Charles University in Prague
Faculty of Pharmacy in Hradec Králové
Department of Pharmaceutical Chemistry and Drug Control

Derivatives of Rhodanine as Potential Antifungal Drugs

Hradec Králové, 2010 Marianna El-Zein

This is to declare that this diploma thesis is my own work and I worked on it on my own. All literature sources are properly cited in reference list.

Date: Signature:

The work was supported by the grant SVV-2010-261-001

Acknowledgement

I would like to thank to Assoc. Prof. RNDr. V. Opletalová, Ph.D. for helping me in writing the thesis. I also thank to my parents for their support during my study. I would also like to thank to Ms. I. Vencovská for recording the IR spectra, and to Assoc. Prof. PharmDr. J. Kuneš, CSc. for recording and interpreting the NMR spectra.

CONTENT

1. INTRODUCTION

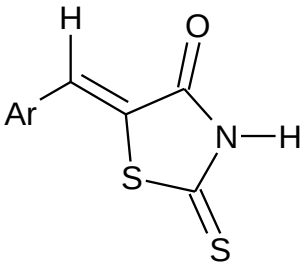
Rhodanine (2-thioxo-1,3-thiazolidin-4-one) forms the basic skeleton of many biologically active substances and potential drugs [1]. Antifungal properties of rhodanine derivatives have been studied since the early 1950s [2]. The first attempts to use rhodanines as mildew-preventing agents were performed by Brown and co-workers. They studied various rhodanine derivatives with the aim to find new and effective compounds for protection of cotton fabrics against cellulolytic fungi and bacteria, *e.g*. *Chaetomium globosum* [3]. Condensation products of rhodanine and *N*-substituted rhodanines with various aldehydes were further studied by Allan et al. [4, 5]. Rhodanine derivatives were also tested against plant fungal pathogens – *Alternaria tenuis* and *Botrytis allii* [6].

5-(5-nitrofurfurylidene)rhodanine was the first rhodanine derivative tested against fungi that cause mycoses in mammals (*Candida albicans* and *Trichophyton mentagrophytes*) [7].

Since that time, plenty of various rhodanine derivatives have been studied as potential antifungal agents [1, 8–12]. Sortino and co-workers [13] tested a series of (*Z*5)-arylmethylidene rhodanines **2** against a panel of both standardized and clinical opportunistic pathogenic fungi. They have found that

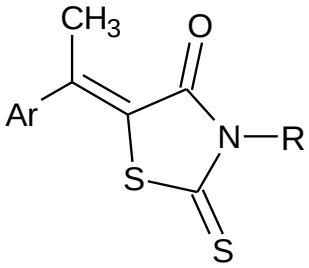
- the 2-thioxothiazolidin-4-one part itself, nor the presence of enone linkage is sufficient for antifungal activity
- 5-(subst.)benzylidenerhodanines were active, but 5-pyridylmethylidene-rhodanines (all three positional isomers) were inactive
- within the 5-(subst.)benzylidenerhodanines the type of substituents on the benzene ring plays an important role in activity
- the most active compounds (F- and CF_3-substituted benzylidenerhodanines) posses high log *P* values and low polarizability; replacement of fluorine with chlorine decreases potency, and substitution with bromine results in the loss of antifungal activity.

Independently of the research performed by Sortino et al. [13], antifungal effects of rhodanine derivatives were studied at the Charles University, Faculty of Pharmacy in Hradec Králové. In addition to (*Z*)-5-arylmethylidenerhodanines **1**, (*Z*)-5-(1-arylethylidene)rhodanines **2** derived from acetophenone, acetylpyridines and acetylpyrazine, and their analogs derived from 3-substituted rhodanines were prepared and tested against a panel of opportunistic pathogenic fungi (*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. The results of these studies were more or less similar to those of Sortino et al. [14–20].



1

Ar = (subst.)phenyl, 2-pyridyl, 3-pyridyl, 4-pyridyl



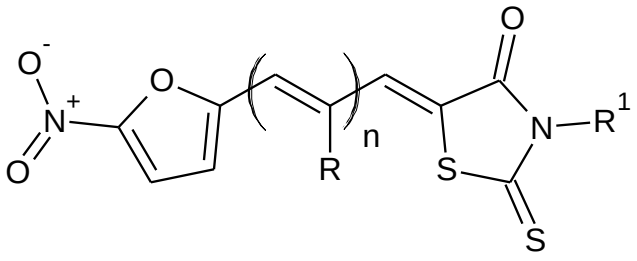
2

Ar = (subst.)phenyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrazinyl, 2-furyl

R = H; NH_2, CH_2CH_2OH, CH_2COOH

2. AIM OF THE WORK

According to previous reports [3, 5, 6], 5-monosubstituted rhodanines are usually, but not always, more effective than their 3-alkyl or 3-phenyl analogs. In 1971, 3-substituted rhodanines of general formula **3** were patented as potential antimicrobial compounds. The derivatives, where n = 0, R = H and R^1 = methyl, ethyl, allyl, cyclopropyl, 2-OH-cyclohexyl, benzyl, 4-Cl-benzyl, inhibited growth of *Candida albicans* and *Epidermophyton floccosum* (MIC = ≤ 0.5 – 20 μg/ml). Unfortunately, MICs for other derivatives are not given in the patent [21, 22].



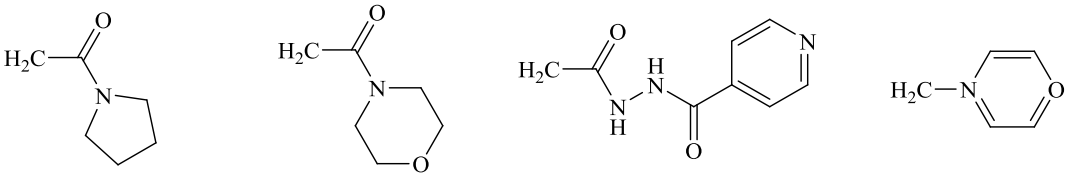
3

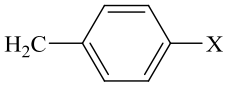
n = 0, 1

R = H, Br

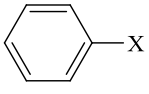
R^1 = n-alkyl, allyl, cyclopropyl, 2-OH-cyclohexyl, CH_2OH,C_2H_4OH, CH_2COOH,

$CH_2COO^-NH_4^+$, $CH(CH_3)COO^-NH_4^+$, $CH_2COOC_2H_5$, CH_2CONH_2,

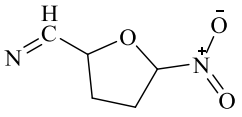




X = H, Cl



X = SO_2NH_2, Cl, COOH



A series of 5-substituted-3-polynitrophenylrhodanines inhibited the germination of spores of *Helmithosporium sativum*, *Alternaria tenuis*, *Aspergilus niger* and *Fusarium oxysporum* [8].

3-(2-Hydroxyethyl)rhodanines that have been studied at the Faculty of Pharmacy in Hradec Králové had better solubility in water, but lower antifungal potency than their 3-unsubstituted congeners [23].

Within the series of *N*-glycosylrhodanines the best activity against *Candida albicans* and *Aspergilus niger* was shown by *N*-(2,3,5-*O*-acetyl-β-D-glucopyranosyl)rhodanine, which had comparable potency to that of rhodanine itself. The corresponding 5-benzylidene derivative was active against only *C. albicans* [24].

3-Aminorhodanines represent another type of 3-substituted derivatives. 3-Amino-rhodanine (but not 3-acetylamino and 3-benzoylaminorhodanine) had better antifungal effectiveness than rhodanine [11]. Anilinorhodanines reported by Brown and co-workers [25] inhibited growth of *Aspergilus niger*. Within this series, 3-[(4-halogenophenyl)-amino]-2-thioxo-1,3-thiazolidin-4-ones exhibited best potencies. Antifungal properties of

condensation products of 3-aminorhodanine with aldehydes and ketones have not yet been studied in detail. (5*Z*)-3-Amino-5-(pyridin-2-ylmethylidene)-2-thioxo-1,3-thiazolidin-4-on prepared by Hiršová [19] was ineffective.

5-(Arylalkylidene-4-oxo-2-thioxo-1,3-thiazolidin-3-yl)alkanoic acids have also been reported as potential antifungal agents [12, 26]. Surprisingly, in a series of rhodanineacetic acids prepared at the Faculty of Pharmacy in Hradec Králové antifungal activity was only observed with {(5*Z*)-[4-oxo-5-(pyridin-2-ylmethylidene)-2-thioxo-1,3-thiazolidin-3-yl]}acetic acid [27].

Antifungal properties have also been reported for Mannich bases derived from 5-arylalkylidenerhodanines [28, 29]. This diploma project is a pilot study the aim of which is to study and optimize reaction conditions for Mannich reaction of (5*Z*)-5-(subst.)benzylidenes-2-thioxo-1,3-thiazolidin-4-ones with various amines.

3. THEORETICAL PART

3.1 Mycoses

During the past 20 years, the incidence of fungal infections in humans has risen in considerably. Fungal infections are categorized in two groups: topical and systemic fungal infections.

- Topical fungal infections can be chronic and often resistant to treatment. Examples of these infections include athlete's foot, which is the most common fungal infection worldwide, and *Candida* infections of the mouth (oral candidiasis or thrush), which can result in periodontitis and gingival diseases.
- Systemic fungal infections can occur in an organ or in the whole body and are transferred via the bloodstream. The two most important factors that are associated with the increase of fungal infections are the growth of immunocompromised patient population and the increased use of invasive devices (such as central venous catheters) and implants (prosthetic cardiac valves). Systemic infections affect deeper tissues and organs. The commonest systemic fungal infections are blastomycosis, histoplasmosis, coccidiomycosis and paracoccidiomycosis [30–34].

Fungi behave as opportunistic invaders and can cause life threatening infections in immunocompromised patients associated with modern high-intensity chemotherapy regimens [35], the acquired immunodeficiency syndrome (AIDS) pandemic, the more widespread use of solid organ transplantation [35], autologous and allogenic stem cell transplantation for haematologic malignancies [36, 37], and the use of aggressive supportive care in high-risk populations, especially those in intensive care units, burn patients [38] and premature neonates [39].

3.1.1 Advances and challenges in management of invasive mycoses

Invasive mycoses pose a major diagnostic and therapeutic challenge. Advances in antifungal agents and diagnostic methods offer the potential for improved outcomes in patients with these infections, which are often lethal. Many fungal pathogens occur

almost exclusively in opportunistic settings. Several areas of ongoing challenge remain, including the emergence of resistant organisms and the absence of reliable markers for early identification of patients at risk of developing invasive fungal disease

3.1.1.1 Candida

Candida is among the leading causes of nosocomial blood stream infections worldwide. Although risk factors for invasive candidosis are well known, including candida colonisation, length of hospital stay, abdominal surgery, and use of parenteral nutrition, antibiotics, or central vascular illness, few assesment strategies can predict a population at high risk of infection. Crude mortality rates for candidaemia have ranged from 30% to 61%, with significant attributable mortality related to *Candida*. The changing epidemiology of nosocomial candidaemia is due to several factors, including the widespread use of antifugals, particularly fluconazole. By contrast, *Candida crusei* is intrinsically resistant to fluconazole and itraconazole but usually susceptible to the newer triazoles and echinocandins. *Candida glabrata*, which has increased in many medical centres, shows dose-dependent susceptibility for many strains to fluconazole and itraconazole.

3.1.1.2 Aspergillus

Mortality rates associated with invasive aspergillosis are still extremely high, particularly in the most extensively immunosuppressed patients and those who develop disseminated infection. Species of *Aspergillus* other than *Aspergillus fumigatus* have been increasingly isolated, including *Aspergillus terreus,* a soil-related species that is often resistant to antifungal therapy, including amphotericin B. This species are generally more susceptible to echinocandins and newer azole antifungals, such as voriconazole and posaconazole, which appear to be better therapeutic options.

3.1.1.3 Zygomycosis

Zygomycosis (also known as mucormycosis) has recently received increased interest because of reports of these infections in severely immunosuppressed patients,

particularly those on long-term prophylaxis with voriconazole. Zygomycetes typically cause a syndrome of vascular invasion, thrombosis, and necrosis, which often presents as rhinocerebral infection but also as pulmonary or disseminated disease. The organisms causing this syndrome are in the order Mucorales, although *Mucor* spp. are uncommon causes of infection; genera include *Rhizopus*, *Absidia*, *Cunninghamella*, *Apophysomyces elegans*, and *Saksenea vasiformis*. The organisms form broad ribbonlike hyphae in tissued and are generally regarded as nonseptate, although rare septae may occur. These organisms may fail to grow from homogenized tissue, which can be a clue to their diagnosis. The organisms are identified by fruiting structures and presence and location of rhizoids-root like structures along the hyphae [40].

3.2 Drugs used for fungal infections

3.2.1. Classes of antifungal agents

Most antifungal drugs interfere with biosynthesis or integrity of ergosterol, the major sterol in the fungal cell membrane. Others cause disruption of the cell wall. Based on their mechanism of action the four main classes of antifungal drugs are the **polyenes, azoles, allylamines** and **echinocandins.**

- Clinically useful "older" agents include topical azole formulations for superficial yeast and dermatophyte infections, first-generation triazoles (fluconazole and itraconazole) for a range of superficial and invasive fungal infections, amphotericin B formulations (for a broad range of invasive fungal infections and terbinafine for dermatophyte infections.
- Clinically important "newer" agents include members of the echinocandin class (e.g., caspofungin, micafungin and anidulafungin) and second-generation triazoles (e.g., voriconazole and posaconazole, ravuconazole, albaconazole and isavuconazole).
- Voriconazole and posaconazole have broad-spectrum activity against yeasts and moulds, including *Aspergillus* spp. Posaconazole is the only azole drug with activity against zygomycete fungi.

- Caspofungin and other echinocandins (micafungin and anidulafungin are effective in treating *Candida* and *Aspergillus* infections [41].

3.2.2 Polyenes

Polyenes have a very broad range of activity against most pathogenic fungi. Polyene macrolides are a class of poorly absorbed, large macrocyclic polyketides that interact with membrane sterols. They are produced by *Streptomyces* spp, and characterized by large lactone rings, containing three to eight conjugated double bonds which are generally combined with one sugar moiety [42].

3.2.2.1. Mechanism of the action

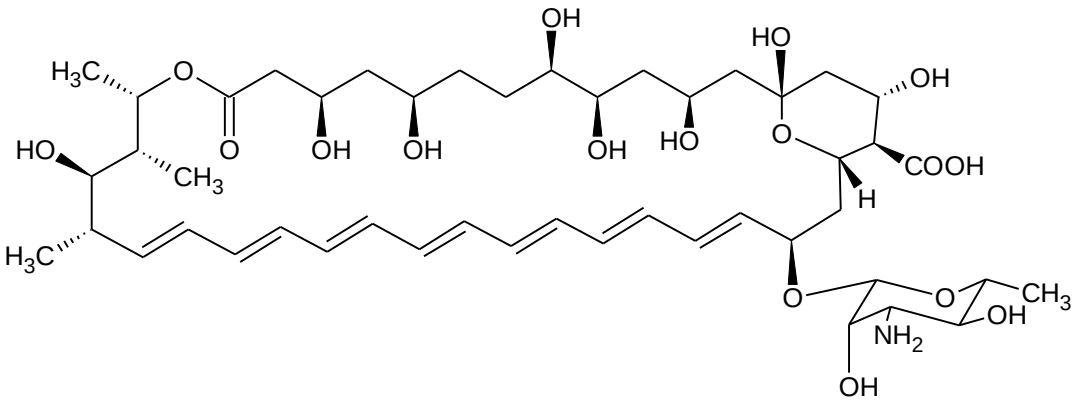
The mode of action of polyenes is to increase the permeability of the cytoplasmic membrane, leading to leakage of the cell contents and eventually death. This action is dependent on binding of the antibiotic to sterols present in the cell membrane. Organisms, such as bacteria, which do not contain sterols, are inherently resistant to the polyenes. The different polyenes have differing affinities for sterols, and amphotericin has a higher affinity for ergosterol present in fungal cytoplasmic membranes than cholesterol, which is found in mammalian cell membranes [43].

3.2.2.2 Polyene Antifungals

Amphotericin B (AmB) is the most widely used member of this group. It has been for many years the only antifungal polyene that could be administered systematically to treat a visceral infection. AmB is useful in treating cases of candidosis, cryptococcosis. This natural compound was isolated from *Streptomyces nodosus* in 1955 [44]. Amphotericin B is still considered the gold standard for the treatment of most life-threatening fungal infections [42]. Locally it is used only for therapy of candidoses; intravenously it is used for therapy of infections cause by *Cryptococcus neoformans*, by *Aspergillus* but mainly infections caused by *Candida*. Amphotericin B has to be administered intravenously because peroral administation is not effective. There is a

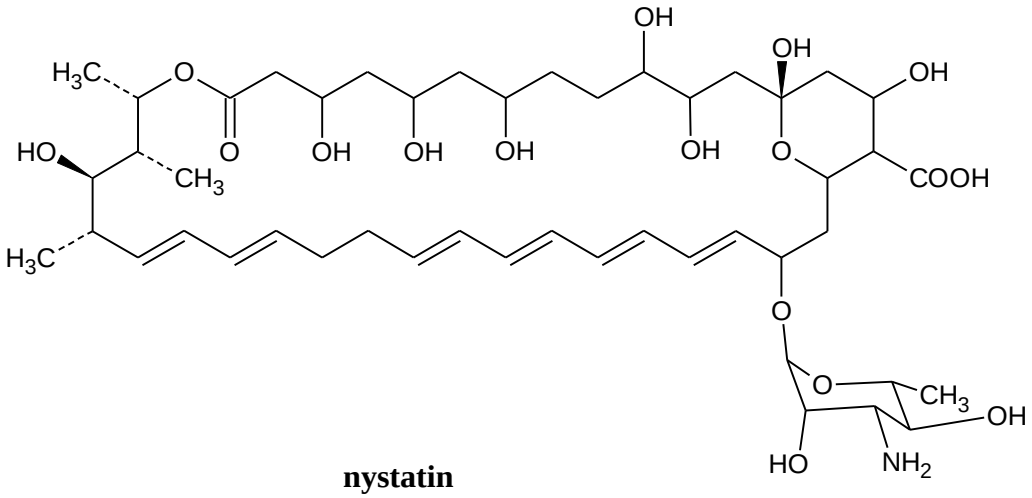
danger of neophrotoxicity that's why the dosage of amphotericin B should be proper [44].

AmBisome is a medicinal product in which AmB is integrated into unilamellar liposomes. The ingredients are supplied lyophilized as a powder and must be reconstituted in water directly before use, producing liposomes with a mean diameter of 60-70 nm. The liposomal material consists if hyfrogenated soy bean lecitine, phosphocholine, cholesterol, and sucrose for an isotonic milieu, with α-tocopherol and disodium succinate hydrate. When AmB containing liposomes come into contact with fungal cells or *Leishmania* organisms, the liposomal matrix is degraded and AmB is set free to bind preferentially to ergosterol in the cell membrane, leading its disintegration [45].



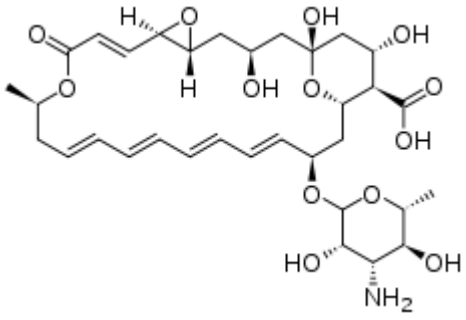
amphotericin B

Nystatin is obtained from *Streptomyces noursei* and is used only locally against candidosis. It can be administered even in form of aerosol [44].



nystatin

Natamycin is obtained from *Streptomyces natalensis* and it is used for local therapy as same as nystatin [44].



natamycin

3.2.2.3 Mechanisms of Resistance

Resistance to AmB is quite rare and is often caused by a decrease in the amount of ergosterol in the plasma membrane leading to a decrease in the binding of AmB. Moreover, some fungal cells have a mutation in their ergosterol biosynthesis pathway such that instead of producing ergosterol they can produce ergosterol-like compounds, for which AmB has lower binding affinity [42].

3.2.3. Azoles

Until the 1940s, relatively few agents were available for the treatment of systemic fungal infections. The development of the polyene antifungals represented a major advance in medical mycology. Although amphotericin B quickly became the mainstay of therapy for serious infections, its use was associated with infusion-related side-effects and dose-limiting nephrotoxicity. The continued search for new and less toxic antifungals led to the discovery of the azoles several decades later. Azole antifungals are divided into the imidazoles (e.g. miconazole and ketoconazole) and the triazoles (e.g. itraconazole, fluconazole, voriconazole) [46].

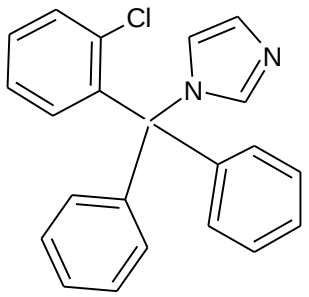
3.2.3.1. Mechanism of action

The azoles inhibit the fungal cytochrome P450 3A (CYP3A) enzyme, lanosterol 14α-demethylase which is responsible for converting lanosterol to ergosterol, the main sterol in fungal cell membrane. The resulting depletion of ergosterol alters the fluidity of the membrane and this interferes with the action of membrane-associated enzymes. The net effect is an inhibition of replication. Azoles also inhibit the transformation of candidal yeast cells into hyphae – the invasive and pathogenic form of the parasite. The depletion of membrane ergosterol reduces the binding sites for amphotericin [47].

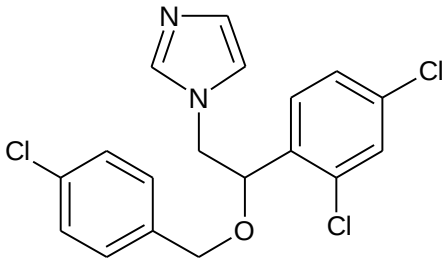
3.2.3.2 Azole antifungals

Azole antifungals have two active groups which contain imidazole or triazole cycle. Imidazole derivatives are mainy used locally. They are used for treatment of infections caused by dermatophytes as *Trichophyton*, *Microsporon* and *Epidermophyton*; they are applicable also to candidosis and infections caused by *Cryptococcus* spp. Imidazole derivatives are administered in the form of creams or solutions. Treatment by itself takes weeks or even months. **Miconazole** is mostly given intravenously or orally for infections of the gastrointestinal tract or also topically [47]. It is not used so much because of tolerance in the body. There are more side effects (dizziness, iritations, even fever) than positive effects [44]. **Clotrimazole, econazole, tioconazole** and **sulconazole** are azole antifungal agents used only for topical application. Clotrimazole interferes

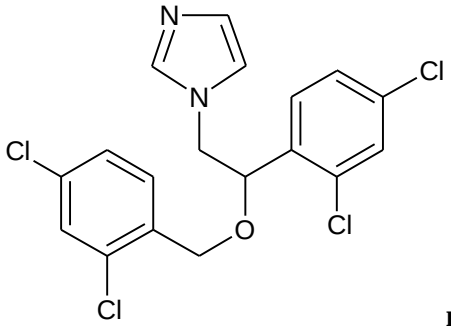
with amino acid transport into the fungus by an action on the cell membrane. It is active against a wide range of fungi, including candidal organisms [47].



clotrimazole

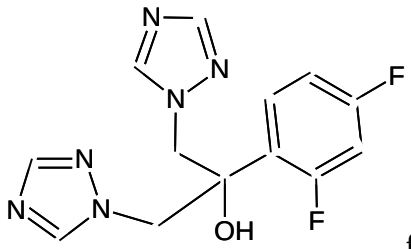


econazole



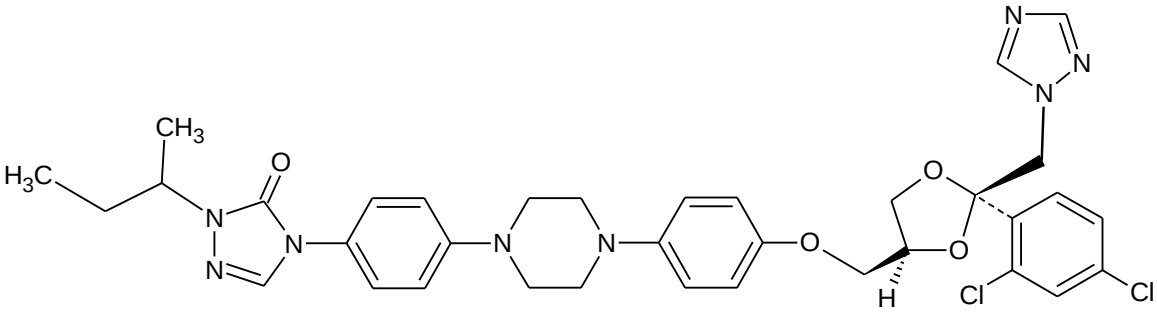
miconazole

Fluconazole has good overall activity against *Candida* spp. and *Cryptococcus neoformans*. However, resistance to the drug is encountered in certain non-albicans *Candida* spp. such as *C. crusei* and some isolates of *C. glabrata*. It is available as oral and intravenous formulations. Fluconazole is commonly given once daily, but can be prescribed twice daily for larger total daily doses.



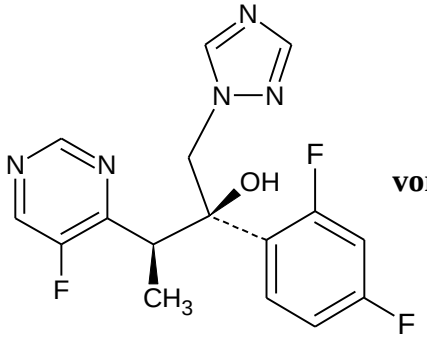
fluconazole

Itraconazole has activity against yeasts and some moulds (including *Aspergillus),* but is disadvantaged by variable bioavailability and an unpleasant taste. Two oral formulations are available: capsule and oral solution. The bioavailability of the capsule form is highly influenced by concomitant food intake, and there is considerable intra- and inter- patient variability in plasma drug concentrations; the solution form has a more favourable pharmacokinetic profile. Itraconazole is usually given twice daily [41].

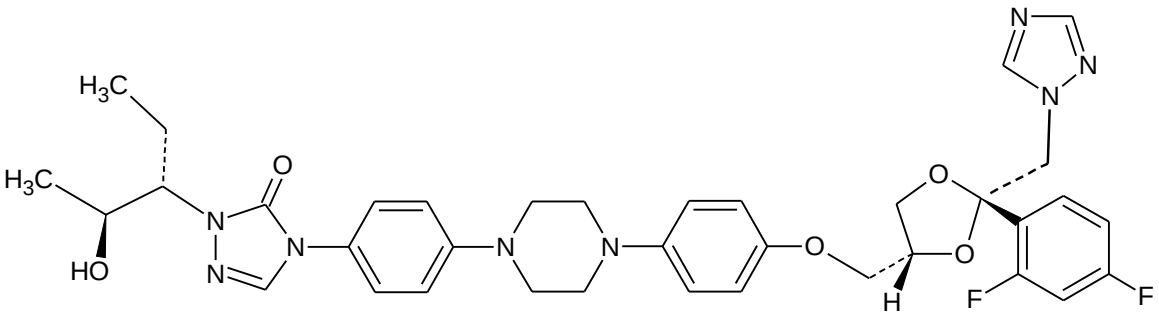


itraconazole

Voriconazole and **posaconazole** have an extended spectrum of activity against yeasts, e.g. *C. neoformans,* and moulds, including *Aspergillus*, *Scedosporium,* and *Fusarium* spp. Voriconazole is active against fluconazole-resistant *Candida* spp. [41].

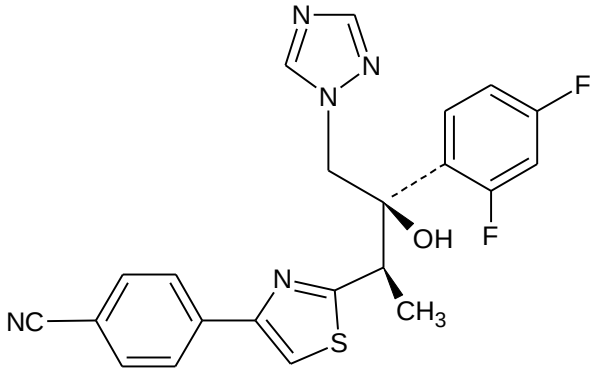


voriconazole



posaconazole

Ravuconazole is under investigation for the treatment of systemic fungal infections [48].



ravuconazole

3.2.3.3 Spectrum of antifungal activity

Azole antifungals have a broad spectrum of antifungal activity. They are active against *Candida* spp., *C. neoformans* and dimorphic fungi. Some azole antifungals are, however, more active than others in different cases. For example, fluconazole is relatively inactive against *C. krusei* as opposed to itraconazole. Azole antifungals are only fungistatic against most yeast species with the exception of *C. neoformans*. Mature *Candida* biofilms exhibit profound resistance to azoles [49].

3.2.3.4 Mechanism of resistance

Different mechanisms have been implicated in the development of MDR, including over expression of genes encoding drug efflux pumps, alteration of membrane sterol composition and overproduction or mutation in the target enzyme of the azoles, lanosterol-14α-demethylase [49].

3.2.4 Allyamines and Thiocarbamates

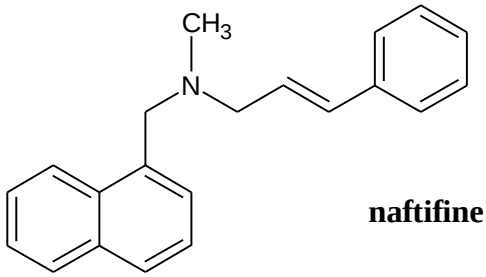
Two classes of synthetic antimycotics, namely allylamines (notably terbinafine and naftifine) and the structurally related thiocarbamates (such as tolnaftate) are mainly used for the treatment of topical infections caused by dermatophytes. The prototype of the allylamines, naftifine, proved to be an efficacious antimycotic for topical aplications in dermatophytes and was the starting point for studies on structure-activity relationships, resulting in the development of terbinafine, an allylamine with considerably enhanced antifungal properties [42].

3.2.4.1 Mechanism of action

Compounds of this class act by selectively inhibiting the enzyme squalene epoxidase which is involved in the synthesis of ergosterol from squalene in the fungal cell wall. The accumulation of squalene within the cell is toxic to the organism [47].

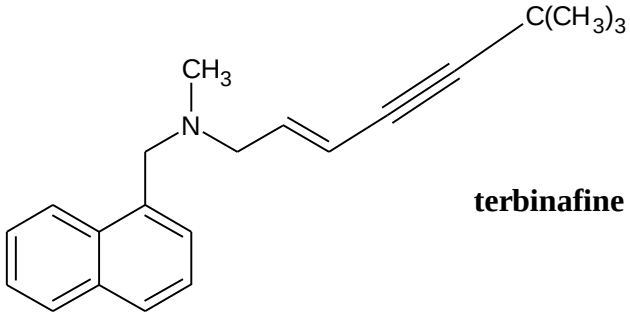
3.2.4.2 Antifungals

Naftifine is a topical allylamine that is effective and safe in the management of superficial dermatomycoses. It is moderately active in vitro against moulds, but is generally less active against yeasts, including *Candida albicans*. However, it has proved reasonably effective in the treatment of patients with cutaneous candidiasis [50].



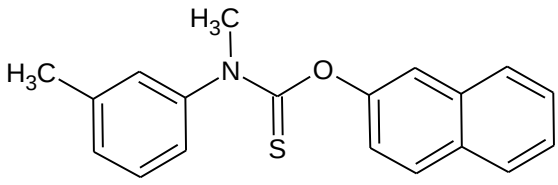
naftifine

Terbinafine is the first orally active compound in the allylamine family. It has fungicidal activity against dermatophytes, moulds and certain dimorphic fungi, and fungistatic activity against *Candida albicans*. Following oral administration the terbinafine is absorbed rapidly [51, 52].



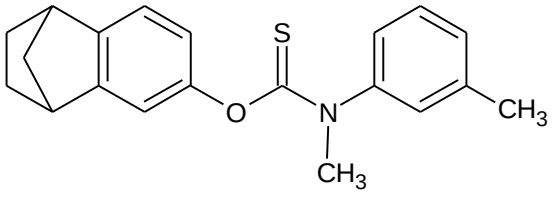
terbinafine

Tolnaftate is a thiocarbamate that is active only against growing dermatophytes. The mode of action is the inhibition of squalene epoxidase. It is used topically in the treatment or prophylaxis of superficial dermatophyte infections and of pityriasis versicolor [52].

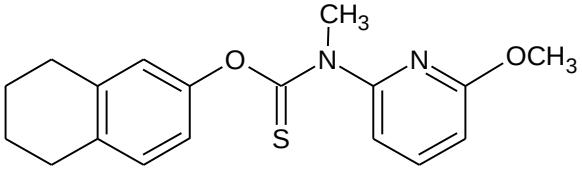


tolnaftate

Another thiocarbamate derivative is **tolciclate** which is used for skin mycoses (tinea corporis, tinea cruris, tinea pedis, tinea manuum and pityriasis versicolors versicolor) [53, 54]. Also **liranaftate** is an efficacious agent for the treatment of dermatophytes. It has been developed in Japan [55].



tolciclate



liranaftate

3.2.4.3 Spectrum of antifungal activity

Terbinafine is mainly active on dermatophytes, including *Trichophyton rubrum*, *T. verrucosum, Microsporum canis* and *Epidermophyton floccosum*. Terbinafine also has in vitro activity against most *Candida* spp., *Aspergillus* spp., *Penicillium marneffei*, *Malassezia furfur, Cryptococcus neoformans, Trichosporon* spp. and *Blastoschizomyces* spp. The use of tolnaftate is essentially restricted to dermatophyte infections such as *M. canis* and *T. mentagrophytes* [30].

3.2.4.4 Mechanism of resistance

Resistance to allylamines has been reported only rarely. However, the potential to develop resistance by the action of multidrug efflux transporters does exist. Resistance to thiocarbamates has not been extensively studied. A study on the mode of

action of tolnaftate in *M. gypseum* revealed a higher content of total phospholipids in tolnaftate-resistant cells compared to wild type cells [42].

3.2.5 Echinocandins

Echinocandins were discovered in 1970s and are inhibitors of the cell wall formation. They are derivatives of fatty acids and cyclic hexapeptides. The various echinocandins differ in having different substituents in the hexapeptide ring or a distinct fatty acid chain [56–59].

3.2.5.1 Mechanism of action

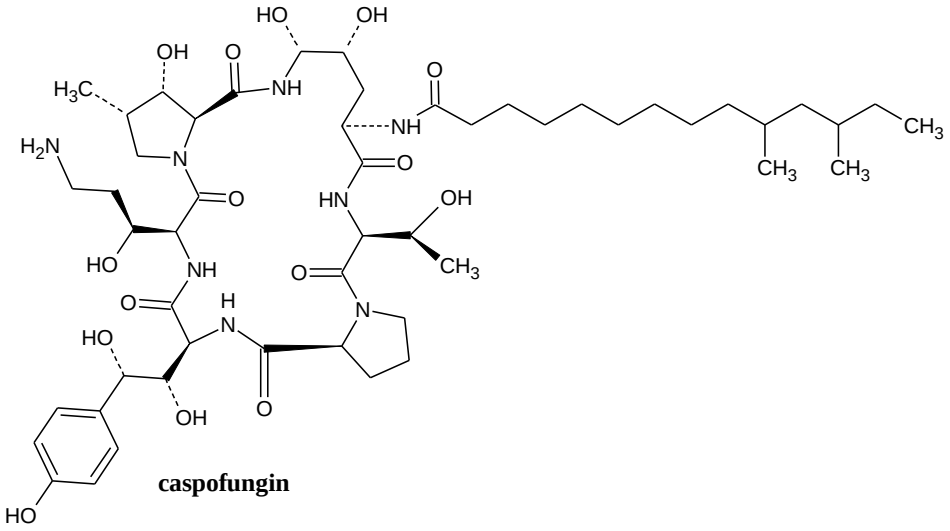
Echinocandins are secondary metabolites comprising a cyclic hexapeptide core with a lipid side chain responsible for antifungal activity. The target for echinocandins is the complex of proteins responsible for synthesis of cell wall β-1,3-glucan polysaccharides. The target of echinocandins does not exist in mammalian cells, so their toxicity is minimal. The first of the class to be licensed was caspofungin for refractory invasive aspergillosis and the second was micafungin. [56–59].

3.2.5.2 Echinocandin antifungals

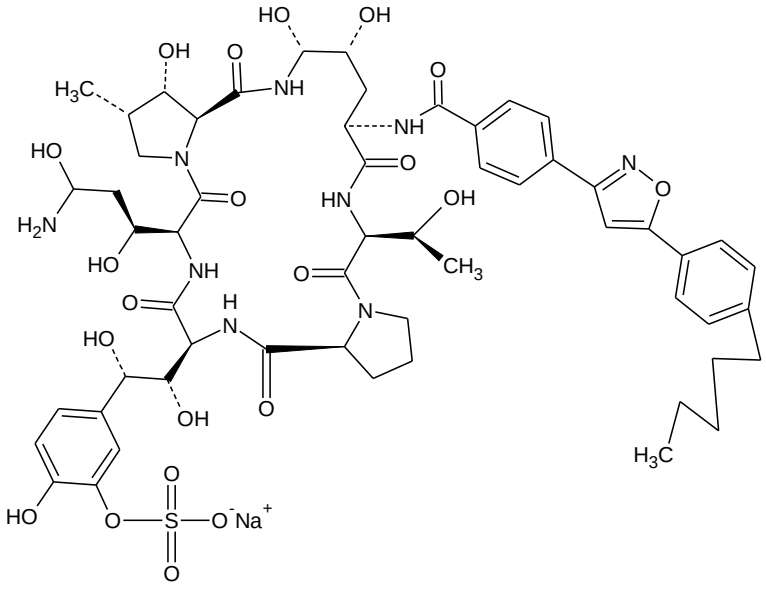
Caspofungin was approved by the FDA in 2001. It is highly active in vitro against most isolates of *Candida* spp. It also has in vitro inhibitory activity against Aspergillus spp nad moderaze activity against some other moulds. It is also active against *Pneumocystis carinii*. In contrast, it has no activity against *Cryptococcus neoformans*, *Trichosporon* spp., *Sporothrix schenckii*, zygomycetes and hyalohyphomycetes. Caspofungin appears to be a suitable antifungal agent in patients with oesophageal candidiasis refractory to fluconazole [58].

Micafungin became available in 2005. It was approved for the treatment of oesophageal candidiasis as well as prophylaxis in patients undergoing stem cell transplantation [58].

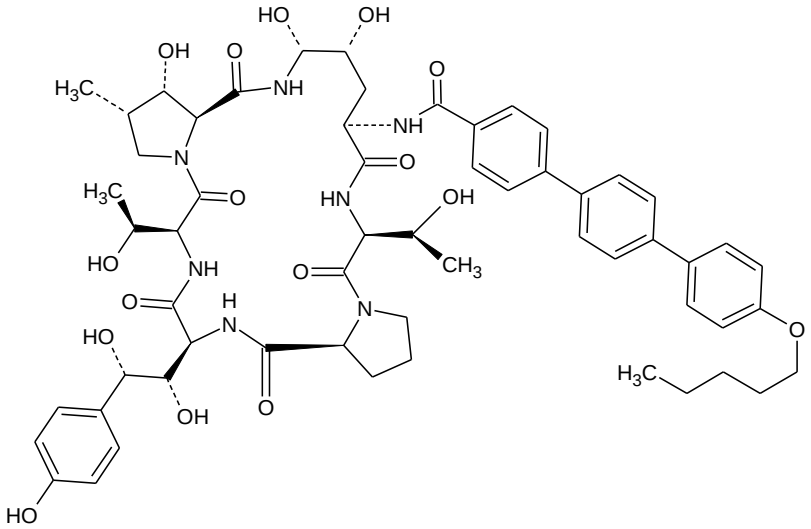
Anidulafungin was approved in 2006 for use in the treatment of oesophageal candidiasis, candidaemia, peritonitis and untra-abdominal abscesses due to *Candida* spp. It slowly degrades in humans, it has a half-live of approximately 25 hours [58].



caspofungin



micafungin



anidulafungin

3.2.5.3 Spectrum of antifungal activity

In vitro and in vivo, echinocandins are rapidly fungicidal against most *Candida* spp. and fungistatic against *Aspergillus* spp. Echinocandins are not active at clinically relevant concentrations against *C. neoformans or Fusarium* spp. Echinocandins show activity against Candida biofilms, which exhibit resistance to other antifungal agents such as azoles [42].

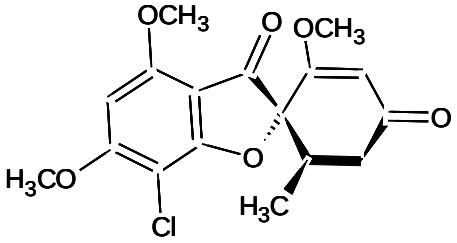
3.2.5.4 Mechanism of resistance

The resistance to echinocandins is very rare [42]. However, as patient exposure to echinocandin drugs broadens, the number of infecting strains with reduced susceptibility is expected to rise. Although a prospective global surveillance study (2001–2006) did not observe echinocandin resistance in invasive candidiasis isolates, cases of breakthrough mycoses during echinocandin use or of echinocandin-resistant isolates are being increasingly reported [60, 61]. Unfortunately, the relationship between reduced *in vitro* susceptibility to echinocandin drugs and clinical failure is ambiguous The (1,3)-β-glucan synthase is a multi-subunit enzyme complex responsible for fungal cell wall construction and division septum deposition. The enzyme catalyses the transfer of sugar moieties from activated donor molecules to specific acceptor molecules forming

glycosidic bonds in the reaction UDP-glucose + {(1,3)-β-D-glucosyl}(N) → UDP + {(1,3)-β-D-glucosyl}(N+1). . The UDP glycosyltransferases are one group of enzymes that carry out this reaction and over 100 members of this protein family are known. The enzyme complex has a minimum of two subunits, Fks1 and Rho. Fks1 appears to be the catalytic subunit. Recent work has shown that spontaneous mutations can arise in two hot spot regions of Fks1 that reduce the enzyme's sensitivity to the drug. However, other strains have been isolated in which the sequence of Fks1 is unaltered yet the fungus has decreased sensitivity to echinocandins. In addition it has been shown that echinocandin-treatment can induce cell wall salvage mechanisms that result in the compensatory upregulation of chitin synthesis in the cell wall. This salvage mechanism strengthens cell walls damaged by exposure to echinocandins. Therefore, fungal resistance to echinocandins can arise due to the selection of either stable mutational or reversible physiological alterations that decrease susceptibility to these antifungal agents [62].

3.2.6 Griseofulvin

The earliest inhibitory agent specific to fungal species was griseofulvin. The precise mechanism of action of this compound is still unknown, but the favoured explanation is that it interferes with microtubule assembly. Its spectrum of action is restricted mainly to the dermatophyte, i. e. fungi causing ringworm and athlete's foot [56, 63].

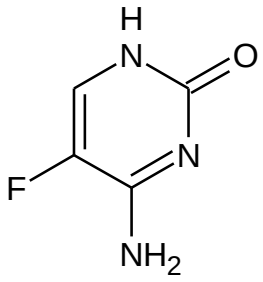


griseofulvin

3.2.7 Flucytosine

Flucytosine works as an antifungal agent through conversion to 5-fluorouracil within the target cells. Fluorouracil becomes incorporated into RNA, causing premature

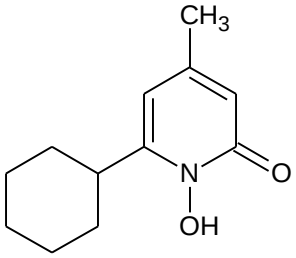
chain termination, and it inhibits DNA synthesis through effects on thymidylate synthase. The useful spectrum of flucytosine is restricted to pathogenic yeasts such as *Candida* spp. and *C. neoformans.* Flucytosine is used as adjunctive, rather than primary therapy, in the clinic, because primary and secondary resistance may occur at a high frequency [56].



flucytosine

3.2.8 Ciclopirox

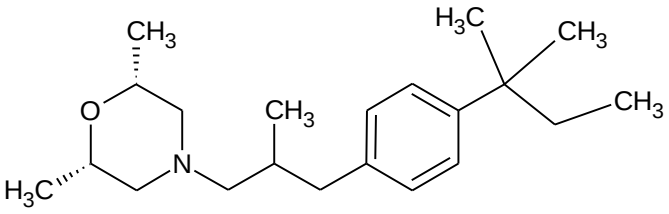
Ciclopirox is a member of the hydroxypyridine family. It is believed to work by inhibiting metal-dependent enzymes by chelating the polyvalent cations (Fe^{3+} or Al^{3+}). This affects intracellular energy production and toxic peroxide degradation. It also inhibits fungal nutrient uptake, resulting in decreased nucleotides and a reduction in protein synthesis [64, 65]. **Ciclopirox** 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. It has also been used in the treatment of vaginal candidiasis. It is applied twice daily for skin infections, as a cream, gel, suspension, solution, or powder; both the base and the olamine salt have been used. A lacquer containing 8% ciclopirox base is applied once daily for nail infections. A shampoo containing 1% ciclopirox base is used twice weekly for the treatment of seborrhoeic dermatitis [66].



ciclopirox

3.2.9 Amorolfine

Amorolfine is another type of local antimycotics. It is a morfoline derivate, which inhibits synthesis of sterols in fungi. Amorolfine has wide spectrum of action against dermatophytes, various filamentous and dematiaceous fungi, yeasts and dimorphic fungi. Its activity is fungicidal for most species [64]. It is applied topically as the hydrochloride in the treatment of fungal nail and skin infections For the treatment of nail infections caused by dermatophytes, yeasts, and moulds a lacquer containing the equivalent of 5% amorolfine is painted onto the affected nail once or sometimes twice weekly until the nail has regenerated. Treatment generally needs to be continued for 6 to 12 months. For skin infections a cream containing the equivalent of 0.25% amorolfine is applied once daily for at least 2 to 3 weeks (up to 6 weeks for foot infections) and continued for 3 to 5 days after clinical cure is achieved [66].



amorfoline

4. EXPERIMENTAL PART

Commercially available substances were used for the preparation of (5*Z*)-5-arylmethylidene-2-thioxo-1,3-thiazolidin-4-ones

- benzaldehyde, rein (FERAK Laborat, Berlin, Germany)
- 4-nitrobenzaldehyde (Lachema, Neratovice, Czech Republic)
- 4-hydroxybenzaldehyde zur Synthese (Merck, Darmstadt, Germany)
- rhodanine, puriss. p. a. (Fluka, International)

For the preparation of Mannich bases, formaldehyde solution, 37% (Sigma, International), diethylamine, purum (Fluka, International) and morpholine, p. a. (International Enzymes, Windsor, Berks., UK) were used.

TLC was performed on TLC aluminium sheets, Silica gel 60 F_{254} (Merck, Darmstadt, Germany). Light petroleum + ethyl acetate 80:20 (v/v) and 60:40 were used as a mobile phases.

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

Melting points were determined using Boëtius apparatus HMK (VEB Analytik, Dresden, Germany) and are uncorrected.

IR spectra were recorded using the spectrophotometer NICOLET 6700. Wavenumbers are given in cm^{-1}.

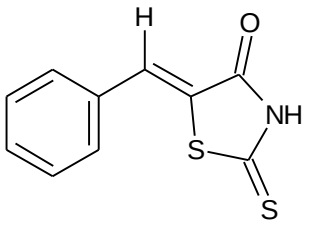
^{1}H-NMR and ^{13}C-NMR spectra were recorded with the VARIAN Mercury-V_xBB 300. Chemical shifts are given in δ, ppm and interaction constants *J* in Hz.

4.1 Preparation of (5*Z*)-5-arylmethylidene-2-thioxo-1,3-thiazolidin-4-ones

4.1.1 General Procedure

An equimolar amount of the aldehyde and rhodanine (0.015 mol) was heated under reflux condenser with ethanol (15 ml) and concentrated ammonia solution (1.1 ml) 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 glass, washed with distilled water (50 ml) and then with 50% ethanol (50 ml). The product was crystallized from anhydrous ethanol.

4.1.1.1 (5*Z*)-5-Benzylidene-2-thioxo-1,3-thiazolidin-4-one

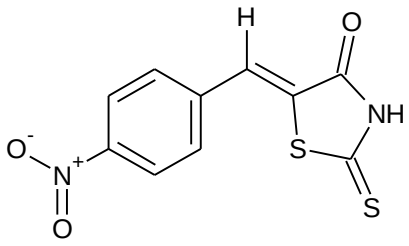


Yield: 86%

Yellow crystalline compound

M. p. 205 – 207 °C (206 – 207 °C [15])

4.1.1.2 (5*Z*)-5-(4-Nitrobenzylidene)-2-thioxo-1,3-thiazolidin-4-one

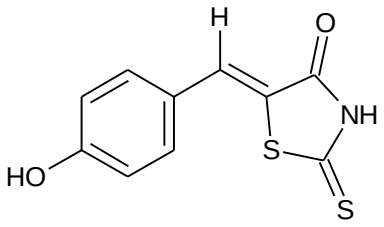


Yield: 82%

Orange crystalline compound

M. p. 273 – 274 °C (272 – 276 °C [16])

4.1.1.3 (5*Z*)-5-(4-Hydroxybenzylidene)-2-thioxo-1,3-thiazolidin-4-one

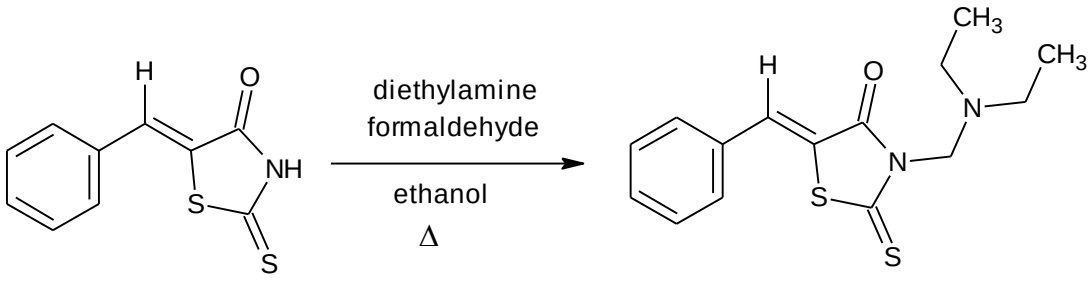


Yield: 84%

Orange crystalline compound

M. p. 285 – 290 °C (294 – 295 °C [15])

4.2 Preparation of (5*Z*)-5-benzylidene-3-[(diethylamino)-methyl]-2-thioxo-1,3-thiazolidin-4-one I.



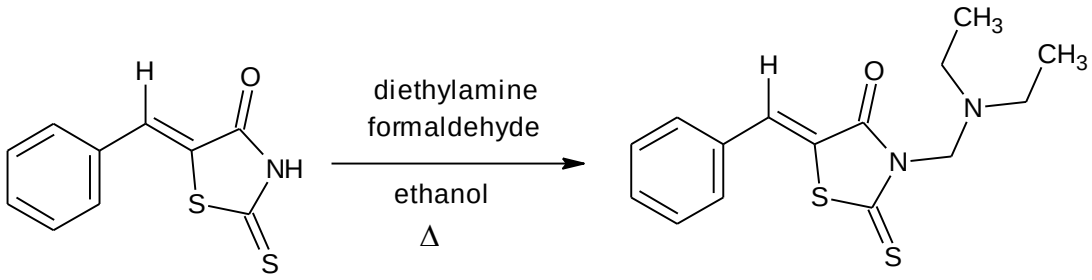
(5*Z*)-5-Benzylidene-2-thioxo-1,3-thiazolidin-4-one (2.21 g, 0.01 mol) was suspended in hot ethanol (30 ml) and formaldehyde solution (37%, 2 ml) was added, followed by diethylamine (0.73 g, 0.01 mol). The reaction mixture was heated on a glycerin bath for 15 minutes under stirring and left overnight at room temperature. Since no solid separated under these conditions, the solution was refrigerated for several days. Only a very small amount of a yellow compound precipitated. Approximately half of ethanol was evaporated under reduced pressure. After cooling, 0.94 g of yellow powder was obtained. Crystallization from the anhydrous ethanol then yielded 0.43 g of yellow, finely crystalline substance. The substance was identified to be starting (5*Z*)-5-benzylidene-2-thioxo-1,3-thiazolidin-4-one.

R_f = 0.43 (light petroleum + ethyl acetate 80:20)

M. p. = 205 – 210 °C (206 – 207 °C [15])

Chromatography of combined mother liquors yielded 0.73 g of yellow substance, R_f of which corresponded to starting (5*Z*)-5-benzylidene-2-thioxo-1,3-thiazolidin-4-one, and several smaller fractions. Based on NMR results, none of these fractions contained (*Z*)-5-benzylidene-3-[(dimethylamino)methyl]-2-thioxothiazolidin-4 one.

4.3 Preparation of (5*Z*)-5-benzylidene-3-[(diethylamino)-methyl]-2-thioxo-1,3-thiazolidin-4-one II.



(5*Z*)-5-benzylidene-2-thioxo-1,3-thiazolidin-4-one (2.21 g, 0.01 mol) was suspended in hot ethanol (30 ml) and formaldehyde solution (37%, 2 ml) was added, followed by diethylamine (0.73 g, 0.01 mol). The reaction mixture was heated on a glycerin bath for 10 hours under stirring and left overnight at room temperature. Since no solid separated under these conditions, the solution was refrigerated for several days. A small amount of a solid precipitated. The solid was filtered off, washed with a small amount of cold ethanol and dried. According to NMR, it was not the required Mannich base.

Mother liquors contained a mixture of compounds that were separated using column chromatography.

Chromatographic conditions:

Silica gel 60, 0.043 – 0.060 mm, Merck – 100 g

Volume of a fraction – 25 ml

TLC for all fractions performed in light petroleum + ethyl acetate 80:20

Fractions	**Mobile phase**	**R_f**	**Weight (g)**	**Characterization**
1 – 7	light petroleum + ethyl acetate 80:20	-----	-----	returned to the column

8 – 11	light petroleum + ethyl acetate 80:20	0.73	0.11	reddish-brown oil; it does not contain the required Mannich (based on NMR results)
12 – 13	light petroleum + ethyl acetate 80:20	0.48	0.02	yellow oil, it does not contain the required Mannich (based on NMR results)
14 – 25	light petroleum + ethyl acetate 80:20	0.63	1.66	Yellow powder; a mixture of compounds in which starting (5*Z*)-5-benzylidene-2-thioxo-1,3-thiazolidin-4-one prevails;
26 – 37	light petroleum + ethyl acetate 60:40	**0.43** 0.25		
37 – 56	light petroleum + ethyl acetate 60:40	0.43 0.10	0.18	yellow oil; it does not contain the required Mannich (based on NMR results)
57 – 100	methanol	0.00	0.10	semisolid substance

Crystallization of combined fractions 14 – 37

The crude yellow substance was crystallized from the anhydrous ethanol. The crystallization yielded 0.48 g of yellow, finely crystalline substance that was identified to be starting (5*Z*)-5-benzylidene-2-thioxo-1,3-thiazolidin-4-one.

R_f = 0.43 (light petroleum + ethyl acetate 80:20)

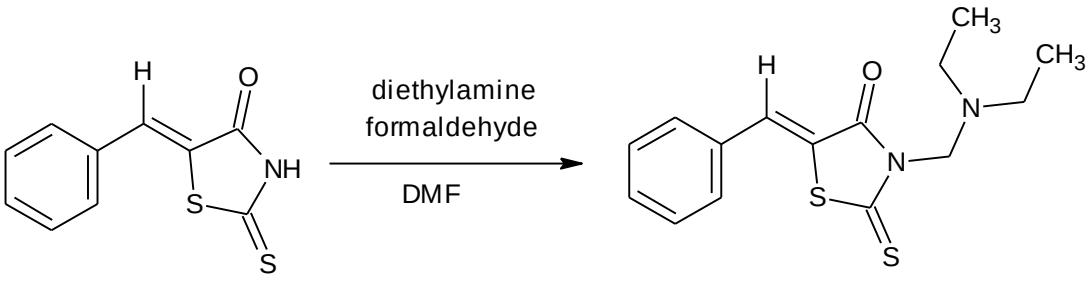
M. p. = 205 – 207 °C (206 – 207 °C [15]).

Crystallization of combined fractions 57 – 100

The semisolid substance was refluxed with 75 ml of anhydrous ethanol, but it did not dissolved completely. After cooling, the solid was filtered off and dried. 0.06 g of an off-white substance melting at 155 – 165 °C.

Unfortunately, NMR of the compound did not correspond to the structure of the required Mannich base.

4.4 Preparation of (5*Z*)-5-benzylidene-3-[(diethylamino)-methyl]-2-thioxo-1,3-thiazolidin-4-one III.

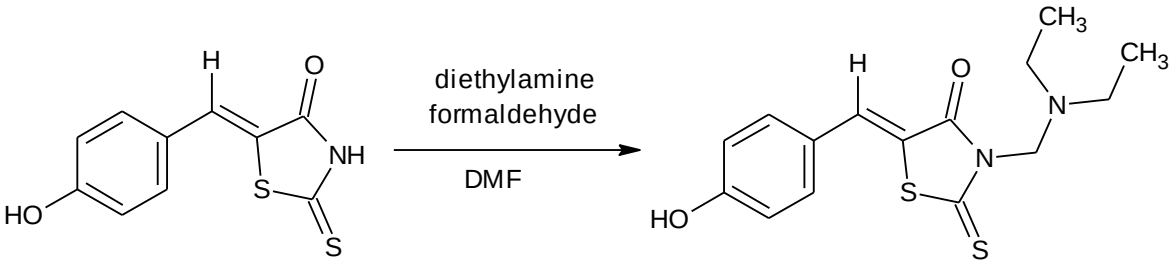


(5*Z*)-5-benzylidene-2-thioxo-1,3-thiazolidin-4-one (2.21 g, 0.01 mol) was suspended in dimethylformamide (30 ml) and diethylamine (0.73 g, 0.01 mol) was added, followed by formaldehyde solution (37%, 1.1 ml). The resulting mixture was stirred for 11 hours at room temperature. Then it was poured into cold water (50 ml) and refrigerated overnight. The separated solid was filtered off and dried. A yellow substance (0.19 g) was obtained. According to TLC, it was a mixture of the starting (5*Z*)-5-benzylidene-2-thioxo-1,3-thiazolidin-4-one (major zone) and another substance (minor zone). Crystallization from anhydrous ethanol gave 0.06 g of a yellow powder that was identified to be starting (5*Z*)-5-benzylidene-2-thioxo-1,3-thiazolidin-4-one.

R_f = 0.43 (light petroleum + ethyl acetate 80:20)

M.p = 205 – 209 °C (206 – 207 °C [15]).

4.5 Preparation (5*Z*)-3-[(diethylamino)methyl]-5-(4-hydroxy-benzylidene)-2-thioxo-1,3-thiazolidin-4-one



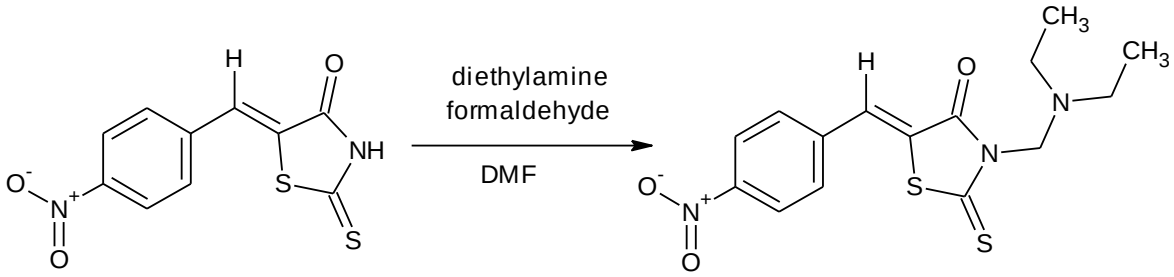
(5*Z*)-5-(4-Hydroxybenzylidene)-2-thioxo-1,3-thiazolidin-4-one (1.19 g, 5 mmol) was suspended in dimethylformamide (15 ml) and diethylamine (0.37 g, 5 mmol) was then added, followed by formaldehyde solution (37%, 0.55 ml). The resulting mixture was stirred for 11 hours at room temperature. Then it was poured into cold water (25 ml) and refrigerated overnight. The separated solid was filtered off and subjected to column chromatography using silica gel 60, 0.043 – 0.060 mm (Merck) as the adsorbent and light petroleum + ethyl acetate 60:40 as the eluent. Volume of a fraction was 20 ml.

Crystalization of fractions 17 – 23 gave 0.23 g of an orange compound that was identified to be starting (5*Z*)-5-(4-hydroxybenzylidene)-2-thioxo-1,3-thiazolidin-4-one.

R_f = 0.40 (light petroleum + ethyl acetate 60:40)

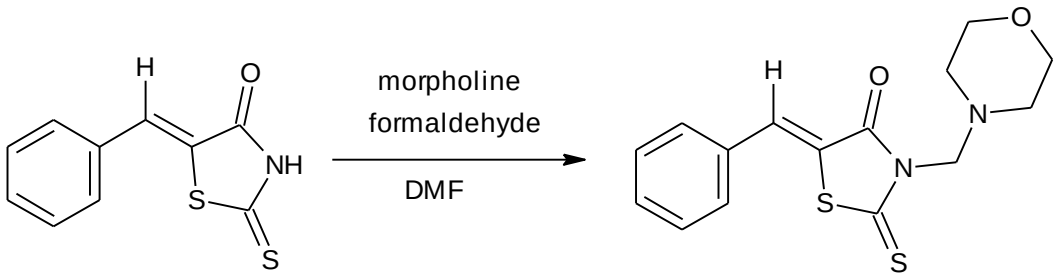
M.p = 288 – 295 °C (294 – 295 °C [15]).

4.6 Preparation (5*Z*)-3-[(diethylamino)methyl]-5-(4-nitro-benzylidene)-2-thioxo-1,3-thiazolidin-4-one



(5*Z*)-5-(4-Nitrobenzylidene)-2-thioxo-1,3-thiazolidin-4-one (3.51 g, 0.01 mol) was suspended in dimethylformamide (30 ml) and diethylamine (0.73 g, 0.01 mol) was added, followed by formaldehyde solution (37%, 1.1 ml). The resulting mixture was stirred for 11 hours at room temperature. Then it was poured into cold water (50 ml) and refrigerated overnight. Orange-brown substance (3.43 g) was obtained after filtration and drying. According to TLC, it was a mixture of the starting (5*Z*)-5-(4-nitrobenzylidene)-2-thioxo-1,3-thiazolidin-4-one (major zone) and some other compounds. Crystallization from anhydrous ethanol did not yield an identifiable compound.

4.7 Preparation of (5*Z*)-5-benzylidene-3-(morpholin-4-yl-methyl)-2-thioxo-1,3-thiazolidin-4-one



(5*Z*)-5-benzylidene-2-thioxo-1,3-thiazolidin-4-one (2.21 g, 0.01 mol) was suspended in dimethylformamide (30 ml) and morpholine (0.87 g, 0.01 mol) was added, followed by formaldehyde solution (37%, 1.1 ml). The mixture was stirred for 11 hours at room temperature. Then it was poured into cold water (50 ml) and refrigerated overnight. The separated solid was filtered off and dried. Yellow crystals (4.54 g) were obtained. Crystallization from anhydrous ethanol gave 2.88 g (90%) of yellow powder that was identified as the required Mannich base.

Molecular weight: 320.43 ($C_{15}H_{16}N_2O_2S_2$)

Melting point: 120 – 125 °C

IR spectrum (ATC, cm^{-1}): 2949, 2850 (CH) 1704 (C=O)

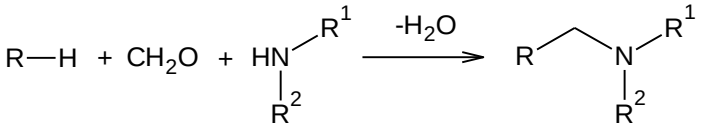
^{1}H NMR (300 MHz, DMSO) δ 7.75 (1H, s, CH), 7.66 – 7.59 (2H, m, H2′, H6′), 7.59 – 7.48 (3H, m, H3′, H4′, H5′), 4.90 (2H, s, NCH_2), 3.51 (4H, t, J = 4.6, OCH_2), 2.64 (4H, t, J = 4.6 Hz, NCH_2)

^{13}C NMR (75 MHz, DMSO) δ 196.1, 168.9, 133.3, 132.6, 131.0, 130.8, 130.8, 129.7, 66.2, 65.4, 51.5

5. DISCUSSION

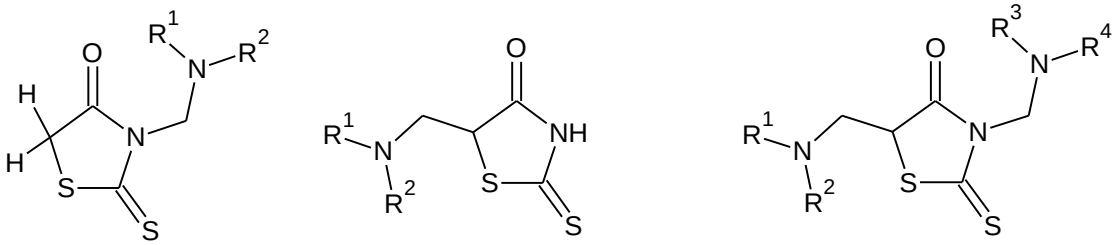
The first Mannich reaction was made in 1912 by Carl Mannich by reaction of salicylantipyrine and hexamethylenetetramine Mannich published more than 60 papers on this topic, one fifth of his wide scientific production, which ended with his death, in 1947. Since then, Mannich bases have been subjects of growing interest [67].

The Mannich reaction is a three-component condensation in which a compound containing an active hydrogen atom (substrate) is allowed to react with formaldehyde and an NH-amine derivative



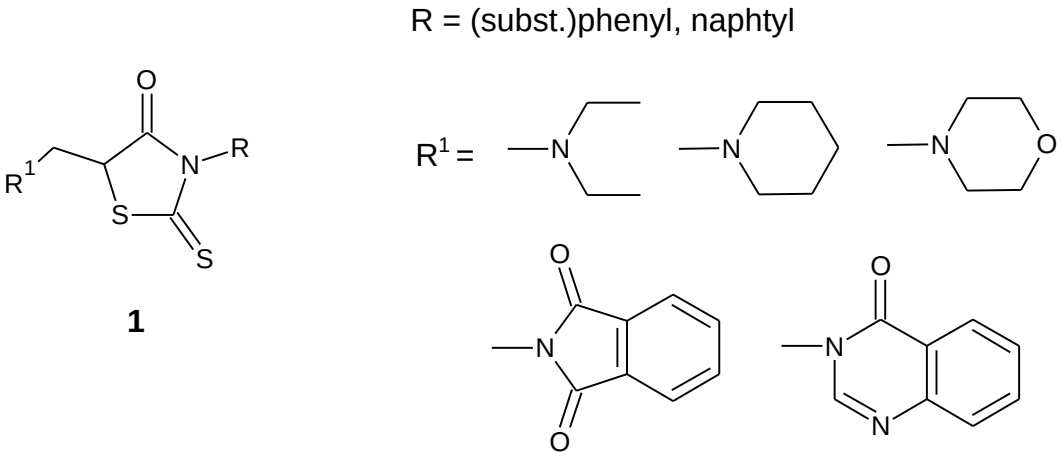
The resulting product (the Mannich base) is an amine compound having the *N*-atom linked to the R-substrate through methylene group. The substrate may belong to a number of different classes of compounds [67].

Unsubstituted rhodanine can react with formaldehyde in either position 3 or position 5 or both forming theoretically three types of Manich bases



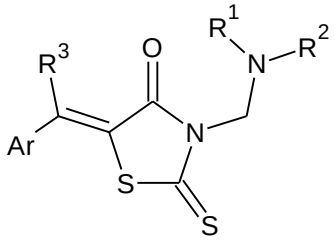
Mannich bases of type A were reported by Indian researchers [68], whilst Mannich bases of type B and C have not been reported so far.

Mannich bases of 3-substituted rhodanines of general formula **1** were studied by Das et al. [69].



1

The most common type of 5-substituted rhodanines are (5*Z*)-5-arylalkylidene-2-thioxo-1,3-thiazolidin-4-ones that form Mannich bases of general formula **2** [28, 29, 70–78].



2

The aim of my diploma thesis was to find suitable reaction conditions for Mannich reaction of (5*Z*)-5-(subst.)benzylidenes-2-thioxo-1,3-thiazolidin-4-ones with various amines. To manage to produce some Mannich reaction I have worked with benzylidenerhodanines which were reacting with the compounds necessary for making Mannich base.

I have first attempted to prepare (5*Z*)-5-benzylidene-3-[(diethylamino)-methyl]-2-thioxo-1,3-thiazolidin-4-one using the method reported by Sen Gupta et al. [28] who used ethanol as the reaction solvent, but only unreacted (5*Z*)-5-benzylidene-2-thioxo-1,3-thiazolidin-4-one was obtained (part. 4.2).

The second experiment (part 4.3) was made after the same authors but the reaction mixture was heated on a glycerin bath for 10 hours. However, this modification did not resulted in the required product either.

The 3rd experiment according to Gaikwad and Gautam [77] where (5*Z*)-5-benzylidene-2-thioxo-1,3-thiazolidin-4-one was suspended in dimethylformamide (part 4.4) was unsuccessful, and only unreacted (5*Z*)-5-benzylidene-2-thioxo-1,3-thiazolidin-4-one was obtained.

Similar results were then achieved in the experiment 4.5 with (5*Z*)-5-(4-hydroxybenzylidene)-2-thioxo-1,3-thiazolidin-4-one.

The reaction mixture from the experiment 4.6. was not chromatographically separated due to the lack of time. Nonetheless, it can be expected that only starting (5*Z*)-5-(4-nitrobenzylidene)-2-thioxo-1,3-thiazolidin-4-one would be obtained.

These results are rather surprising since (5*Z*)-5-benzylidene-3-[(diethylamino)-methyl]-2-thioxo-1,3-thiazolidin-4-one was previously described by Das et al. [68] and by Gaikwad and Gautam [77]. (5*Z*)-3-[(Diethylamino)methyl]-5-(4-nitrobenzylidene)-2-thioxo-1,3-thiazolidin-4-one was prepared by Shukla and co-workers [72]. Only (5*Z*)-3-[(diethylamino)methyl]-5-(4-hydroxybenzylidene)-2-thioxo-1,3-thiazolidin-4-one has not been reported in literature so far.

In the last experiment, (5*Z*)-5-benzylidene-2-thioxo-1,3-thiazolidin-4-one was treated with morpholine and formaldehyde, and the required (5*Z*)-5-benzylidene-3-(morpholin-4-yl-methyl)-2-thioxo-1,3-thiazolidin-4-one was successfully obtained. Its identity was corroborated with IR and NMR results. The product was also submitted to elemental analysis to determine its purity, but the results have not yet been at my disposal. This compound was reported previously [29, 68] and exhibited antibacterial activity and acceptable toxicity [79]. Hence, Mannich bases derived from (5*Z*)-5-arylalkylidene-2-thioxo-1,3-thiazolidin-4-ones deserve further attention as potential antimicrobial agents.

6. CONCLUSIONS

The aim of my diploma thesis was to find suitable reaction conditions for Mannich reaction of (5*Z*)-5-(subst.)benzylidenes-2-thioxo-1,3-thiazolidin-4-ones with various amines.

The attempts to prepare Mannich bases using diethylamine as the basic component were unsuccessful.

(5*Z*)-5-benzylidene-3-(morpholin-4-yl-methyl)-2-thioxo-1,3-thiazolidin-4-one was obtained by treating (5*Z*)-5-benzylidene-2-thioxo-1,3-thiazolidin-4-one with formaldehyde and morpholine in dimethylformamide.

Based on the data obtained from literature, Mannich bases derived from (5*Z*)-5-arylalkylidene-2-thioxo-1,3-thiazolidin-4-ones deserve further attention as potential antimicrobial agents.

7. REFERENCES

1. TOMAŠIĆ, T., MAŠIČ, L. P. Rhodanine as a privileged scaffold in drug discovery. *Curr. Med. Chem.* **2009**, *16*, 1596–1629.
2. BROWN, F. C., BRADSHER, C. K. Mildew-preventing activity of rhodanine derivatives. *Nature.* **1951**, *168*, 171.
3. BROWN, F. C., BRADSHER, C. K., BOND, S. M. et al. Mildew-preventing activity of rhodanine derivatives. *Ind. Eng. Chem.* **1954**, *46*, 1508–1012.
4. ALLAN, F. J., ALLAN, G. G., Mc´NEIL, C. M. F. Mothicidal rhodanine derivatives. *Nature.* **1959**, *184*, 1637.
5. ALLAN, F. J., ALLAN, G. G., CRANK, G. Fungitoxicity of rhodanine derivatives. *J. Appl. Chem*. **1961**, *11*, 68–70.
6. MATOLCSY, G., BORDÁS, B., HAMRÁN, M. Studies on antimicrobial action of compounds containing 1-oxo-2-methylene, 1,2-dioxo and derived groupings. III. Derivatives of isoxazol-5-one, thiohydantoin, pseudothiohydantoin, and rhodanine. *Acta Phytopathol. Acad. Sci. Hung.* **1969**, *4*, 345 –351.
7. MALLICK, S. K., MARTIN, A. R., LINGARD, R. G. Synthesis and antimicrobial evaluation of some 5-(5-nitrofurylidene)rhodanines, 5-(5-nitrofurylidene)-thiazolidine-2,4-diones, and their vinylogs. *J. Med. Chem.* **1971**, *14*, 528–532.
8. GUPTA, S. P., RANI, S. Synthesis and fungitoxicity of some 5-substituted-3-polynitrophenyl rhodanines. *J. Indian. Chem. Soc.* **1978**, *55*, 483–485.
9. MITRA, P., MITRA, A. S. Heterocyclic fungicides. Part IV. *J. Indian. Chem. Soc.* **1984**, *61*, 77–78.
10. SREE LAKSHMI, V. Antimicrobial effects of *o*-nitrobenzaldene rhodanine. *Egypt J. Microbiol*. **1989**, *24*, 247–250.
11. INAMORI, Y, OKAMOTO, Y. TAKEGAWA, Y. et al. Insecticidal and antifungal activities of aminorhodanine derivatives. *Biosci. Biotechnol. Biochem.* **1998**, *62*, 1025–1027.

12. ORCHARD, M. G., NEUSS, J. C., GALLEY, C. M. et al. Rhodanine-3-acetic acid derivatives as inhibitors of fungal protein mannosyl transferase 1 (PMT1). *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3975–3978.
13. SORTINO, M., DELGADO, P., JUÁREZ, S. et al. Synthesis and antifungal activity of (*Z*)-5-arylidenerhodanines. *Bioorg. Med. Chem.* **2007**, *15*, 484–494.
14. DOLEŽEL, J. *Kondenzační produkty acetylpyrazinů s rhodaninem jako potenciální léčiva* (*Condensation Products of Acetylpyrazines with Rhodanine as Potential Drugs*). Diploma thesis. Hradec Králové: Charles University, Faculty of Pharmacy, **2004**.
15. ŠPANINGEROVÁ, E. *Kondenzační produkty aldehydů s rhodaninem jako potenciální léčiva* (*Condensation Products of Aldehydes with Rhodanine as Potential Drugs*). Diploma thesis. Hradec Králové: Charles University, Faculty of Pharmacy, **2006**.
16. KEŠETOVIČOVÁ, D. *Deriváty rhodaninu jako potenciální léčiva I* (*Derivatives of Rhodanine as Potential Drugs I*). Diploma thesis. Hradec Králové: Charles University, Faculty of Pharmacy, **2007**.
17. VLČKOVÁ, M. *Deriváty rhodaninu jako potenciální léčiva II* (*Derivatives of Rhodanine as Potential Drugs II*). Diploma thesis. Hradec Králové: Charles University, Faculty of Pharmacy, **2007**.
18. MAJD, M. M. *Derivatives of rhodanine as potential antifungal and antimycobacterial drugs.* Diploma thesis. Hradec Králové: Charles University, Faculty of Pharmacy, **2007**.
19. HIRŠOVÁ, P. *Deriváty thiazolu jako potenciální léčiva* (*Derivatives of Thiazole as Potential Drugs*). Diploma thesis. Hradec Králové: Charles University, Faculty of Pharmacy, **2008**.
20. KALAFUTOVÁ, S. *Deriváty rhodaninu jako potenciální léčiva III* (*Derivatives of Rhodanine as Potential Drugs III*) Diploma thesis. Hradec Králové: Charles University, Faculty of Pharmacy, **2009**.
21. MOUSSERON, M. *Dérivés de thiazoles et leur preparation.* FR 1604530, **1971**-11-29 [accessed 2010-07-28].

Available from: http://v3.espacenet.com/publicationDetails/originalDocument?CC=FR&NR=1604530A&KC=A&FT=D&date=19711129&DB=EPODOC&locale=en_EP

22. MOUSSERON, M. *Substituted thiazolidine-4-ones.* US 3704296, 1972-11-28 [accessed 2010-07-28]. Available from: http://www.freepatentsonline.com/3704296.pdf
23. PETRIK, P., KUNEŠ, J., VEJSOVÁ, M. et al. *Antifungal and antimycobacterial properties of 5-arylmethylidenerhodanines and their N^3-substituted analogues.* Book of Abstracts of the 5th International Postgraduate Research Symposium on Pharmaceutics (IPORSIP – 2007), Istanbul, September 13–15, 2007. In: *Acta Pharmaceutica Sciencia.* **2007**, *49* (Suppl.), 77.
24. FOYE, W. O., AN, S. H. Synthesis of *N*-glycosylthioureas, *N*-glycosylrhodanines, and *N*-glycosyl-2-aminothiazoles and their antimicrobial activity. *J. Pharm. Sci.* **1981**, *70*, 1059–1063.
25. BROWN, F. C., BRADSHER, C., MOSER, B. F. et al. Structure and antimicrobial activity of the 3-aminorhodanines. *J. Org. Chem.* **1959**, *24*, 1056–1060.
26. ORCHARD, M., NEUSS, J. C. *Thiazolidine derivatives and its use as antifungal agent.* US 6740670. **2004**-05-25 [accessed 2010-07-28]. Available from: http://www.patentstorm.us/patents/6740670.html
27. DOLEŽEL, J., HIRŠOVÁ, P., OPLETALOVÁ, V. et al. Rhodanineacetic acid derivatives as potential drugs: Preparation, hydrophobic properties and antifungal activity of (5-arylalkylidene-4-oxo-2-thioxo-1,3-thiazolidin-3-yl)acetic acids. *Molecules.* **2009**, *14*, 4197-4212.
28. SEN GUPTA, A. K., HAJELA, K., SHANKER, K. et al. Antimicrobial and anticholinesterase activities of 5-(4-substituted phenyl)methylene-4-oxo-2-thioxo-3-thiazolidinylmethylamino derivatives. *Ind. J. Pharm. Sci.* **1983**, *45*, 165–168.

29. NGUYEN, Q. D., DINH, T. T. H., VU, T. A. et al. Synthesis and bioactivities of some rhodanine derivatives. *Tap Chi Duoc Hoc.* **2008**, *48*, 31-34. In SciFinder® [database online]. © American Chemical Society [accessed 2010-07-28]. Available from http://www.cas.org/products/scifindr/index.html
30. Doctor Fungus [online]. © Doctor Fungus Corporation [accessed 2010-07-28]. Available from http://www.doctorfungus.org/mycoses/index.htm.
31. HAY, R. J. Fungal infections. *Clin. Dermatol.* **2006**, *24*, 201–212.
32. AMEEN, M. Epidemiology of superficial fungal infections. *Clin. Dermatol.* **2010**, *28*, 197–201.
33. DENNING, D. W., HOPE, W. W. Therapy of fungal diseases: opportunities and priorities. *Trends Microbiol.* **2010**, *18*, 195–204.
34. BONN D. New antifungals make mayhem for mycoses. *Lancet* **1997**, *35*, 870.
35. PIZZO, P. A. Management of fever in patients with cancer and treatment-induced neutropenia. *N. Engl. J. Med.* **1993**, *328*, 1323–1332.
36. HADLEY S., KARCHMER, A. W. Fungal infections in solid organ transplant recipients. *Dis. Clin. North Am.* **1995**, *9*, 1045–1074.
37. WILLIAMSON, E. C., MILLAR, M. R. STEWARD, C. G. et al. Infections in adults undergoing unrelated donor bone marrow transplantation. *Br. J. Haematol.* **1999**, *104*, 560–568.
38. WINGARD, J. R. Opportunistic infections after blood and marrow transplantation. *Transpl. Infect. Dis*. **1999**, *1*, 3–20.
39. BLUMBERG, H. M.**,** JARVIS, W.R., SOUCIE, J. M. et al. Risk factors for candidal bloodstream infections in surgical intensive care unit patients: the NEMIS prospective multicenter study. The national epidemiology of mycosis survey. *Clin. Infect. Dis.* **2001**, *33*, 177–186.
40. PATTERSON, T. F. Advances and challenges in management of invasive mycoses. *Lancet* **2005**, *366*, 1013–1025.
41. CHEN, S. C. A., SORRELL, T. C. Antifungal agents. *Med. J. Aust.* **2007**, *187*, 404–409.

42. FRANCOIS, I. E. J. A., AERTS, A. M., CAMMUE, B. P. A., et al. Currently used antimycotics: Spectrum, mode of action and resistance occurrence. *Curr. Drug Targets* **2005**, *6*, 895–907.
43. WALKER, R., WHITTLESEA, C. *Clinical pharmacy and therapeutics*. 4th Ed. Livingston: Elsevier/Churchill, 2007.
44. LÜLLMAN, H., MOHR, K., WEHLING, M. *Farmakologie a toxikologie*. 1. vyd. Praha: Grada, 2004.
45. LEMKE, A., KIDERLEN, A. F., KAYSER, O. Amphotericin B. *Appl. Microbiol. Biotechnol.* **2005**, *68*, 151–162.
46. MAERTENS, J. A. History of the development of azole derivatives. *Clin. Microbiol. Infect.* **2004**, *10*, 1–10.
47. RANG, H. P. et al. *Pharmacology*. 6th Ed. Livingston: Elsevier/Churchill, 2007.
48. PASQUALOTO, A. C., DENNING, D. W. New and emerging treatments for fungal infections. *J. Antimicrob. Chemother.* **2008**, *61*(*Suppl. 1*), i19–i30.
49. CALDERONE, R. A. et al. *Candidiasis*. 1st Ed. Washington, D. C.: ASM Press, 2002.
50. MONK, J. P., BROGDEN, R. N. Naftifine. A review of its antimicrobial activity and therapeutic use in superficial dermatomycoses. *Drugs* **1991**, *42*, 659–672.
51. GIANNI, C. Update on antifungal therapy with terbinafine. *G. Ital. Dermatol. Venereol.* **2010**, *145*, 415–424.
52. DEGREEF, H. J., DeDONCKER, P. R. G. Current therapy of dermatophytosis. *J. Am. Acad. Dermatol.* **1994**, *31*, S25–S30.
53. BIANCHI, A., MONTI, G., de CARNERI, I. Tolciclate: further antimycotic studies. *Antimicrob. Agents Chemother.* **1977**, *12*, 129–430.
54. RYDER, N. S., FRANK, I., DUPONT, M.-C. Ergosterol biosynthesis inhibition by the thiocarbamate antifungal agents tolnaftate and tolciclate. *Antimicrob. Agents Chemother.* **1986**, *29*, 858–860.
55. OKU, Y., TAKAHASHI, N., YOKOYAMA, K. Fungicidal activity of liranaftate against dermatophytes. *Jpn. J. Med. Mycol.* **2009**, *50*, 9–13.

56. ODDS, F. C., BROWN, A. J. P., GOW, N. A. R. Antifungal agents: mechanism of action. *Trends Microbiol*. **2003**, *11*, 272–279.
57. DENNING, D. W. Echinocandin antifungal drugs. *Lancet* **2003**, *362*, 1142–1151.
58. PETRIKKOS, G., SKIADA, A. Recent advances in antifungal chemotherapy. *Int. J. Antimicrob. Agents* **2007**, *30*, 108–117.
59. SUCHER, A. J. Echinocandins: The newest class of antifungals. *Ann. Pharmacother*. **2009**, *43*, 1647–1657.
60. PERLIN, D. S. Resistance to echinocandin-class antifungal drugs. *Drug. Resist. Updat.* **2007**, *10*, 121–130.
61. SUN, H.-Y., SINGH, N. Characterisation of breakthrough invasive mycoses in echinocandin recipients: an evidence-based review. *Int. J. Antimicrob. Agents* **2010**, *35*, 211–218.
62. WALKER, L. A., GOW, N. A. R., MUNRO, C. A. Fungal echinocandin resistance. *Fungal Genet. Biol.* **2010**, *47*, 117–126.
63. DEVELOUX, M. Griseofulvin. *Ann. Dermatol. Venereol*. **2001**, *128*, 1317–1325.
64. WELSH, O., VERA-CABRERA, L., WELSH, E. Onychomycosis. *Clin. Dermatol.* **2010**, *28*, 151–159.
65. GUPTA A. K., BARAN, R. Ciclopirox nail lacquer solution 8% in the 21st century. *J. Am. Acad. Dermatol.* **2000**, *43*, S96–S102.
66. Micromedex® (Health Series) [database online]. © Thomson Reuters [accessed 2010-07-28]. Available from http://www.thomsonhc.com/home/dispatch
67. TRAMONTINI, M., ANGIOLINI, L. Mannich Bases: Chemistry and uses. 1st Ed. Boca Raton: CRC Press, 1994.
68. DAS, P. K., SINGH, G. B., DEBNATH, P. K. et al. Anticonvulsant activity of *N*-substituted derivatives of succinimides, thiazolidinediones, and their structural congeners. *Ind. J. Med. Res. (1913 – 1988)* **1975**, *63*, 286–301. In SciFinder® [database online]. © American Chemical Society [accessed 2010-07-28]. Available from http://www.cas.org/products/scifindr/index.html

69. DAS, K., PANDA, D., DASH, B. Rhodanine derivatives. *J. Indian. Chem. Soc.* **1990**, *67*, 58–60.
70. BORISOVA, M. A., GINAK, A. I., SOCHILIN, E. G. Rhodanines in the Mannich reaction. *Zh. Org. Khim.* **1970**, *6*, 1738–1744.
71. HUSAIN. M. I., SHUKLA, M. K., MISTRA, N. D. Search for potent anthelmintics. Part XII. 5-arylidene-3.substituted aminimethyl-4-thiazolidinone-2-thione. *Indian. J. Pharm. Sci.***1980**, *42*, 171–173.
72. SHUKLA, S. K., SINGH, S. P., AWASTHI. L. P. et al. Synthesis of substituted 3-aminomethylidene-4-thiazolidinone-2-thiones as potent antiviral agents. *Indian J. Pharm. Sci.* **1982**, *44*, 153–155.
73. PANDE, A., SAXENA, V. K. Synthesis of 3-(substituted aminomethyl)-5-(nitrobenzylidene)-2-thiones as potential antiviral agents. *Curr. Sci.* **1986**, *55*, 488–491.
74. HUSAIN, M. I., SHUKLA, S. Synthesis and pharmacological studies of some 3-(*N*,*N*-disubstituted aminomethyl)-5-(fluorobenzylidene)-4-oxothiazolidine-2-thiones. *Acta Pharm. Jugosl.* **1986**, *36*, 311–317.
75. WANG, Z., SHI, H., ZHENG, H. et al. Synthesis of 2-thioxo-4-thiazolidinone derivatives. *Youji Huaxue* **1994**, *14*, 190–194. In SciFinder® [database online]. © American Chemical Society [accessed 2010-07-28].
Available from http://www.cas.org/products/scifindr/index.html
76. DIKOVA, I., WESSELINOVA, D., NATOVA, L. Synthesis and biological activity of rhodanine derivatives containing piperazine ring in the molecule. *Fatmatsiya (Sofia, Bulgaria)* **2001**, *48*, 3–9.
77. GAIKWAD N. J., GAUTAM, P. Mannich reaction products of 5-benzylidene-2-thioxo-4-thiazolidine as hypoglycemic agents. *Indian J. Heterocycl. Chem.* **2002**, *12*, 181–182.
78. WESSELINOVA, D., DIKOVA, I. Measurement on the impact of new synthesizes rhodanine derivatives containing piperazine cycle in the molecule on living cells. *Dokl. Bolg. Akad. Nauk.* **2003**, *56*, 89–94.

79. PETERNEL, L., KOTNIK, M., PREŽELJ. A. et al. Comparison of 3 cytotoxicity screening assays and their application to the selection of novel antibacterial hits. *J. Biomol. Screen.* **2009**, *14*, 142–150.

ABSTRACT

Charles University in Prague

Faculty of Pharmacy in Hradec Králové

Department of Pharmaceutical Chemistry and Drug Control

Student: Mariana El-Zein

Consultant: Assoc. Prof. RNDr. Veronika Opletalová, Ph.D.

Title of Thesis: Derivatives of Rhodanine as Potential Antifungal Drugs

Rhodanine (2-thioxo-1,3-thiazolidin-4-one) forms the basic skeleton of many biologically active substances and potential drugs. Antifungal properties of rhodanine derivatives have been studied since the early 1950s.

Theoretical part of the thesis deals with mycoses and drugs that are currently used to treat them. The aim of the experimental work was to find suitable reaction conditions for Mannich reaction of (5*Z*)-5-(subst.)benzylidenes-2-thioxo-1,3-thiazolidin-4-ones with various amines. The attempts to prepare Mannich bases using diethylamine as the basic component were unsuccessful. (5*Z*)-5-benzylidene-3-(morpholin-4-yl-methyl)-2-thioxo-1,3-thiazolidin-4-one was successfully obtained by treating (5*Z*)-5-benzylidene-2-thioxo-1,3-thiazolidin-4-one with formaldehyde and morpholine in dimethylformamide. This compound was reported previously and exhibited antibacterial activity and acceptable toxicity. Hence, Mannich bases derived from (5*Z*)-5-arylalkylidene-2-thioxo-1,3-thiazolidin-4-ones deserve further attention as potential antimicrobial agents.