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Synthesis of novel tetrazole derivatives and evaluation of their antifungal activity

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ABSTRACT

With the appearance of the antifungal resistance, novel antifungal agents need to be identified. In this context new 2,5-disubstituted tetrazole derivatives containing benzothiazole, benzoxazole or phenylsulfonyl moiety were synthesized by *N*-alkylation of aryltetrazole with 2-[(3-chloropropyl)sulfanyl]-1,3-benzothiazole or 2-[(3-chloropropyl)sulfanyl]-1,3-benzoxazole and Michael-type addition of aryltetrazole to phenyl vinyl sulfone. The chemical structures of the synthesized compounds were confirmed by means of ^{1}H NMR, ^{13}C NMR, IR and HRMS spectral data. The compounds were tested against the moulds: *Fusarium sambucinum*, *Fusarium oxysporum*, *Colletotrichum coccodes*, *Aspergillus niger*, and the yeast *Candida albicans*. The results showed that among the moulds only *C. coccodes* was significantly sensitive to all the structures examined. All the tetrazole derivatives acted at the same level against *C. albicans* and demonstrated a high cell growth inhibition (97–99%) at the concentrations ranging from 16 to 0.0313 μg/mL. The mode of action of 2-({3-[5-(4-chlorophenyl)-2*H*-tetrazol-2-yl]propyl}sulfanyl)-1,3-benzoxazole (**5c**) and 2-({3-[5-(2-chlorophenyl)-2*H*-tetrazol-2-yl]propyl}sulfanyl)-1,3-benzoxazole (**5d**) was established by verifying fungal growth in the presence of osmotic protector-sorbitol. The effect of compound **5c** or **5d** combined with Fluconazole was determined using the checkerboard method. The calculated fractional inhibitory concentration index (FIC) indicated antagonism (FIC >1). Additionally, survival experiments with lepidopteran *Galleria mellonella* treated with compounds **5c** and **5d** were performed and demonstrated the lack of toxicity of these compounds.

1. Introduction

Fungal infections constitute a worldwide health problem and the increase in the number of human diseases caused by pathogenic fungi is observed. These infections are difficult to treat and especially dangerous for elderly people and patients with an impaired immune system due to neutropenia, AIDS, diabetes, cancer and induced immunosuppression after an organ transplant.[1] Another common factor contributing to fungal infections is a long-term treatment with antibiotics and contraceptives. Among many types of infections the most common are these caused by *Candida* spp., *Aspergillus* spp. and *Cryptococcus* spp., which lead to candidiasis, aspergillosis and cryptococcosis, respectively.[2–4] The most frequent fungal pathogens are *Candida albicans*, *Aspergillus fumigatus*, *Aspergillus niger* and *Cryptococcus neoformans*.[2–4] The currently used antifungal drugs display various mechanisms of action. Azoles (e.g., Ketoconazole, Itraconazole, Fluconazole, Voriconazole, Posoconazole, Ravuconazole),[2,4–6] and allylamines (e.g. Terbinafine) inhibit synthesis of ergosterol, which is the major component of the fungal cell membrane.[2] Polyene antibiotics (e.g., Amphotericin B, Nystatin) interact with ergosterol and thus disrupt the fungal membrane and increase its permeability.[2,4,5] Other compounds inhibit synthesis of the major components of the cell wall e.g. inhibitors of glucan synthesis (e.g., Echinocandins, Caspofungin, Micafungin) and inhibitors of chitin synthesis (e.g., Nikkomycin, Polyoxins).[2,4,5] We also know antifungal agents that inhibit synthesis of nucleic acids (e.g. Flucytosine) or proteins (e.g. Sordarins) and many others with a more complex mechanism of action.[2,5] The treatment of fungal infections is problematic because of an emerging drugs resistance in fungi,[5,7–11] therefore studies on the design and synthesis of new effective antifungal compounds are conducted and focus on tetrazole derivatives. These azoles significantly inhibit the growth of many pathogenic strains. For instance, 1-(2,4-dihydroxythiobenzoyl)tetrazoles and tetrazoles containing hydrazone moiety exhibit a high antifungal

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activity against *Candida* spp.[12–15] The tetrazole derivatives bearing both hydrazone and thiazoline moieties display a significant antifungal activity against: *Trichoderma harzianum*, *Aspergillus ochraceus*, *Fusarium solani*, *Fusarium moniliforme*, *Fusarium culmorum*, and *C. albicans*.[16] It was found that the tetrazole derivatives containing triazine dendrimeric chalcones exhibit a high fungicidal activity against *C. albicans*, *A. niger*, *A. fumigatus* and *Saccharomyces cerevisiae*,[17] whereas the tetrazoles with quinolone scaffold are effective against *C. albicans* and *A. niger*.[18–20] Furthermore, it is worth mentioning that 2-(1-methyl-1*H*-1,2,3,4-tetrazol-5-yl)sulfanyl-*N*-(5-methylisoxazol-3-yl)acetamide and 2-(1-phenyl-1*H*-1,2,3,4-tetrazol-5-yl)sulfanyl-*N*-(5-methylisoxazol-3-yl)acetmide inhibit the growth of *C. albicans*,[21] 3-aryl-1-(5-phenyl-1*H*-tetrazol-1-yl)prop-2-en-1-one exhibits an activity against *C. albicans* and *A. niger*,[22] pyridyl and pyrimidyl tetrazole derivatives display antifungal properties against *C. albicans*, *A. fumigatus*, and *A. niger*,[23–25] and selected 5-thiosubstituted tetrazole derivatives show an activity against *Trichophyton mentagrophytes*, *Penicillium marneffei*, *Aspergillus flavus* and *A. fumigatus* comparable to that of Itraconazole.[26] Moreover, tetrazolyl derivatives of purine,[27] thiophene,[28] catecholthioethers,[29] and organomercurials exhibit an antifungal activity.[30] Besides, 1-aryltetrazole derivatives containing *N*-substituted piperazine ring with antifungal properties against *A. flavus*, *A. fumigatus*, *P. marneffei,* and *T. mentagrophytes*[31] are known; the tetrazole derivatives bearing both *N*-substituted piperazine and triazole rings are active against *C. neoformans*, *Candida* spp. and *Aspergillus* spp.,[32,33] as well as many others.[34–39]

Herein, as a continuation of our previous work,[40,41] we present the synthesis of novel tetrazole derivatives containing benzothiazole, benzoxazole or phenylsulfonyl scaffold, and evaluation of their antifungal activity against the yeast *C. albicans* and selected mould stains: *A. niger*, *C. coccodes*, *F. sambucinum*, *F. oxysporum*. Two tetrazole derivatives exhibiting the highest anti-*Candida* activity, 2-({3-[5-(4-chlorophenyl)-2*H*-tetrazol-2-yl]propyl}sulfanyl)-1,3-benzoxazole **5c** and 2-({3-[5-(2-chlorophenyl)-2*H*-tetrazol-2-yl]propyl}sulfanyl)-1,3-benzoxazole **5d**, were chosen for further studies including the mode of action and the type of interaction in combination with the synthetic antifungal agent—Fluconazole.

2. Results and discussion

2.1. Synthetic chemistry

New tetrazole derivatives were obtained in two types of reactions including *N*-alkylation and Michael-type addition. The starting 5-aryltetrazoles **1a**–**d** were synthesized from corresponding nitriles, NaN_3 and NH_4Cl in DMF according to the described method.[42] The 2-[(3-chloropropyl)sulfanyl]-1,3-benzothiazole **2** and 2-[(3-chloropropyl)sulfanyl]-1,3-benzoxazole **3** were obtained by *S*-alkylation of 2-mercapto-1,3-benzothiazole or 2-mercapto-1,3-benzoxazole. The reactions were carried out in two phase system 10% NaOH/toluene in the presence of tetrabutylammonium bromide (TBAB). Both products were obtained with satisfactory yields of 76% and 82%, respectively. The phenyl vinyl sulfone **6** was prepared according to the described method.[43]

The study on the preparation of new tetrazole derivatives began with the synthesis of 2-{[3-(5-phenyl-2*H*-tetrazol-2-yl)propyl]sulfanyl}-1,3-benzothiazole **4a**. The first experiment was performed in acetonitrile with K_2CO_3 as a base at reflux (Scheme 1). The progress of the reaction was monitored by thin-layer chromatography. After 47 h the reaction was stopped and the product was purified on column chromatography with silica gel. The purification was hampered and repeated three times because of the presence of numerous by-products. The isolation of the by-products in pure form and their identification was impossible. The yield of the reaction reached only 38%, therefore the synthesis was performed again in THF in the presence of NaOH and TBAB at reflux and in two phase system 10% NaOH/toluene in the presence of TBAB at reflux. The product was obtained with lower yield of 32% and 27%, respectively. Other tetrazole derivatives **4b**–**d** and **5a**–**d** were synthesized using K_2CO_3 and acetonitrile (Scheme 1).

All the reactions were monitored on TLC plates and stopped when the conversion of 2-[(3-chloropropyl)sulfanyl]-1,3-benzothiazole **2** or 2-[(3-chloropropyl)sulfanyl]-1,3-benzoxazole **3** reached approximately 100%. In all the cases formation of many by-products was detected, which significantly complicated the isolation and purification of the main products. In order to purify the product, column chromatography was repeated several times and in some cases (compounds: **4b**–**d**, **5a**, **5d**) additional preparative thin-layer chromatography was done, which undoubtedly decreased the yield of each reaction (Table 1). All the reactions took place regioselectively and 2,5-disubstituted tetrazoles were formed. However, taking into account the large number of the by-products, the formation of a trace amounts of 1,5-disubstituded tetrazoles could not be excluded. As may be seen from the results presented in Table 1, tetrazole derivatives containing benzoxazole moiety (**5a**–**d**) were obtained with higher yields than their benzothiazole analogs (**4a**–**d**).

Significantly better results were obtained in Michael-type addition of 5-aryltetrazoles **1a**–**d** to phenyl vinyl sulfone **6** (Scheme 1). The reactions were performed in isopropanol in the presence of Et_3N. The first experiment, synthesis of 5-phenyl-2-[2-(phenylsulfonyl)ethyl]-2*H*-tetrazole **7a**, was performed at room temperature. The reaction was sluggish and even after 120 h of stirring the conversion was below 100%, and the product was obtained with low yield of 44%. When the same experiment was performed at reflux, the complete conversion was already achieved after 2.5 h, and the product **7a** was obtained with the yield of 76%. Consequently, other tetrazole derivatives containing phenylsulfonyl moiety **7b**–**d** were synthesized at reflux. The time and yields of these reactions were summarized in Table 1.

Comparing the results presented in Table 1 it may be concluded that *N*-alkylation reactions took place with lower yields and significantly longer reaction time than Michael-type additions. Moreover, the influence of the substituent at the benzene ring of tetrazole derivatives **1a**–**d** on the rate of *N*-alkylation reactions was observed. The synthesis time of benzothiazole derivatives **4a**–**d** and benzoxazole derivatives **5a**–**d** ranged from 47–48 h to 120 h. For comparison the rates of Michael-type addition reactions were independent of the structure of tetrazole derivatives **1a**–**d**. The reactions took place in a shorter time (2.5–3 h) and with higher yields (59–76%). In all the reactions the formation of 2,5-disubstituted tetrazole derivatives was preferred.

2.2. Biological activity

A panel of synthesized tetrazole derivatives was screened against the reference yeast *C. albicans* strain ATCC 90028 and the mould strains: *A. niger* ATCC 16404, *C. coccodes* MC 1, *F. sambucinum* MF 1 and *F. oxysporum* MF 5. Moreover, the cytotoxicity of compounds **5c** and **5d** was determined against the invertebrate host *Galleria mellonella* model.

The susceptibility of *C. albicans* to tetrazole derivatives: **4b**, **4d**, **5c**–**d**, **7a**, **7c**–**d** was evaluated at the concentrations ranging from 0.0313 to 16 μg/mL using the method M27-A3 (CLSI).[44] The obtained results were summarized in Table 2. Although compounds provided a high cell growth inhibition (from 97% to 99%) at all the concentrations tested, they did not show MICs at this range (visually assessed quality in the method M27-A3[44]). Thus,