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Short communication

Synthesis and antimicrobial activity of 4-phenyl/cyclohexyl-5-(1-phenoxyethyl)-3-[*N*-(2-thiazolyl)acetamido]thio-4H-1,2,4-triazole derivatives

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Abstract

The increasing clinical importance of drug-resistant fungal and bacterial pathogens has lent additional urgency to microbiological research and new antimicrobial compound development. For this purpose, new thiazole derivatives of triazoles were synthesized and evaluated for antifungal and antibacterial activity.

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-[*N*-(2-thiazolyl)acetamido]thio-4H-1,2,4-triazole derivatives were synthesized by reacting the mercaptotriazoles with 2-chloro-*N*-(2-thiazolyl)acetamide. The chemical structures of the compounds were elucidated by IR, ^{1}H-NMR, FAB$^+$-MS spectral data. Their antimicrobial activities against *Candida albicans* (two strains), *Candida glabrata*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* were investigated. The results showed that some of the compounds have very strong antifungal activity.

Keywords: Thiazole; Triazole; Antifungal activity; Antibacterial activity

1. Introduction

The development of resistance to current antibacterial therapy continues to search for more effective agents. In addition, primary and opportunistic fungal infections continue to increase rapidly because of the increased number of immunocompromised patients (AIDS, cancer and transplants). Several reviews have appeared illustrating the problems encountered by today's infectious disease clinicians [1–3].

As known, not only biochemical similarity of the human cell and fungi forms a handicap for selective activity, but also the easily gained resistance is the main problem encountered in developing safe and efficient antifungals. The azole antifungals may be regarded as a new class providing truly 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 [4,5]. The imidazole antifungals, such as clotrimazole, miconazole, and ketoconazole, showed good topical activity, but were only of limited value for systematic administration. Triazole derivatives are the other 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 imidazole antifungals [6–11].

Triazoles, in particular, 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 antimicro-

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bial agents [12–22]. Substitutions including thio [23,24], alkylthio and alkenylthio [25,26] derivatives have been carried out primarily at the 3-position of the 1,2,4-triazole ring, as potential antimicrobial agents those will overcome the above mentioned resistance problems.

In view of these data, we aimed the synthesis of new 1,2,4-triazole derivatives as novel antimicrobial agents. The triazoles contain thiazole ring system and, in additionally, some of these thiazoles include ester residue. Thiazole moiety was selected as it is well known with its antimicrobial activity [27–30] and ester residue, i.e. easily hydrolyze to acid function, was especially preferred refer to the data discussed as a part of the effect of acidic functions on nonspecific antifungal agents [31].

2. Chemistry

The synthetic route of compounds is outlined in Scheme 1. In the present work, 2-chloro-*N*-(2-thiazolyl)acetamides (**I**) were prepared for the first time by reacting 2-aminothiazoles with chloroacetyl chloride in accordance with the method described in Ref. [32].

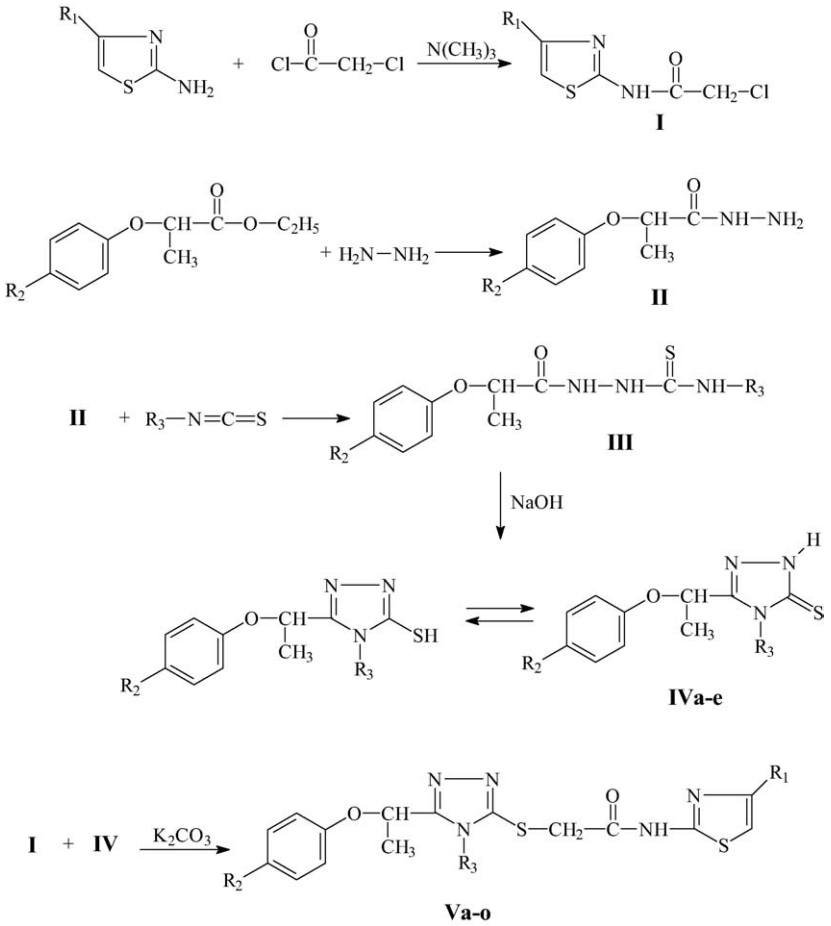
The 2-phenoxypropionic acid hydrazides (**II**) were prepared by reacting ethyl 2-phenoxypropionates with hydrazine hydrate [33,34]. The condensation of the acid hydrazides (**II**) with appropriate phenyl/cyclohexyl isothiocyanate resulted in the formation of 1-(2-phenoxypropionyl)-4-phenyl/cyclohexyl-3-thiosemicarbazides (**III**) [35,36].

The thiosemicarbazides, on refluxing with 2 N sodium hydroxide solution, were cyclized into corresponding 4-phenyl/cyclohexy-5-(1-phenoxyethyl)-2,4-dihydro-3H-1,2,4-triazol-3-thione (**IVa–e**) in which, two of them (**IVd** and **IVe**) are reported for the first time [36–38]. Treatment of equimolar quantities of these triazoles with 2-chloro-*N*-(2-thiazolyl)acetamides (**I**) in the presence of anhydrous potassium carbonate resulted in the formation of the title compounds (**Va–o**). Some characterizations of **IVa–e** and **Va–o** were given in Table 1.

3. Biology

3.1. Antimicrobial activity

Antimicrobial activities of compounds were tested using microbroth dilution method [39,40]. Tested microorganism strains were; *Candida albicans* (NRRL Y-27077), *Candida glabrata* (ATCC 36583), *C. albicans* (isolate obtained from



Scheme 1. The general synthesis reactions.

Table 1
Some characterizations of the compounds

Compounds	R_1	R_2	R_3	M.p. (°C)	Yield (%)	Molecular formula
IVa	–	H	C_6H_5	171–173	50	$C_{16}H_{15}N_3OS$
IVb	–	Cl	C_6H_5	136–138	55	$C_{16}H_{14}ClN_3OS$
IVc	–	CH_3	C_6H_5	183–185	52	$C_{17}H_{17}N_3OS$
IVd	–	H	C_6H_{11}	177–179	46	$C_{16}H_{21}N_3OS$
IVe	–	CH_3	C_6H_{11}	194–196	45	$C_{17}H_{23}N_3OS$
Va	H	H	C_6H_5	202–204	73	$C_{21}H_{19}N_5O_2S_2$
Vb	$COOC_2H_5$	H	C_6H_5	235–238	65	$C_{24}H_{23}N_5O_4S_2$
Vc	$CH_2COOC_2H_5$	H	C_6H_5	140–142	72	$C_{25}H_{25}N_5O_4S_2$
Vd	H	Cl	C_6H_5	222–224	62	$C_{21}H_{18}ClN_5O_2S_2$
Ve	$COOC_2H_5$	Cl	C_6H_5	177–179	72	$C_{24}H_{22}ClN_5O_4S_2$
Vf	$CH_2COOC_2H_5$	Cl	C_6H_5	190–191	65	$C_{25}H_{24}ClN_5O_4S_2$
Vg	H	CH_3	C_6H_5	208–210	71	$C_{22}H_{21}N_5O_2S_2$
Vh	$COOC_2H_5$	CH_3	C_6H_5	206–208	68	$C_{25}H_{25}N_5O_4S_2$
Vi	$CH_2COOC_2H_5$	CH_3	C_6H_5	175–177	63	$C_{26}H_{27}N_5O_4S_2$
Vj	H	H	C_6H_{11}	161–164	72	$C_{21}H_{25}N_5O_2S_2$
Vk	$COOC_2H_5$	H	C_6H_{11}	218–220	68	$C_{24}H_{29}N_5O_4S_2$
Vl	$CH_2COOC_2H_5$	H	C_6H_{11}	153–155	69	$C_{25}H_{31}N_5O_4S_2$
Vm	H	CH_3	C_6H_{11}	201–203	72	$C_{22}H_{27}N_5O_2S_2$
Vn	$COOC_2H_5$	CH_3	C_6H_{11}	232–234	75	$C_{25}H_{31}N_5O_4S_2$
Vo	$CH_2COOC_2H_5$	CH_3	C_6H_{11}	188–190	81	$C_{26}H_{33}N_5O_4S_2$

Faculty of Medicine, Osmangazi University), *Escherichia coli* (ATCC 10798), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 7700). Chloramphenicol and ketoconazole were used as control drugs. The observed data on the antimicrobial activity of the compounds and control drugs were given in Table 2.

4. Results, discussion and conclusion

In the present work, five triazolinethiones (**IVa–e**) (two of them original) and 15 new compounds (**Va–o**) which are thioether derivatives of **IV** were synthesized. The structures of the obtained compounds were elucidated by spectral data.

Table 2
MIC values of the compounds as μg ml^{-1}

Compounds	*C. albicans* [a]	*C. albicans* [b]	*C. glabrata*	*E. coli*	*S. aureus*	*P. aeruginosa*
IVa	16	16	8	100	200	100
IVb	2	>16	1	100	200	200
IVc	1	16	0.25	100	200	100
IVd	16	>16	8	100	200	100
IVe	0.5	>16	0.125	100	200	100
Va	16	>16	8	100	200	100
Vb	>16	>16	8	100	200	100
Vc	4	>16	4	100	200	100
Vd	8	>16	4	400	400	400
Ve	>16	>16	8	100	100	100
Vf	8	8	8	100	200	100
Vg	16	>16	16	100	200	100
Vh	8	16	16	100	200	100
Vi	16	>16	16	200	200	200
Vj	>16	>16	16	200	200	200
Vk	16	16	16	100	>400	100
Vl	16	>16	>16	100	200	100
Vm	16	>16	8	100	200	100
Vn	>16	>16	8	200	200	200
Vo	>16	>16	16	200	200	200
A	4	4	8	–	–	–
B	–		–	50	12.5	200

A: Ketoconazole, **B**: chloramphenicol.
[a] *C. albicans* (NRRL Y-27077).
[b] *C. albicans* (isolate obtained from Faculty of Medicine, Osmangazi University).

According to the IR spectroscopic data of the compounds **IVa–e** which have triazoline-3-thione structure, the observation of C=S stretching bands at 1399–1373 cm^{-1} and the absence of an absorption about in 2600–2550 cm^{-1} region cited [41] for SH group have proved that these compounds were in the thionic form. NH stretching bands of **IVa–e** were observed at 3421–3088 cm^{-1} region. The IR spectra of thioether derivatives (**Va–o**) showed characteristic C=O (amide) stretching bands in 1700–1660 cm^{-1} region. In addition, compounds which are including ester group, showed characteristic C=O (ester) bands in 1745–1715 cm^{-1} region.

In the ^{1}H-NMR spectra of compounds **IV**, NH peaks were observed as singlets at about 13.70–14.04 ppm region. Therefore, it was proved that they were found in thionic form. In thioether derivatives (**V**), the signal due to $COCH_2$ methylene protons, presented in all compounds, appeared at 4.05–4.30 ppm, as singlets. NHCO proton was observed at 12.45–12.90 ppm as a singlet or broad band. All the other aromatic and aliphatic protons were observed at expected regions.

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

The most important part of the results was those which are obtained from antifungal activity screening. Most of the compounds were effective against *C. albicans* (NRRL Y-27077). When compared with ketoconazole; especially **IVb**, **IVc**, **IVe** showed strong, **Vc** similar to reference agent, and **IVa**, **VId**, **Va**, **Vd**, **Vf**, **Vg**, **Vh**, **Vi**, **Vk**, **Vl**, **Vm** moderate activity.

Similar results obtained from *C. glabrata*. Compounds **IVb**, **IVc**, **IVe**, **Vc** and **Vd** show great, **IVa**, **VId**, **Va**, **Vb**, **Ve**, **Vf**, **Vn**, **Vm** similar and **Vg**, **Vh**, **Vi**, **Vj**, **Vk** and **Vo** moderate activity when compared with ketoconazole.

SAR observations showed that triazolethiols are more active then their thioether derivatives. Besides this, the most effective derivatives of triazolethiols were *p*-methylphenoxy derivatives.

The only resistant strain against almost all of the compounds, except **IVa**, **IVc**, **Vf**, **Vh**, **Vk**, was the clinic isolate of *C. albicans*. Among these five active compounds, the most and the only effective compound against clinic isolate of *C. albicans* was **Vf**, which includes chlorine subsistent on phenoxy moiety while other effective compounds does not have. However, it was also observed that the ester substations on the thiazole ring had no determining influence on the antifungal activity.

The result of antibacterial screening of newly prepared compounds **IVe** and **Va–o** expressed as the MIC are summarized in Table 2. The antibacterial assessment revealed that the compounds posses only a moderate or slight activity. The MIC values are generally within the range of 100–400 μg ml^{-1}, most of often between 100 and 200 μg ml^{-1} against all evaluated strains. By comparing their MIC values with chloramphenicol, the compounds were less active against *E. coli* and *S. aureus*. On the other hand the compounds exhibited comparable or better activities against *P. aeruginosa* then those of chloramphenicol.

As we consider all results obtained from antifungal and antibacterial tests together we can say that entire compounds tested are more active towards fungi then bacteria.

5. Experimental

5.1. 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.

5.1.1. General procedure for synthesis of the compounds

*5.1.1.1. 2-Chloro-*N*-(2-thiazolyl)acetamides (**I**).* Chloroacetyl chloride (20 mmol) and triethylamine (20 mmol) were added to a solution of 2-amino-4-substituted-thiazole (20 mmol) in anhydrous benzene and the mixture was treated as described in literature.

*5.1.1.2. 2-Phenoxypropionic acid hydrazides (**II**).* These compounds were prepared according to the previously reported method, by reacting ethyl 2-phenoxypropionates with hydrazine hydrate.

*5.1.1.3. 1-(2-Phenoxypropionyl)-4-phenyl/cyclohexyl-3-thiosemicarbazides (**III**).* Equimolar quantities of acid hydrazide (30 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.

*5.1.1.4. 4-Phenyl/cyclohexy-5-(1-phenoxyethyl)-2,4-dihydro-3H-1,2,4-triazol-3-thione (**IVa–e**).* 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.

*5.1.1.5. 4-Phenyl/cyclohexyl-5-(1-phenoxyethyl)-3-[*N*-(2-thiazolyl)acetamido]thio-4H-1,2,4-triazole (**Va–o**).* A mixture of the 2-chloro-*N*-(2-thiazolyl)acetamides (**I**) (10 mmol), appropriate triazoles (**IV**) and anhydrous potassium carbonate in acetone was 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.

IVa:

IR (KBr) υ_{maks} (cm^{-1}): 3099 (N–H), 1589–1408 (C=C and C=N), 1374 (C=S), 1238–1134 (C–O).

^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 1.45 (3H, d [J = 6.44 Hz], CH–CH_3), 5.30 (1H, q [J = 6.43 Hz], CH–CH_3), 6.60 (2H, d [J = 7.85 Hz], C_2, C_6 protons of O-phenyl), 6.85 (1H, t [J = 7.33 Hz], C_4 proton of O-phenyl), 7.20 (2H, d [J = 7.50 Hz], C_3, C_5 protons of O-phenyl), 7.35–7.50 (5H, m, protons of N-phenyl), 14.00 (1H, s, N–H).

MS (FAB) [M + 1]: m/z 298.

Anal. Calc. for $C_{16}H_{15}N_3OS$: C, 64.62; H, 5.08; N, 14.13. Found: C, 64.30; H, 5.20; N, 14.25.

IVb:

IR (KBr) υ_{maks} (cm^{-1}): 3260–3201 (N–H), 1598–1434 (C=C and C=N), 1373 (C=S), 1236–1170 (C–O).

^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 1.45 (3H, d [J:6.48 Hz], CH–CH_3), 5.25 (1H, q [J = 6.44 Hz], CH–CH_3), 6.75 (2H, d [J = 6.78 Hz], C_2, C_6 protons of O-phenyl), 7.10–7.45 (5H, m, protons of N-phenyl), 7.25 (2H, d [J = 6.79 Hz], C_3, C_5 protons of O-phenyl).

Anal. Calc. for $C_{16}H_{14}ClN_3OS$: C, 57.92; H, 4.25; N, 12.66. Found: C, 57.82; H, 4.30; N, 12.41.

IVc:

IR (KBr) υ_{maks} (cm^{-1}): 3421–3099 (N–H), 1610–1419 (C=C and C=N), 1382 (C=S), 1240–1080 (C–O).

^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 1.50 (3H, d [J = 6.45 Hz], CH–CH_3), 2.20 (3H, s, phenyl-CH_3), 5.30 (1H, q [J = 6.41 Hz], CH–CH_3), 6.60 (2H, d [J = 8.54 Hz], C_2, C_6 protons of O-phenyl), 7.05 (2H, d [J = 8.36 Hz], C_3, C_5 protons of O-phenyl), 7.35–7.50 (5H, m, protons of N-phenyl), 14.05 (1H, s, N–H).

MS (FAB) [M + 1]: m/z 312.

Anal. Calc. for $C_{17}H_{17}N_3OS$: C, 65.57; H, 5.50; N, 13.49. Found: C, 65.30; H, 5.30; N, 13.49.

IVd:

IR (KBr) υ_{maks} (cm^{-1}): 3088 (N–H), 1600–1446 (C=C and C=N), 1399 (C=S), 1228–1083 (C–O).

^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 0.85–1.75 (10H, m, protons of cyclohexyl), 1.55 (3H, d [J = 6.33 Hz], CH–CH_3), 4.15 (1H, br, proton of cyclohexyl), 5.75 (1H, q [J = 6.34 Hz], CH–CH_3), 6.90–7.05 (3H, m, C_2, C_4,C_6 protons of O-phenyl), 7.25–7.35 (2H, t [J = 7.44 Hz], C_3, C_5 protons of O-phenyl), 13.70 (1H, s, N–H).

MS (FAB) [M + 1]: m/z 312.

Anal. Calc. for $C_{16}H_{21}N_3OS$: C, 63.34; H, 6.98; N, 13.85. Found: C, 63.34; H, 7.18; N, 13.80.

IVe:

IR (KBr) υ_{maks} (cm^{-1}): 3240–3095 (N–H), 1614–1456 (C=C and C=N), 1375 (C=S), 1255–1072 (C–O).

Anal. Calc. for $C_{17}H_{23}N_3OS$: C, 64.32; H, 7.30; N, 13.24. Found: C, 64.32; H, 7.55; N, 13.60.

Va:

IR (KBr) υ_{maks} (cm^{-1}): 3185 (NH), 1685 (C=O), 1575–1443 (C=C and C=N), 1223–1072 (C–O).

^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 1.60 (3H, d [J = 6.46 Hz], CH–CH_3), 4.25 (2H, s, S–CH_2), 5.50 (1H, q [J = 6.46 Hz], CH–CH_3), 6.70 (2H, d [J = 7.86 Hz], C_2, C_6 protons of O-phenyl), 6.90 (1H, t [J = 7.29], C_4 proton of O-phenyl), 7.20 (2H, t [J = 8.48 Hz] C_3, C_5 protons of O-phenyl), 7.25–755 (7H, m, protons of N-phenyl and C_4, C_5 protons of thiazole), 12.45 (1H, s, N–H).

MS (FAB) [M + 1]: m/z 438.

Anal. Calc. for $C_{21}H_{19}N_5O_2S_2$: C, 57.65; H, 4.38; N, 16.01. Found: C, 57.45; H, 4.55; N, 16.20.

Vb:

IR (KBr) υ_{maks} (cm^{-1}): 3120 (NH), 1721 (ester C=O), 1664 (amide C=O), 1580–1463 (C=C and C=N), 1231–1098 (C–O).

^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 1.30 (3H, t [J = 7.10 Hz], COO–CH_2–CH_3), 1.60 (3H, d [J = 6.46 Hz], CH–CH_3), 4.20 (2H, s, S–CH_2), 4.30 (2H, q [J = 7.05 Hz], COO–CH_2–CH_3), 5.50 (1H, q [J = 6.48 Hz], CH–CH_3), 6.70 (2H, d [J = 7.86 Hz], C_2, C_6 protons of O-phenyl), 6.90 (1H, t [J = 7.35], C_4 proton of O-phenyl), 7.20 (2H, t [J = 7.45 Hz], C_3, C_5 protons of O-phenyl), 7.40–7.55 (5H, m, protons of N-phenyl), 8.10 (1H, s, C_5 protons of thiazole), 12.90 (1H, s, N–H).

MS (FAB) [M + 1]: m/z 510.

Anal. Calc. for $C_{24}H_{23}N_5O_4S_2$: C, 56.57; H, 4.55; N, 13.74. Found: C, 56.75; H, 4.50; N, 13.60.

Vc:

IR (KBr) υ_{maks} (cm^{-1}): 3163–3105 (NH), 1731 (ester C=O), 1676 (amide C=O), 1585–1454 (C=C and C=N), 1255–1080 (C–O).

Anal. Calc. for $C_{25}H_{25}N_5O_4S_2$: C, 57.34; H, 4.81; N, 13.37. Found: C, 57.34; H, 4.91; N, 13.55.

Vd:

IR (KBr) υ_{maks} (cm^{-1}): 3179 (NH), 1684 (amide C=O), 1595–1488 (C=C, C=N), 1228–1080 (C–O).

^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 1.55 (3H, d [J = 6.44 Hz], CH–CH_3), 4.20 (2H, s, S–CH_2), 5.50 (1H, q [J = 6.45 Hz], CH–CH_3), 6.70 (2H, d [J = 9.20 Hz], C_2, C_6 protons of O-phenyl), 7.15–755 (9H, m, aromatic protons), 12.45 (1H, s, N–H).

MS (FAB) [M + 1]: m/z 472.

Anal. Calc. for $C_{21}H_{18}ClN_5O_2S_2$: C, 53.55; H, 3.84; N, 14.84. Found: C, 53.35; H, 3.90; N, 14.60.

Ve:

IR (KBr) υ_{maks} (cm^{-1}): 3169 (NH), 1737 (ester C=O), 1701 (amide C=O), 1562–1461 (C=C, C=N), 1235–1091 (C–O).

^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 1.30 (3H, t [J = 7.11 Hz], COO–CH_2–CH_3), 1.55 (3H, d [J = 6.46 Hz], CH–CH_3), 4.05 (2H, s, S–CH_2), 4.20 (2H, q [J = 7.13 Hz], COO–CH_2–CH_3), 5.50 (1H, q [J = 6.49 Hz], CH–CH_3), 6.80 (2H, d [J = 9.02 Hz], C_2, C_6 protons of O-phenyl), 7.25 (2H, d [J = 8.97 Hz], C_3, C_5 protons of O-phenyl), 7.40–7.60 (5H, m, protons of N-phenyl), 7.65 (1H, s, C_5 proton of thiazole).

Anal. Calc. for $C_{24}H_{22}ClN_5O_4S_2$: C, 52.99; H, 4.08; N, 12.87. Found: C, 53.25; H, 4.20; N, 12.60.

Vf:

IR (KBr) υ_{maks} (cm^{-1}): 3164 (NH), 1735 (ester C=O), 1671 (amide C=O), 1582–1455 (C=C, C=N), 1259–1077 (C–O).

^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 1.20 (3H, t [J = 7.09 Hz], COO–CH_2–CH_3), 1.55 (3H, d [J = 6.44 Hz], CH–CH_3), 3.70 (2H, s, CH_2–COO–C_2H_5), 4.10 (2H, q

[J = 7.10 Hz], COO–CH_2–CH_3), 4.20 (2H, s, S–CH_2), 5.50 (1H, q [J = 6.46 Hz], CH–CH_3), 6.70 (2H, d [J = 8.97 Hz], C_2, C_6 protons of O-phenyl), 7.00 (1H, s, C_5 proton of thiazole), 7.25 (2H, d [J = 8.95 Hz], C_3, C_5 protons of O-phenyl), 7.35–7.55 (5H, m, protons of N-phenyl), 12.50 (1H, s, N–H).

MS (FAB) [M + 1]: m/z 558.

Anal. Calc. for $C_{25}H_{24}ClN_5O_4S_2$: C, 53.81; H, 4.33; N, 12.55. Found: C, 53.81; H, 4.43; N, 1280.

Vg:

IR (KBr) υ_{maks} (cm^{-1}): 3180 (NH), 1686 (amide C=O), 1575–1435 (C=C, C=N), 1225, 1068 (C–O).

^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 1.55 (3H, d [J = 6.46 Hz], CH–CH_3), 2.20 (3H, s, phenyl-CH_3), 4.25 (2H, s, S–CH_2), 5.45 (1H, q [J = 6.47 Hz], CH–CH_3), 6.60 (2H, d [J = 8.53 Hz], C_2, C_6 protons of O-phenyl), 7.00 (2H, d [J = 8.45 Hz], C_3, C_5 protons of O-phenyl), 7.25–760 (7H, m, aromatic protons), 12.45 (1H, s, N–H).

MS (FAB) [M + 1]: m/z 452.

Anal. Calc. for $C_{22}H_{21}N_5O_2S_2$: C, 58.52; H, 4.69; N, 15.51. Found: C, 58.65; H, 4.80; N, 15.40.

Vh:

IR (KBr) υ_{maks} (cm^{-1}): 3123 (NH), 1725 (ester C=O), 1663 (amide C=O), 1575–1463 (C=C, C=N), 1232–1096 (C–O).

^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 1.25 (3H, t [J = 7.09 Hz], COO–CH_2–CH_3), 1.50 (3H, d [J = 6.46 Hz], CH–CH_3), 2.15 (3H, s, phenyl-CH_3), 4.15 (2H, s, S–CH_2), 4.25 (2H, q [J = 7.08 Hz], COO–CH_2–CH_3), 5.40 (1H, q [J = 6.45 Hz], CH–CH_3), 6.55 (2H, d [J = 8.54 Hz], C_2, C_6 protons of O-phenyl), 6.90 (2H, d [J = 8.43 Hz], C_3, C_5 protons of O-phenyl), 7.30–7.50 (5H, m, protons of N-phenyl), 8.05 (1H, s, C_5 proton of thiazole), 12.70 (1H, s, N–H).

MS (FAB) [M + 1]: m/z 524.

Anal. Calc. for $C_{25}H_{25}N_5O_4S_2$: C, 57.34; H, 4.81; N, 13.37. Found: C, 57.65; H, 4.70; N, 13.40.

Vi:

IR (KBr) υ_{maks} (cm^{-1}): 3164, 3105 (NH), 1745 (ester C=O), 1668 (amide C=O), 1580–1450 (C=C, C=N), 1255–1081 (C–O).

^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 1.15 (3H, t [J = 7.09 Hz], COO–CH_2–CH_3), 1.50 (3H, d [J = 6.46 Hz], CH–CH_3), 2.15 (3H, s, phenyl-CH_3), 3.65 (2H, s, CH_2–COO–C_2H_5), 4.05 (2H, q [J = 7.11 Hz], COO–CH_2–CH_3), 4.15 (2H, s, S–CH_2), 5.35 (1H, q [J = 6.47 Hz], CH–CH_3), 6.55 (2H, d [J = 8.51 Hz], C_2, C_6 protons of O-phenyl), 6.90–7.00 (3H, m, aromatic protons), 7.30–7.55 (5H, m, aromatic protons), 12.45 (1H, s, N–H).

MS (FAB) [M + 1]: m/z 538.

Anal. Calc. for $C_{26}H_{27}N_5O_4S_2$: C, 58.08; H, 5.06; N, 13.03. Found: C, 58.18; H, 5.20; N, 13.32.

Vj:

IR (KBr) υ_{maks} (cm^{-1}): 3165 (NH), 1682 (amid C=O), 1585–1447 (C=C, C=N), 1218–1072 (C–O).

^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 0.95–1.35 (3H, m, protons of cyclohexyl), 1.50–2.10 (7H, m, protons of cyclohexyl), 1.70 (3H, d (J = 6.38 Hz), CH–CH_3), 4.05–4.20 (1H, m, C_1 proton of cyclohexyl), 4.30 (2H, s, S–CH_2), 5.90 (1H, q [J = 6.50 Hz], CH–CH_3), 6.90–7.55 (7H, m, aromatic protons), 12.45 (1H, s, N–H).

MS (FAB) [M + 1]: m/z 444.

Anal. Calc. for $C_{21}H_{25}N_5O_2S_2$: C, 56.86; H, 5.68; N, 15.79. Found: C, 56.66; H, 5.70; N, 15.30.

Vk:

IR (KBr) υ_{maks} (cm^{-1}): 3122 (NH), 1723 (ester C=O), 1663 (amide C=O), 1579–1460 (C=C, C=N), 1230–1097 (C–O).

^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 0.90–1.20 (3H, m, protons of cyclohexyl), 1.25 (3H, t [J = 7.11 Hz], COO–CH_2–CH_3), 1.50–2.05 (7H, m, protons of cyclohexyl), 1.65 (3H, d [J = 6.41 Hz], CH–CH_3), 4.00–4.15 (1H, m, C_1 proton of cyclohexyl), 4.20–4.30 (4H, m, S–CH_2 ve COO–CH_2–CH_3), 5.85 (1H, q [J = 6.46 Hz], CH–CH_3), 6.90–7.00 (3H, m, C_2, C_4, C_6 protons of O-phenyl), 7.25 (2H, t [J = 7.52 Hz], C_3, C_5 protons of O-phenyl), 8.05 (1H, s, C_5 protons of thiazole), 12.85 (1H, s, N–H).

MS (FAB) [M + 1]: m/z 516.

Anal. Calc. for $C_{24}H_{29}N_5O_4S_2$: C, 55.90; H, 5.67; N, 13.58. Found: C, 55.90; H, 5.60; N, 13.58.

Vl:

IR (KBr) υ_{maks} (cm^{-1}): 3166 (NH), 1736 (ester C=O), 1672 (amid C=O), 1576–1449 (C=C, C=N), 1223–1086 (C–O).

^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 0.90–1.30 (3H, m, protons of cyclohexyl), 1.15 (3H, t [J = 7.14 Hz], COO–CH_2–CH_3), 1.45–1.80 (5H, m, protons of cyclohexyl), 1.65 (3H, d [J = 6.41 Hz], CH–CH_3), 1.90–2.05 (2H, m, protons of cyclohexyl), 3.60 (2H, s, CH_2–COO–C_2H_5), 3.95–4.10 (3H, m, COO–CH_2–CH_3 and proton of cyclohexyl), 4.15 (2H, s, S–CH_2), 5.85 (1H, q [J = 6.91 Hz], CH–CH_3), 6.70 (1H, s, C_5 protons of thiazole), 6.90–7.05 (3H, m, C_2, C_4, C_6 protons of O-phenyl), 7.30 (2H, t [J = 7.35 Hz], C_3, C_5 protons of O-phenyl).

MS (FAB) [M + 1]: m/z 530.

Anal. Calc. for $C_{25}H_{31}N_5O_4S_2$: C, 56.69; H, 5.90; N, 13.22. Found: C, 56.60; H, 5.95; N, 13.20.

Vm:

IR (KBr) υ_{maks} (cm^{-1}): 3166 (NH), 1671 (amide C=O), 1574–1454 (C=C, C=N), 1225–1080 (C–O).

^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 1.00–1.30 (3H, m, protons of cyclohexyl), 1.60–1.85 (5H, m, protons of cyclohexyl), 1.65 (3H, d [J = 6.41 Hz], CH–CH_3), 1.90–2.05 (2H, m, protons of cyclohexyl), 2.20 (3H, s, phenyl-CH_3), 4.05–4.25 (1H, m, C_1 proton of cyclohexyl), 4.30 (2H, s, S–CH_2), 5.80 (1H, q [J = 6.51 Hz], CH–CH_3), 6.90 (2H, d [J = 8.57 Hz], C_2, C_6 protons of O-phenyl), 7.05 (2H, d [J = 8.39 Hz], C_3, C_5 protons of O-phenyl), 7.25 (1H, d [J = 3.53 Hz], C_4 proton of thiazole), 7.50 (1H, d [J = 3.54 Hz], C_5 proton of thiazole), 12.45 (1H, s, N–H).

MS (FAB) [M + 1]: m/z 458.

Anal. Calc. for $C_{22}H_{27}N_5O_2S_2$: C, 57.74; H, 5.95; N, 15.30. Found: C, 57.64; H, 6.20; N, 15.37.

Vn:

IR (KBr) υ_{maks} (cm^{-1}): 3132 (NH), 1715 (ester C=O), 1679 (amide C=O), 1578–1451 (C=C, C=N), 1226–1075 (C–O).

^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 1.00–1.20 (3H, m, protons of cyclohexyl), 1.25 (3H, t [J = 7.11 Hz], COO–

CH_2–*CH_3*), 1.55–1.70 (5H, m, protons of cyclohexyl), 1.60 (3H, d [J = 6.44 Hz], CH–*CH_3*), 1.85–2.05 (2H, m, protons of cyclohexyl), 2.20 (3H, s, phenyl-CH_3), 4.05–4.15 (1H, m, C_1 proton of cyclohexyl), 4.20–4.30 (4H, m, S–CH_2 ve COO–*CH_2*–CH_3), 5.80 (1H, q [J = 6.51 Hz], *CH*–CH_3), 6.85 (2H, d [J = 8.59 Hz], C_2, C_6 protons of O-phenyl), 7.10 (2H, d [J = 8.38 Hz], C_3, C_5 protons of O-phenyl), 8.05 (1H, s, C_5 proton of thiazole), 12.85 (1H, s, N–H).

MS (FAB) [M + 1]: *m/z* 530.

Anal. Calc. for $C_{25}H_{31}N_5O_4S_2$: C, 56.69; H, 5.90; N, 13.22. Found: C, 56.80; H, 6.25; N, 13.55.

Vo:

IR (KBr) υ_{maks} (cm^{-1}): 3158 (NH), 1741 (ester C=O), 1673 (amide C=O), 1571–1451 (C=C, C=N), 1228–1074 (C–O).

^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 1.00–1.10 (3H, m, protons of cyclohexyl), 1.15 (3H, t [J = 7.11 Hz], COO–CH_2–*CH_3*), 1.50–1.80 (5H, m, protons of cyclohexyl), 1.65 (3H, d [J = 6.28 Hz], CH–*CH_3*), 1.85–2.10 (2H, m, protons of cyclohexyl), 2.20 (3H, s, phenyl-CH_3), 3.65 (2H, s, *CH_2*–COO–C_2H_5), 4.10 (1H, q [J = 7.10 Hz], COO–*CH_2*–CH_3), 4.10–4.20 (1H, m, C_1 proton of cyclohexyl), 4.25 (2H, s, S–CH_2), 5.80 (1H, q [J = 6.49 Hz], *CH*–CH_3), 6.85 (2H, d [J = 8.54 Hz], C_2, C_6 protons of O-phenyl), 7.00 (1H, s, C_5 proton of thiazole), 7.05 (2H, d [J = 8.47 Hz], C_3, C_5 protons of O-phenyl), 12.45 (1H, s, N–H).

Anal. Calc. for $C_{26}H_{33}N_5O_4S_2$: C, 57.44; H, 6.12; N, 12.88. Found: C, 57.68; H, 6.53; N, 12.71.

5.2. Biology

5.2.1. Antimicrobial activity

Microdilution broth susceptibility assay was used for the antibacterial evaluation of the compounds [39], whereas antifungal susceptibility of the fungus yeasts were examined according to NCCLS reference method for broth dilution antifungal susceptibility testing of yeasts [40]. Chloramphenicol was used as standard antibacterial agent and ketoconazole was used as antifungal agent. And both are prepared as described in the related references.

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