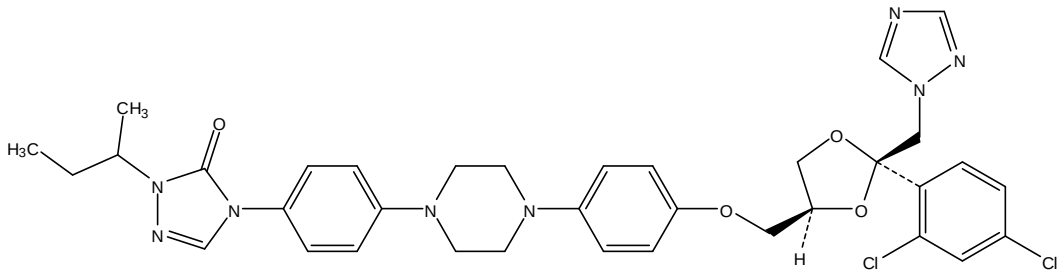
terconazole

Fluconazole is a widely used bis-triazole antifungal agent with systemic action. As with other triazoles, it has five-membered ring structures containing three nitrogen atoms. It is active against *Blastomyces dermatitidis*, *Candida* spp., *Coccidioides immitis*, *Cryptococcus neoformans*, *Epidermophyton* spp., *Histoplasma capsulatum*, *Microsporum* spp., and *Trichophyton* spp. Resistance has developed in some *Candida* spp. following long-term prophylaxis with fluconazole, and cross-resistance with other azoles has been reported [31, 32, 33].



fluconazole

Itraconazole is also a triazole antifungal agent. It has a slightly wider spectrum of activity than ketoconazole. It is active against *Aspergillus* spp., *Blastomyces dermatitidis, Candida* spp., *Coccidioides immitis, Cryptococcus neoformans, Epidermophyton* spp., *Histoplasma capsulatum*, *Malassezia furfur*, *Microsporum* spp., *Paracoccidioides brasiliensis*, *Sporothrix schenckii*, and *Trichophyton* spp. It also has some antiprotozoal activity against *Leishmania* spp. Acquired resistance to itraconazole is rare but ketoconazole-resistant strains of *C. albicans* have been found to be cross resistant to itraconazole [31, 32, 33].

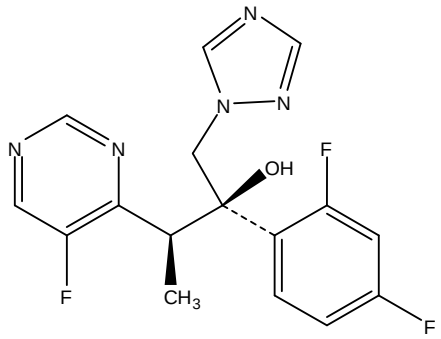


itraconazole

Of the three newest triazole antifungals, voriconazole and ravuconazole are structurally related to fluconazole, whereas posaconazole bears a close resemblance to itraconazole, but with dioxolene ring altered to a tetrahydrofuran. The structural differences might seem small, but they dictate antifungal potency and spectrum, bioavailibility and drug interaction and toxic potential [31, 32].

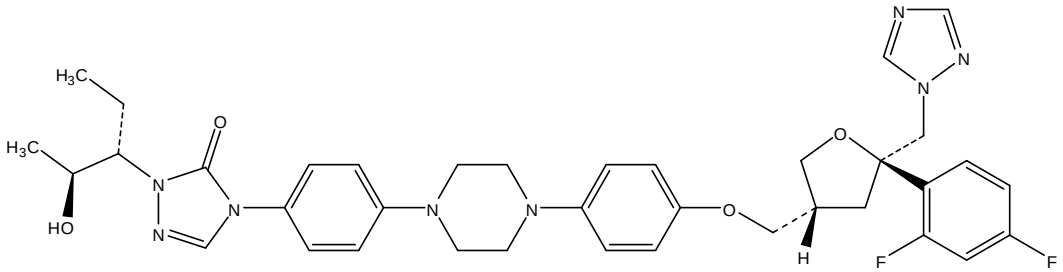
Voriconazole, which is the first approved and so far the most fully characterised of the three new triazoles. Voriconazole has a broad spectrum of activity against all *Candida* species, including fluconazole-resistant strains, as

well as *Aspergillus* spp., *Scedosporium* spp., and *Fusarium* spp. and is even fungicidal against some isolates of filamentous species [31, 32, 33, 35, 36].



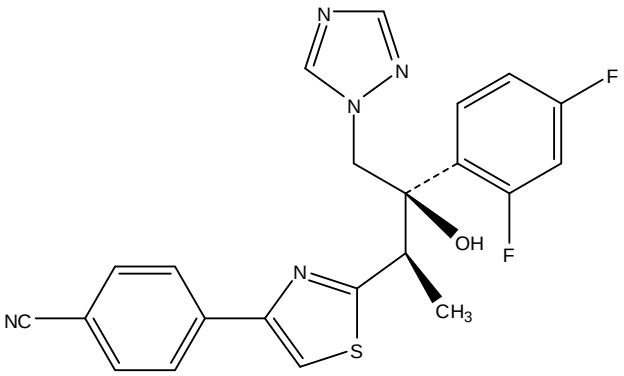
voriconazole

Posaconazole also acts against a broad spectrum of susceptible fungi, (*Candida* spp., *Aspergillus* spp., *Coccidioides immitis, Fonsecaea pedrosoi*, and some species of Fusarium and zygomycetes). It used in the treatment of invasive aspergillosis, chromoblastomycosis, coccidioidomycosis, fusariosis, or mycetoma infections in patients who are resistant to or intolerant of other antifungals [31, 32, 33].



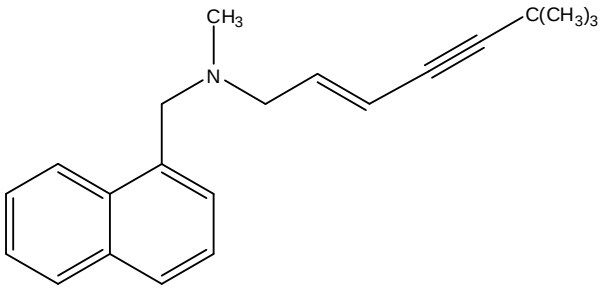
posaconazole

Ravuconazole is a triazole antifungal said to possess a broader spectrum of activity than fluconazole or itraconazole. It is under investigation for the treatment of systemic fungal infections [31, 32, 33].

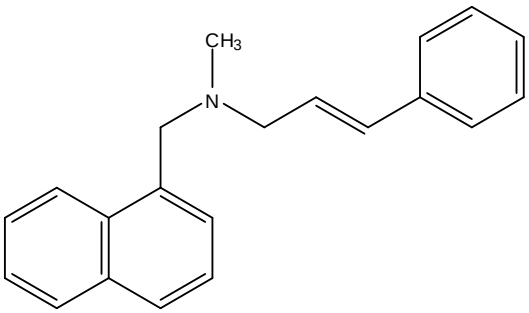


ravuconazole

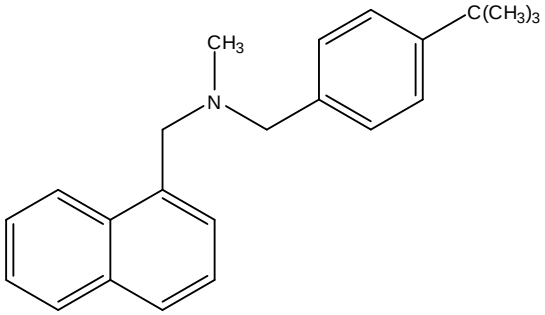
Other sterol synthesis inhibitors are allylamines. The allylamines, notably **terbinafine**, inhibit squalene epoxidase, an early step in the pathway, with fungicidal consequences in susceptible species. These include many filamentous fungi but few pathogenic yeasts. Although terbinafine is not approved for treatment of visceral mycoses, there is interest in the possibility of combining terbinafine with other ergosterol synthesis inhibitors to achieve synergistic inhibitory effects [31, 32, 33]. **Naftifine** and **butenafine** are derivatives with actions similar to those of the terbinafine. They are applied topically once or twice daily for fungal skin infections, particularly dermatophytosis [31, 33, 37].



terbinafine

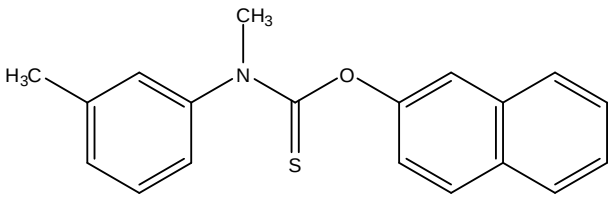


naftifine

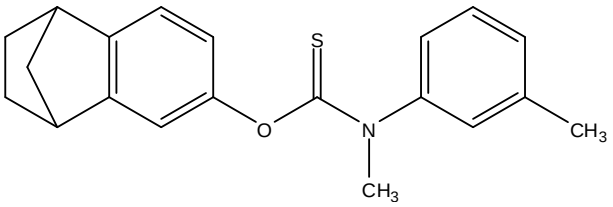


butenafine

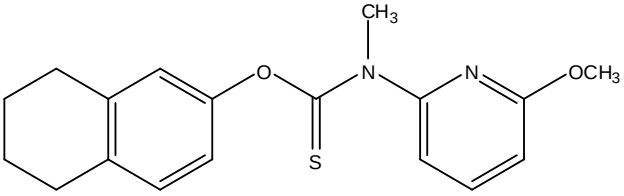
Squalene epoxidase is also inhibited by antifungal agents of thiocarbamate type. **Tolnaftate** inhibits the growth of the dermatophytes, but is not active against *Candida* spp. It used topically in the treatment or prophylaxis of superficial dermatophyte infections and of pityriasis versicolor. **Tolciclate** and **liranaftate** are newer thiocarbamate derivatives [31, 33].



tolnaftate

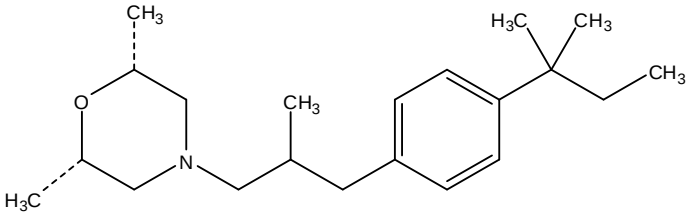


tolciclate



liranaftate

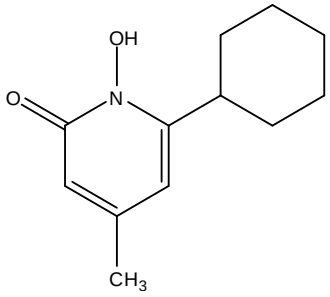
Amorolfine is a structurally unique, topically active antifungal agent, which possesses both fungistatic and fungicidal activity in vitro. Its spectrum of in vitro activity includes dermatophyte, dimorphic, some dematiaceous and filamentous fungi, and some yeasts. It interferes with ergosterol biosynthesis at two steps: the Δ14-reduction and the Δ7-8 isomerisation. As a consequence of this inhibition, the Δ14 sterol ignosterol is accumulated in the cell membrane and ergosterol is depleted. The cell wall thickness is significantly increased and chitin deposits are included inside and outside. In experimental models of systemic mycosis amorolfine shows no significant activity. This lack of systemic activity may be due to strong protein binding and/or rapid metabolism [31, 33, 38, 39].



amorolfine

Ciclopirox (used as olamine) is a hydroxypyridone antifungal that is structurally unrelated to other antifungal agents. It has a wide spectrum of antifungal activity. It inhibits most *Candida*, *Epidermophyton*, *Microsporum*, and

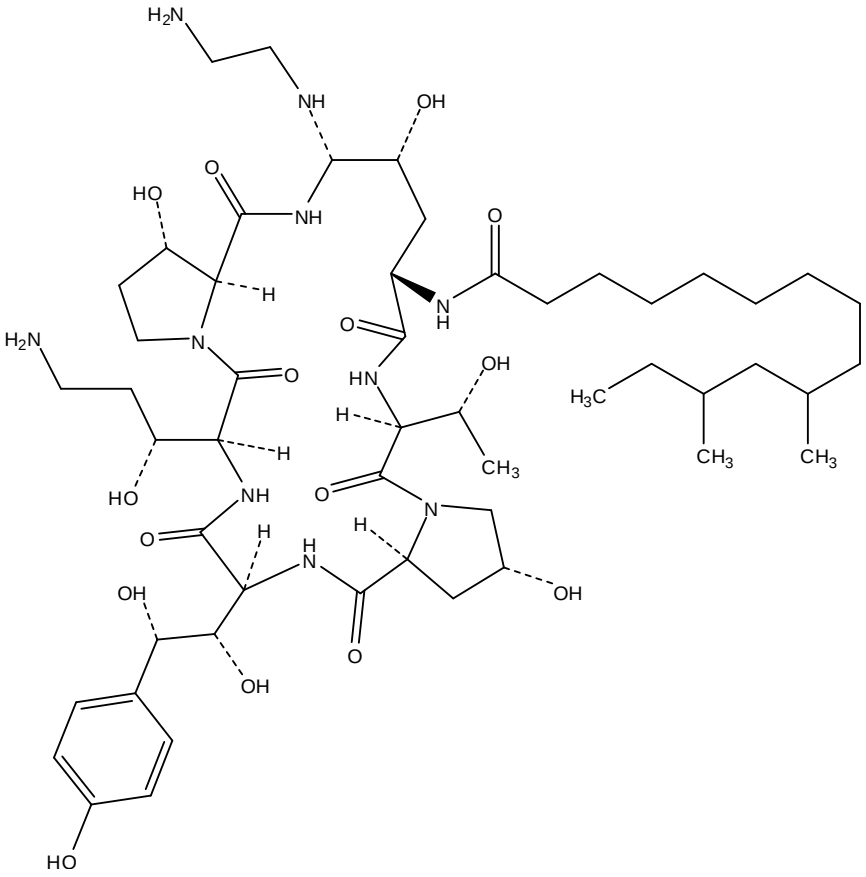
Trichophyton spp. and is also active against *Malassezia furfur*. It is applied topically in the treatment of fungal skin and nail infections [31, 33, 40, 41].



ciclopirox

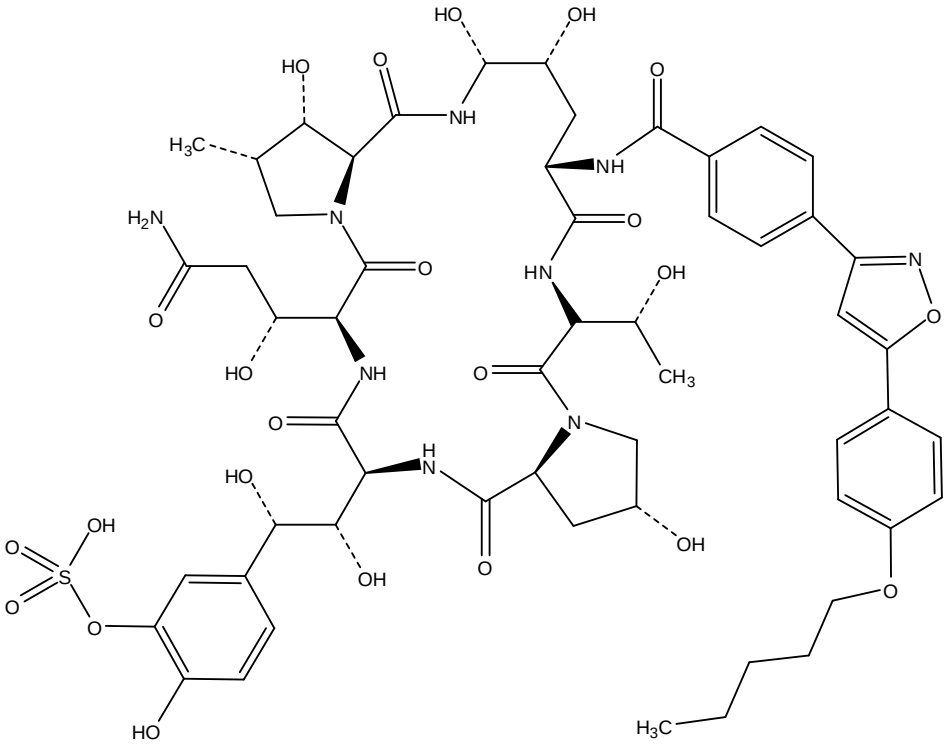
The echinocandins are fungal secondary metabolites comprising a cyclic hexapeptide core with a lipid side chain responsible for antifungal activity. Antifungal activity in the prototype, echinocandin B and aculeacin A, was discovered by random screening in the 1970´s. A modified form of echinocandin B, cilofungin, was developed to the point of phase 2 trials, but was abandoned when its formulation showed toxicity to the patients. In the late 1990´s, three echinocandine-class compounds, caspofungin, anidulafungin, and micafungin all entered clinical development. The target for the echinocandins is complex of proteins responsible for the synthesis of cell wall β-(1,3)-D-glucan polysaccharides. Echinocandins appear to demonstrate favourable activity in vitro against a variety of yeasts (including both *C. albicans* and non-albicans *Candida*) as well as selected moulds (including *Aspergillus* spp.). In general, all echninocandins demonstrate a favourable safety profile and require once-daily parenteral administration. Their metabolism is independent of hepatic cytochrome P450 enzymes, which minimizes drug interactions [32, 42, 43].

Caspofungin (approved 2001) is the first of these agents to be available and is approved for empirical antifungal therapy in febrile neutropenic patients, candidaemia and some forms of invasive candidiasis, and for management of invasive aspergillosis in patients refractory to or intolerant of other therapies. It is used as acetate [32, 33, 44].



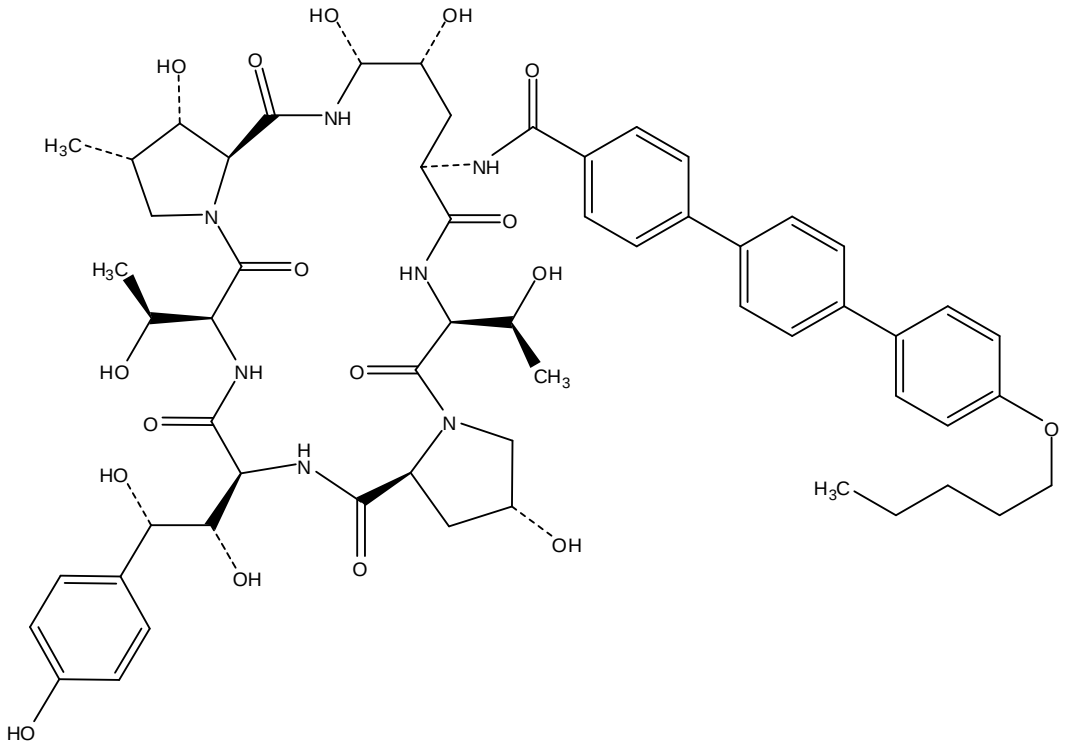
caspofungin

Micafungin was approved in 2005 for treatment of oesophageal candidiasis, and for the prophylaxis of fungal infections in haematopoietic stem cell transplant recipients. It is also in the treatment of aspergillosis. It is given as the sodium salt by intravenous infusion [32, 33].



micafungin

Anidulafungin was approved in 2006 for the treatment of candidemia, other forms of candida infections (intra-abdominal abscess and peritonitis), and esophageal candidiasis. It is a water soluble, semisynthetic, cyclic lipopeptide derivative of echinocandin B. Like all echinocandins, anidulafungin is only available as an intravenous formulation due to its large molecular weight. Its long half-life allows for once-daily dosing intervals. The efficacy of anidulafungin against specific fungal pathogenes varies. Anidulafungin compares favorably with other antifungal agents. Because of the fungistatic effect of anidulafungin against *Aspergillus*, this antifungal may prove to be beneficial when used as part of combination therapy [32, 33, 45].



anidulafungin

Aminocandin (HMR-3702, IP-960) is an investigational agent, with published experience limited to in vitro studies and animal models of infection [42].

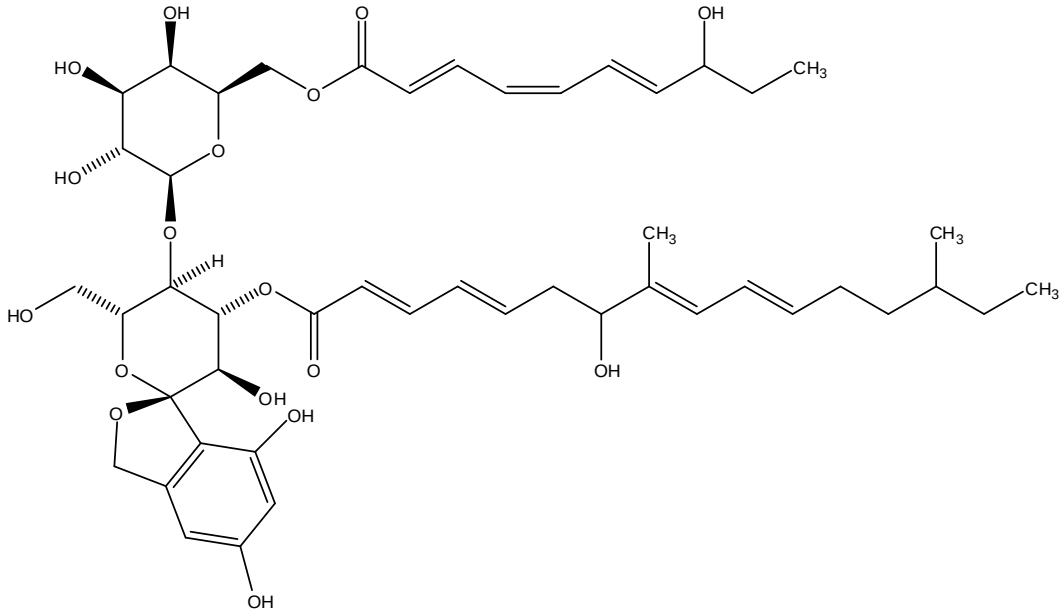
2.3.3.2. New structural classes of antimycotic agents

In recent years a new threat has emerged due to the appearance of fungal strains with multidrug resistance. The substances with the different target structures have been studied intensively as potential new antimycotic agents [46, 47]. According to the mechanism of effect they can be classified as

- novel inhibitors of β-(1,3)-D-glucan synthesis
- inhibitors of chitin synthesis
- selective inhibitors of proteosynthesis
- compounds exerting antifungal activities via lysis

Novel inhibitors of β-(1,3)-D-glucan synthesis are represented by papulacandins, a class of unusual spirocyclic natural products originally isolated

from *Papularia sphaerosperma*. The papulacandins displayed potent in vitro activity against yeasts, particularly *C. albicans* as well as modest efficacy in vivo via s. c. administration (e.g. **populacandin B**). Oral activity against systematic fungal infections has remained elusive. Since the discovery of papulacandins, other structurally related natural products have been reported, including chaetiacandin, saricandin, furanocandin, corynecandin and fusacandin A [48].

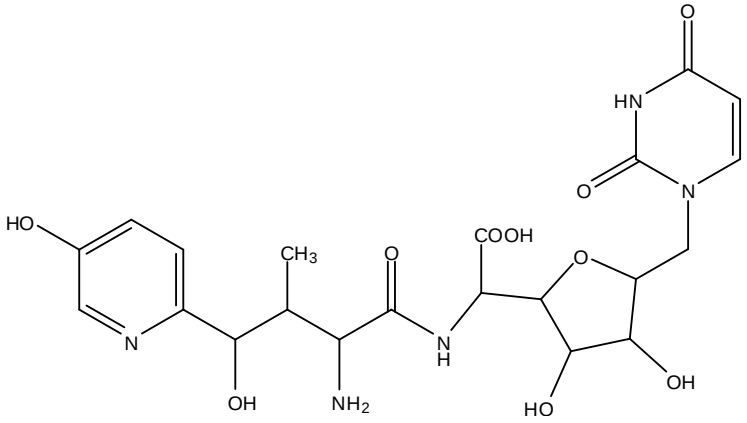


papulacandin B

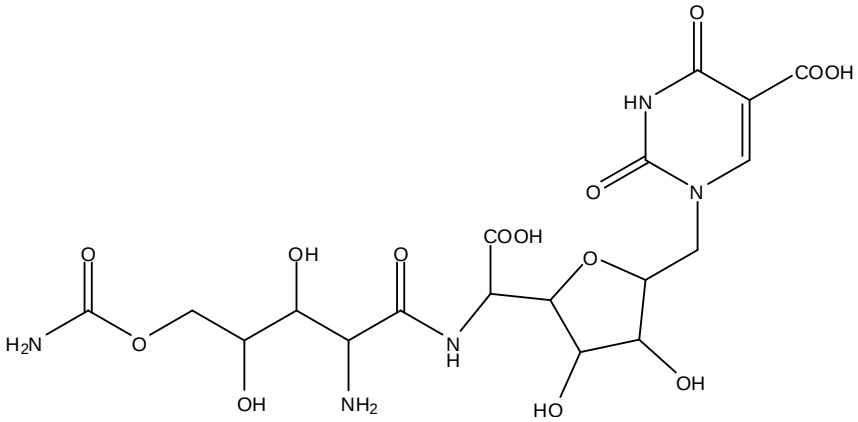
FR-901469 belongs to a new family of natural products inhibiting fungal β-(1,3)-D-glucan synthesis. FR-901469 has shown potent antifungal activity in the murine systemic candidiasis model. However, hepatotoxicity was observed with this compound after multiple dosing in mice. In light of this drawback, chemical modifications were inducted on FR-901469 with the aim of improving the in vivo antifungal activity and reducing hepatotoxicity [48, 49].

Polyoxins and **nikkomycins** belong to the agents affecting chitin synthesis. They are natural products capable of inhibiting chitin synthase, an enzyme that catalyzes the polymerization of *N*-acetylglucosamine to form a major component of the fungal cell wall. Chitin is absent in vertebrates, hence these compounds lack the mammalian toxicity. All these agents are nucleoside peptides. Direct clinical application of the natural peptidyl nucleosides is compromised by

their attenuated in vivo activity, apparently due to their hydrolytic lability and inefficient fungal cell wall permeability. Thus, extensive efforts have focused on synthesis of natural peptidyl nucleosides, their components and analogues in anticipation of establishing useful structure-activity-relationships (SAR) for the development of new antifungal agents [48, 50 – 52].



nikkomycin Z



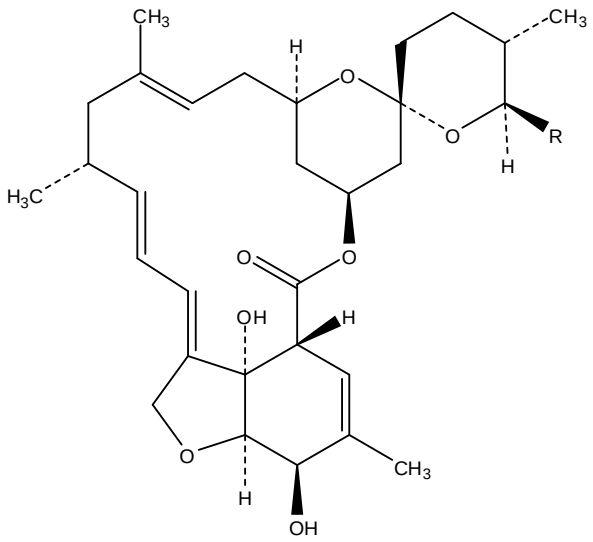
polyoxin D

The **sordarin** antifungal class, although not developed for clinical use, merits mention among the new mechanisms of action. They inhibit protein synthesis by blocking the function of fungal translation Elongation Factor 2 (EF2). The class was discovered by routine screening but was abandoned in the early 1970´s. Interest in sordarins was re-awakened as a result of a prospective screen for inhibitors of *C. albicans* protein synthesis in vitro, which pinpointed the nature of the sordarin antifungal effect. When refined experimentation revealed

EF2 as the specific target of the sordarins, the result engendered surprise because *C. albicans* EF2 displays more than 85% amino acid sequence identity to the human equivalent, and EF2 would never have emerged as a potential target from genomics-based screening. Different sordarin derivatives have different spectra of susceptible species, for reasons that are not yet clear. This might reflect problems of penetration of these agents into target fungi. Nevertheless, their high specificity for the fungal target and the relative ease with which new sordarin variants can be synthesized holds promise for positive future developments with this series [32, 53].

Syringotoxins, **syringomycins**, **syringostatins** and **pseudomycins** are cyclic lipodepsinonapeptides produced by the plant bacterium *Pseudomonas syringae* pv. *syringae*. These metabolites are phytotoxic and growth inhibitory against a broad spectrum of fungi. It has been shown that they target the fungal plasma membrane and alter several membrane functions. They are also known as necrosis-inducing lipodepsipeptide toxins. Members of the syringomycins class are pore-forming cytotoxins that act by promoting passive transmembrane ion flux [48, 54].

The rising occurence of resistance should be coped with inhibitors of the efflux pump, e.g. **milbemycine** [47].



milbemycin

Multidrug resistence in fungi can also be mediated by MDR1 P-glycoprotein. **Aureobasidins**, produced by *Aureobasidium pullulans*, are cyclic depsipeptides. They were found to interfere with ATP-binding cassete transporters, particularly that of MDR1 P-glycoprotein. They exhibited potent antifungal activity against *Candida* spp. and *C. neoformans*, but not *A. fumigatus*. It was also found that aureobasidins inhibit inositol phosphorylceramide synthase, which has recently been identified as a potential target for the development of potential new antifungal drugs [32, 48, 55, 56].

There are hundreds of potential antifungals with unknown mode of action, which are presently under study, e.g. viscosinamide, glomosporin, jaspamides, cyclolithistide A, halolitoralins and lobocyclamides [48, 57,58].

New alternatives to treating mycoses are required to circumvent the often poorly efficient fungal treatments. Human trials using antibody mediated immunity are ongoing against *C. albicans* and *C. neoformans* infections. Other efforts favour the elicitation of protective cellular immune responses. One exciting discovery is the identification of good and bad antibodies that either protect or harm patients. Another challenge in vaccination against fungi is the protection of immunocompromised patients at risk. The challenge of the next several years remains to link the biological and ecological properties of the fungus with the immunosuppresive status of the host in order to understand virulence. There is no doubt that genomic and post genomic approaches will help our understanding of the multigenic character of virulence [59, 60].

3. EXPERIMENTAL PART

Commercially available substances were used for the preparation of arylmethylidenerhodanines:

- 2-methoxybenzaldehyde, purum (Fluka)
- 3-methoxybenzaldehyde (Sigma)
- 4-methoxybenzaldehyde, purum (Fluka)
- 2-bromobenzaldehyde, purum (Fluka)
- pyridine-2-carboxaldehyde, 99% (Aldrich)
- rhodanin, puriss. p. a. (Fluka)

TLC was performed on Silufol UV 254 (Kavalier Votice) plates. Light petroleum + ethyl acetate 60:40 (v/v) was used as a mobile phase.

For analysis, the samples of compounds were dried 24 hours in the dessicator at 1.33 kPa.

Melting points were determined using Boëtius apparatus and are uncorrected.

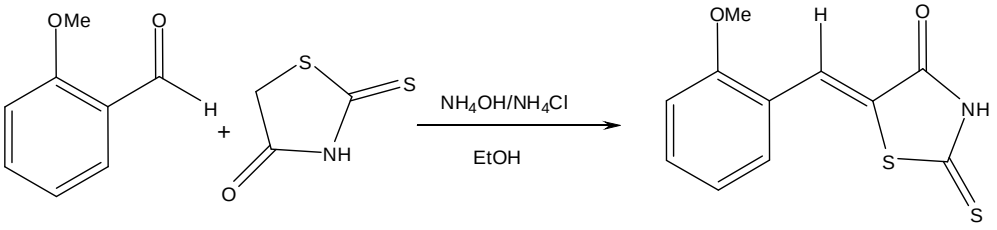
Elemental analyses were performed with the EA 1110 CHNS Analyzer (Carlo Erba).

HPLC analyses were performed as follows:
Separation module: Waters Alliance 2695 XE with the Millennium$^{32®}$ Chromatography Manager Software, Waters 2004.
Column: Symmetry® C_{18} 5 μm, 4.6 × 250 mm.
Mobile phase: methanol + water 70:30 (v/v), flow 0.9 ml/min, injection 30 μl
Detection: Waters Photodiode Array Detector 2996, 210 nm

IR spectra were recorded using the spectrophotometer NICOLET IMPACT 400. Wavenumbers are given in cm^{-1}.

^{1}H-NMR and ^{13}C-NMR spectra were recorded with the VARIAN Mercury-V_xBB 300. Chemical shifts are given in δ, ppm and interaction constants J in Hz.

3.1. Synthesis of 5-(2-methoxybenzylidene)-2-thioxo-1,3-thiazolidin-4-one



A mixture of 2-methoxybenzaldehyde (2.04 g; 0.015 mol), rhodanine (2.00 g, 0.015 mol), concentrated ammonia solution (1.1 ml) and ethanol (15 ml) was heated under reflux condenser until all solid components dissolved. Solution of ammonium chloride (1.00 g) in 2 ml of hot (80 °C) distilled water was then added, and the reaction mixture was refluxed for 2 hours. After cooling, the separated solid was filtered through a sintered filter, washed with distilled water (50 ml) and then with 50% ethanol (50 ml).

3.58 g (95%) of dry crude product was obtained.

For analysis, the product was crystallized from absolute ethanol.

Molecular weight: 251.32

Appearance: yellow, crystalline solid

Melting point: 258 °C

Melting points reported previously

- 251 °C (configuration unknown, ethanol) [61]
- 198 – 200 °C (configuration unknown, methanol) [61]

Elemental analysis for $C_{11}H_9NO_2S_2$:

	% C	**% H**	**% N**	**% S**
Calculated:	52.57	3.61	5.57	25.52
Found:	52.87	3.34	5.67	25.09

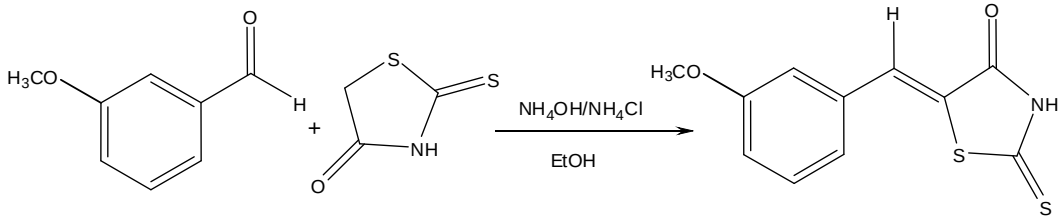
HPLC purity: 98.33 %

IR spectrum (KBr): 3447, 3141(NH); 1705 (C=O)

^{1}H NMR (300 MHz, DMSO): δ 13.76 (1H, bs, NH), 7.78 (1H, s, CH), 7.53 –7.44 (1H, m, H4′), 7.37 (1H, dd, *J* = 7.7 Hz, *J* = 1.7 Hz, H6′), 7.14 (1H, d, *J*= 7.7 Hz, H3′), 7.08 (1H, t, *J* = 7.7 Hz, H5′), 3.88 (3H, s, OCH_3)

^{13}C NMR (75 MHz, DMSO) δ 196.3, 169.6, 158.3, 133.2, 129.9, 126.9, 125.5, 121.5, 121.4, 112.2, 56.0

3.2. Synthesis of 5-(3-methoxybenzylidene)-2-thioxo-1,3-thiazolidin-4-one



A mixture of 3-methoxybenzaldehyde (2.04 g, 0.015 mol), rhodanine (2.00 g, 0.015 mol), concentrated ammonia solution (1.1 ml) and ethanol (15 ml) was heated under reflux condenser until all solid components dissolved. Solution of ammonium chloride (1.00 g) in 2 ml of hot (80 °C) distilled water was then added, and the reaction mixture was refluxed for 2 hours. After cooling, the separated solid was filtered through a sintered filter, washed with distilled water (50 ml) and then with 50% ethanol (50 ml).

3.06 g (81%) of dry crude product was obtained.

For analysis, the product was crystallized from absolute ethanol.

Molecular weight: 251.32

Appearance: yellow, crystalline solid

Melting point: 235 °C

Melting points reported previously

- 232 °C (configuration unknown, ethanol) [61]
- 227 °C (configuration unknown, acetic acid) [61]

- 229 – 230 °C (configuration unknown, nitrobenzene) [61]
- 165 – 170 °C (configuration unknown, methanol) [61]

Elemental analysis for $C_{11}H_9NO_2S_2$:

	% C	**% H**	**% N**	**% S**
Calculated:	52.57	3.61	5,57	25.22
Found:	52.73	5.43	5.65	25.87

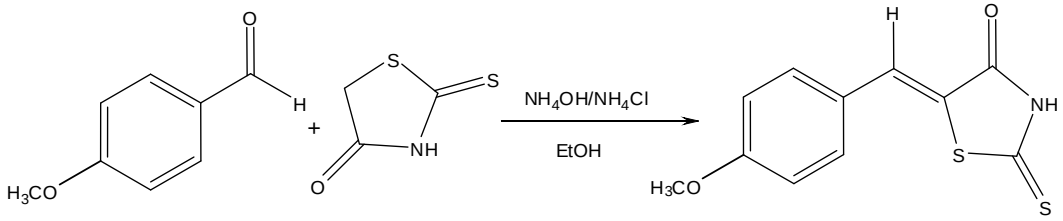
HPLC purity: 93.71%

IR spectrum (KBr): 3151 (NH); 1698 (C=O)

^{1}H NMR (300 MHz, DMSO): δ 13.82 (1H, bs, NH), 7.60 (1H, s, CH), 7.44 (1H, t, J = 8.1 Hz, H5′), 7.16 – 7.03 (3H, m, H2′, H4′, H6′), 3.79 (3H, s, OCH_3)

^{13}C NMR (75 MHz, DMSO): δ 195.8, 169.5, 159.9, 134.5, 131.8, 130.7, 126.0, 122.6, 116.9 115.8, 55.5

3.3. Synthesis of 5-(4-methoxybenzylidene)-2-thioxo-1,3-thiazolidin-4-one



A mixture of 4-methoxybenzaldehyde (2.04 g, 0.015 mol), rhodanine (2.00 g, 0.015 mol), concentrated ammonia solution (1.1 ml) and ethanol (15 ml) was heated under reflux condenser until all solid components dissolved. Solution of ammonium chloride (1.00 g) in 2 ml of hot (80 °C) distilled water was then added, and the reaction mixture was refluxed for 2 hours. After cooling, the separated solid was filtered through a sintered filter, washed with distilled water (50 ml) and then with 50% ethanol (50 ml).

3.40 g (90%) of dry crude product was obtained.

For analysis, the product was crystallized from absolute ethanol.

Molecular weight: 251.32

Appearance: yellow, crystalline solid

Melting point: 260 – 261 °C

Melting points reported previously

- 262 °C (*Z*-isomer) [61]
- 257 – 259 °C (*Z*-isomer, ethanol) [61]
- 242.5 °C (*Z*-isomer, DMF) [61]
- 233 – 235 °C (*E*-isomer, ethyl acetate, hexane) [61]
- 261 – 262 °C (configuration unknown, water, DMF) [61]
- 252 °C (configuration unknown) [61]
- 250 – 251 °C (configuration unknown, ethanol) [61]
- 249 – 254 °C (configuration unknown, ethanol) [61]
- 248 – 251 °C (configuration unknown) [61]
- 245 °C (configuration unknown) [61]
- 230 – 242 °C (configuration unknown, acetic acid) [61]
- 207 – 208 °C (configuration unknown, methanol) [61]

Elemental analysis for $C_{11}H_9NO_2S_2$:

	% C	**% H**	**% N**	**% S**
Calculated:	52.57	3.61	5.57	25.52
Found:	52.25	3.60	5.75	27.94

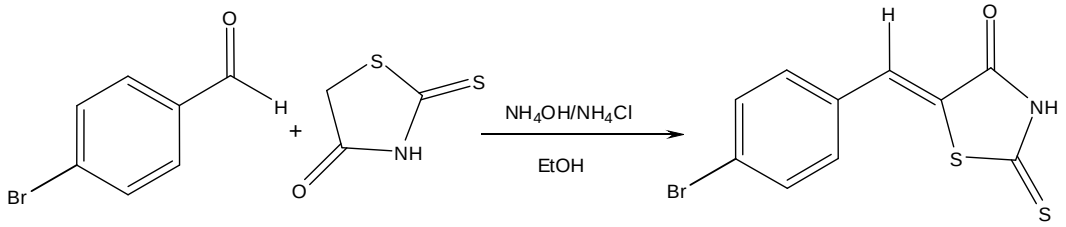
HPLC purity: 86.24%

IR spectrum (KBr): 3448, 3137 (NH); 1687 (C=O)

^{1}H NMR (300 MHz, DMSO): δ 13.72 (1H, bs, NH), 7.59 (1H, s, CH), 7.58 – 7.50 (2H, m, AA´, BB´, H2´, H6´), 7.13 – 7.05 (2H, m, AA´, BB´, H3´, H5´), 3.82 (3H, s, OCH_3)

^{13}C NMR (75 MHz, DMSO): δ 195.7, 169.6, 161.5, 132.9, 132.1, 125.7, 122.4, 115.3, 55.8

3.4. Synthesis of 5-(4-bromobenzylidene)-2-thioxo-1,3-thiazolidin-4-one



A mixture of 4-bromobenzaldehyde (2.78 g, 0.015 mol), rhodanine (2.00 g, 0.015 mol), concentrated ammonia solution (1.1 ml) and ethanol (15 ml) was heated under reflux condenser until all solid components dissolved. Solution of ammonium chloride (1.00 g) in 2 ml of hot (80 °C) distilled water was then added, and the reaction mixture was refluxed for 2 hours. After cooling, the separated solid was filtered through a sintered filter, washed with distilled water (50 ml) and then with 50% ethanol (50 ml).

4.15 g (92%) of dry crude product was obtained.

For analysis, the product was crystallized from absolute ethanol.

Molecular weight: 300.19

Appearance: yellow, crystalline solid

Melting point: 238 °C

Melting points reported previously

- 231 °C (*Z*-isomer, ethanol) [62]
- 238 °C (configuration unknown) [61]
- 237 – 238 °C (configuration unknown, acetic acid) [61]
- 219 °C (configuration unknown) [61]
- 219 °C (configuration unknown, ethanol) [61]

Elemental analysis for $C_{10}H_6BrNOS_2$:

	% C	**% H**	**% N**	**% S**
Calculated:	40.01	2.01	4.67	21.36
Found:	39.95	1.87	4.64	21.78

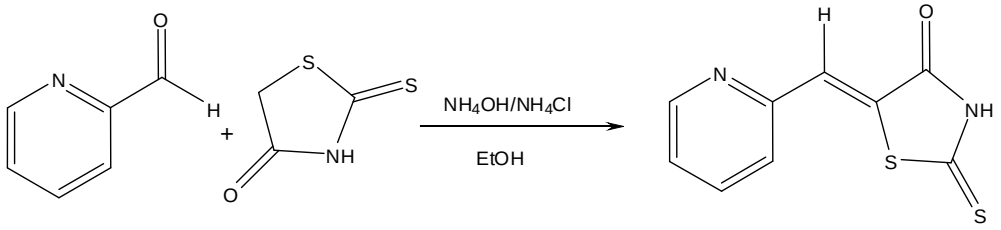
HPLC purity: 89.11%

IR spectrum (KBr): 3442, 3150 (NH); 1708 (C=O)

^{1}H NMR (300 MHz, DMSO): δ 7.76 – 7.69 (2H, m, AA′, BB′, H2′, H6′), 7.60 (1H, s, CH), 7.55 – 7.49 (2H, m, AA′, BB′, H3′, H5′)

^{13}C NMR (75 MHz, DMSO): δ 195.6, 169.5, 132.6, 132.4, 132.4, 130.5, 126.5, 124.5

3.5. Synthesis of 5-(pyridin-2-ylmethylidene)-2-thioxo-1,3-thiazolidin-4-one



A mixture of pyridine-2-carboxaldehyde (1.61 g, 0.015 mol), rhodanine (2.00 g, 0.015 mol), concentrated ammonia solution (1.1 ml) and ethanol (15 ml) was heated under reflux condenser until all solid components dissolved. Solution of ammonium chloride (1.00 g) in 2 ml of hot (80 °C) distilled water was then added, and the reaction mixture was refluxed for 2 hours. After cooling, the separated solid was filtered through a sintered filter, washed with distilled water (50 ml) and then with 50% ethanol (50 ml).

2,35 g (70%) of dry crude product was obtained.

For analysis, the product was crystallized from absolute ethanol.

Molecular weight: 222.29

Appearance: green-yellow, crystalline solid

Melting point: 269 – 272 °C

Melting points reported previously

- 257 – 260 °C (*Z*-isomer, ethanol) [61]
- 253 – 258 °C (configuration unknown, methanol) [61]

- 247 – 250 °C (configuration unknown, ethanol) [61]
- 243 – 245 °C (configuration unknown, ethanol) [61]

Elemental analysis for $C_9H_6N_2OS_2$:

	% C	**% H**	**% N**	**% S**
Calculated:	48.63	2.72	12.60	28.85
Found:	48.26	2.65	12.82	28.90

HPLC purity: 93.99%

IR spectrum (KBr): 3412 (NH); 1726 (C=O)

^{1}H NMR (300 MHz, DMSO): δ 13.66 (1H, bs, NH), 8.77 (1H, d, J = 4.7 Hz, H6′), 7.94 (1H, dt, J = 7.6 Hz, J = 1.8 Hz, H4′), 7.88 (1H, d, J = 7.6 Hz, H3′), 7.67 (1H, s, CH), 7.45 – 7.39 (1H, m, H5′)

^{13}C NMR (75 MHz, DMSO): δ 202.2, 169.5, 151.3, 149.7, 137.8, 129.9, 128.3, 127.6, 124.2

3.6. Evaluation of in vitro antifungal activity

Antifungal activity of all compounds against *Candida albicans* ATCC 44859, *Candida tropicalis* 156, *Candida krusei* E 28, *Candida glabrata* 20/I, *Trichosporon asahii* 1188, *Aspergillus fumigatus* 231, *Absidia corymbifera* 272 and *Trichophyton mentagrophytes* 445 was evaluated by the microdilution broth method. All strains were subcultured on Sabouraud dextrose agar (SDA, Difco) and maintained on the same medium at 4 °C. Prior to testing, each strain was passaged onto SDA and fungal inocula were prepared by suspending yeasts or conidia or sporangiospores in sterile 0.85% saline. The cell density was adjusted, using the Bürker´s chamber, to yield a stock suspension of $(1.0 \pm 0.2) \times 10^5$ CFU/ml. The final inoculum was made by 1:20 dilution of the stock suspension with the test medium. The compounds were dissolved in dimethyl sulfoxide (DMSO) and antifungal activity was determined in the tissue culture medium RPMI 1640 (Sevapharma, Prague, Czech Republic) buffered to pH 7.0 with 0.165 M 3-morpholinopropane-1-sulfonic acid (Sigma). Controls were included as well. The final concentration of DMSO in the test medium did not exceed 1% (v/v) of the total solution composition. The minimum inhibitory concentration (MIC), defined as 80% inhibition of fungal growth compared to control, were determined after 24 and 48 h of static incubation at 35 °C. In the case of *T. mentagrophytes* the MICs were recorded after 72 and 120 h. Amphotericin B and fluconazole were used as reference antifungal drugs. The results are given in Table 1.

Table 1. Minimum inhibitory concentrations of the tested compounds

STRAIN		**COMPOUND – MIC/IC$_{80}$** (μmol.l^{-1})						
		3.1.	***3.2.***	***3.3.***	***3.4.***	***3.5.***	***AMP-B***	***FLU***
CA	*24h*	>125	>125	>500	62.5	62.5	0,002	0,82
	48h	>125	>125	>500	>125	>125	0,068	1,63
CT	*24h*	>125	>125	>500	>125	>125	0,068	1,63
	48h	>125	>125	>500	>125	>125	0,068	> 417,90
CK	*24h*	>125	>125	>500	>125	>125	0,135	52,24
	48h	>125	>125	>500	>125	>125	0,135	104,47
CG	*24h*	>125	>125	>500	>125	>125	0,034	13,06
	48h	>125	>125	>500	>125	>125	0,135	52,24
TA	*24h*	>125	>125	>500	>125	>125	1,082	3,26
	48h	>125	>125	>500	>125	>125	2,164	6,53
AF	*24h*	>125	>125	>500	>125	>125	0,271	> 417,90
	48h	>125	>125	>500	>125	>125	0,135	> 417,90
AC	*24h*	>125	>125	>500	>125	>125	1,082	> 417,90
	48h	>125	>125	>500	>125	>125	2,164	> 417,90
TM	*72h*	>125	>125	250	31.25	>125	1,082	26,12
	120h	>125	>125	500	62.5	>125	1,082	52,24

4. DISCUSSION

In the theoretical part of my diploma thesis some issues concerning tuberculosis and mycoses are discussed. Experimental part focused on the preparation of the following compounds:

- 5-(2-methoxybenzylidene]-2-thioxo-1,3-thiazolidin-4-one (**3.1.)**
- 5-(3-methoxybenzylidene]-2-thioxo-1,3-thiazolidin-4-one (**3.2.)**
- 5-(4-methoxybenzylidene]-2-thioxo-1,3-thiazolidin-4-one (**3.3.)**
- 5-(4-bromobenzylidene]-2-thioxo-1,3-thiazolidin-4-one (**3.4.)**
- 5-pyridin-2-ylmethyliden-2-thioxo-1,3-thiazolidin-4-one (**3.5.)**

All these compounds have been reported in literature previously. Nonetheless, their characterization has been incomplete so far. NMR spectra are often not available, and configuration on the exocyclic double bond has only been determined in some cases [61, 62].

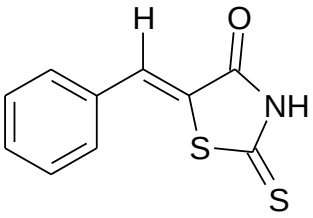
5-(2-Methoxybenzylidene]-2-thioxo-1,3-thiazolidin-4-one has been known since 1941 [63]. 5-(3-Methoxybenzylidene]-2-thioxo-1,3-thiazolidin-4-one was prepared by Avison and Morrison in 1950 [64]. Since then, these compounds have been prepared by many other research groups, but to the best of my knowledge their antifungal effects have not been studied so far [61, 62].

5-(4-Methoxybenzylidene]-2-thioxo-1,3-thiazolidin-4-one was reported at the beginning of the 20th century [61, 65] and 5-(4-bromobenzylidene]-2-thioxo-1,3-thiazolidin-4-one has been known since 1948 [66]. Antifungal activity of these two compounds was studied by Brown et al. [67] and Sortino et al. [68].

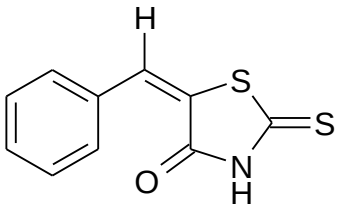
Regarding the heterocyclic derivative, 5-pyridin-2-ylmethyliden-2-thioxo-1,3-thiazolidin-4-one, the synthetic priority belongs to Lapière [69], and its antifungal effects were studied by Allan and co-workers [70] and Sortino et al. [68].

Arylmethylidenerhodanines can form two isomers. According to references, syntheses of these compounds result in *Z*-izomer. Configuration on the exocyclic double bond can be determined on the basis of NMR spectra. ^{1}H-NMR

signals of the methine-group hydrogens for *Z*-izomers are more downfield compared to *E*-izomers [71 – 73].



***Z*-izomer**



***E*-izomer**

The signals of these hydrogens in the rhodanine derivatives studied in the present paper were calculated and compared with the reported and experimental values (Table 2).

Table 2. Comparison of ^{1}H NMR signals of the methine-group

Compound	***E*-isomer**	***Z*-isomer**[a]	**Published values**	**Experimental values**
3.1	7.07[a] 7.99[b]	7.69[a] 8.07[b]	not available	7.78
3.2	6.80[a] 7.72[b]	7.42[a] 7.80[b]	not available	7.60
3.3	6.80[a] 7.72[b]	7.42[a] 7.80[b]	7.52[c]	7.59
3.4	6.80[a] 7.72[b]	7,42[a] 7.80[b]	not available	7.60
3.5	7.01[a] 7.01[b]	7.63[a] 7.63[b]	7.65[c]	7.67

[a] CS ChemOffice 7.0 (*CambridgeSoft, Cambridge, MA, U.S.A.*); [b] CS ChemOffice 10.0 (*CambridgeSoft, Cambridge, MA, U.S.A.*); [c] *Z*-isomer [68]

Based on the experimental ^{1}H-NMR signals, it can be concluded that all arylmethylidenerhodanines reported in the present paper were obtained as single isomers. Comparing the calculated, reported and experimental data shows that 5-(4-methoxybenzylidene]-2-thioxo-1,3-thiazolidin-4-one (3.3) and 5-pyridin-2-ylmethyliden-2-thioxo-1,3-thiazolidin-4-one (3.5.) have *Z*-configuration. In case

of the remaining products, a definitive determination of the configuration would require additional experiments.

The products have already been tested for antifungal effects. The assay was performed at the Department of Biological and Medical Sciences of the Faculty of Pharmacy in Hradec Králové. Unfortunately, no interesting antifungal activity was found. This in agreement with the results of Sortino et al. who studied the same compounds simultaneously with me and found that a substituted benzene ring is a necessary moiety for these compounds to possess antifungal activity, whilst the pyridine analogues are inactive. The type of substituent on the benzene ring appears to play an important role. The most active compounds were F- and CF_3-substituted benzylidenerhodanines. On the other hand, rhodanines with Br atom or with donor substituents (CH_3, OCH_3, O-CH_2-O) showed marginal or null activity [68].

The antimycobaterial properties of the compounds **3.1**. – **3.5**. will be tested later, preferably in the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) [74].

5. CONCLUSIONS

The following compounds were prepared within my experimental work:

- 5-(2-methoxybenzylidene]-2-thioxo-1,3-thiazolidin-4-one (**3.1.)**
- 5-(3-methoxybenzylidene]-2-thioxo-1,3-thiazolidin-4-one (**3.2.)**
- 5-(4-methoxybenzylidene]-2-thioxo-1,3-thiazolidin-4-one (**3.3.)**
- 5-(4-bromobenzylidene]-2-thioxo-1,3-thiazolidin-4-one (**3.4.)**
- 5-pyridin-2-ylmethyliden-2-thioxo-1,3-thiazolidin-4-one (**3.5.)**

The compounds were obtained as single isomers, most probably with *Z*-configuration on the exocyclic double bond.

They have been tested for antifungal effects against 8 pathogenic strains of fungi, but did not exhibit an interesting activity. In the future, their antimycobacterial potency will be evaluated.

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MORID MAHMOUDI MAJD: Derivatives of Rhodanine as Potential Antifungal and Antimycobacterial Drugs". Diploma Thesis, Department of Pharmaceutical Chemistry and Drug Control, Charles University in Prague, Faculty of Pharmacy in Hradec Králové, 2007

Abstract

In the theoretical part of my diploma thesis some issues concerning tuberculosis and mycoses are discussed. Experimental part focused on the preparation of the following compounds

- 5-(2-methoxybenzylidene]-2-thioxo-1,3-thiazolidin-4-one
- 5-(3-methoxybenzylidene]-2-thioxo-1,3-thiazolidin-4-one
- 5-(4-methoxybenzylidene]-2-thioxo-1,3-thiazolidin-4-one
- 5-(4-bromobenzylidene]-2-thioxo-1,3-thiazolidin-4-one
- 5-pyridin-2-ylmethyliden-2-thioxo-1,3-thiazolidin-4-one.

The compounds were prepared by the condensation of rhodanine with aromatic aldehydes in ethanol using a mixture NH_4OH/NH_4Cl as the catalyst. Their purity was checked by the elemental analysis and HPLC. The products were characterized by IR and NMR spectra.

The susceptibility of 8 pathogenic fungal strains (*Candida albicans* ATCC 44859, *Candida tropicalis* 156, *Candida krusei* E 28, *Candida glabrata* 20/I, *Trichosporon asahii* 1188, *Aspergillus fumigatus* 231, *Absidia corymbifera* 272 and *Trichophyton mentagrophytes* 445) to these substances was determined by the microdilution broth method. No interesting activity was found. The compounds will further be tested for antimycobacterial effects.