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Original article

SYNTHESIS OF SOME 4-PHENYL/CYCLOHEXYL-5-(1-PHENOXYETHYL)-3-[(3,5-DIARYL-2-PYRAZOLINYL)ACETYL]THIO-4H-1,2,4-TRIAZOLE DERIVATIVES AND THEIR ANTIFUNGAL ACTIVITY

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Abstract

Triazole derivatives are the major chemical group of antifungal azole derivatives. Nowadays, the most frequently used triazoles are fluconazole and itraconazole. They posses a broad spectrum of antifungal activity and reduced toxicity when compared with the other antifungals. In this study, we aimed the synthesis of new 1,2,4-triazole derivatives as novel antifungal agents. The reaction of propionic acid hydrazides with various aryl/alkyl isothiocyanates gave thiosemicarbazides which furnished the mercaptotriazoles by alkali cyclization. The 4-phenyl/cyclohexyl-5-(1-phenoxyethyl)-3-[(3,5-diaryl-2-pyrazolin-1-yl)acetyl]thio-4H-1,2,4-triazole derivatives were synthesized by reacting the mercaptotriazoles with 1-(2-chloroacetyl)-3,5-diaryl-2-pyrazoline. The chemical structures of the compounds were elucidated by IR, ^{1}H-NMR, FAB-MS spectral data and elemental analysis. Their antifungal activities against Candida albicans (two strains), Candida glabrata, Candida tropicalis, Candida krusei , Candida utilis, Geotrichum candidum were investigated. The results showed that some of the compounds have strong antifungal activity.

Key words: *Triazole, Pyrazoline, Antifungal activity*

Bazı 4-Fenil/siklohegzil-5-(1-fenoksietil)-3-[(3,5-diaril-2-pirazolinil)asetil]tiyo-4H-1,2,4-triazol Türevlerinin Sentezleri ve Antifungal Etkileri

Triazol türevleri, azol antifungallerin en büyük kimyasal grubudur. Günümüzde flukonazol ve itrakonazol en sık kullanılan triazol türevleridir. Diğer antifungaller ile kıyaslandığında, düşük toksisite ve geniş etki spektrumuna sahiptirler. Bu çalışmada, yeni antifungal bileşikler olarak 1,2,4-triazol türevlerini sentezlemeyi amaçladık. Alkali siklizasyonla merkaptotrizolleri oluşturan tiyosemikarbazidleri, propiyonik asid hidrazidi ile aril/alkil izotiyosiyanatların reaksiyonu 31 verdi. Merkaptotriazoller ile 1-(2-kloroasetil)-3,5-diaril-2-pirazolinin reaksiyonu ile 4-fenil/siklohegzil-5-(1-fenoksietill)-3-[(3,5-diaril-2-pirazolin-1-il)asetil]tiyo-4H-1,2,4-triazol türevleri sentezlendi. Bileşiklerin kimyasal yapıları ve saflık tayinleri IR, ^{1}H-NMR, FAB-MS spectral verileri ve elemental analiz ile aydınlatıldı. Bileşiklerin antifungal aktiviteleri Candida albicans (2 suş), Candida glabrata, Candida tropicali, Candida krusei , Candida utilis, Geotrichum candidum'a karşı araştırıldı. Sonuçlar, bazı bileşiklerin güçlü antifungal etkiye sahip olduklarını gösterdi.

Anahtar kelimeler: *Triazol, Pirazolin, Antifungal etki*

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INTRODUCTION

Over the last three decades there has been a dramatic increase in the incidence of fungal infections. Discovery of new drugs for the treatment of systemic mycoses is a major challenge in infectious disease research. There is an urgent need for new antifungal remedies with novel modes of action due to a decreased antifungal susceptibility of newly emerging fungi in growing setting of the immunocompromised patients (e.g., HIV-positive and neutropenic patients). As known, not only biochemical similarity of the human cell and fungi forms is a handicap for selective activity, but also the easily gained resistance is the main problem encountered in developing safe and efficient antifungals. While the incidence of systemic fungal infections has been increasing, the choice of suitable antifungal agents remains relatively limited although the advent of the new echinocandin class is a welcome development as is the expansion of members of the azole antifungals (1-4). The azole antifungals may be regarded as a new class providing truely effective drugs those are reported to inhibit fungi by blocking the biosynthesis of certain fungal lipids, especially ergosterol in cell membranes, and by additional mechanisms (5,6). Triazole derivatives are the major chemical groups of antifungal azole derivatives. Nowadays, the most frequently used triazoles are fluconazole and itraconazole.

They possess a broad spectrum of antifungal activity and show reduced toxicity when compared with the other antifungals (7–11).

Substituted-1,2,4-triazoles and the open-chain thiosemicarbazide counterparts of 1,2,4-triazole, are among the various heterocycles that have received the most attention during the last two decades as potential antifungal and antibacterial agents (12–18). Substitutions including thio (19,20) alkylthio and alkenylthio (21) derivatives have been carried out primarily at the 3-position of the 1,2,4-triazole ring, as potential antifungal and antibacterial agents those will overcome the above mentioned problems.

In view of these data, we aimed to synthesize of new 3-alkylthio-1,2,4-triazole derivatives as novel antifungal agents. As a substituent for obtaining diversity in these novel derivatives, pyrazoline moiety was selected because it is well known with its antifungal activity (22-25).

EXPERIMENTAL

Chemistry

All melting points (m.p.) were determined in open capillaries on a Gallenkamp apparatus and are uncorrected. The purity of the compounds was routinely checked by thin layer chromatography (TLC) using silica gel 60G (Merck). Spectroscopic data were recorded by the following instruments. IR: Shimadzu IR-435 spectrophotometer; ^{1}H-NMR: Bruker 250 MHz spectrometer, MS-FAB: VG Quattro Mass spectrometer. Elemental analyses were recorded on Perkin Elmer EAL 240 spectrometer.

General procedure for synthesis of the compounds
1-(Chloroacetyl)-3-(2-thienyl)-5-aryl-2-pyrazolines ***(I)****.*

Chloroacetyl chloride (50 mmol) and triethylamine (50 mmol) were added to a solution of 5-aryl-3-(2-thienyl)-2-pyrazoline (50 mmol) in anhydrous acetone and the mixture was treated as described in literature (24-25).

2-Phenoxypropionic acid hydrazides ***(II)****.*

These compounds were prepared according to the previously reported method, by reacting ethyl 2-phenoxypropionates with hydrazine hydrate (26,27).

*1-(2-Phenoxypropionyl)-4-phenyl/cyclohexyl-3-thiosemicarbazides (**III**).*

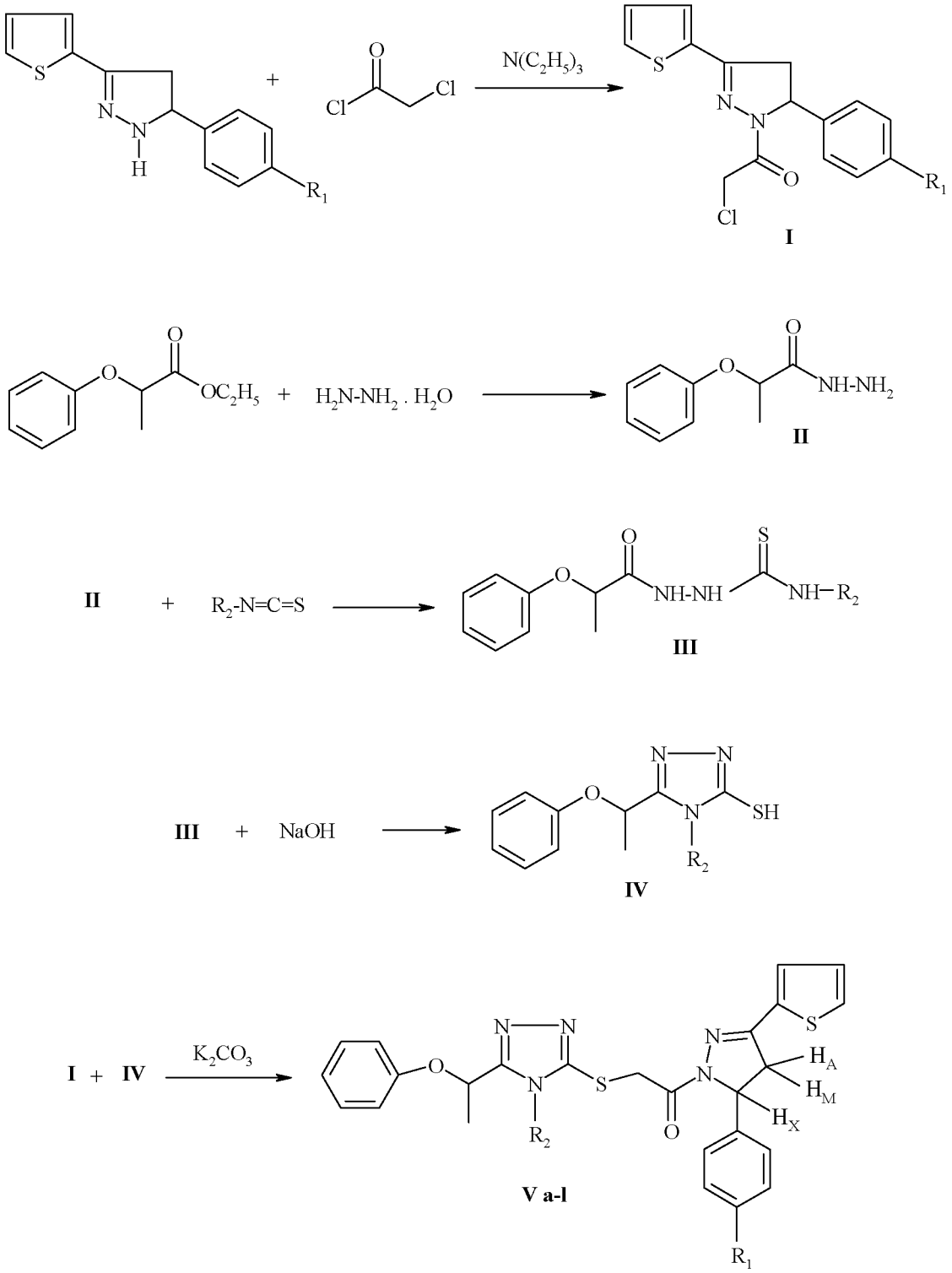
Equimolar quantities of acid hydrazide *(**II**)* (50 mmol) and phenyl/cyclohexyl isothiocyanate in 25 ml of absolute ethanol were refluxed for 3–5 h. The resulting solid was filtered and recrystallized from ethanol (28).

*3-Mercapto-4-phenyl/cyclohexyl-5-(1-phenoxyethyl)-4H-1,2,4-triazole (**IV**)*

Suitable substituted thiosemicarbazides **(III)** (20 mmol) were dissolved in 2 N sodium hydroxide and the resulting solution was heated under reflux for 3 h. The solution was cooled and acidified to pH 2–3 with hydrochloric acid solution and recrystallized from ethanol (29,30).

*4-Phenyl/cyclohexyl-5-(1-phenoxyethyl)-3-[(3-thienyl-5-aryl-2-pyrazoline-1 -yl)acetyl]thio-4H-1,2,4-triazole (**Va–l**).*

A mixture of the 1-(chloroacetyl)-3-(2-thienyl)-5-aryl-2-pyrazolines **(I)** (10 mmol), appropriate triazoles **(IV)** and anhydrous potassium carbonate in acetone were mixtured at room temperature for 6 h. The mixture was filtered, the filtrate was evaporated until dryness. The residue was washed with water and recrystallized from ethanol.



Scheme 1

Table 1. Some characterisations of the compounds

	R_1	R_2	M.p. °C	Yield %	Mol. Formula	M.W.
Va	H	C_6H_5	98	68	$C_{31}H_{27}N_5O_2S_2$	565
Vb	$N(CH_3)_2$	C_6H_5	90	72	$C_{33}H_{32}N_6O_2S_2$	608
Vc	F	C_6H_5	104	65	$C_{31}H_{26}FN_5O_2S_2$	583
Vd	Cl	C_6H_5	89	69	$C_{31}H_{26}ClN_5O_2S_2$	599,5
Ve	OCH_3	C_6H_5	84	78	$C_{32}H_{29}N_5O_3S_2$	595
Vf	CH_3	C_6H_5	85	69	$C_{32}H_{29}N_5O_2S_2$	579
Vg	H	C_6H_{11}	110	72	$C_{31}H_{33}N_5O_2S_2$	571
Vh	$N(CH_3)_2$	C_6H_{11}	114	75	$C_{33}H_{38}N_6O_2S_2$	614
Vi	F	C_6H_{11}	106	69	$C_{31}H_{32}FN_5O_2S_2$	589
Vj	Cl	C_6H_{11}	92	70	$C_{31}H_{32}ClN_5O_2S_2$	605,5
Vk	OCH_3	C_6H_{11}	88	65	$C_{32}H_{35}N_5O_3S_2$	601
Vl	CH_3	C_6H_{11}	96	65	$C_{32}H_{35}N_5O_2S_2$	685

Va: IR (KBr) υ_{maks} (cm^{-1}): 1695 (C=O), 1599–1453 (C=C and C=N)
^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 1.55 (3H, dd, J= 6.4, 1.9 Hz, CH_3), 3.19 (1H, dd, J_{AM}= 18.1 Hz, J_{AX}= 4.5 Hz, C_4-H_A of pyrazoline), 3.91 (1H, dd, J_{MA}= 18.1 Hz, J_{MX}=12.0 Hz, C_4-H_M of pyrazoline), 4.40-4.60 (2H, m, S-CH_2), 5.4-5.6 (2H, m, O-CH and C_5-H_X of pyrazoline), 6.65-7.78 (18H, m, aromatic protons)
MS (FAB); *m/z*: 566 [M + 1]
Anal. Calc. $C_{31}H_{27}N_5O_2S_2$: C, 65.82; H, 4.81; N, 12.38. Found: C, 65.85; H, 4.81; N, 12.39

Vb: IR (KBr) υ_{maks} (cm^{-1}): 1705 (C=O), 1605–1473 (C=C and C=N)
^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 1.56 (3H, dd, J= 6.8, 1.3 Hz, CH_3), 2.84 (6H, s, $N(CH_3)_2$), 3.15 (1H, dd, J_{AM} = 18.1 Hz, J_{AX}= 4.1 Hz, C_4-H_A of pyrazoline), 3.83 (1H, dd, J_{MA}= 18.1 Hz, J_{MX}=12.0 Hz, C_4-H_M of pyrazoline), 4.45-4.60 (2H, m, S-CH_2), 5.4-5.55 (2H, m, O-CH and C_5-H_X of pyrazoline), 6.60-7.80 (17H, m, aromatic protons)
MS (FAB); *m/z*: 609 [M + 1]
Anal. Calc. $C_{33}H_{32}N_6O_2S_2$: C, 65.11; H, 5.30; N, 13.80. Found: C, 65.13; H, 5.33; N, 13.83.

Vc: IR (KBr) υ_{maks} (cm^{-1}): 1710 (C=O), 1590–1450 (C=C and C=N)
^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 1.55 (3H, dd, J= 6.4 , 2.6 Hz, CH_3), 3.20 (1H, dd, J_{AM}= 18.1 Hz, J_{AX}=4.5 Hz, C_4-H_A of pyrazoline), 3.90 (1H, dd, J_{MA}= 17.7 Hz, J_{MX}=11.7 Hz, C_4-H_M of pyrazoline), 4.45-4.65 (2H, m, S-CH_2), 5.4-5.6 (2H, m, O-CH and C_5-H_X of pyrazoline), 6.70-7,79 (17H, m, aromatic protons).
MS (FAB); *m/z*: 584 [M + 1]
Anal. Calc. $C_{31}H_{26}FN_5O_2S_2$: C, 63.79; H, 4.49; N, 12.00. Found: C, 63.80; H, 4.45; N, 12.03.

Vd: IR (KBr) υ_{maks} (cm^{-1}): 1600 (C=O), 1605–1463 (C=C and C=N)
^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 1.55 (3H, dd, J= 6.4, 2.3 Hz, CH_3), 3.20 (1H, dd, J_{AM} = 17.9 Hz, J_{AX}=4.6 Hz, C_4-H_A of pyrazoline), 3.90 (1H, dd, J_{MA} = 17.9 Hz, J_{MX}=11.8 Hz, C_4-H_M of pyrazoline), 4.40-4.60 (2H, m, S-CH_2), 5.4-5.6 (2H, m, O-CH and C_5-H_X of pyrazoline), 6.65-7.79 (17H, m, aromatic protons)
MS (FAB); *m/z*: 600 [M + 1]
Anal. Calc. $C_{31}H_{26}ClN_5O_2S_2$: C, 62.04; H, 4.37; N, 11.67. Found: C, 62.06; H, 4.35; N, 11.63.

Ve: IR (KBr) υ_{maks} (cm^{-1}): 1680 (C=O), 1602–1456 (C=C and C=N)
^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 1.56 (3H, dd, J= 6.4, 1.5 Hz, CH_3), 3.17 (1H, dd, J_{AM} = 17.9 Hz, J_{AX}=4.3 Hz, C_4-H_A of pyrazoline), 3.72 (3H, s, OCH_3), 3.87 (1H, dd, J_{MA} = 17.9 Hz, J_{MX}=11.5 Hz, C_4-H_M of pyrazoline), 4.40-4.65 (2H, m, S-CH_2), 5.4-5.6 (2H, m, O-CH and C_5-H_X of pyrazoline), 6.65-7.78 (17H, m, aromatic protons).
MS (FAB); *m/z*: 596 [M + 1]
Anal. Calc. $C_{32}H_{29}N_5O_3S_2$: C, 64.52; H, 4.91; N, 11.76. Found: C, 64.55; H, 4.90; N, 11.80.

Vf: IR (KBr) υ_{maks} (cm^{-1}): 1702 (C=O), 1601–1453 (C=C and C=N)
^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 1.55 (3H, dd, J= 6.4, 1.9 Hz, CH_3), 2.26 (3H, s, pheny-CH_3), 3.16 (1H, dd J_{AM} =17.9 Hz, J_{AX}=4.3 Hz, C_4-H_A of pyrazoline), 3.88 (1H, dd, J_{MA} = 17.9 Hz, J_{MX}=11.7 Hz, C_4-H_M of pyrazoline), 4.45-4.60 (2H, m, S-CH_2), 5.4-5.6 (2H, m, O-CH and C_5-H_X of pyrazoline) 6.65-7.78 (17H, m, aromatic protons)
MS (FAB); *m/z*: 580 [M + 1]
Anal. Calc. $C_{32}H_{29}N_5O_2S_2$: C, 66.30; H, 5.04; N, 12.08. Found: C, 66.31; H, 5.08; N, 12.10.

Vg: IR (KBr) υ_{maks} (cm^{-1}): 1708 (C=O), 1604–1450 (C=C and C=N)
^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 0.9-2.1 (13H, m, CH_3 and C_2 ,C_3 ,C_4 ,C_5 ,C_6 protons of cyclohexyl), 3.19 (1H, dd, J_{AM}= 17.9 Hz, J_{AX}= 4.3 Hz, C_4-H_A of pyrazoline), 3.92 (1H, dd, J_{MA}= 17.7 Hz, J_{MX}=11.7 Hz, C_4-H_M of pyrazoline), 4.13 (1H, m, C_1 proton of cyclohexyl), 4.50-4.70 (2H, m, S-CH_2), 5.59 (1H, dd, J_{MX}= 11.7 Hz, J_{AX}= 4.5 Hz, C_5-H_X of pyrazoline), 5.85-5.88 (1H, m, O-CH), 6.9-7.4 (13H, m, aromatic protons).
MS (FAB); *m/z*: 572 [M + 1]
Anal. Calc. $C_{31}H_{33}N_5O_2S_2$: C, 65.12; H, 5.82; N, 12.25. Found: C, 65.12; H, 5.80; N, 12.24.

Vh: IR (KBr) υ_{maks} (cm^{-1}): 1704 (C=O), 1599–1451 (C=C and C=N)
^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 0.9-2.1 (13H, m, CH_3 and C_2 ,C_3 ,C_4 ,C_5 ,C_6 protons of cyclohexyl), 2.85 (6H, s, $N(CH_3)_2$), 3.16 (1H, dd, J_{AM}= 17.7 Hz, J_{AX}= 3.9 Hz, C_4-H_A of pyrazoline), 3.85 (1H, dd, J_{MA}= 17.7 Hz, J_{MX}=11.7 Hz, C_4-H_M of pyrazoline), 4.12 (1H, m, C_1 proton of cyclohexyl), 4.55-4.70 (2H, m, S-CH_2), 5.46 (1H, dd, J_{MX}= 11.7 Hz, J_{AX}= 4.0 Hz, C_5-H_X of pyrazoline), 5.85-5.88 (1H, m, O-CH), 6.65-7.78 (12H, m, aromatic protons).
MS (FAB); *m/z*: 615 [M + 1]
Anal. Calc. $C_{33}H_{38}N_6O_2S_2$: C, 64.47; H, 6.23; N, 13.67. Found: C, 64.45; H, 6.20; N, 13.68.

Vi: IR (KBr) υ_{maks} (cm^{-1}): 1703 (C=O), 1603–1469 (C=C and C=N)
^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 0.9-2.1 (13H, m, CH_3 and C_2 ,C_3 ,C_4 ,C_5 ,C_6 protons of cyclohexyl), 3.20 (1H, dd, J_{AM}= 18.1 Hz, J_{AX}= 4.5 Hz, C_4-H_A of pyrazoline), 3.91 (1H, dd, J_{MA}= 18.1 Hz, J_{MX}=11.7 Hz, C_4-H_M of pyrazoline), 4.13 (1H, m, C_1 proton of cyclohexyl), 4.50-4.75 (2H, m, S-CH_2), 5.60 (1H, dd, J_{MX}= 11.7 Hz, J_{AX}= 4.5 Hz, C_5-H_X of pyrazoline), 5.85-5.88 (1H, m, O-CH), 6.9-7.79 (12H, m, aromatic protons).
MS (FAB); *m/z*: 590 [M + 1]
Anal. Calc. $C_{31}H_{32}FN_5O_2S_2$: C, 63.14; H, 5.47; N, 11.87. Found: C, 63.17; H, 5.50; N, 11.89.

Vj: IR (KBr) υ_{maks} (cm^{-1}): 1695 (C=O), 1589–1462 (C=C and C=N)
^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 0.9-2.1 (13H, m, CH_3 and C_2 ,C_3 ,C_4 ,C_5 ,C_6 protons of cyclohexyl), 3.20 (1H, dd, J_{AM}= 18.1 Hz, J_{AX}= 4.5 Hz, C_4-H_A of pyrazoline), 3.91 (1H, dd, J_{MA}= 18.1 Hz, J_{MX}=11.8 Hz, C_4-H_M of pyrazoline), 4.13 (1H, m, C_1 proton of cyclohexyl), 4.55-4.75 (2H, m, S-CH_2), 5.59 (1H, dd, J_{MX}= 11.8 Hz, J_{AX}= 4.5 Hz, C_5-H_X of pyrazoline), 5.85-5.88 (1H, m, O-CH), 6.9-7.80 (12H, m, aromatic protons)
MS (FAB); *m/z*: 606 [M + 1]
Anal. Calc.$C_{31}H_{32}ClN_5O_2S_2$: C, 61.42; H, 5.32; N, 11.55. Found: C, 61.45; H, 5.35; N, 11.53.

Vk: IR (KBr) υ_{maks} (cm^{-1}): 1690 (C=O), 1595–1469 (C=C and C=N)
^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 0.9-2.1 (13H, m, CH_3 and C_2 ,C_3 ,C_4 ,C_5 ,C_6 protons of cyclohexyl), 3.18 (1H, dd, J_{AM}= 18.1 Hz, J_{AX}= 4.5 Hz, C_4-H_A of pyrazoline), 3.72 (3H, s, OCH_3), 3.88 (1H, dd, J_{MA}= 18.1 Hz, J_{MX}=12.1 Hz, C_4-H_M of pyrazoline), 4.12 (1H, m, C_1 proton of cyclohexyl), 4.50-4.70 (2H, m, S-CH_2), 5.53 (1H, dd, J_{MX}= 11.5 Hz, J_{AX}= 4.5 Hz, C_5-H_X of pyrazoline), 5.85-5.88 (1H, m, O-CH), 6.87-7.78 (12H, m, aromatic protons)
MS (FAB); *m/z*: 602 [M + 1]
Anal. Calc. $C_{32}H_{35}N_5O_3S_2$: C, 63.87; H, 5.86; N, 11.64. Found: C, 63.83; H, 5.90; N, 11.61.

Vl: IR (KBr) υ_{maks} (cm^{-1}): 1695 (C=O), 1600–1473 (C=C and C=N)
^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 0.9-2.1 (13H, m, CH_3 and C_2 ,C_3 ,C_4 ,C_5 ,C_6 protons of cyclohexyl), 2.26 (3H, s, phenyl-CH_3), 3.16 (1H, dd, J_{AM}= 18.1 Hz, J_{AX}= 4.4 Hz, C_4-H_A of pyrazoline), 3.89 (1H, dd, J_{MA}= 18.1 Hz, J_{MX}=12.0 Hz, C_4-H_M of pyrazoline), 4.13 (1H, m, C_1 proton of cyclohexyl), 4.55-4.70 (2H, m, S-CH_2), 5.54 (1H, dd, J_{MX}= 11.5 Hz, J_{AX}= 4.4 Hz, C_5-H_X of pyrazoline), 5.85 -5.88 (1H, m, O-CH), 6.90-7.79 (12H, m, aromatic protons)
MS (FAB); *m/z*: 586 [M + 1]
Anal. Calc. $C_{32}H_{35}N_5O_2S_2$: C, 65.61; H, 6.02; N, 11.96. Found: C, 65.65; H, 6.00; N, 11.99.

Biology
Microdilution broth susceptibility assay

Microdilution broth susceptibility assay was used for the antifungal evaluation of the compounds (31-32). Stock solutions of the samples were prepared in dimethylsulfoxide (DMSO, Carlo-Erba, France). Dilution series using sterile distilled water were prepared from 4 mg/ml to 0.007 mg/ml in micro-test tubes (Eppendorf) that were transferred to 96-well microtiter plates. Overnight grown fungus suspensions in double-strength Mueller-Hinton broth were standardised to 10^8 CFU/ml using McFarland No: 0.5 standard solution. 100 μl of each microorganism suspension was then added into the wells. The last well-chain without microorganism was used as a negative control. Sterile distilled water and the medium served as a positive growth control. After incubation at 37°C for 18-24 h the first well without turbidity was determined as the minimal inhibitory concentration (MIC). Ketoconazole and fluconazole were used as control drugs.
Tested microorganism strains were; *Candida albicans* (Y-12983), *C. albicans* (isolate obtained from Faculty of Medicine, Osmangazi University), *C. glabrata* (isolate obtained from Faculty of Medicine, Osmangazi University), *C. tropicalis* (Y-12968), *C. krusei* (Y-7179), *C. utilis* (Y-900), *Geotrichum candidum* (T-552). The observed data on the antifungal activity of the compounds and control drug were given in Table 2.

Table 2. Antifungal activities of the compouns (μg/mL)

Comp.	A	B	C	D	E	F	G
Va	4	4	4	2	4	4	4
Vb	4	4	4	1	2	4	4
Vc	4	4	4	2	4	2	2
Vd	4	4	4	4	4	4	4
Ve	4	4	4	0,5	2	4	4
Vf	4	4	4	1	4	4	4
Vg	4	4	4	4	4	4	4
Vh	4	2	2	2	4	4	2
Vi	4	4	4	1	4	2	4
Vj	4	4	4	2	4	2	4
Vk	2	2	4	2	4	4	4
Vl	4	4	4	2	4	2	4
Ref.-1	4	4	4	2	4	2	4
Ref.-2	2	4	4	4	2	4	4

Reference 1:Fluconazole, **Reference 2**: Ketoconazole
A: *C. albicans* (Y-12983), **B:** *C. albicans* (isolate obtained from Faculty of Medicine, Osmangazi University), **C:** *C. glabrata* (isolate obtained from Faculty of Medicine, Osmangazi University), **D:** *C. tropicalis* (Y-12968), **E:** *C. krusei* (Y-7179), **F:** *C. utilis* (Y-900), **G:** *G. candidum* (T-552)

RESULTS AND DISCUSSION

In the present work, 12 new triazole derivatives were synthesized. The structures of the obtained compounds were elucidated by spectral data. According to the IR spectroscopic data of the compounds **Va–l** showed characteristic C=O (amide) stretching bands and C=C, C=N bands in 1710–1680 cm^{-1} and 1605-1450 cm^{-1} regions respectively. In the ^{1}H-NMR spectra of compounds (**Va-l**), H_A, H_B and H_X protons of the pyrazoline ring were seen as doublet of doublets at about 3.15-3.20 ppm, 3.83-3.91 ppm and 5.00-5.60 ppm respectively. The methylene protons signal due to S-CH_2, present in all compounds, appeared at 4.40–4.75 ppm, as multiplets. All other aromatic and aliphatic protons were observed at the expected regions. Although it is easy to clarify the chemical shifts of the aromatic protons of our compounds, i.e.

12-13 protons for compounds **Va-f** and 17-18 protons for compounds **Vg-l**, the situation is quite complicated to differentiate those protons in aromatic region. This is not an unexpected case interpreting NMR results at a resolution of 250 MHz. However, integral values of those aromatic protons correspond to expected values for these compounds.

Mass spectra (MS (FAB)) of compounds showed M+1 peaks, in agreement with their molecular formula.

MIC's were recorded as the minimum inhibitory concentration of compounds, which inhibits the growth of tested microorganisms. All tested compounds showed important antifungal activity. The MIC values are generally within the range of 0.5-4 μg/mL against all evaluated strains. Tested compounds are at least as much active as control drugs. Especially **Vb**, **Ve**, **Vf**, **Vi** against *C. tropicalis*; **Vc**, **Vh** against *G. candidum*; **Vh, Vk** against *C. albicans* (clinical isolate) and **Vh** against *C. glabrata* (clinical isolate) showed strong activity when compared with reference agents.

CONCLUSION

When the structure and activity relationship is taken in consideration R_2 substitution seems to have no effect on activity, R_1 substitution has effect on activity. Especially the $N(CH_3)_2$ and OCH_3 substitions on R_1 have positive effect on activity. As a result we can say that these compounds could be taken in consideration as high potent antifungal agents with their strong activity.

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