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Research paper

Synthesis and biological evaluation of new naphthalene substituted thiosemicarbazone derivatives as potent antifungal and anticancer agents

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ABSTRACT

New thiosemicarbazone derivatives (**1**–**10**) were obtained *via* the reaction of 4-(naphthalen-1-yl)thiosemicarbazide with fluoro-substituted aromatic aldehydes. The synthesized compounds were evaluated for their *in vitro* antifungal effects against pathogenic yeasts and molds using broth microdilution assay. Ames and umuC assays were carried out to determine the genotoxicity of the most effective antifungal derivatives. Furthermore, all compounds were evaluated for their cytotoxic effects on A549 human lung adenocarcinoma and NIH/3T3 mouse embryonic fibroblast cell lines using XTT test. Among these derivatives, 4-(naphthalen-1-yl)-1-(2,3-difluorobenzylidene)thiosemicarbazide (**1**) and 4-(naphthalen-1-yl)-1-(2,5-difluorobenzylidene)thiosemicarbazide (**3**) can be identified as the most promising antifungal derivatives due to their notable inhibitory effects on *Candida* species and no cytotoxicity against NIH/3T3 mouse embryonic fibroblast cell line. According to Ames and umuC assays, compounds **1** and **3** were classified as non-mutagenic compounds. On the other hand, 4-(naphthalen-1-yl)-1-(2,4-difluorobenzylidene)thiosemicarbazide (**2**) can be considered as the most promising anticancer agent against A549 cell line owing to its notable inhibitory effect on A549 cells with an IC_{50} value of 31.25 μg/mL when compared with cisplatin (IC_{50} = 16.28 μg/mL) and no cytotoxicity against NIH/3T3 cells.

1. Introduction

Fungal infections have emerged as a serious health problem in industrialized countries. In particular, immunocompromised patients are highly susceptible to life-threatening infections caused by opportunistic fungi. Immunosuppressant drugs and anticancer agents have provided an opportunity for fungi to cause serious infections. Additionally, resistance and toxicity problems of currently available antifungal agents have reinforced the requirement for discovering new safe antifungal agents [1].

On the other hand, cancer has emerged as the second leading cause of death after cardiovascular disorders. The ongoing battle against cancer is far from over. Altering cell fate with small synthetic compounds is one of the key strategies in cancer therapy. The discovery of new chemotherapeutics has become one of the most important goals in medicinal chemistry. Cytotoxicity and genotoxicity of anticancer agents to healthy cells are major problems in cancer therapy and engender the risk of inducing secondary malignancy. The dose of anticancer drug sufficient to kill tumor cells is often toxic to healthy cells and leads to many adverse effects, which in turn, limits its treatment efficacy. In recent years, there has been a concerned search for the discovery and development of new selective antifungal and anticancer agents, devoid of unpleasant side effects of conventional antifungal and anticancer agents [2,3].

Azomethines (Schiff bases) have been widely studied as they possess many interesting features, including photochromic and thermochromic properties, proton transfer tautomeric equilibria, biological and pharmacological activities, as well as suitability for analytical applications [4]. They exhibit biological effects including antifungal and antitumor activity [5–10].

Thiosemicarbazone (TSC) is considered as one of the most important scaffolds and is embedded in many anticancer agents.

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Many aliphatic, aromatic, and heteroaromatic carbaldehyde TSCs were synthesized and evaluated for their antitumor activity [11–17].

TSCs are well-known chelators of metal ions and many of their biological activities have been attributed to their ability to form biologically active complexes [18]. The antineoplastic activity of TSCs is most often attributed to the ability of the compounds to inhibit mammalian ribonucleotide reductase (RR). RR is a multi-subunit enzyme responsible for the reduction of ribonucleotides to deoxyribonucleotides, which are the building blocks for DNA replication and repair in all living cells. TSCs, known iron chelators, can destabilize or damage the non-heme iron-stabilized tyrosyl free radical and thus inhibit the catalytic function of RR [10,19–21].

Some naphthalene derivatives have also been reported as potent apoptosis inducers, P-glycoprotein inhibitors, microtubule inhibitors or glutamamide analogues [22–29]. On the other hand, terbinafine and naftifine, allylamine antifungal agents bearing a naphthalene moiety, are widely used for the treatment of fungal infections, particularly those caused by dermatophytes [30,31].

In recent decades, the introduction of fluorine into a molecule has become increasingly prevalent in the design of drug candidates. Fluorine substitution can improve potency and target selectivity by affecting pK_a, modulating conformation, hydrophobic interactions and lipophilicity, or a combination of these properties [32–34].

In the current work, we carried out the synthesis of a new series of naphthalene-based thiosemicarbazones and investigated their antifungal and cytotoxic effects on A549 human lung adenocarcinoma and NIH/3T3 mouse embryonic fibroblast cell lines. Among these compounds, the most effective antifungal compounds were evaluated for their genotoxicity using Ames and umuC assays.

2. Results and discussion

The synthesis of thiosemicarbazone derivatives (**1**–**10**) followed the general pathway depicted in Scheme 1. Initially, 4-(naphthalen-1-yl)thiosemicarbazide (**A**) was synthesized *via* the reaction of 1-naphthyl isothiocyanate with hydrazine hydrate. The reaction of 4-(naphthalen-1-yl)thiosemicarbazide (**A**) with fluoro-substituted aromatic aldehydes afforded thiosemicarbazone derivatives (**1**–**10**). The structures of compounds **1**–**10** were confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectral data and elemental analyses.

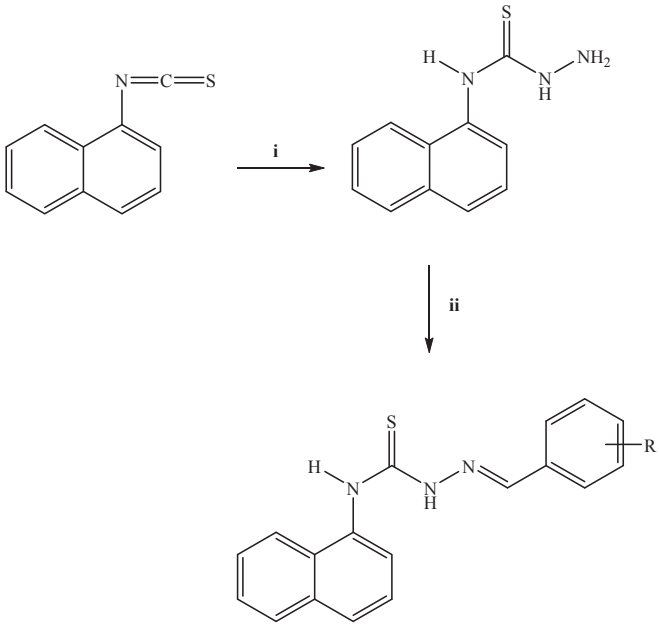
The synthesized compounds (**1**–**10**) were tested *in vitro* against pathogenic yeasts and molds. As shown in Table 1, generally the compounds exhibited more significant antifungal activity against *Candida* species than *Aspergillus* species.

Among the pathogenic fungi species, *Candida zeylanoides* was the most susceptible yeast to the tested compounds. All compounds exhibited remarkable antifungal activity against *C. zeylanoides* with a MIC value of 250 μg/mL when compared with ketoconazole (MIC = 250 μg/mL). The microbiological results revealed that the antifungal effects of the compounds on *C. zeylanoides* did not depend on the substituents.

Among all compounds, compounds **1** and **3** showed the highest antifungal activity against *Candida glabrata* with a MIC value of 125 μg/mL when compared with ketoconazole (MIC = 62.50 μg/mL). This result clearly indicated that the position of fluoro substituent on benzene ring is important for antifungal activity against *C. glabrata*.

Considering the antifungal effects of compounds **1** and **3** against pathogenic yeasts and molds, further studies were performed to determine their genotoxicity.

In umuC test procedure, the whole test is considered valid if the positive controls reach an induction ratio (IR) of 2. The average OD_{600} value of the negative controls of the second plate should increase by a factor of 2 during 2 h incubation (growth control). Our



Compound	R
1	2,3-diF
2	2,4-diF
3	2,5-diF
4	2,6-diF
5	3,4-diF
6	3,5-diF
7	2,4,6-triF
8	3-Cl-4-F
9	2-Cl-6-F
10	4-(4-Fluorophenoxy)

Scheme 1. The synthetic route for the preparation of the thiosemicarbazone derivatives (**1**–**10**). Reagents and conditions: (i) $NH_2NH_2.H_2O$, ethanol, rt, 4 h; (ii) ArCHO, ethanol, reflux, 6 h.

experiments provided these validity criteria. According to umuC test, a sample dilution is considered genotoxic if the IR is ≥1.5 and the growth factor (G) is ≥0.5 and also it is recommended that a dose response should be observed [35]. Our results were presented in Table 2. Compounds **1** and **3** did not show an IR ≥ 1.5, while their growth factors (G) were <0.5, so they were not determined as genotoxic compounds according to umuC assay (Fig. 1).

Ames assay was performed to investigate the genotoxicity of compounds **1** and **3**. In Ames MPF assay, more than 25 positive wells were observed with positive controls, which complied with the requirements of the validity criteria. Negative controls also showed less than 8 positive wells in the presence and absence of S9 with TA98 and TA100, which complied with the requirements for validation in the Ames MPF assay manual and previous studies [36]. Our results were presented in Table 3.

Compounds **1** and **3** had a baseline of 3.56 with TA 98 in the presence of S9 and 1.94 in the absence of S9 with related negative control values. Furthermore, fold inductions over baseline were less than 2 in each concentrations of the compounds and the significant different results obtained did not show a dose–response tendency. Compounds **1** and **3** were found to be non-mutagenic against TA 98.

Compounds **1** and **3** showed a baseline of 6.88 and 5.51 against TA100 with/without S9, respectively. Fold inductions over baseline were not more than 2 and statistically different results did not reveal a dose–response tendency. According to these findings,