



# Synthesis, antimicrobial activity, and determination of the lipophilicity of ((cyclohex-3-enylmethylene)hydrazinyl)thiazole derivatives

Anna Biernasiuk<sup>1</sup> · Magdalena Kawczyńska<sup>2</sup> · Anna Berecka-Rycerz<sup>3</sup> · Beata Rosada<sup>2</sup> · Anna Gumieniczek<sup>3</sup> · Anna Malm<sup>1</sup> · Katarzyna Dzitko<sup>4</sup> · Krzysztof Z. Łączkowski<sup>2</sup>

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## Abstract

Synthesis and investigation of antimicrobial activity of fifteen novel thiazoles containing cyclohexene moiety are presented. Among the derivatives, compounds **3a–3d**, **3f**, **3n**, and **3o** showed very strong activity against the reference *Candida* spp. strains with MIC = 0.015–3.91 µg/ml. The activity of these compounds is similar and even higher than the activity of nystatin used as positive control. Compounds **3d**, **3f**, **3n**, **3o** showed the highest activity with very strong effect towards most of yeasts isolated from clinical materials with MIC = 0.015–7.81 µg/ml. The cytotoxicity studies for the most active compounds showed that *Candida* spp. growth was inhibited at noncytotoxic concentrations for the mammalian L929 fibroblast. In addition, a good correlation was obtained between lipophilicity of compounds determined using reversed phase thin-layer chromatography and their antifungal activity.

**Keywords** Thiazole · Antimicrobial activity · *Candida* spp. · Lipophilicity · Thin-layer chromatography

## Introduction

Nowadays, the incidence of Invasive Fungal Infection is increasing worldwide. They are mostly caused by yeasts belonging to *Candida* spp., especially *Candida albicans*, but

most recently also by other species like *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis*, or *Candida krusei* called as non-*albicans* *Candida* species (Ishida et al. 2011; Silva et al. 2017; Pappas et al. 2016). These fungi are a part of the microbiota colonizing mucocutaneous areas, with a reported prevalence 15–75%, mainly within the oral cavity, upper airways, gastrointestinal tract, and vagina of healthy individuals (Ghannoum et al. 2010). They are one of the most important opportunistic fungal pathogens causing mucosal and systemic infections (Hacioglu et al. 2018). Invasive candidiasis are considered the life-threatening infections associated with high morbidity and mortality (Pappas et al. 2016; Hacioglu et al. 2018). The mortality rate of bloodstream infections caused by these yeasts was reported to be as high as 40–60% among immunosuppressed and hospitalized patients (Sun et al. 2015; Li et al. 2018; Turecka et al. 2018).

The incidence of fungal infection is increasing, especially in populations of immunosuppressed individuals (persons with HIV/AIDS or primary immune deficiency) and due to the continuous development of modern medicines, such as organ transplantation, hemodialysis, parenteral nutrition, extensive applicability of catheters or medical implants, cancer chemotherapy, and the widespread use of broad-spectrum antibiotics, glucocorticoids, and

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✉ Anna Biernasiuk  
anna.biernasiuk@umlub.pl

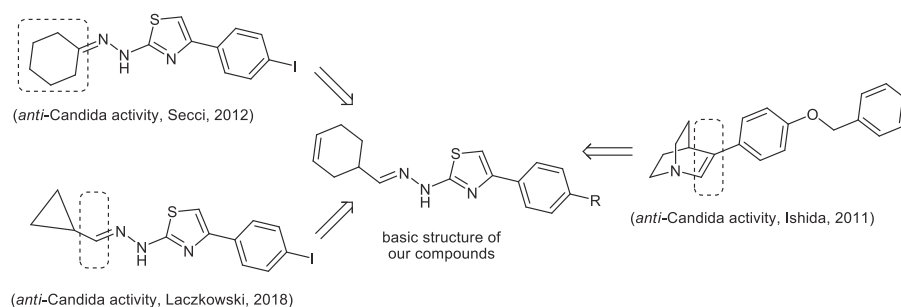
<sup>1</sup> Department of Pharmaceutical Microbiology with Laboratory for Microbiological Diagnostics, Faculty of Pharmacy, Medical University of Lublin, Chodźki 1, Lublin 20-093, Poland

<sup>2</sup> Department of Chemical Technology and Pharmaceuticals, Faculty of Pharmacy, Nicolaus Copernicus University, Juraszka 2, Bydgoszcz 85-089, Poland

<sup>3</sup> Department of Medicinal Chemistry, Faculty of Pharmacy, Medical University of Lublin, Jaczewskiego 4, Lublin 20-090, Poland

<sup>4</sup> Department of Immunoparasitology, Faculty of Biology and Environmental Protection, University of Lodz, Banacha 12/16, Lodz 90-237, Poland

**Fig. 1** Rational design of the target compounds **3a–3o**



immunosuppressants (Turecka et al. 2018; Roemer and Krysan 2014; Gomes da Silva Dantas et al. 2018). In addition to the predisposing factors of the host, the pathogenicity of *Candida* species is affected by their virulence factors, such as the polymorphism (growth in two forms, unicellular and filamentous), production of extracellular enzymes (proteases, phospholipases, and hemolysins), as well as the expression of adhesins, which is related to the biofilm formation (on host tissues or on medical device surfaces) (Hacioglu et al. 2018; Sun et al. 2015; Li et al. 2018; Gomes da Silva Dantas et al. 2018).

At present, the available antifungal drugs are limited to three major classes: polyenes (amphotericin B or nystatin), which bind fungal cell membrane ergosterol leading to cell lysis; azoles (fluconazole or posaconazole) that inhibit ergosterol biosynthesis; and echinocandins (caspofungin or micafungin) inhibiting fungal (1,3)- $\beta$ -D-glucan cell wall synthesis (Ishida et al. 2011; Pappas et al. 2016; Li et al. 2018; Turecka et al. 2018; Roemer and Krysan 2014; Ahmad et al. 2017). Some characteristics of these drugs restrict their use in prophylaxis and clinical practice, such as fungistatic activity (azoles), which extends the required time of treatment, high toxicity (polyenes) and nonspecific interaction with other drugs, which increases their side effects (Gomes da Silva Dantas et al. 2018; Ahmad et al. 2017). Moreover, as a consequence of the wide use of these antifungals, drug resistance of *Candida* spp. is increasing, which poses a serious threat to antifungal therapy (Li et al. 2018). Thus, discovering new antifungal agents or overcoming drug resistance become a hot topic in the antifungal field (Li et al. 2018; Ahmad et al. 2017).

The last years of the research have confirmed that thiazole scaffold has diverse biological properties that can be used to treat many diseases, such as microbial infections (Bikobo et al. 2017; Secci et al. 2012; Łączkowski et al. 2018a, 2018b; Zaki et al. 2018; Ansari et al. 2018), Parkinson's disease (Carradori et al. 2018), cancer (de Santana et al. 2018; Łączkowski et al. 2014, 2016a, 2016b; Piechowska et al. 2019; Gomha et al. 2018), epilepsy (Siddiqui and Ahsan 2010; Ahangar et al. 2011; Łączkowski et al. 2016a, 2016b, 2017), toxoplasmosis (Schultz et al. 2014; Łączkowski et al. 2018a, 2018b), and antioxidants (Geronikaki et al. 2013; Djukic et al. 2018). Considering the above-

mentioned findings, we decided to design and synthesize the structure of hybrids incorporating both the thiazole ring and the cyclohexenyl moiety. Our earlier studies as well as literature data suggested that such hybrid structures will show some synergistic effects responsible for achieving high antimicrobial activity (Secci et al. 2012). The investigated compounds have been rationalized, so that they contain such substituents that in earlier studies were responsible for the highest antimicrobial activity (Fig. 1). Thanks to the use of electron-donating as well as electron-withdrawing character of substituents we were able to modify the hydrophilic and hydrophobic properties of the designed molecules.

Next, the synthesized compounds were evaluated for their antimicrobial activity against a panel of 32 different strains of microorganisms, including Gram-positive bacteria, Gram-negative bacteria, and fungi belonging to yeasts. These microorganisms came from American Type Culture Collection (ATCC), routinely used for the evaluation of antimicrobials. Moreover, the isolates of *Candida* spp. from clinical materials were included.

## Materials and methods

### Chemistry

All experiments were carried out under air atmosphere unless stated otherwise. Reagents were generally the best quality commercial-grade products and were used without further purification.  $^1\text{H}$  NMR (400 MHz) and  $^{13}\text{C}$  NMR (176 MHz) spectra were recorded on a Bruker Avance III multinuclear instrument. Melting points were determined in open glass capillaries and are uncorrected. Analytical TLC was performed using Macherey-Nagel Polygram Sil G/UV<sub>254</sub> 0.2 mm plates. 3-Cyclohexene-1-carboxaldehyde, thiosemicarbazide, and appropriate bromoketones were commercial materials (Aldrich).

### 2-(Cyclohex-3-enylmethylene)hydrazinecarbothioamide (2)

Thiosemicarbazide (1.82 g, 20.0 mmol) was added to a stirred solution of 3-cyclohexene-1-carboxaldehyde (1) (2.20 g,

20.0 mmol) in absolute ethyl alcohol (30 ml) and then (1.0 ml) of acetic acid was added. The reaction mixture was stirred under reflux for 20 h under nitrogen atmosphere. Next, the reaction mixture was added to water (100 ml) and neutralized with  $\text{NaHCO}_3$  solution. The product was filtered off and subsequently washed with water to afford the desired product to yield 2.93 g (80%); mp 122–123 °C; eluent: dichloromethane/methanol (95:5),  $R_f = 0.74$ .  $^1\text{H}$  NMR (DMSO- $d_6$ , 700 MHz)  $\delta$  (ppm): 1.41–1.46 (m, 1H, CH); 1.78–1.84 (m, 1H, CH); 2.00–2.06 (m, 3H, CH,  $\text{CH}_2$ ); 2.08–2.14 (m, 1H, CH); 2.41–2.44 (m, 1H, CH); 5.66 (s, 2H, 2CH); 7.39 (d, 1H, CH,  $J = 5.0$  Hz); 7.44 (bs, 1H, NH); 7.98 (bs, 1H, NH); 11.05 (bs, 1H, NH).  $^{13}\text{C}$  NMR (176 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 24.29 ( $\text{CH}_2$ ); 26.06 ( $\text{CH}_2$ ); 28.28 ( $\text{CH}_2$ ); 36.32 (CH); 125.95 (CH); 127.18 (CH); 150.54 (CH=N); 178.18 (C=S). *Anal.* Calcd. for  $\text{C}_8\text{H}_{13}\text{N}_3\text{S}$ : C, 52.43; H, 7.15; N, 22.93. Found: C, 52.45; H, 7.20; N, 22.98.

#### 2-(2-(Cyclohex-3-enylmethylene)hydrazinyl)-4-(4-fluorophenyl)thiazole (3a). Typical procedure

Carbothioamide **2** (0.183 g, 1.0 mmol) was added to a stirred solution of 2-bromo-1-(4-fluorophenyl)ethanone (0.217 g, 1.0 mmol) in absolute ethyl alcohol (15 ml). The reaction mixture was stirred at room temperature for 20 h. Next, the reaction mixture was added to water (50 ml) and neutralized with  $\text{NaHCO}_3$  solution. The product was extracted with dichloromethane ( $2 \times 100$  ml), the solvent was evaporated in vacuo, and the product was purified on silica gel column chromatography (230–400 mesh) using (dichloromethane,  $R_f = 0.51$ ) to afford the desired product: 0.27 g, 90%; mp 153–155 °C.  $^1\text{H}$  NMR (DMSO- $d_6$ , 400 MHz)  $\delta$  (ppm): 1.41–1.46 (m, 1H, CH); 1.78–1.84 (m, 1H, CH); 2.00–2.06 (m, 3H, CH,  $\text{CH}_2$ ); 2.08–2.14 (m, 1H, CH); 2.46–2.49 (m, 1H, CH); 5.69 (s, 2H, 2CH); 7.15–7.24 (m, 3H, 3CH); 7.34 (d, 1H, CH,  $J = 5.0$  Hz); 7.84–7.87 (m, 2H, 2CH); 11.59 (bs, 1H, NH).  $^{13}\text{C}$  NMR (176 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 23.90 ( $\text{CH}_2$ ); 25.84 ( $\text{CH}_2$ ); 28.17 ( $\text{CH}_2$ ); 36.01 (CH); 102.78 (CH); 115.45 (d, 2CH;  $J_{\text{C-F}} = 19.7$  Hz); 125.53 (CH); 126.87 (CH); 127.50 (d, 2CH,  $J_{\text{C-F}} = 6.2$  Hz); 131.51 (C); 148.77 (C); 160.90 (CH=N); 162.27 (C-F); 168.78 (C-NH). *Anal.* Calcd. for  $\text{C}_{16}\text{H}_{16}\text{FN}_3\text{S}$ : C, 63.76; H, 5.35; N, 13.94. Found: C, 63.74; H, 5.40; N, 13.99.

#### 4-(4-Bromophenyl)-2-(2-(cyclohex-3-enylmethylene)hydrazinyl)thiazole (3b)

2-Bromo-1-(4-bromophenyl)ethanone was reacted with **2**. Yield: 0.29 g, 80%, (dichloromethane,  $R_f = 0.72$ ); mp 165–167 °C.  $^1\text{H}$  NMR (DMSO- $d_6$ , 400 MHz)  $\delta$  (ppm): 1.41–1.46 (m, 1H, CH); 1.78–1.84 (m, 1H, CH); 2.00–2.06 (m, 3H, CH,  $\text{CH}_2$ ); 2.08–2.14 (m, 1H, CH); 2.46–2.49 (m, 1H, CH); 5.69 (s, 2H, 2CH); 7.31 (s, 1H, CH); 7.35–7.38

(d, 1H, CH,  $J = 5.0$  Hz); 7.59 (d, 2H, 2CH,  $J = 8.0$  Hz); 7.77 (d, 2H, 2CH,  $J = 8.0$  Hz); 11.64 (bs, 1H, NH).  $^{13}\text{C}$  NMR (176 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 23.85 ( $\text{CH}_2$ ); 25.76 ( $\text{CH}_2$ ); 28.04 ( $\text{CH}_2$ ); 36.05 (CH); 104.31 (CH); 120.88 (C); 125.46 (CH); 126.87 (CH); 127.74 (2CH); 131.61 (2CH); 132.97 (C); 147.50 (C); 150.54 (CH=N); 168.91 (C-NH). *Anal.* Calcd. for  $\text{C}_{16}\text{H}_{16}\text{BrN}_3\text{S}$ : C, 53.04; H, 4.45; N, 11.60. Found: C, 53.02; H, 4.50; N, 11.66.

#### 4-(4-Chlorophenyl)-2-(2-(cyclohex-3-enylmethylene)hydrazinyl)thiazole (3c)

2-Bromo-1-(4-chlorophenyl)ethanone was reacted with **2**. Yield: 0.28 g, 88%, (dichloromethane,  $R_f = 0.36$ ); mp 155–158 °C.  $^1\text{H}$  NMR (DMSO- $d_6$ , 400 MHz)  $\delta$  (ppm): 1.41–1.46 (m, 1H, CH); 1.78–1.84 (m, 1H, CH); 2.00–2.06 (m, 3H, CH,  $\text{CH}_2$ ); 2.08–2.14 (m, 1H, CH); 2.46–2.49 (m, 1H, CH); 5.69 (s, 2H, 2CH); 7.29 (s, 1H, CH); 7.36 (d, 1H, CH,  $J = 5.0$  Hz); 7.45 (d, 2H, 2CH,  $J = 8.0$  Hz); 7.84 (d, 2H, 2CH,  $J = 8.0$  Hz); 11.61 (bs, 1H, NH).  $^{13}\text{C}$  NMR (176 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 23.90 ( $\text{CH}_2$ ); 25.88 ( $\text{CH}_2$ ); 28.17 ( $\text{CH}_2$ ); 36.01 (CH); 103.86 (CH); 125.53 (CH); 126.87 (CH); 127.25 (2CH); 128.63 (2CH); 131.86 (C); 133.73 (C); 148.87 (CH=N); 149.21 (C); 168.82 (C-NH). *Anal.* Calcd. for  $\text{C}_{16}\text{H}_{16}\text{ClN}_3\text{S}$ : C, 60.46; H, 5.07; N, 13.22. Found: C, 60.50; H, 5.01; N, 13.18.

#### 2-(2-(Cyclohex-3-enylmethylene)hydrazinyl)-4-*p*-tolylthiazole (3d)

2-Bromo-1-(4-methylphenyl)ethanone was reacted with **2**. Yield: 0.29 g, 97%, (dichloromethane,  $R_f = 0.41$ ); mp 156–158 °C.  $^1\text{H}$  NMR (DMSO- $d_6$ , 400 MHz)  $\delta$  (ppm): 1.41–1.46 (m, 1H, CH); 1.78–1.84 (m, 1H, CH); 2.00–2.06 (m, 3H, CH,  $\text{CH}_2$ ); 2.08–2.14 (m, 1H, CH); 2.31 (s, 3H,  $\text{CH}_3$ ); 2.41–2.44 (m, 1H, CH); 5.70 (s, 2H, 2CH); 7.13 (s, 1H, CH); 7.20 (d, 2H, 2CH,  $J = 8.0$  Hz); 7.34 (d, 1H, CH,  $J = 5.0$  Hz); 7.70 (d, 2H, 2CH,  $J = 8.0$  Hz); 11.56 (bs, 1H, NH).  $^{13}\text{C}$  NMR (176 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 20.89 ( $\text{CH}_3$ ); 23.90 ( $\text{CH}_2$ ); 25.91 ( $\text{CH}_2$ ); 28.22 ( $\text{CH}_2$ ); 36.00 (CH); 102.11 (CH); 125.49 (CH); 125.55 (2CH); 126.87 (CH); 129.19 (2CH); 132.25 (C); 136.71 (C); 148.58 (C); 150.51 (CH=N); 168.57 (C-NH). *Anal.* Calcd. for  $\text{C}_{17}\text{H}_{19}\text{N}_3\text{S}$ : C, 68.65; H, 6.44; N, 14.13. Found: C, 68.70; H, 6.45; N, 14.18.

#### 2-(2-(Cyclohex-3-enylmethylene)hydrazinyl)-4-(4-(trifluoromethyl)phenyl)thiazole (3e)

2-Bromo-1-(4-trifluoromethylphenyl)ethanone was reacted with **2**. Yield: 0.31 g, 89%, (dichloromethane,  $R_f = 0.54$ ); mp 132–134 °C.  $^1\text{H}$  NMR (DMSO- $d_6$ , 400 MHz)  $\delta$  (ppm): 1.41–1.46 (m, 1H, CH); 1.78–1.84 (m, 1H, CH); 2.00–2.06

(m, 3H, CH, CH<sub>2</sub>), 2.08–2.14 (m, 1H, CH); 2.41–2.44 (m, 1H, CH); 5.70 (s, 2H, 2CH); 7.37 (d, 1H, CH, *J* = 5.0 Hz); 7.47 (s, 1H, CH); 7.75 (d, 2H, 2CH, *J* = 8.0 Hz); 8.03 (d, 2H, 2CH, *J* = 8.0 Hz); 11.68 (bs, 1H, NH). <sup>13</sup>C NMR (176 MHz, DMSO-d<sub>6</sub>) δ (ppm): 23.87 (CH<sub>2</sub>); 25.86 (CH<sub>2</sub>); 28.15 (CH<sub>2</sub>); 36.00 (CH); 105.89 (CH); 125.51 (CH); 125.61 (2CH); 126.07 (2CH); 126.87 (CH); 127.52 (q, C, *J*<sub>C-F</sub> = 29.5 Hz); 138.47 (C); 148.92 (C); 149.05 (CH=N); 168.93 (C–NH). *Anal.* Calcd. for C<sub>17</sub>H<sub>16</sub>F<sub>3</sub>N<sub>3</sub>S: C, 58.11; H, 4.59; N, 11.96. Found: C, 58.08; H, 4.63; N, 12.01.

#### 2-(2-(Cyclohex-3-enylmethylene)hydrazinyl)-4-(4-methoxyphenyl)thiazole (3f)

2-Bromo-1-(4-methoxyphenyl)ethanone was reacted with **2**. Yield: 0.28 g, 88%, (dichloromethane, *R*<sub>f</sub> = 0.23); mp 121–123 °C. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 400 MHz) δ (ppm): 1.41–1.53 (m, 1H, CH); 1.81–1.89 (m, 1H, CH); 1.98–2.23 (m, 4H, 2CH, CH<sub>2</sub>), 2.46–2.49 (m, 1H, CH); 3.77 (s, 3H, OCH<sub>3</sub>); 5.69 (s, 2H, 2CH); 6.96 (d, 2H, 2CH, *J* = 8.0 Hz); 7.04 (s, 1H, CH); 7.34 (d, 1H, CH, *J* = 5.0 Hz); 7.75 (d, 2H, 2CH, *J* = 8.0 Hz); 11.55 (bs, 1H, NH). <sup>13</sup>C NMR (176 MHz, DMSO-d<sub>6</sub>) δ (ppm): 24.28 (CH<sub>2</sub>); 26.31 (CH<sub>2</sub>); 28.59 (CH<sub>2</sub>); 36.38 (CH); 55.57 (CH<sub>3</sub>); 101.28 (CH); 114.38 (2CH); 125.94 (CH); 127.26 (2CH); 128.18 (CH); 133.37 (C); 148.95 (C); 150.63 (CH=N); 159.16 (C–OCH<sub>3</sub>); 168.93 (C–NH). *Anal.* Calcd. for C<sub>17</sub>H<sub>19</sub>N<sub>3</sub>OS: C, 65.15; H, 6.11; N, 13.41. Found: C, 65.20; H, 6.10; N, 13.46.

#### 2-(2-(Cyclohex-3-enylmethylene)hydrazinyl)-4-(4-nitrophenyl)thiazole (3g)

2-Bromo-1-(4-nitrophenyl)ethanone was reacted with **2**. Yield: 0.31 g, 93%, (dichloromethane, *R*<sub>f</sub> = 0.33); mp 145–147 °C. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 400 MHz) δ (ppm): 1.41–1.46 (m, 1H, CH); 1.78–1.84 (m, 1H, CH); 2.00–2.06 (m, 3H, CH, CH<sub>2</sub>), 2.08–2.14 (m, 1H, CH); 2.41–2.44 (m, 1H, CH); 5.70 (s, 2H, 2CH); 7.39 (d, 1H, CH, *J* = 5.0 Hz); 7.63 (s, 1H, CH); 8.09 (d, 2H, 2CH, *J* = 9.0 Hz); 8.26 (d, 2H, 2CH, *J* = 9.0 Hz); 11.74 (bs, 1H, NH). <sup>13</sup>C NMR (176 MHz, DMSO-d<sub>6</sub>) δ (ppm): 24.28 (CH<sub>2</sub>); 26.22 (CH<sub>2</sub>); 28.52 (CH<sub>2</sub>); 36.41 (CH); 108.39 (CH); 124.52 (2CH); 125.90 (CH); 126.73 (2CH); 127.26 (CH); 141.25 (C); 146.60 (C); 148.83 (C); 149.69 (CH=N); 169.49 (C–NH). *Anal.* Calcd. for C<sub>16</sub>H<sub>16</sub>N<sub>4</sub>O<sub>2</sub>S: C, 58.52; H, 4.91; N, 17.06. Found: C, 58.50; H, 4.93; N, 17.08.

#### 4-(2-(2-(Cyclohex-3-enylmethylene)hydrazinyl)thiazol-4-yl)benzotrile (3h)

4-(Bromoacetyl)benzotrile was reacted with **2**. Yield: 0.30 g, 98%, (dichloromethane, *R*<sub>f</sub> = 0.26); mp 180–182 °C.

<sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 400 MHz) δ (ppm): 1.41–1.46 (m, 1H, CH); 1.78–1.84 (m, 1H, CH); 2.00–2.06 (m, 3H, CH, CH<sub>2</sub>); 2.08–2.14 (m, 1H, CH); 2.41–2.44 (m, 1H, CH); 5.70 (s, 2H, 2CH); 7.37 (d, 1H, CH, *J* = 5.0 Hz); 7.54 (s, 1H, CH); 7.85 (d, 2H, 2CH, *J* = 8.5 Hz); 7.99 (d, 2H, 2CH, *J* = 8.5 Hz); 11.69 (bs, 1H, NH). <sup>13</sup>C NMR (176 MHz, DMSO-d<sub>6</sub>) δ (ppm): 23.88 (CH<sub>2</sub>); 25.83 (CH<sub>2</sub>); 28.13 (CH<sub>2</sub>); 36.01 (CH); 106.98 (CH); 109.54 (C); 119.08 (C); 125.50 (CH); 126.14 (2CH); 126.86 (CH); 132.72 (2CH); 138.93 (C); 148.74 (C); 149.15 (CH=N); 168.98 (C–NH). *Anal.* Calcd. for C<sub>17</sub>H<sub>16</sub>N<sub>4</sub>S: C, 66.21; H, 5.23; N, 18.17. Found: C, 66.28; H, 5.25; N, 18.22.

#### 2-(2-(Cyclohex-3-enylmethylene)hydrazinyl)-4-(2,4-difluorophenyl)thiazole (3i)

2-Bromo-1-(2,4'-difluorophenyl)ethanone was reacted with **2**. Yield: 0.30 g, 94%, (dichloromethane, *R*<sub>f</sub> = 0.52); mp 130–132 °C. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 400 MHz) δ (ppm): 1.41–1.46 (m, 1H, CH); 1.78–1.84 (m, 1H, CH); 2.00–2.06 (m, 3H, CH, CH<sub>2</sub>); 2.08–2.14 (m, 1H, CH); 2.41–2.44 (m, 1H, CH); 5.70 (s, 2H, 2CH); 7.11–7.19 (m, 2H, 2CH); 7.30–7.38 (m, 2H, 2CH); 7.95–8.04 (m, 1H, CH); 11.63 (bs, 1H, NH). <sup>13</sup>C NMR (176 MHz, DMSO-d<sub>6</sub>) δ (ppm): 24.27 (CH<sub>2</sub>); 26.25 (CH<sub>2</sub>); 28.55 (CH<sub>2</sub>); 36.33 (CH); 104.92 (t, C, *J*<sub>C-F</sub> = 47.0 Hz); 107.56 (d, C, *J*<sub>C-F</sub> = 27.0 Hz); 112.19 (dd, C, *J*<sub>C-F</sub> = 5.9 Hz, *J*<sub>C-F</sub> = 37.0 Hz); 112.19 (dd, C, *J*<sub>C-F</sub> = 6.8 Hz, *J*<sub>C-F</sub> = 19.2 Hz); 125.91 (CH); 127.26 (CH); 130.81 (dd, C, *J*<sub>C-F</sub> = 8.5 Hz, *J*<sub>C-F</sub> = 16.4 Hz); 143.69 (C); 149.36 (CH=N); 159.53 (dd, C, *J*<sub>C-F</sub> = 23.0 Hz, *J*<sub>C-F</sub> = 289.0 Hz); 162.02 (dd, C, *J*<sub>C-F</sub> = 23.0 Hz, *J*<sub>C-F</sub> = 281.0 Hz); 168.54 (C–NH). *Anal.* Calcd. for C<sub>16</sub>H<sub>15</sub>F<sub>2</sub>N<sub>3</sub>S: C, 60.17; H, 4.73; N, 13.16. Found: C, 60.21; H, 4.69; N, 13.19.

#### 4-(4-Azidophenyl)-2-(2-(cyclohex-3-enylmethylene)hydrazinyl)thiazole (3j)

2-Bromo-1-(4-azidophenyl)ethanone was reacted with **2**. Yield: 0.33 g, 99%, (dichloromethane, *R*<sub>f</sub> = 0.34); mp 124–126 °C. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 400 MHz) δ (ppm): 1.41–1.46 (m, 1H, CH); 1.78–1.84 (m, 1H, CH); 2.00–2.06 (m, 3H, CH, CH<sub>2</sub>); 2.08–2.14 (m, 1H, CH); 2.41–2.44 (m, 1H, CH); 5.70 (s, 2H, 2CH); 7.15 (d, 2H, 2CH, *J* = 8.5 Hz); 7.23 (s, 1H, CH); 7.35 (d, 1H, CH, *J* = 5.0 Hz); 7.87 (d, 2H, 2CH, *J* = 8.5 Hz); 11.61 (bs, 1H, NH). <sup>13</sup>C NMR (176 MHz, DMSO-d<sub>6</sub>) δ (ppm): 23.89 (CH<sub>2</sub>); 25.88 (CH<sub>2</sub>); 28.16 (CH<sub>2</sub>); 36.02 (CH); 102.10 (CH); 119.38 (2CH); 125.53 (CH); 126.87 (CH); 127.17 (2CH); 131.77 (C); 138.28 (C); 149.07 (CH=N); 149.25 (C); 168.73 (C–NH). *Anal.* Calcd. for C<sub>16</sub>H<sub>16</sub>N<sub>6</sub>S: C, 59.24; H, 4.97; N, 25.91. Found: C, 59.25; H, 5.01; N, 25.95.

### 2-(2-(Cyclohex-3-enylmethylene)hydrazinyl)-4-(3,4-dichlorophenyl)thiazole (3k)

2-Bromo-1-(3'4'-dichlorophenyl)ethanone was reacted with **2**. Yield: 0.22 g, 63%, (dichloromethane,  $R_f = 0.50$ ); mp 117–120 °C.  $^1\text{H}$  NMR (DMSO- $d_6$ , 400 MHz)  $\delta$  (ppm): 1.41–1.46 (m, 1H, CH); 1.81–1.89 (m, 1H, CH); 1.98–2.10 (m, 3H, CH, CH<sub>2</sub>); 2.11–2.20 (m, 1H, CH); 2.42–2.50 (m, 1H, CH); 5.70 (s, 2H, 2CH); 7.36 (d, 1H, CH,  $J = 5.0$  Hz); 7.44 (s, 1H, CH); 7.64 (d, 1H, CH,  $J = 8.5$  Hz); 7.80 (dd, 1H, CH,  $J = 3.0$  Hz,  $J = 9.0$  Hz); 8.04 (d, 1H, CH,  $J = 2.0$  Hz); 11.65 (bs, 1H, NH).  $^{13}\text{C}$  NMR (176 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 24.27 (CH<sub>2</sub>); 26.24 (CH<sub>2</sub>); 28.54 (CH<sub>2</sub>); 36.39 (CH); 105.73 (CH); 125.90 (C); 125.97 (C); 127.25 (C); 127.58 (C); 130.06 (C); 131.25 (C); 131.85 (C); 137.76 (C); 148.15 (CH=N); 149.59 (C); 169.29 (C–NH). *Anal.* Calcd. for C<sub>16</sub>H<sub>15</sub>Cl<sub>2</sub>N<sub>3</sub>S: C, 54.55; H, 4.29; N, 11.93. Found: C, 54.57; H, 4.32; N, 11.96.

### 4-(Chloromethyl)-2-(2-(cyclohex-3-enylmethylene)hydrazinyl)thiazole (3l)

1,3-Dichloroacetone was reacted with **2**. Yield: 0.14 g, 55%, (dichloromethane/methanol (95:5),  $R_f = 0.73$ ); mp 127–129 °C.  $^1\text{H}$  NMR (DMSO- $d_6$ , 400 MHz)  $\delta$  (ppm): 1.41–1.46 (m, 1H, CH); 1.78–1.84 (m, 1H, CH), 2.00–2.06 (m, 3H, CH, CH<sub>2</sub>); 2.08–2.14 (m, 1H, CH); 2.41–2.44 (m, 1H, CH); 4.57 (s, 2H, 2CH); 5.68 (s, 2H, CH<sub>2</sub>); 6.84 (s, 1H, CH); 7.30 (d, 1H, CH,  $J = 6.0$  Hz); 11.50 (bs, 1H, NH).  $^{13}\text{C}$  NMR (176 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 23.85 (CH<sub>2</sub>); 25.84 (CH<sub>2</sub>); 28.15 (CH<sub>2</sub>); 35.96 (CH); 42.01 (CH<sub>2</sub>-Cl); 107.58 (CH); 125.51 (CH); 126.87 (CH); 147.87 (C–CH<sub>2</sub>Cl); 148.84 (CH=N); 169.10 (C–NH). *Anal.* Calcd. for C<sub>11</sub>H<sub>14</sub>ClN<sub>3</sub>S: C, 51.66; H, 5.52; N, 16.43. Found: C, 51.67; H, 5.55; N, 16.46.

### 2-(2-(Cyclohex-3-enylmethylene)hydrazinyl)-4-(adamant-1-yl)thiazole (3m)

1-Adamantyl bromomethyl ketone was reacted with **2**. Yield: 0.31 g, 92%, (dichloromethane,  $R_f = 0.51$ ); mp 155–157 °C.  $^1\text{H}$  NMR (DMSO- $d_6$ , 400 MHz)  $\delta$  (ppm): 1.41–1.53 (m, 1H, CH); 1.65–1.76 (m, 7H, 7CH); 1.80–1.88 (m, 7H, 7CH); 1.98–2.09 (m, 6H, 6CH); 2.11–2.20 (m, 1H, CH); 5.69 (s, 2H, 2CH); 6.40 (s, 1H, CH); 7.49 (d, 1H, CH,  $J = 5.0$  Hz); 11.69 (bs, 1H, NH).  $^{13}\text{C}$  NMR (176 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 24.12 (CH<sub>2</sub>); 26.01 (CH<sub>2</sub>); 28.18 (6CH<sub>2</sub>); 28.30 (CH<sub>2</sub>); 36.00 (CH); 36.47 (CH); 36.54 (2CH); 41.14 (C); 100.83 (CH); 125.72 (CH); 127.28 (CH); 153.77 (CH=N); 169.25 (C–NH). *Anal.* Calcd. for C<sub>20</sub>H<sub>27</sub>N<sub>3</sub>S: C, 70.34; H, 7.97; N, 12.30. Found: C, 70.34; H, 7.99; N, 12.35.

### Ethyl 2-(2-(2-(cyclohex-3-enylmethylene)hydrazinyl)thiazol-4-yl)acetate (3n)

Ethyl 4-chloro-3-oxobutanoate was reacted with **2**. Yield: 0.20 g, 68%, (dichloromethane,  $R_f = 0.51$ ); mp 125–127 °C.  $^1\text{H}$  NMR (DMSO- $d_6$ , 400 MHz)  $\delta$  (ppm): 1.17 (t, 3H, CH<sub>3</sub>,  $J = 7.0$  Hz); 1.41–1.46 (m, 1H, CH); 1.78–1.84 (m, 1H, CH); 2.00–2.06 (m, 3H, CH, CH<sub>2</sub>); 2.08–2.14 (m, 1H, CH); 2.41–2.44 (m, 1H, CH); 3.53 (s, 2H, CH<sub>2</sub>); 4.05 (q, 2H, CH<sub>2</sub>,  $J = 7.0$  Hz); 5.68 (s, 2H, 2CH); 6.53 (s, 1H, CH); 7.28 (d, 1H, CH,  $J = 6.0$  Hz); 11.39 (bs, 1H, NH).  $^{13}\text{C}$  NMR (176 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 14.20 (CH<sub>3</sub>); 23.86 (CH<sub>2</sub>); 25.87 (CH<sub>2</sub>); 28.19 (CH<sub>2</sub>); 35.95 (CH); 37.15 (CH<sub>2</sub>); 60.26 (CH<sub>2</sub>-O); 104.89 (CH); 125.53 (CH); 126.86 (CH); 145.00 (C); 148.44 (CH=N); 168.52 (C=O); 170.13 (C–NH). *Anal.* Calcd. for C<sub>14</sub>H<sub>19</sub>N<sub>3</sub>O<sub>2</sub>S: C, 57.31; H, 6.53; N, 14.32. Found: C, 57.28; H, 6.56; N, 14.36.

### 2-(2-(Cyclohex-3-enylmethylene)hydrazinyl)-4-methylthiazole (3o)

Chloroacetone was reacted with **2**. Yield: 0.12 g, 52%, (dichloromethane,  $R_f = 0.29$ ); mp 128–129 °C.  $^1\text{H}$  NMR (DMSO- $d_6$ , 400 MHz)  $\delta$  (ppm): 1.39–1.52 (m, 1H, CH); 1.78–1.88 (m, 1H, CH); 1.96–2.01 (m, 3H, CH, CH<sub>2</sub>); 2.15–2.20 (m, 1H, CH); 2.33 (s, 3H, CH<sub>3</sub>); 2.39–2.49 (m, 1H, CH); 5.68 (s, 2H, 2CH); 6.28 (s, 1H, CH); 7.29 (d, 1H, CH,  $J = 5.5$  Hz); 11.24 (bs, 1H, NH).  $^{13}\text{C}$  NMR (176 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 17.65 (CH<sub>3</sub>); 24.28 (CH<sub>2</sub>); 26.34 (CH<sub>2</sub>); 28.64 (CH<sub>2</sub>); 36.34 (CH); 102.27 (CH); 125.95 (CH); 127.25 (CH); 148.02 (C–CH<sub>3</sub>); 148.78 (CH=N); 168.70 (C–NH). *Anal.* Calcd. for C<sub>11</sub>H<sub>15</sub>N<sub>3</sub>S: C, 59.69; H, 6.83; N, 18.99. Found: C, 59.71; H, 6.83; N, 19.04.

## Microbiology

### Microbial material

The examined 1,3-tiazole derivatives **3a–3o** were screened in vitro for antibacterial and antifungal activities using the broth microdilution method according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) (EUCAST 2003) and Clinical and Laboratory Standards Institute (CLSI) guidelines (Wayne Clinical and Laboratory Standards Institute 2012) against reference strains of microorganisms from American Type Culture Collection (ATCC), including Gram-positive bacteria (*Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 43300, *Micrococcus luteus* ATCC 10240, *Bacillus cereus* ATCC 10876, and *Bacillus subtilis* ATCC 6633), Gram-negative bacteria (from the *Enterobacteriaceae* family: *Escherichia coli* ATCC 25922,

*Klebsiella pneumoniae* ATCC 13883, *Proteus mirabilis* ATCC 12453, *Salmonella typhimurium* ATCC 14028 and other: *Pseudomonas aeruginosa* ATCC 9027 and *Bordetella bronchiseptica* ATCC 4617) and fungi belonging to yeasts (*Candida albicans* ATCC 2091, *Candida albicans* ATCC 10231, *Candida glabrata* ATCC 90030, *Candida parapsilosis* ATCC 22019, and *Candida krusei* ATCC 14243).

In the study of antifungal activity of the compounds **3a–3o** 14 clinical strains of different species of yeasts from *Candida* species were also used, namely *C. albicans*, *C. dubliniensis*, *C. famata*, *C. glabrata*, *C. guilliermondii*, *C. inconspicua*, *C. kefyr*, *C. krusei*, *C. lambica*, *C. lusitanae*, *C. parapsilosis*, *C. pulcherrima*, *C. sake*, and *C. tropicalis*. These fungi were isolated by the authors (from Department of Pharmaceutical Microbiology of Medical University in Lublin, Poland) from various clinical materials, e.g., from upper respiratory tract of hospitalized patients including cancer persons (i.e., with lung cancer or hematological malignancies), patients with chronic hepatitis C, patients with diabetes and elderly people, aged of 65 years old or older. The Ethical Committee of the Medical University of Lublin approved the study protocol (No. KE-0254/75/2011). These isolates were identified by standard diagnostic methods—biochemical microtests, e.g., API Candida, API 20 C AUX and ID 32 C (bioMérieux) on the basis of assimilation of different substrates.

### Antimicrobial activity

All the used microbial cultures were first subcultured on appropriate nutrient media. Microbial suspensions were prepared in 0.85% NaCl with an optical density of 0.5 McFarland standard scale. The surface of Mueller–Hinton agar and RPMI 1640 with MOPS were inoculated with the suspensions of bacterial or fungal species, respectively. The examined compounds **3a–3o** were first dissolved in dimethyl sulfoxide (DMSO). Next, suspensions were plated on solid media containing 2 mg/ml of the tested compounds followed incubation under appropriate conditions. The inhibition of microbial growth was assessed by comparison with a control culture prepared without any sample tested. Ciprofloxacin or nystatin (Sigma) were used as a reference antibacterial or antifungal compounds, respectively.

Subsequently minimal inhibitory concentration (MIC) of these compounds was examined by the two-fold microdilution broth method in Mueller-Hinton broth (for bacteria) and RPMI 1640 broth with MOPS (for fungi) prepared in 96-well polystyrene plates. Final concentrations of the compounds in the liquid media ranged from 1000 to 0.0038 µg/ml. Microbial suspensions were added per each well containing broth and various concentrations of the examined compounds. After incubation, the MIC was

determined spectrophotometric as the lowest concentration of the samples showing complete bacterial or fungal growth inhibition. Appropriate DMSO, sterile, and growth controls were carried out. The media with no tested substances were used also as controls.

The minimal bactericidal concentration (MBC) or minimal fungicidal concentration (MFC) are defined as the lowest concentration of the compound that is required to kill a particular bacterial or fungal species. MBC or MFC of substances **3a–3o** were assessed by removing the culture using for MIC determinations from each well and spotting onto appropriate agar medium. The plates were incubated. The lowest compounds concentrations with no visible growth observed were assessed as a bactericidal or fungicidal concentrations. All the experiments were repeated three times and representative data are presented (Wiegand et al. 2008).

In this study was defined: no bioactivity (MIC > 1000 µg/ml), mild (MIC = 501–1000 µg/ml), moderate (MIC = 126–500 µg/ml), good (MIC = 26–125 µg/ml), strong (MIC = 10–25 µg/ml), and very strong bioactivity (MIC < 10 µg/ml). The MBC/MIC or MFC/MIC ratios were calculated in order to determine bactericidal/fungicidal (MBC/MIC ≤ 4, MFC/MIC ≤ 4) or bacteriostatic/fungistatic (MBC/MIC > 4, MFC/MIC > 4) effect of the tested compounds (O'Donnell et al. 2010).

### Cytotoxic assay

#### Preparation of compounds and drugs

Selected compounds **3a**, **3d**, **3f**, **3n**, and **3o** were dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO, USA) to 50 mg/ml. Dilution of the all compounds were freshly prepared before the cells were exposed. The final concentration of DMSO in the compounds dilutions was not higher than 1.00%.

#### Cell viability assay

Cell viability assay were performed according to international standards (ISO 10993-5:2009(E)), using the tetrazolium salt [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT, Sigma-Aldrich) and mouse adherent fibroblasts L929 cells (ATTC® CCL-1™). Briefly, 1 × 10<sup>4</sup>/100 µL/well of L929 cells placed into 96-well plates were incubated 24 h in 37 °C and 10% CO<sub>2</sub> to achieve a confluent monolayer. Afterwards, the growth medium (GM) was replaced by 100 µL of the compounds dilutions in culture medium (CM) and the cells were treated next 24 h. In addition, cells were treated with a 4.0–0.03% concentration of DMSO and 1 M NaOH as the compound solvent (data not shown). Then, 50 µL of 1 mg/ml of MTT solution in CM was added to

each well for more 2 h (37 °C, 10% CO<sub>2</sub>) incubation. Following, CM was carefully aspirated and 150 µL of DMSO and 25 µL 0.1M glycine buffer (pH = 10.5) (Sigma-Aldrich) was added. The optical density at 570 nm on the ELISA reader (Multiskan EX, Labsystems, Vienna, VA, USA), was read. The results were expressed as a percentage of viability compared with untreated cells. All experiments were performed in triplicate.

**Growth Medium (GM)**—RPMI 1640 media R8758 (Sigma-Aldrich), supplemented with 5% fetal bovine serum (FBS, ATCC<sup>®</sup> 30-2020<sup>™</sup>), 100 U/ml penicillin and 100 µg/ml streptomycin (Penicillin–Streptomycin Solution ATCC<sup>®</sup> 30-2300<sup>™</sup>).

**Culture Medium (CM)**—RPMI 1640, without phenol red (Biowest, Nuaille, France), supplemented with 5% fetal bovine serum (FBS, ATCC<sup>®</sup> 30-2020<sup>™</sup>), 2 mM L-glutamine (Sigma-Aldrich), 100 U/ml penicillin and 100 µg/ml streptomycin (Penicillin–Streptomycin Solution ATCC<sup>®</sup> 30-2300<sup>™</sup>).

### Lipophilicity

Experimental lipophilicity of the synthesized thiazole derivatives **3a–3o** was determined using reversed phase thin-layer chromatography (RP-TLC–reversed phase thin-layer chromatography) on 10 × 10 cm HPTLC plates coated with C18 silica F254 (Merck, Darmstadt, Germany). Binary solvents were prepared by mixing appropriate volumes of water and one polar modifier (60–80% acetone, 60–90% acetonitrile, 60–85% 1,4-dioxane, 70–95% methanol, in volumes increasing by 5%). All organic modifiers were of analytical grade and they were supplied by POCH (Gliwice, Poland) and Merck (Darmstadt, Germany). The examined compounds **3a–3o** and reference substances with known lipophilicity: 2-aminophenol (S1), 8-hydroxyquinoline (S2), 2-naphthol (S3), diphenylamine (S4), 3,4-benzopyrene (S5)

were dissolved in methanol to obtain the concentrations 2.0 mg/ml. Volumes of 0.2 µl were spotted to the plates. The chromatograms were developed to a distance of 9 cm from the origin, in a horizontal Teflon chamber with an eluent distributor (Chromdes, Lublin, Poland) at 23 ± 1 °C. After developing, the spots were located under ultraviolet (UV) illumination at 254 nm.

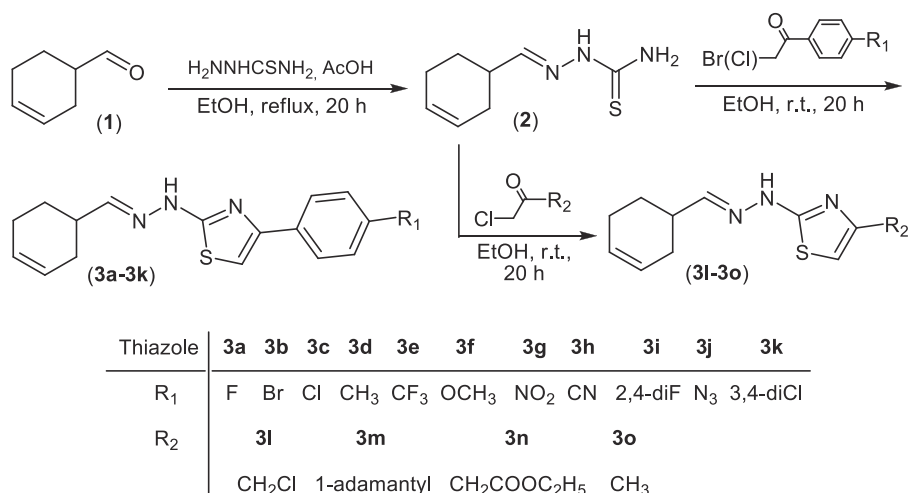
## Results and discussion

### Chemistry

Synthesis of the title compounds was accomplished following the well known Hantzsch cyclization reaction. In the first step 2-(cyclohex-3-enylmethylene)hydrazinecarbothioamide (**2**) was synthesized with 80% yield through condensation of 3-cyclohexene-1-carboxaldehyde with thiosemicarbazide in absolute ethyl alcohol containing catalytic amount of glacial acetic acid. In the next step cyclization of hydrazinecarbothioamide **2** with variety of *para*-substituted bromoacetophenones in ethanolic solution and under room temperature produced (2-(cyclohex-3-enylmethylene)-hydrazinyl)thiazole **3a–3o** with high yield (52–99%) and with high chemical purity. The reaction pathway has been summarized in Scheme 1. All obtained products were purified on silica gel column chromatography, and fully characterized spectroscopically using <sup>1</sup>H and <sup>13</sup>C NMR, and elemental analyses.

In the <sup>1</sup>H NMR spectrum of compound **2**, three characteristic signals derived from the NH<sub>2</sub> and NH groups at 7.44, 7.98 and 11.05 ppm can be observed. These three signals characteristic for hydrazinecarbothioamides are the result of the exchange of a hydrogen atom between the NH<sub>2</sub> group and the sulfur atom. The <sup>13</sup>C NMR of carbon atoms present in C=N and C–SH groups resonate around 150 and

**Scheme 1** Synthesis of the target compounds **3a–3o**



178 ppm, respectively. Also  $^1\text{H}$  NMR spectra of (2-(cyclohex-3-enylmethylene)hydrazinyl)thiazole (**3a–3o**) showed characteristic singlet at  $\delta$  (6.28–7.63) ppm due to the presence of the H-5 atom in the thiazole ring and broadened hydrazine NH singlet at  $\delta$  (11.24–11.74) ppm. The  $^{13}\text{C}$  NMR of carbon atoms present in C=N and C–NH groups resonate around 150 and 170 ppm, respectively, which proves that the conversion of substrates to the expected products was successful. Purity of the products was confirmed by the elemental analyses, whose results were in good agreement with the calculated values. All reactions were repeated at least two times and are fully reproducible.

## Biological evaluation

### Antifungal activity

According to the results presented in Table 1, on the basis of minimal inhibitory concentration values obtained by the broth microdilution method, it was shown that compounds **3a–3d**, **3f**, **3n**, and **3o** exhibited very strong activity against reference *Candida* spp. with MIC = 0.015–3.91  $\mu\text{g/ml}$ . The activity of these compounds is similar and even higher than the activity of nystatin used as positive control. The compounds **3n** and **3o** showed the highest activity with very strong fungicidal effect towards yeasts with MIC = 0.015–0.03  $\mu\text{g/ml}$  and similar MFC = 0.015–0.06  $\mu\text{g/ml}$  (MFC/MIC = 1–4) (Tables 1 and 2). Moreover, the compounds **3h–3j** indicated very strong anticandidal effect (MIC = 0.24–7.81  $\mu\text{g/ml}$ , MFC = 1.95 to  $\geq 1000$   $\mu\text{g/ml}$ ) towards reference *C. albicans* and *C. krusei* strains. Also substance **3l** with MIC = 1.95–125  $\mu\text{g/ml}$  and MFC = 3.91 to  $> 1000$   $\mu\text{g/ml}$  had the high activity (Tables 1 and 2). The compounds **3e**, **3k**, **3m** did not show any antifungal effect against reference *Candida* spp. strains. All tested compounds were inactive towards *C. glabrata* ATTC 90030, which is naturally insensitive to some antimycotics, especially to the azole drugs (Tumbarello et al. 2008). In addition, compounds **3g–3j** were inactive towards *C. parapsilosis* ATTC 22019.

In view of the fact that these substances were highly active against the reference yeasts, we decided to examine their activity against the clinical isolates of *Candida* spp. The compounds **3n** and **3o** showed the highest activity with very strong antifungal effect towards most of yeasts (*C. albicans*, *C. dubliniensis*, *C. famata*, *C. inconspicua*, *C. krusei*, *C. tropicalis*, *C. lusitaniae*, *C. parapsilosis*, *C. guilliermondii*, and *C. sake*) with MIC = 0.015–3.91  $\mu\text{g/ml}$  and MFC = 0.015–125  $\mu\text{g/ml}$  (Tables 1 and 2). The anticandidal effect was both fungicidal (MFC = 1–4) and fungistatic (MFC = 8–64). The activity of these substances was similar to nystatin. The compounds **3d** and **3f** showed also very strong activity towards *C. albicans*, *C. dubliniensis*, *C.*

*inconspicua*, *C. krusei*, *C. tropicalis*, *C. lusitaniae*, and *C. sake* (MIC = 3.91–7.81  $\mu\text{g/ml}$  and MFC = 7.81 to  $\geq 1000$   $\mu\text{g/ml}$ ). The substances **3a–3c** had slightly lower effect in relation to the same clinical isolates (MIC = 1.95–125  $\mu\text{g/ml}$  and MFC = 7.81 to  $\geq 1000$   $\mu\text{g/ml}$ ). Substances **3a–3d** and **3f** indicated a weaker activity against other strains of *Candida* such as *C. kefyr*, *C. famata*, *C. guilliermondii*, and *C. parapsilosis* with MIC = 31.25–1000  $\mu\text{g/ml}$  and MFC = 500 to  $\geq 1000$   $\mu\text{g/ml}$ . Also compounds **3g–3j** and **3l** showed some anticandidal activity. These studies showed that the most susceptible to substances **3a–3o** was *C. lusitaniae*, while *C. glabrata*, *C. pulcherrima*, and *C. lambica* were the least sensitive to them. The antifungal effect was different - both fungicidal (MFC/MIC = 1–4) and fungistatic (MFC/MIC = 8 to  $> 256$ ) (Table 2). The compounds **3e**, **3k**, and **3m** did not show any activity towards clinical isolates.

### Antibacterial activity

The studies of activity of the compounds **3a–3o** against bacteria demonstrated that compounds **3b**, **3c**, **3g**, **3k–3m**, and **3o** exhibited some activity against reference Gram-positive bacteria and *Bordetella bronchiseptica* ATTC 4617 belonging to Gram-negative microorganisms. Among them, the compound **3k** exhibited moderate or good effect (MIC = 62.5–500  $\mu\text{g/ml}$  and MBC = 500 to  $\geq 1000$   $\mu\text{g/ml}$ ) against these bacteria as well as compound **3o** had very strong effect towards *B. bronchiseptica* ATTC 4617 (MIC = 0.06  $\mu\text{g/ml}$  and MBC = 0.48  $\mu\text{g/ml}$ ), moderate activity against *Bacillus cereus* ATCC 10876 (MIC = 500  $\mu\text{g/ml}$  and MBC  $> 1000$   $\mu\text{g/ml}$ ) and mild effect towards the rest of these bacteria (MIC = 1000  $\mu\text{g/ml}$ , MBC  $\geq 1000$   $\mu\text{g/ml}$ ). The compounds **3l** and **3n** showed strong (MIC = 15.62  $\mu\text{g/ml}$  and MBC  $> 62.5$   $\mu\text{g/ml}$ ) and very strong activity (MIC = 0.06  $\mu\text{g/ml}$  and MBC = 0.24  $\mu\text{g/ml}$ ) towards *B. bronchiseptica* ATTC 4617, respectively.

The structure–activity relationship analysis showed that the type of substituent has a decisive influence on the activity. As can be seen, compounds **3n** and **3o** containing  $\text{CH}_2\text{COOEt}$  and  $\text{CH}_3$  groups directly bound to the thiazole ring show the highest activity against *Candida* spp. Also, compounds **3d** and **3f** containing  $\text{CH}_3$  and  $\text{OCH}_3$  substituents in the phenyl ring show high activity against yeasts. These results show that compounds containing electron-donating groups promote high antifungal activity of compounds. The only exception is the adamantyl group in compound **3m**, which did not show any activity towards *Candida* spp., and the only explanation of this fact is that this group is sterically hindered. Also compounds **3e** and **3k** containing the  $\text{CF}_3$  and 3,4-diCl substituents did not show any activity against *Candida* spp. It can be noticed that the presence of electron-



**Table 1** The activity data of compounds **3a–3o** expressed as MIC [ $\mu\text{g/ml}$ ] against the reference and clinical strains of fungi

| Species                              | MIC [ $\mu\text{g/ml}$ ] of the tested compounds |       |       |      |       |      |      |      |       |       |       |       | Nystatin |
|--------------------------------------|--|-------|-------|------|-------|------|------|------|-------|-------|-------|-------|----------|
|                                      | 3a   | 3b    | 3c    | 3d   | 3f    | 3g   | 3h   | 3i   | 3j    | 3l    | 3n    | 3o    |          |
| <i>C. albicans</i><br>ATCC 2091      | 0.48   | 7.81  | 0.48  | 0.48 | 1.95  | 1000 | 7.81 | 7.81 | 3.91  | 125   | 0.015 | 0.03  | 0.24     |
| <i>C. albicans</i><br>ATCC 10231     | 0.06   | 0.06  | 0.12  | 0.12 | 0.24  | 125  | 0.98 | 0.9  | 0.98  | 15.6  | 0.015 | 0.03  | 0.48     |
| <i>C. parapsilosis</i><br>ATCC 22019 | 0.48   | 3.91  | 0.48  | 0.48 | 3.91  | –    | –    | –    | –     | 31.25 | 0.03  | 0.03  | 0.24     |
| <i>C. glabrata</i><br>ATCC 90030     | –  | –     | –     | –    | –     | –    | –    | –    | –     | –     | –     | –     | 0.24     |
| <i>C. krusei</i><br>ATCC 14243       | 0.06   | 0.98  | 0.12  | 0.06 | 0.48  | 125  | 0.24 | 0.48 | 0.98  | 1.95  | 0.015 | 0.015 | 0.24     |
| <i>C. krusei</i>                     | 31.25  | 15.62 | 15.62 | 7.81 | 3.91  | 1000 | 1000 | 500  | 250   | 125   | 0.06  | 0.06  | 0.24     |
| <i>C. tropicalis</i>                 | 31.25  | 7.81  | 15.62 | 1.95 | 3.91  | 1000 | 125  | 250  | 500   | 250   | 0.06  | 0.06  | 0.015    |
| <i>C. inconspicua</i>                | 31.25  | 15.62 | 15.62 | 1.95 | 7.81  | 500  | 500  | 250  | 62.5  | 62.5  | 0.03  | 0.06  | 0.12     |
| <i>C. lusitanae</i>                  | 7.81   | 1.95  | 3.91  | 7.81 | 3.91  | 500  | 7.81 | 500  | 15.62 | 250   | 0.03  | 0.06  | 0.12     |
| <i>C. sake</i>                       | 31.25  | 31.25 | 15.62 | 3.91 | 1.95  | 500  | 250  | 125  | 125   | 62.5  | 0.06  | 0.12  | 0.06     |
| <i>C. dubliniensis</i>               | 125  | 3.91  | 31.25 | 7.81 | 7.81  | 1000 | 500  | 500  | 1000  | 500   | 0.06  | 0.98  | 0.12     |
| <i>C. albicans</i>                   | 125  | 15.62 | 31.25 | 3.91 | 3.91  | 1000 | 1000 | 500  | 1000  | 500   | 0.015 | 0.03  | 0.24     |
| <i>C. kefyr</i>                      | 1000   | 500   | 500   | 1000 | 250   | 1000 | 500  | 1000 | 1000  | 500   | 3.91  | 62.5  | 0.12     |
| <i>C. famata</i>                     | 500  | 125   | 250   | 1000 | 125   | 500  | 500  | 1000 | 500   | 125   | 3.91  | 1.95  | 0.06     |
| <i>C. guilliermondii</i>             | 1000   | 125   | 500   | 1000 | 250   | –    | 1000 | 1000 | 1000  | 250   | 1.95  | 0.24  | 0.06     |
| <i>C. parapsilosis</i>               | 125  | 31.25 | 125   | –    | 15.62 | 1000 | 1000 | 500  | 1000  | 250   | 0.015 | 0.06  | 0.12     |
| <i>C. glabrata</i>                   | –  | 1000  | –     | –    | –     | 1000 | –    | –    | –     | 1000  | 500   | 125   | 0.48     |
| <i>C. pulcherrima</i>                | –  | 1000  | –     | –    | –     | –    | –    | –    | –     | 1000  | 1000  | 125   | 0.12     |
| <i>C. lambica</i>                    | –  | –     | –     | –    | –     | –    | –    | –    | –     | –     | 500   | 62.5  | 0.24     |

withdrawing groups reduces the activity of compounds. An important result that we have noticed earlier in our research is the lack of activity of derivatives containing the  $\text{CF}_3$  group.

### Cytotoxicity against L929 cells

Selected compounds **3a**, **3d**, **3f**, **3n**, and **3o** were tested for their cytotoxicity against mouse adherent fibroblast cells line (L929 - ATTC<sup>®</sup> CCL-1<sup>™</sup>) according to the international standards: ISO 10993-5:2009(E) using MTT assay. The percent of viable cells  $\pm$  standard derivation in the concentrations range between 0 to 50.00  $\mu\text{g/ml}$  together with the no cytotoxic concentration values are presented in Table 3. The results of the cytotoxicity evaluation showed that *Candida* spp. growth was inhibited at noncytotoxic concentrations for the mammalian L929 fibroblast (NCC 10–50  $\mu\text{g/ml}$ ).

### Lipophilicity determination

On the basis of retardation coefficients ( $R_F$ ) for reference substances and tested compounds,  $R_M$  values were

calculated using the equation:  $R_M = \log(1 - R_F/R_F)$ . Then,  $R_{M0}$  values (equivalent to the retention of a solute extrapolated to pure water as mobile phase) were calculated using the equation:  $R_M = R_{M0} - S\varphi$ , where  $\varphi$  is the volume fraction of the organic modifier in the mobile phase and  $S$  is the slope of the regression curve (Tables 4 and 5).

The calculated  $R_{M0}$  values for the reference substances were correlated with their  $\log P$  values found in the literature (Komsta et al. 2010) and appropriate calibration curves were obtained with sufficient linearity:

$$\log P_{\text{exp}} = 1.2387R_{M0} - 0.4642, r^2 = 0.8719 \text{ (acetone)},$$

$$\log P_{\text{exp}} = 1.6749R_{M0} - 0.1582, r^2 = 0.8542 \text{ (acetonitrile)},$$

$$\log P_{\text{exp}} = 1.4500R_{M0} - 0.8960, r^2 = 0.7668 \text{ (1,4 - dioxane)},$$

$$\log P_{\text{exp}} = 0.9620R_{M0} + 0.2659, r^2 = 0.7526 \text{ (methanol)}.$$

The lipophilicity of the synthesized 1,3-tiazole derivatives **3a–3o** was calculated on the basis of the above calibration equations and their  $R_{M0}$  values. The calculated lipophilicity values were presented in Table 6.

**Table 2** The activity data of compounds **3a–3o** expressed as MFC [ $\mu\text{g/ml}$ ] and (MFC/MIC) against the reference and clinical strains of fungi

| Species                              | MFC [ $\mu\text{g/ml}$ ] and (MFC/MIC) of the tested compounds |               |                |                |               |             |             |               |                |              |             |              | Nystatin  |
|--------------------------------------|--|---------------|----------------|----------------|---------------|-------------|-------------|---------------|----------------|--------------|-------------|--------------|-----------|
|                                      | 3a   | 3b            | 3c             | 3d             | 3f            | 3g          | 3h          | 3i            | 3j             | 3l           | 3n          | 3o           |           |
| <i>C. albicans</i><br>ATCC 2091      | 0.98<br>2  | 7.81<br>1     | 0.98<br>2      | 0.48<br>1      | 3.91<br>2     | >1000<br>>1 | 15.62<br>2  | >1000<br>>128 | >1000<br>>256  | 250<br>2     | 0.03<br>2   | 0.06<br>2    | 0.24<br>1 |
| <i>C. albicans</i><br>ATCC 10231     | 0.12<br>2  | 0.12<br>2     | 0.24<br>2      | 0.24<br>2      | 0.98<br>4     | >1000<br>>8 | 3.91<br>4   | 3.91<br>4     | 1.95<br>2      | 31.25<br>2   | 0.06<br>4   | 0.06<br>2    | 0.48<br>1 |
| <i>C. parapsilosis</i><br>ATCC 22019 | 7.81<br>16   | >1000<br>>256 | >1000<br>>1024 | >1000<br>>1024 | >1000<br>>256 | –<br>–      | –<br>–      | –<br>–        | –<br>–         | >1000<br>>32 | 0.06<br>2   | 0.06<br>2    | 0.48<br>2 |
| <i>C. glabrata</i><br>ATCC 90030     | –  | –             | –              | –              | –             | –           | –           | –             | –              | –            | –           | –            | 0.48<br>2 |
| <i>C. krusei</i><br>ATCC 14243       | 0.24<br>4  | 7.81<br>8     | 0.24<br>2      | 0.24<br>4      | 1.95<br>4     | >1000<br>>8 | 1.95<br>8   | 1.95<br>4     | >1000<br>>1024 | 3.91<br>2    | 0.03<br>2   | 0.015<br>1   | 0.24<br>1 |
| <i>C. krusei</i>                     | 125<br>4   | 125<br>8      | 1000<br>64     | 1000<br>128    | 125<br>32     | >1000<br>>1 | >1000<br>>1 | 1000<br>2     | 1000<br>4      | >1000<br>>8  | 0.06<br>1   | 0.24<br>4    | 0.98<br>4 |
| <i>C. tropicalis</i>                 | 125<br>4   | 15.62<br>2    | 15.62<br>1     | 15.62<br>8     | 15.62<br>4    | >1000<br>>1 | 500<br>4    | 500<br>2      | 1000<br>2      | 1000<br>4    | 0.24<br>4   | 1.95<br>32   | 0.06<br>4 |
| <i>C. inconspicua</i>                | 250<br>8   | 250<br>16     | 1000<br>64     | 1000<br>512    | 31.25<br>4    | >1000<br>>2 | 1000<br>2   | 1000<br>4     | 125<br>2       | 1000<br>16   | 0.06<br>2   | 0.06<br>1    | 0.48<br>4 |
| <i>C. lusitanae</i>                  | 15.62<br>2   | 7.81<br>4     | 15.62<br>4     | 1000<br>128    | 7.81<br>2     | >1000<br>>2 | 1000<br>128 | 1000<br>2     | >1000<br>64    | 500<br>2     | 0.06<br>2   | 0.12<br>2    | 0.24<br>2 |
| <i>C. sake</i>                       | 1000<br>32   | 125<br>4      | >1000<br>>64   | >1000<br>>256  | 15.62<br>8    | >1000<br>>2 | >1000<br>>4 | 250<br>2      | >1000<br>>8    | 1000<br>16   | 0.12<br>2   | 1.95<br>16   | 0.12<br>2 |
| <i>C. dubliniensis</i>               | 500<br>4   | 7.81<br>2     | 500<br>16      | 7.81<br>1      | 7.81<br>1     | >1000<br>>1 | 1000<br>2   | 1000<br>2     | >1000<br>>1    | 1000<br>2    | 0.12<br>2   | 1.95<br>2    | 0.12<br>1 |
| <i>C. albicans</i>                   | 250<br>2   | 62.5<br>4     | 500<br>16      | 15.62<br>4     | 15.62<br>4    | >1000<br>>1 | >1000<br>>1 | 1000<br>2     | >1000<br>>1    | 1000<br>2    | 0.03<br>2   | 0.98<br>32   | 0.24<br>1 |
| <i>C. kefyr</i>                      | >1000<br>>1  | >1000<br>>2   | >1000<br>>2    | >1000<br>>1    | 1000<br>4     | >1000<br>>1 | >1000<br>>2 | >1000<br>>1   | >1000<br>>1    | 1000<br>2    | 125<br>32   | 250<br>4     | 0.24<br>2 |
| <i>C. famata</i>                     | >1000<br>>2  | >1000<br>>8   | >1000<br>>4    | >1000<br>>1    | 1000<br>8     | >1000<br>>2 | >1000<br>>2 | >1000<br>>1   | >1000<br>>2    | 1000<br>8    | 15.62<br>4  | 3.91<br>2    | 0.12<br>2 |
| <i>C. guilliermondii</i>             | >1000<br>>1  | >1000<br>>8   | >1000<br>>2    | >1000<br>>1    | 1000<br>4     | –<br>–      | 1000<br>1   | >1000<br>>1   | 1000<br>1      | 1000<br>4    | 125<br>64   | 3.91<br>16   | 0.12<br>2 |
| <i>C. parapsilosis</i>               | 1000<br>8  | 500<br>16     | >1000<br>>8    | –<br>–         | 250<br>16     | >1000<br>>1 | >1000<br>>1 | >1000<br>>2   | >1000<br>>1    | 1000<br>4    | 0.03<br>2   | 0.24<br>4    | 0.48<br>4 |
| <i>C. glabrata</i>                   | –  | >1000<br>>1   | –<br>–         | –<br>–         | –<br>–        | >1000<br>>1 | –<br>–      | –<br>–        | –<br>–         | >1000<br>>1  | >1000<br>>1 | >1000<br>>8  | 0.48<br>1 |
| <i>C. pulcherrima</i>                | –  | >1000<br>>1   | –<br>–         | –<br>–         | –<br>–        | –<br>–      | –<br>–      | –<br>–        | –<br>–         | >1000<br>>1  | >1000<br>>1 | >1000<br>>8  | 0.24<br>2 |
| <i>C. lambica</i>                    | –  | –             | –              | –              | –             | –           | –           | –             | –              | –            | >1000<br>>2 | >1000<br>>16 | 0.48<br>2 |

It is well known that RP-TLC method allows fast and reproducible determination of lipophilicity values, especially for a wide range of new synthesized compounds. In the present study, determination coefficients ( $r^2$ ) obtained for calibration equations were sufficiently high ( $>0.75$ ) providing accuracy of further lipophilicity determination. At the same time, lipophilicity of the reference substances was highly correlated with their  $R_{M0}$  values (Table 5). As far as

the tested compounds were concerned, the determination coefficients were also sufficiently high ( $>0.95$ ) for all eluents. Lipophilicity values obtained for the tested compounds were similar to each other using eluents containing methanol, acetonitrile, and acetone while higher values were calculated for 1,4-dioxane systems. At the same time, in 1,4-dioxane systems, the calculated  $r^2$  were the highest values (almost all  $r^2 > 0.99$ ). In the literature, methanol and

**Table 3** The viability (%) ± standard deviation (SD) of mammalian L929 cells in the presence of tested compounds (**3a**, **3d**, **3f**, **3n**, and **3o**) in the concentration range between 0 and 50.00 µg/ml

| Thiazoles | Concentration [µg/ml] |              |              |              |              |              | NCC [µg/ml]  |              |               |               |       |
|-----------|-----------------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|---------------|---------------|-------|
|           | 50.0                  | 25.0         | 12.5         | 10.0         | 6.25         | 3.12         |              | 1.56         | 0.78          | 0.39          | 0.15  |
| <b>3a</b> | nt                    | nt           | nt           | 97.43 ± 2.30 | 98.83 ± 1.56 | 93.67 ± 1.42 | 92.63 ± 1.63 | 89.01 ± 0.60 | 95.96 ± 1.89  | 96.46 ± 3.26  | 10.00 |
| <b>3d</b> | nt                    | nt           | nt           | 97.64 ± 0.38 | 98.83 ± 0.98 | 93.67 ± 1.42 | 97.13 ± 2.34 | 89.01 ± 0.60 | 95.96 ± 1.89  | 95.36 ± 0.18  | 10.00 |
| <b>3f</b> | nt                    | nt           | nt           | 97.74 ± 2.12 | 97.34 ± 0.37 | 86.31 ± 1.63 | 86.31 ± 0.69 | 88.03 ± 3.09 | 95.31 ± 1.39  | 98.97 ± 0.88  | 10.00 |
| <b>3n</b> | 96.95 ± 2.75          | 96.69 ± 1.28 | 99.22 ± 1.31 | 95.80 ± 0.61 | 93.96 ± 3.58 | 90.00 ± 1.01 | 89.39 ± 0.42 | 91.55 ± 3.27 | 93.66 ± 0.26  | 98.18 ± 0.88  | 50.00 |
| <b>3o</b> | nt                    | 99.36 ± 3.17 | 99.75 ± 0.12 | 98.30 ± 1.32 | 96.53 ± 1.62 | 89.92 ± 0.04 | 90.72 ± 2.23 | 91.27 ± 3.05 | 101.14 ± 0.68 | 100.47 ± 0.14 | 25.00 |

To calculate the reduction of cells viability in the presence of tested compounds (Co.) compared with the untreated blank the equation was used:  $\text{viability (\%)} = 100\% \times \text{sample OD}_{570} / (\text{the mean value of the measured optical density of the treated cells} / \text{blank OD}_{570})$  (the mean value of the measured optical density of the untreated cells). NCC—No Cytotoxic Concentration—means the maximum concentration of tested compounds at which no cytotoxic effect was observed in vitro. nt—not tested—the solubility of the tested compounds at these concentrations was unsatisfactory

**Table 4** Log *P* and *R*<sub>M0</sub> values of the reference substances **S1–S5**

| Standard           | log <i>P</i> | <i>R</i> <sub>M0</sub> | <i>S</i> | <i>r</i> <sup>2</sup> | <i>φ</i> |
|--------------------|--------------|------------------------|----------|-----------------------|----------|
| Acetone–water      |              |                        |          |                       |          |
| <b>S1</b>          | 0.62         | 1.77                   | −0.03    | 0.9793                | 67.12    |
| <b>S2</b>          | 2.02         | 1.74                   | −0.03    | 0.9650                | 68.97    |
| <b>S3</b>          | 2.7          | 1.87                   | −0.03    | 0.9658                | 73.54    |
| <b>S4</b>          | 3.5          | 3.27                   | −0.04    | 0.8676                | 83.05    |
| <b>S5</b>          | 6.04         | 5.24                   | −0.06    | 0.9615                | 86.98    |
| Acetonitrile–water |              |                        |          |                       |          |
| <b>S1</b>          | 0.62         | 0.81                   | −0.01    | 0.9655                | 59.08    |
| <b>S2</b>          | 2.02         | 2.14                   | −0.03    | 0.9887                | 83.36    |
| <b>S3</b>          | 2.7          | 1.03                   | −0.01    | 0.9671                | 71.63    |
| <b>S4</b>          | 3.5          | 1.94                   | −0.03    | 0.9871                | 69.37    |
| <b>S5</b>          | 6.04         | 3.44                   | −0.03    | 0.9905                | 106.84   |
| 1,4-Dioxane–water  |              |                        |          |                       |          |
| <b>S1</b>          | 0.62         | 2.03                   | −0.04    | 0.9720                | 57.35    |
| <b>S2</b>          | 2.02         | 2.23                   | −0.04    | 0.9901                | 62.31    |
| <b>S3</b>          | 2.7          | 1.89                   | −0.03    | 0.9840                | 59.09    |
| <b>S4</b>          | 3.5          | 2.38                   | −0.03    | 0.9854                | 73.11    |
| <b>S5</b>          | 6.04         | 4.82                   | −0.06    | 0.9912                | 80.46    |
| Methanol–water     |              |                        |          |                       |          |
| <b>S1</b>          | 0.62         | 2.17                   | −0.03    | 0.9877                | 85.52    |
| <b>S2</b>          | 2.02         | 1.76                   | −0.02    | 0.9839                | 77.02    |
| <b>S3</b>          | 2.7          | 1.67                   | −0.02    | 0.9815                | 75.91    |
| <b>S4</b>          | 3.5          | 2.53                   | −0.03    | 0.9546                | 87.82    |
| <b>S5</b>          | 6.04         | 5.96                   | −0.06    | 0.9895                | 105.25   |

The S1 (2-aminophenol), S2 (8-hydroxyquinoline), S3 (2-naphtol), S4 (diphenylamine), and S5 (3,4-benzopyrene) were used as reference substances

1,4-dioxane were recommended as the most suitable organic modifiers for such estimations (Rutkowska et al. 2013).

The lowest lipophilicity values were obtained for the compounds **3n** and **3o** containing the CH<sub>2</sub>COOEt and CH<sub>3</sub> group, respectively. These two compounds also had the highest antifungal activity. Similarly, the compound **3l** with the CH<sub>2</sub>Cl substituent showed low lipophilicity value. However, probably due to the presence of electron-withdrawing chlorine atom, its antifungal activity is only moderate. On the contrary, the highest values of lipophilicity were obtained for the compounds **3e**, **3m**, and **3k**, independently of the organic modifier used in the binary solvent. This substances, as it turned out, showed no antimicrobial activity. As can be seen, while the additional phenyl ring was present in the structure, the lipophilicity increased (**3d**, **3e**, **3f**). When fluoride atoms were introduced instead the chlorine ones, the lipophilicity decreased (**3i** versus **3k**). It was also confirmed that removal of chlorine atom decreased the lipophilicity (**3c** versus **3k**). When CF<sub>3</sub> group was introduced to the structure instead of

**Table 5**  $R_{M0}$  values of the synthesized 1,3-thiazole derivatives **3a–3o**

| Thiazoles          | $R_{M0}$ | $S$   | $r^2$  | $\varphi$ |
|--------------------|----------|-------|--------|-----------|
| Acetone–water      |          |       |        |           |
| <b>3a</b>          | 4.62     | −0.06 | 0.9832 | 79.66     |
| <b>3b</b>          | 5.11     | −0.06 | 0.9778 | 82.69     |
| <b>3c</b>          | 5.28     | −0.06 | 0.9804 | 81.70     |
| <b>3d</b>          | 4.82     | −0.06 | 0.9703 | 80.91     |
| <b>3e</b>          | 5.67     | −0.07 | 0.9695 | 82.12     |
| <b>3f</b>          | 4.68     | −0.06 | 0.9804 | 78.26     |
| <b>3g</b>          | 5.45     | −0.07 | 0.9694 | 80.12     |
| <b>3h</b>          | 4.58     | −0.06 | 0.9568 | 78.42     |
| <b>3i</b>          | 5.14     | −0.06 | 0.9639 | 82.67     |
| <b>3j</b>          | 5.19     | −0.06 | 0.9644 | 82.44     |
| <b>3k</b>          | 5.96     | −0.07 | 0.9709 | 84.69     |
| <b>3l</b>          | 3.43     | −0.05 | 0.9713 | 75.59     |
| <b>3m</b>          | 6.69     | −0.08 | 0.9659 | 85.38     |
| <b>3n</b>          | 3.22     | −0.04 | 0.9861 | 74.54     |
| <b>3o</b>          | 3.29     | −0.04 | 0.9780 | 76.73     |
| Acetonitrile–water |          |       |        |           |
| <b>3a</b>          | 3.08     | −0.03 | 0.9844 | 89.17     |
| <b>3b</b>          | 3.28     | −0.03 | 0.9967 | 95.54     |
| <b>3c</b>          | 3.26     | −0.03 | 0.9887 | 93.97     |
| <b>3d</b>          | 3.21     | −0.03 | 0.9872 | 92.37     |
| <b>3e</b>          | 3.59     | −0.04 | 0.9924 | 91.96     |
| <b>3f</b>          | 3.20     | −0.04 | 0.9963 | 87.90     |
| <b>3g</b>          | 3.19     | −0.04 | 0.9940 | 87.85     |
| <b>3h</b>          | 3.06     | −0.04 | 0.9978 | 85.96     |
| <b>3i</b>          | 3.30     | −0.04 | 0.9902 | 93.42     |
| <b>3j</b>          | 3.34     | −0.04 | 0.9967 | 91.90     |
| <b>3k</b>          | 3.77     | −0.04 | 0.9943 | 100.28    |
| <b>3l</b>          | 2.28     | −0.03 | 0.9978 | 79.01     |
| <b>3m</b>          | 4.67     | −0.05 | 0.9515 | 99.82     |
| <b>3n</b>          | 2.18     | −0.03 | 0.9895 | 77.43     |
| <b>3o</b>          | 2.63     | −0.03 | 0.9913 | 86.38     |
| 1,4-Dioxane–water  |          |       |        |           |
| <b>3a</b>          | 5.04     | −0.07 | 0.9995 | 75.16     |
| <b>3b</b>          | 5.97     | −0.08 | 0.9942 | 76.86     |
| <b>3c</b>          | 5.54     | −0.07 | 0.9969 | 76.65     |
| <b>3d</b>          | 5.30     | −0.07 | 0.9966 | 76.24     |
| <b>3e</b>          | 5.89     | −0.08 | 0.9981 | 77.76     |
| <b>3f</b>          | 4.85     | −0.07 | 0.9992 | 73.79     |
| <b>3g</b>          | 5.36     | −0.07 | 0.9995 | 73.78     |
| <b>3h</b>          | 4.99     | −0.07 | 0.9992 | 72.28     |
| <b>3i</b>          | 5.48     | −0.07 | 0.9955 | 77.07     |
| <b>3j</b>          | 5.49     | −0.07 | 0.9967 | 76.64     |
| <b>3k</b>          | 5.88     | −0.08 | 0.9978 | 78.24     |
| <b>3l</b>          | 3.96     | −0.06 | 0.9979 | 69.80     |
| <b>3m</b>          | 6.81     | −0.08 | 0.9631 | 81.07     |
| <b>3n</b>          | 3.85     | −0.06 | 0.9935 | 69.21     |

**Table 5** (continued)

| Thiazoles      | $R_{M0}$ | $S$   | $r^2$  | $\varphi$ |
|----------------|----------|-------|--------|-----------|
| <b>3o</b>      | 3.39     | −0.05 | 0.9993 | 70.66     |
| Methanol–water |          |       |        |           |
| <b>3a</b>      | 5.65     | −0.06 | 0.9844 | 93.19     |
| <b>3b</b>      | 6.15     | −0.06 | 0.9967 | 96.15     |
| <b>3c</b>      | 5.99     | −0.06 | 0.9887 | 95.46     |
| <b>3d</b>      | 5.92     | −0.06 | 0.9872 | 95.76     |
| <b>3e</b>      | 6.27     | −0.07 | 0.9924 | 94.24     |
| <b>3f</b>      | 5.15     | −0.05 | 0.9963 | 95.26     |
| <b>3g</b>      | 5.24     | −0.06 | 0.9940 | 94.14     |
| <b>3h</b>      | 4.99     | −0.05 | 0.9978 | 91.31     |
| <b>3i</b>      | 5.81     | −0.06 | 0.9902 | 96.27     |
| <b>3j</b>      | 6.15     | −0.06 | 0.9969 | 96.40     |
| <b>3k</b>      | 6.88     | −0.07 | 0.9943 | 97.20     |
| <b>3l</b>      | 3.96     | −0.04 | 0.9978 | 89.39     |
| <b>3m</b>      | 7.04     | −0.07 | 0.9515 | 101.70    |
| <b>3n</b>      | 4.11     | −0.05 | 0.9895 | 88.61     |
| <b>3o</b>      | 3.85     | −0.04 | 0.9913 | 93.41     |

**Table 6** Log  $P_{exp}$  values of the synthesized 1,3-thiazole derivatives **3a–3o** calculated by standardization method

| Thiazoles | log $P_{acetone}$ | log $P_{acetonitrile}$ | log $P_{1,4-dioxane}$ | log $P_{methanol}$ |
|-----------|-------------------|------------------------|-----------------------|--------------------|
| <b>3a</b> | 5.26              | 5.01                   | 6.41                  | 5.70               |
| <b>3b</b> | 5.87              | 5.34                   | 7.76                  | 6.19               |
| <b>3c</b> | 6.07              | 5.29                   | 7.13                  | 6.03               |
| <b>3d</b> | 5.51              | 5.22                   | 6.79                  | 5.96               |
| <b>3e</b> | 6.55              | 5.85                   | 7.64                  | 6.30               |
| <b>3f</b> | 5.33              | 5.21                   | 6.14                  | 5.22               |
| <b>3g</b> | 6.28              | 5.19                   | 6.88                  | 5.31               |
| <b>3h</b> | 5.21              | 4.96                   | 6.34                  | 5.07               |
| <b>3i</b> | 5.91              | 5.37                   | 7.05                  | 5.86               |
| <b>3j</b> | 5.97              | 5.44                   | 7.07                  | 6.18               |
| <b>3k</b> | 6.92              | 6.16                   | 7.63                  | 6.88               |
| <b>3l</b> | 3.79              | 3.66                   | 4.84                  | 4.07               |
| <b>3m</b> | 7.83              | 7.66                   | 8.98                  | 7.04               |
| <b>3n</b> | 3.52              | 3.50                   | 4.69                  | 4.22               |
| <b>3o</b> | 3.60              | 4.25                   | 4.02                  | 3.97               |

chlorine atom, a small increase of lipophilicity occurred (**3e** versus **3c**).

## Conclusions

As a result of our research, we have identified a promising scaffold, (2-(cyclohex-3-enylmethylene)hydrazinyl)thiazole which significantly increases antifungal activity in all tested *Candida* spp. strains. Among the derivatives, compounds

**3a–3d, 3f, 3n, and 3o** showed very strong activity against reference *Candida* spp., and also towards most of yeasts isolated from clinical materials with MIC = 0.015–7.81 µg/ml. The activity of these compounds is similar and even higher than the activity of nystatin used as positive control. The cytotoxicity studies for the most active compounds showed that *Candida* spp. growth was inhibited at non-cytotoxic concentrations for the mammalian L929 fibroblast. In addition, a good correlation was obtained between lipophilicity of compounds determined using reversed phase thin-layer chromatography (RP-TLC) and their antifungal activity. The highest antifungal activity was demonstrated by the compounds **3n** and **3o** containing the CH<sub>2</sub>COOEt and CH<sub>3</sub> group, which are characterized by the lowest lipophilicity values. All these results provide a new understanding of the relationship between the structure of thiazole derivatives, their lipophilicity and antifungal activity.

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### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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