ANTIFUNGAL ACTIVITY OF SOME NOVEL THIADIAZOLE DERIVATIVES

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Abstract - The antibiotic resistance which was expedited by the use presume on antimicrobial drugs has been a significant global challenge for public health. In addition to this candidiasis is the most common fungal infection worldwide causing important morbidity and mortality especially in immunocompromised patients. Literature survey uncovers that the various derivatives of thiadiazole have been synthesized for their pharmalogical activities and many of them supported the finding that piperazine derivatives are potent against various microorganism strains.

Index Terms - Antifungal, thiodiazole, piperazine.

I. INTRODUCTION

Fungal infections represent a serious and presently unresolved health problem, particularly in developed countries. The rate of invasive fungal infections has expanded since the 1980s, particularly in the vast populace of immunocompromised patients as well as those hospitalized with serious underlying disease [1]. Type infections caused by Candida can be classified under two headings as superficial or systemic. Superficial diseases of the cutaneous or mucocutaneous tissues incorporate oropharyngeal candidiasis vaginitis, conjunctivitis, esophagitis, or gastrointestinal candidiasis. Systemic infections include endocarditis, pyelonephritis, esophagitis, meningitis, and disseminated candidiasis [2]. It is separately reported that thiadiazole and piperazine rings have antimicrobial activity [3-5]. In light of this information, it was designed a skeleton composed of thiadiazole and piperazine and synthesized 10 different novel derivatives for checking the antifungal activity of them.

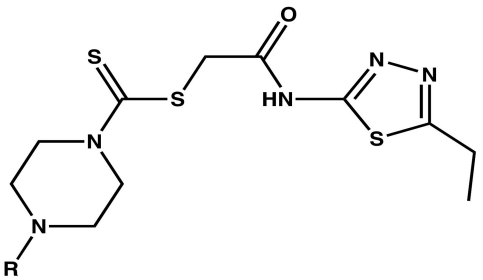


Figure 1. Main skeleton of the synthesized compounds.

The ergosterol biosynthesis pathway is required for generation of a major constituent of the fungal plasma membrane. Ergosterol is the main sterol, and thus is essential of for the growth and normal membrane function of fungal cell.

II. MATERIALS AND METHODS

Chemistry

All chemicals used in the syntheses were purchased from either Sigma-Aldrich Chemicals (Sigma-Aldrich Corp., St. Louis, MO, USA) or Merck Chemicals (Merck KGaA, Darmstadt, Germany). Melting points of the synthesized compounds were measured by MP90 digital melting point apparatus (Mettler Toledo, Ohio, USA) and were presented as uncorrected.

A Bruker 300 MHz and 75 MHz digital FT-NMR spectrometer (Bruker Bioscience, Billerica, MA, USA) in DMSO-d6, respectively recorded ^{1}H NMR and ^{13}C NMR spectra. In the NMR spectra splitting patterns were designated as follows: s: singlet; d: doublet; t: triplet; m: multiplet. Coupling constants (J) were reported as Hertz (Hz). The IR spectra of the compounds were recorded using an IRAffinity-1S Fourier transform IR (FTIR) spectrometer (Shimadzu, Kyoto, Japan). High resolution mass spectra were recorded on an LCMS-IT-TOF instrument (Shimadzu, Kyoto, Japan) by using ESI method. LCMSMS analysis of ergosterol was performed on Shimadzu LCMS-8040 instrument (Shiadzu, Kyoto, Japan) by using ESI technique. The purities of compounds were checked by TLC on silica gel 60 F254 (Merck KGaA, Darmstadt, Germany).

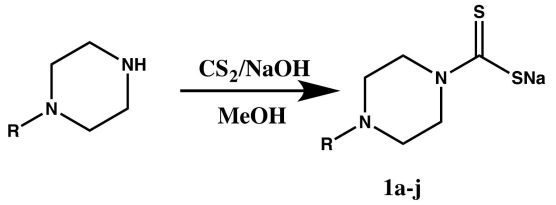


Figure 2. Synthesis pathway of the final compounds.

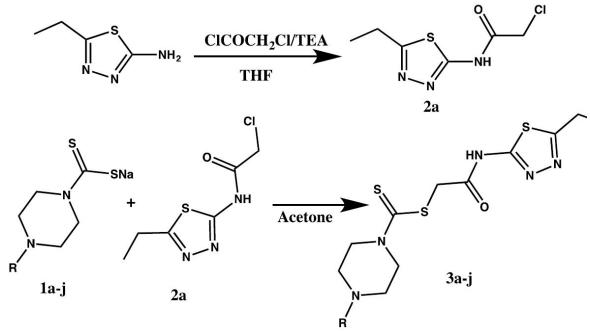
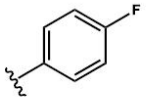
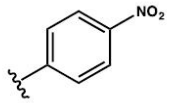
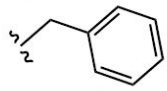
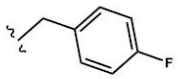
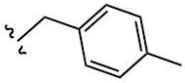
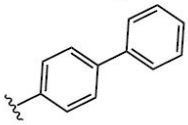
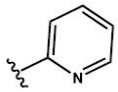
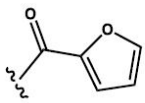


Table 1. Derivatives of synthesized compound.

Compound	R
3a	-CH_3
3b	-CH_2CH_2OH
3c	
3d	
3e	
3f	
3g	
3h	
3i	
3j	

Synthesis of sodium 4-substitutedpiperazine-1 -carbodithioate (1a-1j)

A mixture of 4-substituted piperazine (3 mmol) carbon disulfide (3.6 mmol, 0.216 mL) and NaOH (3.6 mmol, 0.144 g) were added in MeOH and stirred at 0oC for 4 h. After completion of the reaction, the precipitated product was filtered, washed with diethyl ether, dried and recrystallized from ethanol.

Synthesis of 2-Chloro-N-(5-ethyl-1,3,4-thiadiazol-2-yl) acetamide (2a) Chloroacetyl chloride (0.018 mol, 1.39 mL) was added dropwise with stirring to a solution of 5-ethyl-1,3,4-thiadiazol-2-amine (0.017 mol, 2.19 g) and trimethylamine (0.021 mol, 2.93 mL) in THF (50mL). The mixture is stirred at 0°C. After the completion of dropping, the mixture was allowed to stir for 1 h. After completion of the reaction, solvent was removed by means of rotary evaporator. The residue was washed with water, dried and recrystallized from ethanol.

General procedure for the synthesis of target compounds (3a-3j)

Sodium 4-substituted piperazine-1-carbodithioate (1a-1j) (1.47 mmol) and 2-chloro-N-(5-ethyl-1,3,4-thiadiazol-2-yl)acetamide (1.47 mmol, 0.30 g) were stirred for 4h in acetone. After completion of the reaction, the solvent was evaporated. The precipitated product was washed with water in order to removed potassium carbonate, dried and recrystallized from ethanol. And on the below, hydrogen and carbon NMR data of three of the final compounds was given as sample.

2-[(5-Ethyl-1,3,4-thiadiazol-2-yl)amino]-2-oxoethyl-4-(2-hydroxyethyl)piperazine-1-carbodithioate (3b)

^{1}H NMR (300 Mhz, DMSO-d_6, ppm) δ 1.28 (3H, t, J= 7.4 Hz, CH_3), 2.44 (2H, t, J= 6.1 Hz, CH_2-CH_2-OH), 2.52-2.57 (4H, br s, piperazine-H), 2.98 (2H, q, J= 7.5 Hz, CH_2-CH_3), 3.52 (2H, t, J= 6.5 Hz, CH_2-CH_2-OH), 3.93 (2H, br s, piperazine-H), 4.17 (2H, br s, piperazine-H), 4.37 (2H, s, $COCH_2$), 4.50 (1H, s, OH), 12.72 (1H, s, NH). ^{13}C NMR (75 MHz, DMSO-d_6, ppm) δ 14.30 (CH_3), 23.13 (CH_2-CH_3), 40.10, 50.38, 51.68, 52.89, 53.05, 58.95, 59.94, 58.95, 59.94, 158.74, 165.90, 166.69, 194.24 (C=S).

2-[(5-Ethyl-1,3,4-thiadiazol-2-yl)amino]-2-oxoethyl -4-(4-nitrophenyl)piperazine-1-carbodithioate (3d)

^{1}H NMR (300 Mhz, DMSO-d_6, ppm) δ 1.26 (3H, t, J= 7.6 Hz, CH_3), 2.94 (2H, q, J= 7.6 Hz, CH_2-CH_3), 3.72 (4H, br s, piperazine-H), 4.15 (2H, br s, piperazine-H), 4.32 (2H, br s, piperazine-H), 4.35 (2H, s, $COCH_2$), 6.94 (2H, d, J= 9.5 Hz, aromatic-H), 8.10 (2H, d, J= 9.1 Hz, aromatic-H), 12.45 (1H, s, NH). ^{13}C NMR (75 MHz, DMSO-d_6, ppm) δ 14.36 (CH_3), 23.27 (CH_2-CH_3), 41.13, 45.11, 48.84, 50.48, 112.27, 126.28, 137.24, 154.14, 160.55, 165.14, 167.26, 195.17 (C=S).

2-[(5-Ethyl-1,3,4-thiadiazol-2-yl)amino]-2-oxoethyl-4-(2-furoyl)piperazine-1-carbodithioate (3j)

^{1}H NMR (300 Mhz, DMSO-d_6, ppm) δ 1.28 (3H, t, J= 7.5 Hz, CH_2-CH_3), 2.98 (2H, q, J= 7.6 Hz, CH_2-CH_3), 3.86 (4H, br s, piperazine-H), 4.08 (2H, br s,

piperazine-H), 4.27 (2H, br s, piperazine-H), 4.41 (2H, s, $COCH_2$), 6.65-6.66 (1H, m, aromatic-H), 7.07-7.08 (1H, m, aromatic-H), 7.88-7.89 (1H, m, aromatic-H), 12.77 (1H, s, NH). ^{13}C NMR (75 MHz, DMSO-d_6, ppm) δ 14.30 (CH_2-CH_3), 23.13 (CH_2-CH_3), 42.79, 49.55, 51.03, 111.92, 116.64, 145.64, 147.05, 158.63, 158.92, 165.95, 166.57, 194.93 (C=S).

Antifungal Activity Assay

Antifungal Activity Assay The anticandidal effect of compounds 3i–3j were screened in accord with the EUCAST definitive (EDef 7.1) method [6]. Fluconazole was employed as a reference drug. The in vitro growth inhibitory activity of the compounds were tested against Candida albicans ATCC 90028, Candida crusei ATCC 6258, Candida parapsilosis ATCC 22019, Candida tropicalis ATCC 13803, Candida albicans ATCC 10231, Candida glabrata ATCC 2001, Candida famata (Abant İzzet Baysal University Medical Faculty Hospital, clinical izolate) and Candida lusitaniae (Abant İzzet Baysal University Medical Faculty Hospital, clinical izolate). The results were obtained as MIC values and MIC readings were accomplished twice for each chemical agent. After incubating overnight at 37 ◦C, the fungal strains were sustained in Roswell Park Memorial Institute (RPMI) medium. Adjustment of the inoculum density to a 0.5 McFarland turbidity standard was carried out by using a spectrophotometer. The resulting inoculum suspension contained 0.5 $\times 10^5$–2.5 $\times$ 10^5 cfu/mL for fungi. The two-fold serial dilutions technique were applied and the test was carried out in Mueller–Hinton broth and RPMI at pH7 and the test was performed for medium at pH7. The last well on the microplates containing only inoculated broth and the last well with no growth of microorganism represent controls and the MIC50 in μg/mL, respectively. DMSO was used as solvent. Further dilutions of the compounds and standard drugs in the RPMI medium were prepared in the required quantities at contcentrations of 160 - 0.312 μg/mL. After a 24 hμg/mL. After a 24 h incubation of the of the final plates including microorganism strains, MIC50 values were determined with a microplate reader at 490 nm. Each experiment in antifungal assay was performed twice.

Table 2. Minimum inhibitory concentration (MIC50; μg/mL) values of compounds on four strains of Candida (1a-8a)

	1	2	3	4	5	6	7	8
3a	20	20	20	20	20	20	40	20
3b	>160	160	160	160	160	160	>160	160
3c	80	80	80	40	80	40	80	80
3d	20	20	20	20	20	20	20	20
3e	40	20	20	40	40	20	40	80
3f	20	20	20	20	20	20	20	20
3g	80	40	40	40	40	40	80	80
3h	80	160	80	80	160	80	20	40
3i	40	40	20	20	40	40	40	80
3j	20	40	40	40	40	40	80	40
Ref	10	20	10	20	20	20	20	10

1. Candida albicans ATCC 90028, 2. Candida crusei ATCC 6258, 3. Candida parapsilosis ATCC 22019, 4. Candida tropicalis ATCC 13803, 5. Candida albicans ATCC 10231, 6. Candida glabrata ATCC 2001, 7. Candida famata ((Abant İzzet Baysal University Medical Faculty Hospital, clinic izolate)), 8. Candida lusitaniae ((Abant İzzet Baysal University Medical Faculty Hospital, clinic izolate)), Ref: Fluconazol

Measuring Inhibition of Ergosterol Biosynthesis

Ergosterol standard was analyzed by Electrospray ionization (ESI) technique. In positive mode ESI, characteristic mass pattern of ergosterol was observed on the analysis. And one characteristic peak was chosen and forced to fragmentation. Two of the daughter ions collected from the fragmentation used for the multiple reaction monitoring (MRM) method development that is necessary for the quantification of ergosterol. Ergosterol amount in negative control samples was regarded as 100% and other samples collected from the microplates that used on antifungal activity assay were calculated in comparison with that control.

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