Research Article

Synthesis of New [1,2,4]Triazolo[3,4-*b*][1,3,4]thiadiazines and Study of Their Anti-*Candidal* and Cytotoxic Activities

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Received 9 April 2014; Revised 21 May 2014; Accepted 26 May 2014; Published 18 June 2014

Academic Editor: Xinyong Liu

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New triazolothiadiazine derivatives **5a–h** were synthesized from 4-amino-3-(4-pyridyl)-5-mercapto-4*H*-1,2,4-triazole (**3**) with substituted aryl hydrazonoyl chlorides **4a–h**. The compounds were tested *in vitro* against eleven *Candida* species and compared with standard drug ketoconazole. Among these compounds, the compounds bearing *p*-chlorophenyl **5e**, *p*-methoxyphenyl **5c**, phenyl **5a**, and *p*-sulphonamidophenyl **5g** substituents on triazolothiadiazine system were found to be the most effective derivatives against *Candida* species. Compound **5e** was the most effective compound against *C. parapsilosis* (ATCC 22019), *C. albicans* (ATCC 66027), *C. specie* [blood] 12810, and *C. specie* [urine] 300 with MIC value of 6.25 µg/mL, whereas ketoconazole exhibits the inhibitory activity with MIC value of 3–30 µg/mL against all tested strains. It was clear that there is a positive correlation between anti-*Candidal* activity and *p*-chlorophenyl substitution on triazolothiadiazine ring. All the synthesized compounds were also investigated for their potential cytotoxicity on noncancer cell line (MCF-12) using WST-1 assay. Three compounds **5d**, **5a**, and **5h** were found to have the same IC₅₀ value as that of standard drug ketoconazole against noncancer cell line MCF-12 (IC₅₀ ≥ 1.0 × 10⁵ µg/mL).

1. Introduction

Candida species have emerged as the most common cause of systemic fungal infections worldwide over the last two decades. The most frequently implicated risk factors include treatment with broad-spectrum antibiotics, use of central venous catheters and implantable prosthetic devices, parenteral nutrition, prolonged intensive care unit stay, hemodialysis, and immunosuppression [1]. Amphotericin and azole drugs are used, for the treatment of an infection with *Candida* spp. [2]. The prominent drugs bearing triazole rings are fluconazole, itraconazole, voriconazole, and posaconazole, all of which are widely used antifungal drugs for the treatment of systemic fungal infection [3]. Resistance has been developed due to widespread use of these agents in recent years. As a result of this situation, medicinal chemists have focused on the research of new anti-*Candidal* agents [4, 5]. The mercapto and thione which substituted

1,2,4-triazole ring system have been reported to possess a variety of biological activities such as antibacterial [6], antifungal [7], antitubercular [8], anticancer [9], diuretic [10], and hypoglycemic [11]. The amino and mercapto groups are readymade nucleophilic centers for synthesis of condensed heterocyclic rings [12]. The recent literature survey revealed that 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives have promising biological activities such as anti-HIV [13], CNS stimulant [14, 15], antifungal [16], and anti-inflammatory [17]. Studies have also confirmed that triazolothiadiazine derivatives possess anti-*Candidal* activity [18–24].

Pyridine ring is a prominent scaffold present in various bioactive molecules and has played a vital role in the development of different medicinal agents [25–27]. The anti-*Candidal* activity of pyridine has also been reported [28, 29]. There are evidences that hybridization of two or more different bioactive molecules with complimentary pharmacophoric function or with different mechanism of

action often renders synergistic effects [30]. Encouraged by this, we designed a set of compounds by hybridizing two different pharmacophores with the aim of increasing their anti-*Candida* activity. We then synthesized hybrid compounds by linking triazolothiadiazine ring system with the pyridine ring (Figure 1). The compounds were evaluated *in vitro* against various *Candida* species and investigated for their cytotoxic effects.

2. Material and Methods

2.1. Chemistry. All the solvents were of LR grade and were obtained from Merck. The elemental analysis of compounds was performed on the CHN Elementar (Analysensysteme GmbH, Germany) and Vario EL III (Elementar Americas Corporation). The elemental analysis (C, H, N) of compounds was found within a limit of $\pm 0.4\%$ of the theoretical values. The homogeneity of the compounds was checked by TLC performed on Silica gel G coated plates (Merck). Iodine chamber was used for visualization of TLC spots. The FT-IR spectra were recorded in KBr pellets on a (Spectrum BX) Perkin Elmer FT-IR spectrometer. Melting points were determined on a Gallenkamp melting point apparatus, and thermometer was uncorrected. NMR Spectra were scanned in DMSO- d_6 on a Bruker NMR spectrophotometer operating at 500 MHz for ^1H and 125.76 MHz for ^{13}C at the Research Center, College of Pharmacy, King Saud University, Saudi Arabia. Chemical shifts are expressed in δ -values (ppm) relative to TMS as an internal standard and D_2O was added to confirm the exchangeable protons. Mass spectra were measured on Agilent Triple Quadrupole 6410 QQQ LC/MS with ESI (Electrospray ionization) source.

2.1.1. 4-Amino-3-(4-pyridyl)-5-mercapto-4H-1,2,4-triazole (3). Isonicotinic acid hydrazide (**1**) (13.7 g, 0.1 mol) was dissolved in absolute ethanol (200 mL) containing potassium hydroxide (11.2 g, 0.1 mol) at room temperature. To this, carbon disulfide (12.5 mL) was added in parts and the reaction mixture was stirred for 16 h at room temperature. After the reaction completion, diethyl ether (100 mL) was added and the reaction mixture was stirred for a further 3 h. After this hydrazine hydrate (99%, 10.3 g, 0.1 mol) was added gradually to the potassium dithiocarbazinate salt **2** which dissolved in water (100 mL) with stirring and the content was refluxed for 8 h, during which hydrogen sulfide gas evolved and the color of the reaction mixture changed to deep green. It was then cooled and acidified with hydrochloric acid to pH 1. The yellow-colored solid separated out and was filtered and purified by recrystallization from DMF to give compound **3** [31].

2.1.2. (7Z)-7-[2-(Aryl)hydrazinylidene]-6-methyl-3-(pyridin-4-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (5a-h). To a mixture of 4-amino-3-(4-pyridyl)-5-mercapto-4H-1,2,4-triazole (**3**), (0.49 g, 2 mmol), and the appropriate hydrazonoyl chloride (**4a-h**) (2 mmol) in ethanol (20 mL), triethylamine (2 mmol, 0.2 mL) was added. The reaction mixture was heated under reflux for 3 h then left to cool. The

precipitated solid was collected by filtration, washed with ethanol, and dried. Recrystallization of the reaction products from ethanol afforded the corresponding compounds [32].

2.1.3. (7Z)-7-[2-(Phenyl)hydrazinylidene]-6-methyl-3-(pyridin-4-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (5a). IR (KBr) ν_{max} (cm^{-1}): 3180 (NH str.), 1590 (C=N str.), 1450 (C=C str.), 1220 (N-N=C str.), 750 (C-S-C str.); ^1H NMR (DMSO- d_6 , 500 MHz) δ ppm: 2.0 (3H, s, CH_3), 7.32–7.38 (5H, m, Ar-H), 8.02 (2H, d, $J = 4$ Hz, pyridyl H), 8.7 (2H, d, $J = 4$ Hz, pyridyl H), 10.36 (1H, bs, -NH, D_2O exch.). ^{13}C NMR (DMSO- d_6 , 125.76 MHz) δ ppm: 21.09 (CH_3), 115.9, 121.7, 132.8, 150.2. MS (ESI) m/z : 335.1 $[\text{M}]^+$. Anal. Calc. for $\text{C}_{17}\text{H}_{13}\text{N}_7\text{S}$: C, 57.30; H, 3.91; N, 29.23; S, 9.56%. Found: C, 57.5; H, 3.90; N, 29.20; S, 9.53%.

2.1.4. (7Z)-7-[2-(2-Methylphenyl)hydrazinylidene]-6-methyl-3-(pyridin-4-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (5b). IR (KBr) ν_{max} (cm^{-1}): 3000 (NH str.), 1600 (C=N str.), 1450 (C=C str.), 1200 (N-N=C str.), 700 (C-S-C str.); ^1H NMR (DMSO- d_6 , 500 MHz) δ ppm: 2.26 (3H, s, CH_3), 2.51 (3H, s, Ar- CH_3), 7.16–7.28 (4H, m, Ar-H), 8.10 (2H, d, $J = 4$ Hz, pyridyl H), 8.79 (2H, d, $J = 4$ Hz, pyridyl H), 10.26 (1H, bs, -NH, D_2O exch.). ^{13}C NMR (DMSO- d_6 ; 125.76 MHz) δ ppm: 21.1 (CH_3), 114.3, 116.3, 121.69, 129.2, 132.9, 143.6, 147.3, 148.7, 150.1, 167.6. MS (ESI) m/z : 350.1 $[\text{M}]^+$. Anal. Calc. for $\text{C}_{17}\text{H}_{15}\text{N}_7\text{S}$: C, 58.44; H, 4.33; N, 28.06; S, 9.18%. Found: C, 58.67; H, 4.32; N, 28.15; S, 9.17%.

2.1.5. (7Z)-7-[2-(4-Methoxyphenyl)hydrazinylidene]-6-methyl-3-(pyridin-4-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (5c). IR (KBr) ν_{max} (cm^{-1}): 3180 (NH str.), 1600 (C=N str.), 1420 (C=C str.), 1220 (N-N=C str.), 750 (C-S-C str.); ^1H NMR (DMSO- d_6 ; 500 MHz) δ ppm: 2.51 (3H, s, CH_3), 3.73 (3H, s, $-\text{OCH}_3$), 6.93 (2H, d, $J = 9.0$ Hz, Ar-H), 7.31 (2H, d, $J = 8.5$ Hz, Ar-H), 8.09 (2H, d, $J = 3.5$ Hz, pyridyl H), 8.79 (2H, d, $J = 3.5$ Hz, pyridyl H), 10.22 (1H, bs, -NH, D_2O exch.); ^{13}C NMR (DMSO- d_6 , 125.76 MHz) δ ppm: 21.0 (CH_3), 55.2, 62.9, 114.5, 115.6, 121.6, 132.8, 137.2, 138.7, 148.7, 150.2, 152.4, 154.9. MS (ESI) m/z : 366.1 $[\text{M}]^+$. Anal. Calc. for $\text{C}_{17}\text{H}_{15}\text{N}_7\text{SO}$: C, 55.88; H, 4.14; N, 26.83; S, 8.78%. Found: C, 55.68; H, 4.13; N, 26.73; S, 8.76%.

2.1.6. (7Z)-7-[2-(4-Bromophenyl)hydrazinylidene]-6-methyl-3-(pyridin-4-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (5d) [33]. IR (KBr) ν_{max} (cm^{-1}): 3000 (NH str.), 1560 (C=N str.), 1462 (C=C str.), 1220 (N-N=C str.), 780 (C-S-C str.); ^1H NMR (DMSO- d_6 , 500 MHz) δ ppm: 2.6 (3H, s, CH_3), 7.0–7.3 (4H, m, Ar-H), 8.0 (2H, d, $J = 4$ Hz, pyridyl H), 8.7 (2H, d, $J = 4$ Hz, pyridyl H), 10.3 (1H, bs, -NH, D_2O exch.). ^{13}C NMR (DMSO- d_6 , 125.76 MHz) δ ppm: 21.11 (CH_3), 114.3, 116.4, 121.6, 122.2, 129.2, 132.8, 138.7, 143.6, 148.7, 150.2, 152.3. MS (ESI) m/z : 413.90 $[\text{M}]^+$, 415.0 $[\text{M}+2]^+$. Anal. Calc. for $\text{C}_{16}\text{H}_{12}\text{N}_7\text{SBr}$: C, 46.39; H, 2.92; N, 23.67; S, 7.74%. Found: C, 46.47; H, 2.93; N, 23.76; S, 7.73%

FIGURE 1: Structure of the lead compound A and new synthesized compounds 5a-h.

2.1.7. (7Z)-7-[2-(4-Chlorophenyl)hydrazinylidene]-6-methyl-3-(pyridin-4-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (5e). IR (KBr) $\nu_{\max}$ (cm^{-1}): 3000 (NH str.), 1600 (C=N str.), 1400 (C=C str.), 1210 (N-N=C str.), 780 (C-S-C str.); ^1H NMR (DMSO- d_6 , 500 MHz) δ ppm: 2.1 (3H, s, CH_3), 7.48 (2H, d, $J = 8.5$ Hz, Ar-H), 7.79 (2H, d, $J = 9.0$ Hz, Ar-H), 8.10 (2H, d, $J = 4$ Hz, pyridyl H), 8.80 (2H, d, $J = 4$ Hz, pyridyl H), 10.6 (1H, bs, -NH, D_2O exch.). ^{13}C NMR (DMSO- d_6 , 125.76 MHz) δ ppm: 21.0 (CH_3), 113.9, 119.1, 121.5, 121.7, 132.7, 137.1, 138.5, 146.2, 147.3, 148.7, 150.1, 150.2, 152.1, 167.6. MS (ESI) m/z : 370.1 $[\text{M}]^+$, 371.0 $[\text{M}+1]^+$. Anal. Calc. for $\text{C}_{16}\text{H}_{12}\text{N}_7\text{SCl}$: C, 51.96; H, 3.27; N, 26.51; S, 8.67%. Found: C, 51.75; H, 3.26; N, 26.40; S, 8.65%.

2.1.8. (7Z)-7-[2-(4-Fluorophenyl)hydrazinylidene]-6-methyl-3-(pyridin-4-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (5f). IR (KBr) $\nu_{\max}$ (cm^{-1}): 3060 (NH str.), 1600 (C=N str.), 1462 (C=C str.), 1240 (N-N=C str.), 700 (C-S-C str.); ^1H NMR (DMSO- d_6 , 500 MHz) δ ppm: 2.0 (3H, s, CH_3), 8.0–8.2 (6H, m, Ar-H), 8.9 (2H, d, $J = 4.0$ Hz, pyridyl H), 11.1 (1H, bs, -NH, D_2O exch.). ^{13}C NMR (125.76 MHz, DMSO- d_6) δ ppm: 62.9, 116.4, 121.5, 150.1, 167.6. MS (ESI) m/z : 354.1 $[\text{M}]^+$. Anal. Calc. for $\text{C}_{16}\text{H}_{12}\text{N}_7\text{SF}$: C, 54.38; H, 3.42; N, 27.75; S, 9.07%. Found: C, 54.18; H, 3.43; N, 27.65; S, 9.08%.

2.1.9. (7Z)-7-[2-(4-Sulphonamidophenyl)hydrazinylidene]-6-methyl-3-(pyridin-4-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (5g). IR (KBr) $\nu_{\max}$ (cm^{-1}): 3200 (NH str.), 600 (C=N str.), 1450 (C=C str.), 1180 (N-N=C str.), 780 (C-S-C str.). ^1H NMR (DMSO- d_6 , 500 MHz) δ ppm: 1.9 (3H, s, CH_3), 2.1 (2H, s, -NH, D_2O exch.), 7.3–7.8 (4H, m, Ar-H), 8.0 (2H, d, $J = 6$ Hz, pyridyl H), 8.77 (2H, d, $J = 6$ Hz, pyridyl H), 10.30 (1H, bs, -NH, D_2O exch.). ^{13}C NMR (125.76 MHz,

DMSO- d_6) δ ppm: 21.1, 113.9, 121.7, 127.2, 132.9, 137.1, 147.3, 148.7, 150.2, 167.6. MS (ESI) m/z : 415.1 $[\text{M}]^+$. Anal. Calc. for $\text{C}_{16}\text{H}_{14}\text{N}_8\text{S}_2\text{O}_2$: C, 46.37; H, 3.40; N, 27.04; S, 15.47%. Found: C, 46.57; H, 3.41; N, 27.15; S, 15.51%.

2.1.10. (7Z)-7-[2-(4-Methylphenyl)hydrazinylidene]-6-methyl-3-(pyridin-4-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (5h). IR (KBr) $\nu_{\max}$ (cm^{-1}): 3100 (NH str.), 1650 (C=N str.), 1450 (C=C str.), 1200 (N-N=C str.), 700 (C-S-C str.). ^1H NMR (DMSO- d_6 , 500 MHz) δ ppm: 2.0 (3H, s, CH_3), 2.2 (3H, s, CH_3), 7.14–7.26 (4H, m, Ar-H), 8.02 (2H, s, CH, $d, J = 4$ Hz, pyridyl H), 8.77 (2H, d, $J = 4$ Hz, pyridyl H), 10.26 (1H, bs, -NH, D_2O exch.). ^{13}C NMR (DMSO- d_6 , 125.76 MHz) δ ppm: 21.1 (CH_3), 114.0, 115.3, 121.1, 128.0, 131.9, 142.2, 143.4, 147.0, 147.5, 150.0, 165.2. MS (ESI) m/z : 350.1 $[\text{M}]^+$. Anal. Calc. for $\text{C}_{17}\text{H}_{15}\text{N}_7\text{S}$: C, 58.44; H, 4.33; N, 28.06; S, 9.18%. Found: C, 58.67; H, 4.32; N, 28.15; S, 9.17%.

2.2. Anti-Candidal Evaluation. The compounds 5a-h were tested *in vitro* against eleven *Candida* spp. including *C. albicans*, *C. tropicalis*, and *C. parapsilosis*. Among these, *C. albicans* ATCC 10231, 66027, *C. tropicalis* ATCC 66029, and *C. parapsilosis* 22019 were procured from American type culture collection. Moreover, other *Candida* isolates (*Candida* sp. [HVS] 13184; *Candida* sp. [HVS] 11972; *Candida* sp. [HVS] 178; *Candida* sp. [urine] 300; *Candida* sp. [urine] 12341; *Candida* sp. [urine] 12485; and *Candida* sp. [blood] 12810) were collected from Prince Salman Hospital, Riyadh, Saudi Arabia. Isolates were derived from high vaginal swabs, blood, and urine. All isolates were grown on Sabouraud dextrose agar media and were preserved at 4°C till the use. Standard drug ketoconazole was used as positive control. Dimethyl sulfoxide (DMSO) was used as negative control. Stock solution of

SCHEME 1: Synthesis of compounds **5a-h**.

ketoconazole was prepared in (DMSO) (16 mg/1 mL). Other compounds were also dissolved in DMSO (100 mg/1 mL).

2.3. Cytotoxicity Evaluation. The MCF-12 cell line is a nontumorigenic epithelial cell line established from tissue taken at reduction mammoplasty from a nulliparous patient with fibrocystic breast disease that contained focal areas of intraductal hyperplasia. MCF-12 cells were cultured in DMEM/F-12 (Dulbecco's modified Eagle's medium/Ham's nutrient mixture F-12), GIBCO, and were supplemented with 10 µg/mL insulin (Sigma), 500 ng/mL hydrocortisone (Sigma), 10% fetal bovine serum (Lonza), 10 ng/mL EGF (Epidermal growth factor), BD Biosciences, and 1% ABM (Autologous Bone Marrow), GIBCO. Cells were seeded into 96-well plates at 0.6×10^4 /well and incubated overnight. The medium was replaced with fresh one containing the desired concentrations of the compounds. After 48 h, 10 µL of the WST-1 (water soluble tetrazolium salts) reagent was added to each well and the plates were reincubated for 4 h at 37°C. The amount of formazan was quantified using ELISA (enzyme-linked immunosorbent assay) reader at 450 nm.

3. Results and Discussion

3.1. Synthesis. Treatment of isonicotinic hydrazide (**1**) with carbon disulfide, in the presence of potassium hydroxide, afforded the potassium salt of hydrazinecarbodithioate **2**. Furthermore, treatment of the salt **2** with hydrazine hydrate in aqueous ethanol afforded the corresponding 1,2,4-triazole-5-thione derivative **3** [30]. Reaction of the compound **3** with substituted hydrazonoyl chlorides **4a-h** in refluxing ethanol, in the presence of triethylamine, afforded compounds identified as (7Z)-7-[2-(aryl)hydrazinylidene]-6-methyl-3-(pyridine-4-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines (**5a-h**). These

reactions are summarized in Scheme 1. The purity of the compounds was checked by TLC and elemental analysis. The compounds of the series were identified by spectral data. The physicochemical properties of all compounds are given in (Table 1). In the IR spectra of all compounds, NH, C=N, and C=C bands were observed at 3200–3000 cm⁻¹, 1650–1560 cm⁻¹, and 1462–1400 cm⁻¹, respectively. In the ¹H NMR spectra of the compounds, which were taken in DMSO-*d*₆, D₂O exchangeable NH proton was seen as broad singlet at about 10.22–11.1 ppm. The pyridyl protons appear as doublets at 7.3–8.9 ppm and 8.0–9.6 ppm with *J* value of 4 Hz each. The aromatic protons appear as multiplet at about 6.9–8.2 ppm. The signal due to methyl protons, present in all compounds, appeared at 2.0–2.6 ppm, as singlet. Mass spectra (ESI) of the compounds showed molecular ion peaks [M]⁺, in agreement with their molecular formula. In the ¹³C NMR spectra of all compounds **5a-h**, the peaks belonging to triazolothiadiazine carbons appeared at 140–160 ppm. All other aromatic and aliphatic carbons were observed at expected regions. All compounds gave satisfactory elemental analysis. The structures of the compounds were in complete agreement with the previously reported products of similar reactions [34, 35].

3.2. Anti-Candidal Activity. All compounds were screened for their *in vitro* anti-*Candida* activity against eleven strains of *Candida* species (Table 2). Minimum inhibitory concentration (MIC) for *Candida* isolates was determined by microdilution method as per the protocol of CLSI (M27-A3 CLSI 2008) [36]. Compound A (MIC 20–370 µg/mL against ketoconazole) was chosen as the lead compound for further modification [37]. Replacement of cyclohexylethyl moiety by pyridine ring, addition of substituted phenylhydrazine moiety at 6th position of 1,3,4-thiadiazine, and preserving the pharmacophore (triazolothiadiazine ring) were studied. The

TABLE 1: Some properties of the synthesized compounds **5a–h**.

Compound	Ar	Yield (%)	M.p. (°C)	Molecular formula	Molecular weight (g/mol)
5a	C ₆ H ₅	50	295–297	C ₁₆ H ₁₃ N ₇ S	335.38
5b	2-CH ₃ -C ₆ H ₅	60	288–290	C ₁₇ H ₁₅ N ₇ S	349.41
5c	4-OCH ₃ -C ₆ H ₅	65	273–275	C ₁₇ H ₁₅ N ₇ OS	365.41
5d	4-Br-C ₆ H ₅	80	270–272	C ₁₆ H ₁₂ BrN ₇ S	414.28
5e	4-Cl-C ₆ H ₅	75	293–295	C ₁₆ H ₁₂ ClN ₇ S	369.83
5f	4-F-C ₆ H ₅	80	278–280	C ₁₆ H ₁₂ FN ₇ S	353.37
5g	4-SO ₂ NH ₂ -C ₆ H ₅	70	320–322	C ₁₆ H ₁₄ N ₈ O ₂ S ₂	414.46
5h	4-CH ₃ -C ₆ H ₅	60	290–292	C ₁₇ H ₁₅ N ₇ S	349.41

TABLE 2: Anti-*Candidal* activity of the compounds (**5a–h**) as MIC values (μg/mL).

	5a	5b	5c	5d	5e	5f	5g	5h	K [#]
<i>C. tropicalis</i> ATCC 66029	25	>100	12.5	25	12.5	25	>100	>100	3.0
<i>C. parapsilosis</i> ATCC 22019	12.5	>100	25	12.5	6.25	6.25	25	>100	3.0
<i>C. albicans</i> ATCC 10231	25	>100	25	>100	>100	>100	>100	>100	30.0
<i>C. albicans</i> ATCC 66027	>100	>100	25	12.5	6.25	50	12.5	>100	30.0
<i>Candida</i> sp. [HVS*] 13184	12.5	>100	12.5	>100	>100	>100	>100	>100	7.0
<i>Candida</i> sp. [HVS] 11972	6.25	>100	12.5	>100	>100	>100	>100	>100	7.0
<i>Candida</i> sp. [HVS] 178	>100	>100	50	>100	>100	>100	>100	>100	7.0
<i>Candida</i> sp. [urine] 300	6.25	>100	12.5	>100	6.25	25	6.25	>100	15.0
<i>Candida</i> sp. [urine] 12341	6.25	>100	6.25	>100	25	12.5	25	>100	15.0
<i>Candida</i> sp. [urine] 12485	>100	>100	6.25	50	25	25	25	>100	15.0
<i>Candida</i> sp. [blood] 12810	12.5	>100	25	>100	6.25	>100	25	>100	7.0

* HVS: high vaginal swab; K[#]: ketoconazole.

compounds with ortho methylphenyl **5b** and *p*-methylphenyl **5h** were devoid of activity up to 100 μg/mL. The compounds with *p*-bromophenyl **5d** and *p*-fluorophenyl **5f** substituents exhibit inhibitory activity against *Candida* species at (MIC, 12.5 μg/mL). Compounds **5a**, **5c**, **5e**, and **5g** exhibit the highest inhibitory activity against the *Candida* species (MIC, 6.25 μg/mL). The compound with phenyl substitution on triazolothiadiazine ring **5a** was found to be active against *C. specie* [urine] 300, *C. specie* [HVS] 11972, and *C. specie* [urine] 12341. The compound with *p*-methoxyphenyl substituent on triazolothiadiazine ring **5c** was found to be active against *C. specie* [urine] 12485 and *C. specie* [urine] 12341 and the compound with *p*-sulphonamidophenyl substituent on triazolothiadiazine ring **5g** was found to be active against *C. specie* [urine] 300 with MIC value of 6.25 μg/mL, whereas ketoconazole (standard drug) exhibits the inhibitory activity with a MIC value of 3–30 μg/mL against all tested strains. The compound with *p*-chlorophenyl substituent on triazolothiadiazine ring **5e** was found to be the most potent derivative against *C. parapsilosis* (ATCC 22019), *C. albicans* (ATCC 66027), *C. specie* [blood] 12810, and *C. specie* [urine] 300. This could result from increased lipophilicity associated with chlorophenyl group. It can be also attributed to the inductive effect of the chloro substituent. The biological results indicate that *Candida* sp. [urine] 300 was more susceptible to compounds **5a**, **5e**, and **5g**. It is apparent that there is a positive correlation between anti-*Candidal* activity and chlorophenyl substitutions on the triazolothiadiazine ring.

3.3. Cytotoxicity. From a pharmacological point of view, it is important for the studied compounds to exhibit high bioactivity and at the same time show no or low cytotoxicity effects. All the compounds were evaluated against noncancer cell line, MCF-12 (nontumorigenic epithelial cell line) for their cytotoxic properties using WST-1 assay. The biological study indicated that compound **5g** with *p*-sulfonamido phenyl substitution possessed the highest cytotoxicity, whereas compound **5d** with *p*-bromophenyl substitution exhibited the lowest cytotoxicity against MCF-12 cells among the title compounds (Table 3). Three compounds **5d**, **5a**, and **5h** were found to have same IC₅₀ value as that of standard drug ketoconazole against noncancer cell line MCF-12 (IC₅₀ ≥ 1.0 × 10⁵ μg/mL). The most potent compound **5e** against *Candidal* spp. was found to have medium cytotoxicity in comparison to standard drug. The high cytotoxicity of compound **5d** may be because of the sulfonamido group (–SO₂NH₂), which has been reported to show cytotoxicity in literature [38].

4. Conclusions

In conclusion, we focused on the synthesis of new triazolothiadiazine derivatives **5a–h**, which were tested *in vitro* against various *Candida* species. Compound **5e** was the most effective compound against *C. parapsilosis* (ATCC 22019), *C. albicans* (ATCC 66027), *C. specie* [blood] 12810, and *C. specie* [urine] 300 with MIC value of 6.25 μg/mL compared to ketoconazole with MIC value of 3–30 μg/mL against all tested strains. This outcome confirms that *p*-chlorophenyl

TABLE 3: *In vitro* cytotoxicity of compounds (5a–h) on noncancer cell line (MCF-12).

Compound	IC ₅₀ (μg/mL) ^a
5a	$>1.0 \times 10^5$
5b	3.0×10^4
5c	6.6×10^4
5d	$>1.2 \times 10^5$
5e	5.4×10^4
5f	5.4×10^4
5g	2.4×10^4
5h	$>1.0 \times 10^5$
Gentamicin	$>1.0 \times 10^5$
Ketoconazole	$>1.0 \times 10^5$

^aIC₅₀: concentration of the compound (μg/mL) producing 50% cell growth inhibition after 48 h of compound exposure, as determined by the WST-1 assay. Each experiment was run at least three times, and the results are presented as average values.

substituent on triazolthiadiazine ring may have a considerable influence on anti-*Candida* activity. The cytotoxic effects of the compounds were also investigated. Compound 5g possessed the highest cytotoxicity, because of the sulfonamido group (–SO₂NH₂), whereas compounds 5d, 5a, and 5h exhibited the lowest cytotoxicity against MCF-12 cells. Based on the reported results, 5e derivative could also be used in association with azole derivatives to enhance their antifungal activity. Moreover, compound 5e displaying a better activity against *Candida* spp. represents a good template for the development of novel broad-spectrum anti-*Candida* spp. agents.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for funding this research group no. (RG 1435-006).

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