Medicinal Chemistry Research (2019) 28:2023–2036
https://doi.org/10.1007/s00044-019-02433-2

ORIGINAL RESEARCH

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

Anna Biernasiuk[1] · Magdalena Kawczyńska[2] · Anna Berecka-Rycerz[3] · Beata Rosada[2] · Anna Gumieniczek[3] · Anna Malm[1] · Katarzyna Dzitko[4] · Krzysztof Z. Łączkowski[2]

Received: 4 May 2019 / Accepted: 28 August 2019 / Published online: 9 September 2019
© The Author(s) 2019

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 mucocutaneus 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

Supplementary information The online version of this article (https://doi.org/10.1007/s00044-019-02433-2) contains supplementary material, which is available to authorized users.

✉ Anna Biernasiuk
anna.biernasiuk@umlub.pl

[1] Department of Pharmaceutical Microbiology with Laboratory for Microbiological Diagnostics, Faculty of Pharmacy, Medical University of Lublin, Chodźki 1, Lublin 20-093, Poland

[2] Department of Chemical Technology and Pharmaceuticals, Faculty of Pharmacy, Nicolaus Copernicus University, Jurasza 2, Bydgoszcz 85-089, Poland

[3] Department of Medicinal Chemistry, Faculty of Pharmacy, Medical University of Lublin, Jaczewskiego 4, Lublin 20-090, Poland

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

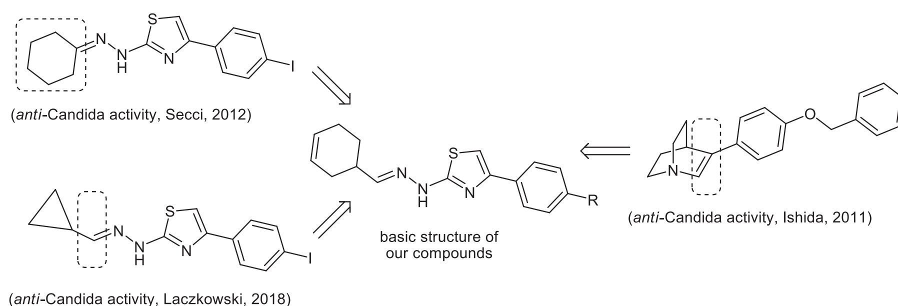


Fig. 1 Rational design of the target compounds **3a–3o**

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)-*β*-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}H NMR (400 MHz) and ^{13}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_{254} 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 $NaHCO_3$ 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), $R_f = 0.74$. ^1H NMR (DMSO-d_6, 700 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_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}C NMR (176 MHz, DMSO-d_6) δ (ppm): 24.29 (CH_2); 26.06 (CH_2); 28.28 (CH_2); 36.32 (CH); 125.95 (CH); 127.18 (CH); 150.54 (CH=N); 178.18 (C=S). *Anal.* Calcd. for $C_8H_{13}N_3S$: 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 $NaHCO_3$ solution. The product was extracted with dichloromethane (2 × 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. ^1H NMR (DMSO-d_6, 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_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}C NMR (176 MHz, DMSO-d_6) δ (ppm): 23.90 (CH_2); 25.84 (CH_2); 28.17 (CH_2); 36.01 (CH); 102.78 (CH); 115.45 (d, 2CH; $J_{C–F} = 19.7$ Hz); 125.53 (CH); 126.87 (CH); 127.50 (d, 2CH, $J_{C–F} = 6.2$ Hz); 131.51 (C); 148.77 (C); 160.90 (CH=N); 162.27 (C–F); 168.78 (C–NH). *Anal.* Calcd. for $C_{16}H_{16}FN_3S$: 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. ^1H NMR (DMSO-d_6, 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_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}C NMR (176 MHz, DMSO-d_6) δ (ppm): 23.85 (CH_2); 25.76 (CH_2); 28.04 (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 $C_{16}H_{16}BrN_3S$: 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. ^1H NMR (DMSO-d_6, 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_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}C NMR (176 MHz, DMSO-d_6) δ (ppm): 23.90 (CH_2); 25.88 (CH_2); 28.17 (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 $C_{16}H_{16}ClN_3S$: 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. ^1H NMR (DMSO-d_6, 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_2); 2.08–2.14 (m, 1H, CH); 2.31 (s, 3H, 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}C NMR (176 MHz, DMSO-d_6) δ (ppm): 20.89 (CH_3); 23.90 (CH_2); 25.91 (CH_2); 28.22 (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 $C_{17}H_{19}N_3S$: 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. ^1H NMR (DMSO-d_6, 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_2), 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). ^{13}C NMR (176 MHz, DMSO-d_6) δ (ppm): 23.87 (CH_2); 25.86 (CH_2); 28.15 (CH_2); 36.00 (CH); 105.89 (CH); 125.51 (CH); 125.61 (2CH); 126.07 (2CH); 126.87 (CH); 127.52 (q, C, $J_{C–F}=29.5$ Hz); 138.47 (C); 148.92 (C); 149.05 (CH=N); 168.93 (C–NH). *Anal.* Calcd. for $C_{17}H_{16}F_3N_3S$: 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, $R_f=0.23$); mp 121–123 °C. ^{1}H NMR (DMSO-d_6, 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_2), 2.46–2.49 (m, 1H, CH); 3.77 (s, 3H, OCH_3); 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). ^{13}C NMR (176 MHz, DMSO-d_6) δ (ppm): 24.28 (CH_2); 26.31 (CH_2); 28.59 (CH_2); 36.38 (CH); 55.57 (CH_3); 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_3); 168.93 (C–NH). *Anal.* Calcd. for $C_{17}H_{19}N_3OS$: 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, $R_f=0.33$); mp 145–147 °C. ^{1}H NMR (DMSO-d_6, 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_2), 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). ^{13}C NMR (176 MHz, DMSO-d_6) δ (ppm): 24.28 (CH_2); 26.22 (CH_2); 28.52 (CH_2); 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 $C_{16}H_{16}N_4O_2S$: 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)benzonitrile (3h)

4-(Bromoacetyl)benzonitrile was reacted with **2**. Yield: 0.30 g, 98%, (dichloromethane, $R_f=0.26$); mp 180–182 °C. ^{1}H NMR (DMSO-d_6, 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_2); 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). ^{13}C NMR (176 MHz, DMSO-d_6) δ (ppm): 23.88 (CH_2); 25.83 (CH_2); 28.13 (CH_2); 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 $C_{17}H_{16}N_4S$: 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, $R_f=0.52$); mp 130–132 °C. ^{1}H NMR (DMSO-d_6, 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_2); 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). ^{13}C NMR (176 MHz, DMSO-d_6) δ (ppm): 24.27 (CH_2); 26.25 (CH_2); 28.55 (CH_2); 36.33 (CH); 104.92 (t, C, $J_{C–F}=47.0$ Hz); 107.56 (d, C, $J_{C–F}=27.0$ Hz); 112.19 (dd, C, $J_{C–F}=5.9$ Hz, $J_{C–F}=37.0$ Hz); 112.19 (dd, C, $J_{C–F}=6.8$ Hz, $J_{C–F}=19.2$ Hz); 125.91 (CH); 127.26 (CH); 130.81 (dd, C, $J_{C–F}=8.5$ Hz, $J_{C–F}=16.4$ Hz); 143.69 (C); 149.36 (CH=N); 159.53 (dd, C, $J_{C–F}=23.0$ Hz, $J_{C–F}=289.0$ Hz); 162.02 (dd, C, $J_{C–F}=23.0$ Hz, $J_{C–F}=281.0$ Hz); 168.54 (C–NH). *Anal.* Calcd. for $C_{16}H_{15}F_2N_3S$: 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%, (dichloromethan, $R_f=0.34$); mp 124–126 °C. ^{1}H NMR (DMSO-d_6, 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_2); 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). ^{13}C NMR (176 MHz, DMSO-d_6) δ (ppm): 23.89 (CH_2); 25.88 (CH_2); 28.16 (CH_2); 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 $C_{16}H_{16}N_6S$: 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}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 1.41–1.46 (m, 1H, CH); 1.81–1.89 (m, 1H, CH); 1.98–2.10 (m, 3H, CH, CH_2); 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}C NMR (176 MHz, DMSO-d_6) δ (ppm): 24.27 (CH_2); 26.24 (CH_2); 28.54 (CH_2); 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 $C_{16}H_{15}Cl_2N_3S$: 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}H NMR (DMSO-d_6, 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_2); 2.08–2.14 (m, 1H, CH); 2.41–2.44 (m, 1H, CH); 4.57 (s, 2H, 2CH); 5.68 (s, 2H, CH_2); 6.84 (s, 1H, CH); 7.30 (d, 1H, CH, $J = 6.0$ Hz); 11.50 (bs, 1H, NH). ^{13}C NMR (176 MHz, DMSO-d_6) δ (ppm): 23.85 (CH_2); 25.84 (CH_2); 28.15 (CH_2); 35.96 (CH); 42.01 (CH_2-Cl); 107.58 (CH); 125.51 (CH); 126.87 (CH); 147.87 (C–CH_2Cl); 148.84 (CH=N); 169.10 (C–NH). *Anal*. Calcd. for $C_{11}H_{14}ClN_3S$: 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}H NMR (DMSO-d_6, 400 MHz) δ (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}C NMR (176 MHz, DMSO-d_6) δ (ppm): 24.12 (CH_2); 26.01 (CH_2); 28.18 (6CH_2); 28.30 (CH_2); 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 $C_{20}H_{27}N_3S$: 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}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 1.17 (t, 3H, CH_3, $J = 7.0$ Hz); 1.41–1.46 (m, 1H, CH); 1.78–1.84 (m, 1H, CH); 2.00–2.06 (m, 3H, CH, CH_2); 2.08–2.14 (m, 1H, CH); 2.41–2.44 (m, 1H, CH); 3.53 (s, 2H, CH_2); 4.05 (q, 2H, CH_2, $J = 7.0$ 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}C NMR (176 MHz, DMSO-d_6) δ (ppm): 14.20 (CH_3); 23.86 (CH_2); 25.87 (CH_2); 28.19 (CH_2); 35.95 (CH); 37.15 (CH_2); 60.26 (CH_2–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 $C_{14}H_{19}N_3O_2S$: 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}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 1.39–1.52 (m, 1H, CH); 1.78–1.88 (m, 1H, CH); 1.96–2.01 (m, 3H, CH, CH_2); 2.15–2.20 (m, 1H, CH); 2.33 (s, 3H, CH_3); 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}C NMR (176 MHz, DMSO-d_6) δ (ppm): 17.65 (CH_3); 24.28 (CH_2); 26.34 (CH_2); 28.64 (CH_2); 36.34 (CH); 102.27 (CH); 125.95 (CH); 127.25 (CH); 148.02 (C–CH_3); 148.78 (CH=N); 168.70 (C–NH). *Anal*. Calcd. for $C_{11}H_{15}N_3S$: 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. lusitaniae, 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 × 104/100 μL/well of L929 cells placed into 96-well plates were incubated 24 h in 37 °C and 10% CO_2 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_2) 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® 30-2020™), 100 U/ml penicillin and 100 µg/ml streptomycin (Penicillin–Streptomycin Solution ATCC® 30-2300™).

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

Lipophilicity

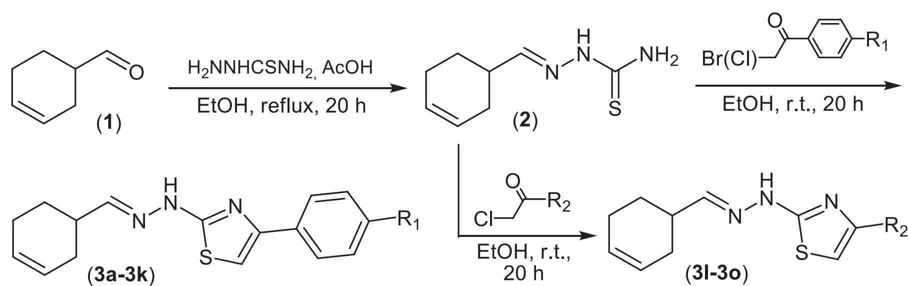
Experimental lipophilicity of the synthesized thiazole derivatives **3a–3o** was determined using reversed phase thin-layer chromatography (RP-TLC–reversed phase thin-layer chromatography) on 10 × 10 cm HPTLC plates coated with C18 silica F254 (Merck, Darmstadt, Germany). Binary solvents were prepared 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-naphtol (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 ^{1}H and ^{13}C NMR, and elemental analyses.

In the ^{1}H NMR spectrum of compound **2**, three characteristic signals derived from the NH_2 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_2 group and the sulfur atom. The ^{13}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**

Thiazole	3a	3b	3c	3d	3e	3f	3g	3h	3i	3j	3k
R_1	F	Br	Cl	CH_3	CF_3	OCH_3	NO_2	CN	2,4-diF	N_3	3,4-diCl
R_2	3l	3m	3n	3o							
	CH_2Cl	1-adamantyl	$CH_2COOC_2H_5$	CH_3							

178 ppm, respectively. Also ^{1}H NMR spectra of (2-(cyclohex-3-enylmethylene)hydrazinyl)thiazole (**3a**–**3o**) showed characteristic singlet at δ (6.28–7.63) ppm due to the presence of the H-5 atom in the thiazole ring and broadened hydrazine NH singlet at δ (11.24–11.74) ppm. The ^{13}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 µg/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 µg/ml and similar MFC = 0.015–0.06 µg/ml (MFC/MIC = 1–4) (Tables 1 and 2). Moreover, the compounds **3h**–**3j** indicated very strong anticandidal effect (MIC = 0.24–7.81 µg/ml, MFC = 1.95 to ≥1000 µg/ml) towards reference *C. albicans* and *C. krusei* strains. Also substance **3l** with MIC = 1.95–125 µg/ml and MFC = 3.91 to >1000 µg/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 µg/ml and MFC = 0.015–125 µg/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 µg/ml and MFC = 7.81 to ≥1000 µg/ml). The substances **3a**–**3c** had slightly lower effect in relation to the same clinical isolates (MIC = 1.95–125 µg/ml and MFC = 7.81 to ≥1000 µg/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 µg/ml and MFC = 500 to ≥1000 µg/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 µg/ml and MBC = 500 to ≥1000 µg/ml) against these bacteria as well as compound **3o** had very strong effect towards *B. bronchiseptica* ATTC 4617 (MIC = 0.06 µg/ml and MBC = 0.48 µg/ml), moderate activity against *Bacillus cereus* ATCC 10876 (MIC = 500 µg/ml and MBC > 1000 µg/ml) and mild effect towards the rest of these bacteria (MIC = 1000 µg/ml, MBC ≥ 1000 µg/ml). The compounds **3l** and **3n** showed strong (MIC = 15.62 µg/ml and MBC > 62.5 µg/ml) and very strong activity (MIC = 0.06 µg/ml and MBC = 0.24 µg/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 CH_2COOEt and CH_3 groups directly bound to the thiazole ring show the highest activity against *Candida* spp. Also, compounds **3d** and **3f** containing CH_3 and 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 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 [µg/ml] against the reference and clinical strains of fungi

Species	MIC [µg/ml] of the tested compounds												Nystatin
	3a	3b	3c	3d	3f	3g	3h	3i	3j	3l	3n	3o	
C. albicans 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
C. albicans 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
C. parapsilosis ATCC 22019	0.48	3.91	0.48	0.48	3.91	–	–	–	–	31.25	0.03	0.03	0.24
C. glabrata ATCC 90030	–	–	–	–	–	–	–	–	–	–	–	–	0.24
C. krusei 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
C. krusei	31.25	15.62	15.62	7.81	3.91	1000	1000	500	250	125	0.06	0.06	0.24
C. tropicalis	31.25	7.81	15.62	1.95	3.91	1000	125	250	500	250	0.06	0.06	0.015
C. inconspicua	31.25	15.62	15.62	1.95	7.81	500	500	250	62.5	62.5	0.03	0.06	0.12
C. lusitaniae	7.81	1.95	3.91	7.81	3.91	500	7.81	500	15.62	250	0.03	0.06	0.12
C. sake	31.25	31.25	15.62	3.91	1.95	500	250	125	125	62.5	0.06	0.12	0.06
C. dubliniensis	125	3.91	31.25	7.81	7.81	1000	500	500	1000	500	0.06	0.98	0.12
C. albicans	125	15.62	31.25	3.91	3.91	1000	1000	500	1000	500	0.015	0.03	0.24
C. kefyr	1000	500	500	1000	250	1000	500	1000	1000	500	3.91	62.5	0.12
C. famata	500	125	250	1000	125	500	500	1000	500	125	3.91	1.95	0.06
C. guilliermondii	1000	125	500	1000	250	–	1000	1000	1000	250	1.95	0.24	0.06
C. parapsilosis	125	31.25	125	–	15.62	1000	1000	500	1000	250	0.015	0.06	0.12
C. glabrata	–	1000	–	–	–	1000	–	–	–	1000	500	125	0.48
C. pulcherrima	–	1000	–	–	–	–	–	–	–	1000	1000	125	0.12
C. lambica	–	–	–	–	–	–	–	–	–	–	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 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® CCL-1™) according to the international standards: ISO 10993-5:2009(E) using MTT assay. The percent of viable cells ± standard derivation in the concentrations range between 0 to 50.00 µg/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 µg/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 φ 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:

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

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

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

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

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 [μg/ml] and (MFC/MIC) against the reference and clinical strains of fungi

Species	MFC [μg/ml] and (MFC/MIC) of the tested compounds												Nystatin
	3a	3b	3c	3d	3f	3g	3h	3i	3j	3l	3n	3o	
C. albicans ATCC 2091	0.98	7.81	0.98	0.48	3.91	>1000	15.62	>1000	>1000	250	0.03	0.06	0.24
	2	1	2	1	2	>1	2	>128	>256	2	2	2	1
C. albicans ATCC 10231	0.12	0.12	0.24	0.24	0.98	>1000	3.91	3.91	1.95	31.25	0.06	0.06	0.48
	2	2	2	2	4	>8	4	4	2	2	4	2	1
C. parapsilosis ATCC 22019	7.81	>1000	>1000	>1000	>1000	–	–	–	–	>1000	0.06	0.06	0.48
	16	>256	>1024	>1024	>256					>32	2	2	2
C. glabrata ATCC 90030	–	–	–	–	–	–	–	–	–	–	–	–	0.48
													2
C. krusei ATCC 14243	0.24	7.81	0.24	0.24	1.95	>1000	1.95	1.95	>1000	3.91	0.03	0.015	0.24
	4	8	2	4	4	>8	8	4	>1024	2	2	1	1
C. krusei	125	125	1000	1000	125	>1000	>1000	1000	1000	>1000	0.06	0.24	0.98
	4	8	64	128	32	>1	>1	2	4	>8	1	4	4
C. tropicalis	125	15.62	15.62	15.62	15.62	>1000	500	500	1000	1000	0.24	1.95	0.06
	4	2	1	8	4	>1	4	2	2	4	4	32	4
C. inconspicua	250	250	1000	1000	31.25	>1000	1000	1000	125	1000	0.06	0.06	0.48
	8	16	64	512	4	>2	2	4	2	16	2	1	4
C. lusitaniae	15.62	7.81	15.62	1000	7.81	>1000	1000	1000	>1000	500	0.06	0.12	0.24
	2	4	4	128	2	>2	128	2	64	2	2	2	2
C. sake	1000	125	>1000	>1000	15.62	>1000	>1000	250	>1000	1000	0.12	1.95	0.12
	32	4	>64	>256	8	>2	>4	2	>8	16	2	16	2
C. dubliniensis	500	7.81	500	7.81	7.81	>1000	1000	1000	>1000	1000	0.12	1.95	0.12
	4	2	16	1	1	>1	2	2	>1	2	2	2	1
C. albicans	250	62.5	500	15.62	15.62	>1000	>1000	1000	>1000	1000	0.03	0.98	0.24
	2	4	16	4	4	>1	>1	2	>1	2	2	32	1
C. kefyr	>1000	>1000	>1000	>1000	1000	>1000	>1000	>1000	>1000	1000	125	250	0.24
	>1	>2	>2	>1	4	>1	>2	>1	>1	2	32	4	2
C. famata	>1000	>1000	>1000	>1000	1000	>1000	>1000	>1000	>1000	1000	15.62	3.91	0.12
	>2	>8	>4	>1	8	>2	>2	>1	>2	8	4	2	2
C. guilliermondii	>1000	>1000	>1000	>1000	1000	–	1000	>1000	1000	1000	125	3.91	0.12
	>1	>8	>2	>1	4		1	>1	1	4	64	16	2
C. parapsilosis	1000	500	>1000	–	250	>1000	>1000	>1000	>1000	1000	0.03	0.24	0.48
	8	16	>8		16	>1	>1	>2	>1	4	2	4	4
C. glabrata	–	>1000	–	–	–	>1000	–	–	–	>1000	>1000	>1000	0.48
		>1				>1				>1	>1	>8	1
C. pulcherrima	–	>1000	–	–	–	–	–	–	–	>1000	>1000	>1000	0.24
		>1								>1	>1	>8	2
C. lambica	–	–	–	–	–	–	–	–	–	–	>1000	>1000	0.48
											>2	>16	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	
3a	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
3d	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
3f	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
3n	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 ± 026	98.18 ± 0.88	50.00
3o	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: viability (%) = 100% × sample OD_{570} (the mean value of the measured optical density of the treated cells)/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_{M0} values of the reference substances **S1–S5**

Standard	log P	R_{M0}	S	r^2	φ
Acetone–water					
S1	0.62	1.77	−0.03	0.9793	67.12
S2	2.02	1.74	−0.03	0.9650	68.97
S3	2.7	1.87	−0.03	0.9658	73.54
S4	3.5	3.27	−0.04	0.8676	83.05
S5	6.04	5.24	−0.06	0.9615	86.98
Acetonitrile–water					
S1	0.62	0.81	−0.01	0.9655	59.08
S2	2.02	2.14	−0.03	0.9887	83.36
S3	2.7	1.03	−0.01	0.9671	71.63
S4	3.5	1.94	−0.03	0.9871	69.37
S5	6.04	3.44	−0.03	0.9905	106.84
1,4-Dioxane–water					
S1	0.62	2.03	−0.04	0.9720	57.35
S2	2.02	2.23	−0.04	0.9901	62.31
S3	2.7	1.89	−0.03	0.9840	59.09
S4	3.5	2.38	−0.03	0.9854	73.11
S5	6.04	4.82	−0.06	0.9912	80.46
Methanol–water					
S1	0.62	2.17	−0.03	0.9877	85.52
S2	2.02	1.76	−0.02	0.9839	77.02
S3	2.7	1.67	−0.02	0.9815	75.91
S4	3.5	2.53	−0.03	0.9546	87.82
S5	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_2COOEt and CH_3 group, respectively. These two compounds also had the highest antifungal activity. Similarly, the compound **3l** with the CH_2Cl 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_3 group was introduced to the structure instead of

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

Thiazoles	R_{M0}	S	r^2	φ
Acetone–water				
3a	4.62	−0.06	0.9832	79.66
3b	5.11	−0.06	0.9778	82.69
3c	5.28	−0.06	0.9804	81.70
3d	4.82	−0.06	0.9703	80.91
3e	5.67	−0.07	0.9695	82.12
3f	4.68	−0.06	0.9804	78.26
3g	5.45	−0.07	0.9694	80.12
3h	4.58	−0.06	0.9568	78.42
3i	5.14	−0.06	0.9639	82.67
3j	5.19	−0.06	0.9644	82.44
3k	5.96	−0.07	0.9709	84.69
3l	3.43	−0.05	0.9713	75.59
3m	6.69	−0.08	0.9659	85.38
3n	3.22	−0.04	0.9861	74.54
3o	3.29	−0.04	0.9780	76.73
Acetonitrile–water				
3a	3.08	−0.03	0.9844	89.17
3b	3.28	−0.03	0.9967	95.54
3c	3.26	−0.03	0.9887	93.97
3d	3.21	−0.03	0.9872	92.37
3e	3.59	−0.04	0.9924	91.96
3f	3.20	−0.04	0.9963	87.90
3g	3.19	−0.04	0.9940	87.85
3h	3.06	−0.04	0.9978	85.96
3i	3.30	−0.04	0.9902	93.42
3j	3.34	−0.04	0.9967	91.90
3k	3.77	−0.04	0.9943	100.28
3l	2.28	−0.03	0.9978	79.01
3m	4.67	−0.05	0.9515	99.82
3n	2.18	−0.03	0.9895	77.43
3o	2.63	−0.03	0.9913	86.38
1,4-Dioxane–water				
3a	5.04	−0.07	0.9995	75.16
3b	5.97	−0.08	0.9942	76.86
3c	5.54	−0.07	0.9969	76.65
3d	5.30	−0.07	0.9966	76.24
3e	5.89	−0.08	0.9981	77.76
3f	4.85	−0.07	0.9992	73.79
3g	5.36	−0.07	0.9995	73.78
3h	4.99	−0.07	0.9992	72.28
3i	5.48	−0.07	0.9955	77.07
3j	5.49	−0.07	0.9967	76.64
3k	5.88	−0.08	0.9978	78.24
3l	3.96	−0.06	0.9979	69.80
3m	6.81	−0.08	0.9631	81.07
3n	3.85	−0.06	0.9935	69.21
3o	3.39	−0.05	0.9993	70.66
Methanol–water				
3a	5.65	−0.06	0.9844	93.19
3b	6.15	−0.06	0.9967	96.15
3c	5.99	−0.06	0.9887	95.46
3d	5.92	−0.06	0.9872	95.76
3e	6.27	−0.07	0.9924	94.24
3f	5.15	−0.05	0.9963	95.26
3g	5.24	−0.06	0.9940	94.14
3h	4.99	−0.05	0.9978	91.31
3i	5.81	−0.06	0.9902	96.27
3j	6.15	−0.06	0.9969	96.40
3k	6.88	−0.07	0.9943	97.20
3l	3.96	−0.04	0.9978	89.39
3m	7.04	−0.07	0.9515	101.70
3n	4.11	−0.05	0.9895	88.61
3o	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 $P_{1,4\text{-dioxane}}$	log $P_{methanol}$
3a	5.26	5.01	6.41	5.70
3b	5.87	5.34	7.76	6.19
3c	6.07	5.29	7.13	6.03
3d	5.51	5.22	6.79	5.96
3e	6.55	5.85	7.64	6.30
3f	5.33	5.21	6.14	5.22
3g	6.28	5.19	6.88	5.31
3h	5.21	4.96	6.34	5.07
3i	5.91	5.37	7.05	5.86
3j	5.97	5.44	7.07	6.18
3k	6.92	6.16	7.63	6.88
3l	3.79	3.66	4.84	4.07
3m	7.83	7.66	8.98	7.04
3n	3.52	3.50	4.69	4.22
3o	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_2COOEt and CH_3 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.

Acknowledgements This study was supported by the Nicolaus Copernicus University (project No. 786/2014).

Compliance with ethical standards

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

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

Ahangar N, Ayati A, Alipour E, Pashapour A, Foroumadi A, Emami S (2011) 1-((2-Arylthiazol-4-yl)methyl)azoles as a new class of anticonvulsants: design, synthesis, in vivo screening, and in silico drug-like properties. Chem Biol Drug Des 78:844–852

Ahmad A, Wani MY, Patel M, Sobrals AJFN, Duse AG, Aqlan FM, Al-Bogami AS (2017) Synergistic antifungal effect of cyclized chalcone derivatives and fluconazole against *Candida albicans*. Med Chem Comm 8:2195–2207

Ansari A, Ali A, Asif M, Rauf MA, Shamsuzzaman OM (2018) Facile one-pot multicomponent synthesis and molecular docking studies of steroidal oxazole/thiazole derivatives with effective antimicrobial, antibiofilm and hemolytic properties. Steroids 134:22–36

Bikobo DSN, Vodnar DC, Stana A, Tiperciuc B, Nastasă C, Douchet M, Oniga O (2017) Synthesis of 2-phenylamino-thiazole derivatives as antimicrobial agents. J Saudi Chem Soc 21:861–868

Carradori S, Ortuso F, Petzer A, Bagetta D, De Monte C, Secci D, De Vita D, Guglielmi P, Zengin G, Aktumsek A, Alcaro S, Petzer JP (2018) Design, synthesis and biochemical evaluation of novel multi-target inhibitors as potential anti-Parkinson agents. Eur J Med Chem 143:1543–1552

Clinical and Laboratory Standards Institute (2012) Reference method for broth dilution antifungal susceptibility testing of yeasts. M27-S4. Clinical and Laboratory Standards Institute, Wayne, PA, USA

Djukic M, Fesatidou M, Xenikakis I, Geronikaki A, Angelova VT, Savic V, Pasic M, Krilovic B, Djukic D, Gobeljic B, Pavlica M, Djuric A, Stanojevic I, Vojvodic D, Saso L (2018) In vitro antioxidant activity of thiazolidinone derivatives of 1,3-thiazole and 1,3,4-thiadiazole. Chem Biol Interact 286:119–131

European Committee for Antimicrobial Susceptibility Testing (EUCAST) determination of minimum inhibitory concentrations (MICs) of antibacterial agents by broth dilution (2003) EUCAST discussion document E. Dis 5.1. Clin Microbiol Infect 9:1–7

Ghannoum MA, Jurevic RJ, Mukherjee PK, Cui F, Sikaroodi M, Naqvi A, Gillevet PM (2010) Characterization of the oral fungal microbiome (mycobiome) in healthy individuals. PLoS Pathog 6:e1000713

Gomha SM, Abdelhamid AO, Abdelrehem NA, Kandeel SM (2018) Efficient synthesis of new benzofuran-based thiazoles and investigation of their cytotoxic activity against human breast carcinoma cell lines. J Heterocycl Chem 55:995–1001

Geronikaki AA, Pitta EP, Liaras KS (2013) Thiazoles and thiazolidinones as antioxidants. Curr Med Chem 20:4460–4480

Gomes da Silva Dantas F, Araújo de Almeida-Apolonio A, Pires de Araújo R, Regiane Vizolli Favarin L, Fukuda de Castilho P, de Oliveira Galvão F, Estivalet Svidzinski TI, Casagrande GA, Pires de Oliveira KM (2018) A promising copper (II) complex as antifungal and antibiofilm drug against yeast infection. Molecules 23:1856–1869

Hacioglu M, Tan ASB, Dosler S, Inan N, Otuk G (2018) In vitro activities of antifungals alone and in combination with tigecycline against *Candida albicans* biofilms. PeerJ 6:e5263

Ishida K, Fernandes Rodrigues JC, Cammerer S, Urbina JA, Gilbert I, de Souza W, Rozental S (2011) Synthetic arylquinuclidine derivatives exhibit antifungal activity against *Candida albicans, Candida tropicalis* and *Candida parapsilopsis*. Ann Clin Microbiol Antimicrob 10:3

Komsta Ł, Skibiński R, Berecka A, Gumieniczek A, Radkiewicz B, Radoń M (2010) Revisiting thin-layer chromatography as a lipophilicity determination tool—a comparative study on several techniques with a model solute set. J Pharm Biomed Anal 53:911–918

Li Y, Sun L, Lu Ch, Gong Y, Li M, Sun S (2018) Promising antifungal targets against *Candida albicans* based on ion homeostasis. Front Cell Infect Microbiol 8:286–298

Łączkowski KZ, Konklewska N, Biernasiuk A, Malm A, Sałat K, Furgała A, Dzitko K, Bekier A, Baranowska-Łączkowska A, Paneth A (2018a) Thiazoles with cyclopropyl fragment as antifungal, anticonvulsant, and anti-*Toxoplasma gondii* agents: synthesis, toxicity evaluation, and molecular docking study. Med Chem Res 27:2125–2140

Łączkowski KZ, Misiura K, Świtalska M, Wietrzyk J, Baranowska-Łączkowska A, Fernández B, Paneth A, Plech T (2014) Synthesis and in vitro antiproliferative activity of thiazole-based nitrogen mustards. The hydrogen bonding interaction between model systems and nucleobases. Anticancer Agents Med Chem 14:1271–1281

Łączkowski KZ, Świtalska M, Baranowska-Łączkowska A, Plech T, Paneth A, Misiura K, Wietrzyk J, Czaplińska B, Mrozek-Wilczkiewicz A, Malarz K, Musioł R, Grela I (2016) Thiazole-based nitrogen mustards: design, synthesis, spectroscopic studies, DFT calculation, molecular docking, and antiproliferative activity against selected human cancer cell lines. J Mol Struct 1119:139–150

Łączkowski KZ, Sałat K, Misiura K, Podkowa A, Malikowska N (2016) Synthesis and anticonvulsant activities of novel 2-

(cyclopentylmethylene)hydrazinyl-1,3-thiazoles in mouse models of seizures. J Enzym Inhib Med Chem 31:1576–1582

Łączkowski KZ, Landowska K, Biernasiuk A, Sałat A, Furgała A, Plech T, Malm A (2017) Synthesis, biological evaluation and molecular docking studies of novel quinuclidinone derivatives as potential antimicrobial and anticonvulsant agents. Med Chem Res 26:2088–2104

Łączkowski KZ, Anusiak J, Świtalska M, Dzitko K, Cytarska J, Baranowska-Łączkowska A, Plech T, Paneth A, Wietrzyk J, Białczyk J (2018b) Synthesis, molecular docking, ctDNA interaction, DFT calculation and evaluation of antiproliferative and anti-*Toxoplasma gondii* activities of 2,4-diaminotriazine-thiazole derivatives. Med Chem Res 27:1131–1148

O'Donnell F, Smyth TJ, Ramachandran VT, Smyth WF (2010) A study of the antimicrobial activity of selected synthetic and naturally occurring quinolones. Int J Antimicrob Agents 35:30–38

Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, Reboli AC, Schuster MG, Vazquez JA, Walsh TJ, Zaoutis TE, Sobel JD (2016) Executive summary: clinical practice guideline for the management of candidiasis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis 15:62 e1–50

Piechowska K, Świtalska M, Cytarska J, Jaroch K, Łuczykowski K, Chałupka J, Wietrzyk J, Misiura K, Bojko B, Kruszewski S, Łączkowski KZ (2019) Discovery of tropinone-thiazole derivatives as potent caspase 3/7 activators, and noncompetitive tyrosinase inhibitors with high antiproliferative activity : rational design, one-pot tricomponent synthesis, and lipophilicity determination. Eur J Med Chem 175:162–171

Roemer T, Krysan DJ (2014) Antifungal drug development: challenges, unmet clinical needs, and new approaches. Cold Spring Harb Perspect Med 4:pii: a019703

Rutkowska E, Pająk K, Jóźwiak K (2013) Lipophilicity—methods of determination and its role in medicinal chemistry. Acta Pol Pharm —Drug Res 70:3–18

Silva S, Rodrigues CF, Araújo D, Rodrigues ME, Henriques M (2017) *Candida* species biofilms' antifungal resistance. J Fungi 3:8–24

Sun L, Liao K, Wang D (2015) Effects of magnolol and honokiol on adhesion, yeast-hyphal transition, and formation of biofilm by *Candida albicans*. PLoS ONE 10:e0117695

Secci D, Bizzarri B, Bolasco A, Carradori S, D'Ascenzio M, Rivanera D, Mari E, Polletta L, Zicari A (2012) Synthesis, anti-*Candida* activity, and cytotoxicity of new (4-(4-iodophenyl)thiazol-2-yl) hydrazine derivatives. Eur J Med Chem 53:246–253

de Santana TI, Barbosa MO, Gomes PATM, da Cruz ACN, da Silva TG, Leite ACL (2018) Synthesis, anticancer activity and mechanism of action of new thiazole derivatives. Eur J Med Chem 144:874–886

Siddiqui N, Ahsan W (2010) Triazole incorporated thiazoles as a new class of anticonvulsants: design, synthesis and in vivo screening. Eur J Med Chem 45:1536–1543

Schultz TL, Hencken CP, Woodard LE, Posner GH, Yolken RH, Jones-Brando L, Carruthers VB (2014) A thiazole derivative of artemisinin moderately reduces *Toxoplasma gondii* cyst burden in infected mice. J Parasitol 100:516–521

Tumbarello M, Sanguinetti M, Trecarichi EM, La Sorda M, Rossi M, De Carolis E, De Gaetano Donati K, Fadda G, Cauda R, Posteraro B (2008) Fungaemia caused by *Candida glabrata* with reduced susceptibility to fluconazole due to altered gene expression: risk factors, antifungal treatment and outcome. J Antimicrob Chemother 62:1379–1385

Turecka K, Chylewska A, Kawiak A, Waleron KF (2018) Antifungal activity and mechanism of action of the Co(III) coordination complexes with diamine chelate ligands against reference and clinical strains of *Candida* spp. Front Microbiol 9:1954–1607

Wiegand I, Hilpert K, Hancock REW (2008) Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc 3:163–175

Zaki YH, Al-Gendey MS, Abdelhamid AO (2018) A facile synthesis, and antimicrobial and anticancer activities of some pyridines, thioamides, thiazole, urea, quinazoline, β-naphthyl carbamate, and pyrano[2,3-d]thiazole derivatives. Chem Cent J 12:70