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Synthesis, characterization and antimicrobial activity of new 2,5-disubstituted-1,3,4-thiadiazole derivatives

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ABSTRACT

In this study, new derivatives of 2,5-dimercapto- 1,3,4-thiadiazole were synthesized by cyclization and coupling reactions, in a satisfactory yield. The reaction of 2,5-dimercapto-1,3,4-thiadiazole with ethyl bromoacetate in absolute ethanol afforded the ester derivative (1), which was treated with hydrazine hydrate to give the corresponding carbohydrazide (2). The carbohydrazide (2) was reacted with two and four equivalents of methyl acetoacetate, and afforded the corresponding methyl pyrazolone (3) and pyranopyrazole (8), derivatives, respectively. Furthermore, the carbohydrazide (2) reacted with acetyl acetone and gave dimethyl pyrazole derivative (4). Refluxing of carbohydrazide (2) with anhydrides; like succinic, maleic and phthalic anhydrides, afforded the compounds (5), (6) and (7) respectively. Treatment of carbohydrazide (2) with tetrahydrofuran in glacial acetic acid gave the compound (9). The reaction of carbohydrazide (2) with phenyl isothiocyanate, furnished compound (10), which is successfully cyclized upon addition of 5% NaOH under reflux, to give the corresponding mercapto triazole derivative (11). Also, a series of amino acid methyl esters (12a-c) and dipeptides (14a-c) attached to the heterocyclic ring were successfully synthesized, starting from amino acid esters and azides (2a,13a), respectively. All the synthesized compounds were screened for their *in vitro* antimicrobial activities against six pathogenic bacterial strains, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and three fungal strains, *Aspergillus niger, Aspergillus terreus and Candida albicans,* by well diffusion method.These compounds showed moderate antibacterial activities, and both compounds (9 and 14c) exhibited the most potent antifungal activities against *Aspergillus terreus*. All the synthesized compounds were in good agreement with elemental and spectral data (FT-IR,^{1}HNMR spectroscopy).

Keywords: 2,5-dimercapto-1,3,4-thiadiazole derivatives, amino acids, antibacterial, antifungal,

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INTRODUCTION

One of the main objectives of organic and medicinal chemistry is the design, synthesis and production of compounds having considerable value as human, therapeutic agents. Thiadiazole is an important scaffold known to be associated with several biological activities. The sulfur atom of thiadiazole imparts improved liposolubility, and the mesoionic nature of thiadiazoles makes these compounds better to cross cellular membranes[1]. The synthesis of new derivatives containing 1,3,4- thiadiazoles has attracted widespread attention due to diverse applications as antibacterial [2-4],antifungal [2,5], antitubercular [2,6,7], antiviral [2,8],antioxiant[3.9],antitumoral [3,10],anti-inflammatory [2,11,12],and anticonvulsant[2,13], etc.Therefore, the present study was undertaken to synthesize new compounds having 2,5-dimercapto thiadiazole ring attached to different moieties, (3-14c).All the new compounds were characterized by elemental and spectral analysis and screened for their *in vitro*, antibacterial and antifungal activities.

MATERIALS AND METHODS

Experimental

All the newly synthesized compounds gave moderate yields. The homogeneity of the synthesized compounds was ascertained by thin layer chromatography (TLC) on silica gel G (Merck) coated plates by using the different solvent system.Iodine chamber and UV lamps were used for visualization of (TLC) spots. The chemicals and solvents were purchased from Fluka, BDH, and Thomas Baker companies. Melting points were determined on Thomas Hoover electric melting points apparatus and are uncorrected. FT-IR spectra (KBr) were recorded on Shimadzu FT-IR-8400S spectrophotometer and ^{1}HNMR spectra measured with 400MHz, Avance III 400-Bruker, using tetramethylsilane (TMS) as an internal standard.The chemical shifts are expressed in ppm δ scale. The percentage of carbon, hydrogen and nitrogen were obtained using a CHNS analyzer (Euro EA3000 elemental analyzer).

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General Methods

The target compounds were synthesized by the following steps.

Synthesis of diethyl 2,2' [(1,3,4-thiadiazole-2,5-diyl) bis (sulfanediyl) acetate (1) [14]

To a solution of 2,5-dimercapto-1,3,4-thiadiazole (0.1 mol,15 g) in (20 ml) of absolute ethanol, (0.2 mol,12 g) of potassium hydroxide, (KOH) was added.The solution was stirred for 30 min., and then ethyl bromoacetate (0.2 mol, 25 ml) was added dropwise to the solution.The reaction mixture was refluxed for 4-5 hrs., Then cooled to room temperature, poured into (100 ml) of ice water.The precipitate was filtered off, washed with water and recrystallized from ethanol to get white fluffy powder. .Yield 79%; m.p. 47-48 °C; **IR (υ cm^{-1}, KBr)**: 2988 ($\underline{CH_2}$-aliph. str) ,1742 ($\underline{C=O}$ str of ester); **^{1}HNMR (400MHz, DMSO-d6, δ ppm)**: 4.22(s,4H,2CH_2,S-$\underline{CH_2}$-C=O), 4.16-4.09 (q,4H,2$\underline{CH_2}$),1.21-1.16 (t, 4H, $\underline{2CH_2}$), 1.21-1.16 (t,6H,2$\underline{CH_3}$), and singlet signal at 2.5 and 3.34 ppm due to the solvent DMSO-d_6 and water dissolved in DMSO-d_6 , respectively [19].

Synthesis of 2, 2'-[(1,3,4-thiadiazole-2,5-diyl)bis(sulfanediyl)]di(acetohydrazide) (2) [19]

A Suspension of compound (2) (0.02 mol, 6.4 g) in (20 ml) of absolute ethanol was stirred for 15 min., at 40 °C until the ester was dissolved , (0.04 mol, 2.2ml) of hydrazine hydrate (80 %) was added and the solution was stirred for 30 min., and refluxed for 4 hrs, cooled, filtered, washed and recrystallized from ethanol (90%) to get white crystals. Yield 74%; m.p.140-142 °C; **IR (υ cm^{-1}, KBr)**: 3319 ($\underline{NH}$ str), 3293,3270 ($\underline{NH_2}$ str), 1694 ($\underline{C=O}$ str).

Synthesis of 2,2'(2,2'-[(1,3,4-thiadiazole-2,5-diyl)bis(sulfaneddiyl)]bis(acetyl)]bis (5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (3) [15]

(0.002mol,0.5 g) of compound (2) was stirred for 30 min., in absolute ethanol (50 ml), then (0.004 mol ,0.5 g) methyl acetoacetate was added, then refluxed for 8 hrs., cool and filtrate the residue which was recrystallized from chloroform to get off white powder. Yield 70%; m.p. 144-146 °C; **IR (υ cm^{-1}, KBr)**: 3199 ($\underline{NH}$ str.) and disappearance of NH_2 band, 1732 ($\underline{C=O}$ str of pyrazolne), 1684 ($\underline{C=O}$ str of acyclic amide); **^{1}HNMR(400MHz, DMSO-d_6 , δ ppm)**: 4.43(s,4H,2$\underline{CH_2}$-pyrazoline ring), 4.06 (s, S-$\underline{CH_2}$), 2.14(s, 2$\underline{CH_3}$); Anal.Calcd. for $C_{14}H_{14}N_6O_4S_3$: C, 39.43; H, 3.31; N,19.71; S, 22.55. Found: C, 38.97; H, 3,52; N, 19.24; S, 22.07

Synthesis of 2,2' [(1,3,4-thiadiazole-2,5-diyl)bis (sulfanediyl)]bis(1-(3,5-dimethyl-1*H*-pyrazol-1-yl)ethan-1-one (4) [16]

(0.002 mol, 0.5 g) of compound (2) was stirred for 30 min., in absolute ethanol (50 ml) then (0.004 mol, 0.4 g) acetylacetone was added, and refluxed for 6 hrs., the residue was collected, after concentration and cooling, the solid product formed was filtered off and recrystallized from benzene, to get white fluffy powder. Yield 50% ; m.p.49-51; **IR (υ cm^{-1}, KBr)**: 2988,2937 ($\underline{CH_2}$ aliph. str), 2955 ($\underline{CH_3}$-aliph.str), disappearance of NH_2 and NH-bands, 1728 ($\underline{C=O}$ str of amide); **^{1}HNMR (400MHz, DMSO-d_6 , δ ppm)**: 2.35, 2.15(2s,12H, 4$\underline{CH_3}$- 3and 5-di-methyl pyrazole ring), 4.22 (s,2H,S-$\underline{CH_2}$); Anal. calcd. for $C_{16}H_{18}N_6O_2S_3$: C, 45.48; H, 4.29;N, 19.89; S, 22.67 . Found : C,44.91; H,4.03; N,19.03; S,22.81

Synthesis of compounds (5), (6) and (7) [17]

(0.002 mol, 0.5 g) of compound (2) stirred for 20 min., in glacial acetic acid then (0.04 mol), (0.34g) succinic anhydride ,(0.33g) maleic anhydride, (0.5g) phthalic anhydride, added and refluxed for 8hrs., then cooled, filtered, and recrystallized from ethyl acetate: DMF, to get the desired product:

Compound (5):White crystals. Yield 65% ; m.p. 232-235°C; IR (υ cm^{-1}, KBr): 3210 (*sec*.$\underline{NH}$ str.), 2986, 2938 ($\underline{CH_2}$ aliph. str), 1691 (sym. and asym str cyclic $\underline{C=O}$ amide); ^{1}HNMR(400MHz, DMSO-d_6 , δ ppm): 9.5(**s,** 2H**,** *sec*. 2$\underline{NH}$ of amide), 4.08(m, S-$\underline{CH_2}$-C=O), 2.45(t,4H,2$\underline{CH_2}$-succinic hydrazide), 2.36 (t, 4H, 2$\underline{CH_2}$-succinic hydrazide); Anal. Calcd for $C_{14}H_{14}N_6O_6S_3$: C, 36.67; H,3.08; N,18.33; S,20.98. Found: C,37.97; H,3.20; N,19.05; S, 20.22 .

Compound (6): Brown crystals. Yield 57% ; m.p.265-267 °C; **IR (υ cm^{-1}, KBr)**: 3211(*sec*.NH str of amide) and absence of (NH_2 str) band, 1785, 1735 (sym. and asym. cyclic C=O str), 1690 (acyclic C=O str of amide); Anal. Calcd. for $C_{14}H_{10}N_6O_6S_3$: C,37.00; H,2.22; N, 18.49; S,21.16. Found:C,36.10; H,2.31; N, 19.08; S, 22.01 .

Compound (7): Off white crystals; Yield 72%; m.p.260-263 °C; **IR (υ cm^{-1}, KBr)**: 3211 (NH str of amide) and absence of (NH_2) band, 3101(aromatic CH-str),1734,1794 (sym and asym. cyclic C=O str); **^{1}HNMR (400MHz, DMSO-d$_6$, δ ppm)**: 7.99-7.93 (m,8H, aromatic protons), 4.11(m, S-CH_2-C=O); Anal.Calcd. for $C_{22}H_{14}N_6O_6S_3$: C, 47.65; H, 2.45; N, 15.15; S, 17.34. Found:C,47.63; H, 2.60; N, 15.85; S, 17.31

Synthesis of 1,1'-(2,2'-[(1,3,4 thiadiazole-2,5-diyl) bis (sulfanediyl) bis (acetyl)] bis (3,4-dimethyl pyrano [2,3-c] pyrazol-6 (1*H*)-one) (8) [18]

(0.002mol , 0.5 g) of compound (2) and (0.008 mol,1 g) of methyl acetoacetate in pyrex test tube, heated in oil bath in 180-185°C for 45 min., with gentle mixing by glass rod then cooled and adding diethyl ether,filtration the brown residue which is washed by dilute HCl (0.1N), reddish brown crystals are obtained, and recrystallized from acetone. Yield 45%; m.p. 230-232 °C; **IR (υ cm^{-1}, KBr)**:

Disappearance of NH_2 bands, 3072,3028 (aromatic CH str), 2845 (sym.CH_3 str),1730 (C=O cyclic ester), 1701 (acyclic C=O amide); **^{1}HNMR (400MHz, DMSO-d$_6$, δ ppm)**: 5.82(s,2H,2CH-pyran ring), 4.24 (s,4H, 2S-CH_2); Anal. Calcd. for $C_{22}H_{18}N_6O_6S_3$: C, 47.30; H, 3.25; N,15.05; S,17.22. Found: C, 48.72; H,3.23; N, 15.53; S, 18.02 .

Synthesis of 2,2'-[(1,3,4-thiadiazole-2,5-diyl) bis (sulfanediyl)]bis (N-(pyrrolidine-1-yl) acetamide (9) [19]

A mixture of compound (2) (0.02 mol, 0.5 g) and tetrahydrofuran (0.04 mol, 0.32g) in glacial acetic acid (15 ml) were refluxed for 8 hrs., the solvent was reduced to one third of its volume under reduced pressure and then cooled. The solid separated on cooling, and was recrystallized from benzene to give the desired product. White crystals. Yield 63%; m.p. 219-221°C; **IR (υ cm^{-1}, KBr)**: 3199 (NH-str), 2920 (CH_2 aliph. str),1674 (C=O amide), 1610 (C=N); **^{1}HNMR (400MHz, DMSO-d6,δ ppm)**: 4.01 (d,4H,2S-CH_2), 2.51 (t, 4H, 2CH_2-pyrrolidine), 1.92 (t,4H, 2CH_2-pyrrolidine) ; Anal. Calcd. for $C_{14}H_{22}N_6O_2S_3$: C, 41.77; H, 5.51; N, 20.88; S, 23.90 . Found: C, 40.67; H,5.29; N, 21.3; S, 23.81 .

Synthesis of 2,2'-(2,2'-[(1,3,4-thiadiazole-2,5-diyl) bis (sulfanediyl)] bis (acetyl))
Bis (N-phenylhydrazine-1-carbothioamide) (10)[20]

(0.004mol, 1g) of compound (2) was stirred for 30 min., in absolute ethanol (30ml) to dissolve it, then (0.008mol, 0.8 g) of phenyl isothiocyanate was added, the mixture was refluxed for 8 hrs., cooled , filtered and recrystallized from ethanol/water (75:25), to get white crystals. Yield 80%; m.p.180-182 °C; **IR (υ cm^{-1}, KBr)**: 3188 (NH str of amide), 2994,2987 (sym and asym. CH aliph.str),1685 (C=O str of amide), 1252 (C=S str); **^{1}HNMR (400MHz, DMSO-d$_6$, δ ppm)**: 10.42 (s,4NH,4H, S=C-NH-Ph) and (S=C-NH-NH), 9.76 (s,2H, 2NH, *sec*. amide NH-C=O), 7.44 -7.17 (m,10H-aromatic protons), 4.13 (s, S-CH_2-C=O); Anal. Calcd. for $C_{20}H_{20}N_8O_2S_5$: C, 42.54; H, 3.57; N, 19.84; S, 28.39. Found: C,43.87; H,3.86; N,19.03; S, 27.61 .

Synthesis of 5,5'-(((1,3,4-thiadiazole-2,5- diyl)bis(sulfanediyl))bis(methylene))bis (4-phenyl-4*H*-1,2,4-triazole-3-thiol) (11) [21]

A mixture of (0.0015 mol, 0.85 g) of compound (10) in (30 ml) of 2M (NaOH) was refluxed for 6 hrs., the solution cooled and acidified by 1N (HCl), the precipitate was filtered , recrystallized from ethyl acetate, to afford white powder. Yield 51%; m.p. 226-229 °C; **IR (υ cm^{-1}, KBr)**: 3119 (NH str.),2920 (CH_2 aliph. str),1610 (C=N str); **^{1}HNMR (400MHz, DMSO-d$_6$, δ ppm)**:13.95(s, 2H,2NH-thione -tatuomerism), 13.08 (s,2H, 2SH) , 7.65-7.26 (m,10H- aromatic protons), 4.2 (s,S-CH_2); Anal. calcd. for $C_{20}H_{16}N_8S_5$: C, 45.44; H, 3.05; N, 21.19; S, 30.32. Found: C,46.30; H 3.11; N,20.56; S,29.90 .

Procedure for azide method: synthesis of amino acid derivatives attached to 2,5-dimercapto-1,3,4-thiadiazole (12a-c) [22]

To a cold solution 0°C of (0.002 mol, 0.5 g) compound (2) in acetic acid (6 ml), 1N HCl (3 ml), and water (20 ml) was added to a solution of $NaNO_2$ (0.006mol, 0.36 g) in cold water (10 ml). The reaction mixture was stirred at 0 °C for 15 min. The yellow syrup formed, was extracted with cold ethyl acetate (30 ml), washed with cold 5% $NaHCO_3$, water, and finally dried over anhydrous (Na_2SO_4). The extract was kept below 5 °C for 24 hr. To this solution (0.004) mol of the amino acid ester, phenylalanine (0.716 g) , leucine (0.636 g) and tyrosine (0.78 g), was stirred with triethylamine (2ml) in ethyl acetate (20ml) for 20 min., then filtered.

The residue was added to the azide (2a), extracted previously with ethyl acetate, and stirred for 24hrs. at 0 °C then other 24 hrs. at room temperature.

The solvent evaporated, and the residue washed with (0.1N) HCl then by acetone, and recrystallized from a suitable solvent, to afford the desired products (**12a-c).**

Compound (12a): White powder, recrystallized from petroleum ether/ethyl acetate. Yield42%; m.p.14-116 °C; **IR (υ cm^{-1}, KBr)**: 3306 (*sec*.NH-str of amide)**,** 3030 (aromatic CH str), 1751(C=O str of ester), 1651(C=O str of amide),1537 (NH-bend.amide); **^{1}HNMR (400MHz, DMSO-d$_6$, δ ppm)**: 8.78 (d,2H,2NH, amide bond), 7.29-7.19 (m, 10H-aromatic protons), 4.52(m, *α*-CH-amino acid),4.02 (d, 2x S-CH$_2$-C=O), 3.61(s,6H, 2x O-CH$_3$); Anal.calcd. for $C_{26}H_{28}N_4O_6S_3$: C, 53.05; H, 4.79; N, 9.52; S, 16.34. Found: C, 54.52; H, 4.63; N, 9.85; S, 16.87.

Compound (12b): Yellow powder, recrystallized from acetone. Yield 60%; m.p.54-56°C; **IR (υ cm^{-1}, KBr)**: 3330,3261 (NH str of amide), 3086 (aromatic CH str), 2985,2930 (sym and asym aliph.CH$_2$ str), 2873 (CH str)**,**1755 (C=O str of ester), 1676 (C=O str of amide); **^{1}HNMR (400MHz, DMSO-d$_6$, δ ppm)**: 8.69 (d,2H,2NH-*sec*.amide), 4.32-4.23 (m, *α*-CH-amino acid), 4.06 (2d,4H,2S-CH$_2$-C=O), 3.62 (s,6H, 2xOCH$_3$), 0.89-0.83 (2d,12H,4CH$_3$ leu.); Anal.calcd. for $C_{20}H_{32}N_4O_6S_3$: C, 46.14; H, 6.19; N, 10.76; S, 18.47. Found: C, 46.30; H;5.96; N, 10.38; S, 18.90 .

Compound (12c): White powder, recrystallized from ethyl acetate/acetone. Yield 39% ; m.p. 164- 165 °C; **IR (υ cm^{-1},KBr)**: 3454 (phenolic OH str), 3327, 3288(*sec*. NH str of amide),3061 , 3037 (aromatic CH str), 2964,2929 (sym and assy. aliph.CH$_2$ str), 1728 (C=O str of ester), 1645 (C=O str of amide); **^{1}HNMR (400MHz, DMSO-d$_6$, δ ppm)**: 8.72 (d, 2H,2NH, *sec*.amide), 6.99 (d,4H, 2-*ortho* aromatic protons to OH gr.), 6.67 (d,4H, 2-*meta* aromatic protons to OH gr.), 4.5 (m, *α*-CH-amino acid), 4.42 (m,4H,2S-CH$_2$-C=O), 3.59 (s, 6H,2xO-CH$_3$) , 3.22 , 2.98 t, (CH$_2$- attached to phenyl ring); Anal. Calcd. for $C_{26}H_{28}N_4O_8S_3$: C, 50.30; H, 4.55; N, 9.03; S, 15.50. Found:C, 51.63; H, 4.61; N, 9.32 ; S, 16.03.

Synthesis of 2,2' [(1,3,4-thiadiazole-2,5-diyl)bid(sulfanediyl)]bis(N-(1-hydrazinyl-3-(4-hydroxyphenyl)-1-oxopropane-2-yl)acetamide (13) [23]

(0,0016 mol,1g) of compound (12c) was stirred in absolute ethanol for 30 min., at (50 °C), (1ml) of hydrazine hydrate (99%) was added slowly and the reaction mixture refluxed for 8 hrs., then stirred overnight, filtered ,the residue (white crystals), recrystallized from ethanol 70%. Yield; 66%; m.p. 225-227 °C; **IR (υ cm^{-1}, KBr)**: 3321 (phenolic OH str), 3292,3269 (NH$_2$ str), 3200 (NH str of amide), 3086 (aromatic CH str) , disappearance of (C=O ester) band at 1755 cm^{-1} , 1689, 1633 (C=O str of amides).

Procedure for azide method: synthesis of peptide derivatives (14a-c) [22]

To (0.0008 mol, 0.5 g) of compound (13) was stirred in acetic acid (6 ml), 1N HCl (3 ml), and water (20 ml) was added to a solution of $NaNO_2$ (0.006mol,0.36 g) in cold water, (10 ml). The reaction mixture was stirred at 0 °C for 15 min. The yellow syrup formed was extracted with cold ethyl acetate (30 ml), washed with cold 5% $NaHCO_3$, H_2O and finally dried over anhydrous (Na_2SO_4), the extract was kept at 0 °C. To this solution amino acid ester, (0.0016 mol), leucine (0.36 g), alanine (0.29 g), glycine (0.2 g) was stirred with (2ml) triethylamine in (20ml) ethyl acetate for 20 min. The solution continues worked up as previously mentioned in the synthesis of (12a-c), to get the desired products.

Compound (14a): Brown crystals , recystallized from ethyl acetate. Yeild 49%; m.p. 90-92 °C; IR **(υ cm^{-1}, KBr)**: 3340 (phenolic OH str), 3277 (*sec*. NH str of amide) , 3086 (aromatic CH str.), 2850 (CH str), 1734 (C=O str of ester), 1645 (C=O str of amide); **^{1}HNMR (400MHz, DMSO-d$_6$, δ ppm)**: 9.18 (s, OH-tyr.), 8.48-8.40 (2d, 4NH, for the peptide bond), 4.48-4.28 (m, *α*-CH-amino acid), 7.01(d, 4H, 2-*ortho*-aromatic protons to OH gr.)**,** 6.63 (d,4H,2-*meta* aromatic protons to OH gr**.)**; Anal. Calcd. for $C_{38}H_{50}N_6O_{10}S_3$: C, 53.88; H, 5.95; N, 9.92; S, 11.35.Found;C, 54.45; H, 5.84; N, 10.20**;** S, 10.99 .

Compund (14 b): Brown crystals, recrystallized fron ethyl acetate /acetone (60/40).Yeild 30%; m.p. 125-127°C; **IR (υ cm^{-1}, KBr)**: **)** 3419 (phenolic OH str.), 3255 (*sec*.NH str of amides), 3043 (aromatic CH str), 2958 ,2921 (sym and asym. aliphaic CH$_2$ str), 1734 (C=O str of ester), 1637 (C=O str. of amide): **^{1}HNMR (400MHz, DMSO-d$_6$, δ ppm)**: 9.18 (s, 2H, 2OH-tyr), 7.8-7.4 (2d,4NH, for the peptide bond), 7.02 (d,4H,2-*ortho*- aromatic protons to OH gr.), 6.64 (d,4H,2*meta*-aromatic protons to OH gr.), 4.5-4.28 (m, *α*-CH-amino acid), 4.02 (d,4H, 2 S-CH$_2$-C=O), 3.62 (s,6H, 2xO-CH$_3$); Anal. calcd**.** for $C_{32}H_{38}N_6O_9S_3$: C, 50.38; H, 5.02; N, 11.02; S, 12.61. Found; C, 51.95; H, 4.89 ; N, 11.30; S, 12.20.

Compound (14 c): Dark brown crystals, recrystallized from acetone. Yeild 35%; m.p. 78-81°C ; **IR (υ cm^{-1}, KBr)**: 3419.0 (phenolic OH str), 3301,3275 (*sec*. NH str of amides), 3057 (aromatic CH str), 2956 ,2929 (sym and asym. aliph. CH$_2$ str), 1712 (C=O str of ester), 1649 (C=O str of amide); **^{1}HNMR (400MHz, DMSO-d$_6$,δ ppm)**: 9.19 (s,2H,2OH-tyr) 8.57-8.52 (2t,4H,4NH, for the peptide bond), 7.02 (d,4H,2- *ortho*- aromatic protons to OH gr.), 6.64

(d,4H,2- *meta*- aromatic protons to OH gr.), 4.02 (d,4H,2 S-CH_2- C=O), 3.63 (s,6H, 2xO-CH**3**); Anal. calcd. for $C_{30}H_{34}N_6O_{10}S_3$: C, 49.04; H, 4.66; N, 11.44; S, 13.09. Found: C, 47.95; H, 4.98; N, 11.82; S,12.78 .

Biological screening. Antimicrobial activity test.

The antimicrobial activity of the synthesized compounds was done *in vitro*, by using well diffusion method [27, 28].All the compounds were screened for their *in vitro* antibacterial activity *against Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumonia* at a concentration of 50 and 100 µ/ml. Netilmicin was used as a reference drug at 30 µg/ml concentration. Similarly, *in vitro* antifungal activity of synthesized compounds was determined by well diffusion method against *Aspergillus niger, Aspergillus terreus,* and *Candida albicans* at concentration of 250µg/ ml in potato dextrose agar (PDA) media. Fluconazole was used as a standard drug at a concentration of 75µg/ml. The freshly prepared bacterial cells and fungal spores were spread onto the Muller-Hinton agar plates and (PDA) medium in laminar air flow chamber, then were cultured and incubated at 37 °C for 24hr., for the bacteria and at 30 °C for 72 hr. for the fungi species. The test compounds that were previously dissolved in DMSO, the zone of inhibition was measured in (mm), after incubation of plates 24 hr. for the antibacterial and 72 hr. for the antifungal and the results are demonstrated in **(Table 1 and 2)**.

RESULTS AND DISCUSSION

CHEMISTRY

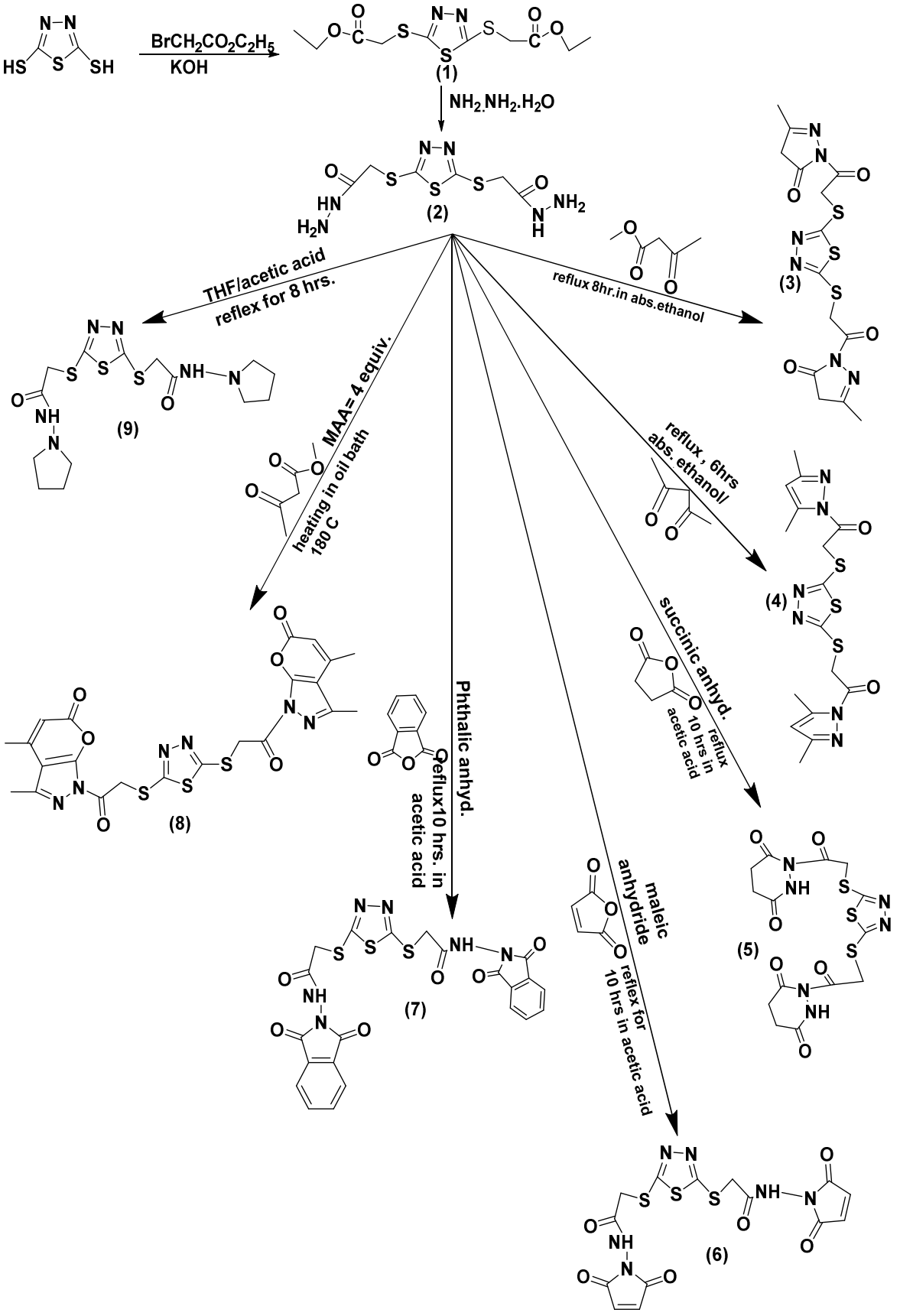
The synthesis of compounds, **(3-9), (10-12c)** and **(13-14c)** was accomplished and outlined in **scheme: 1**, **2** and **3,** respectively. It involves the reaction of 2,5-dimercapto-1,3,4-thiadiazole with ethyl bromoacetate in the presence of a base to form ethyl ester derivative **(**1), then refluxing (1) with hydrazine hydrate to afford the carbohydrazide (2).
The hydrazide (2), was treated with two and four equivalents of methyl acetoacetate, and gave methyl pyrazolone (3) and pyranopyrazol (8), derivatives, respectively. While, The hydrazide (2), when refluxed with acetyl acetone, in ethanol afforded the dimethyl pyrazole derivative (4). Correspondingly, the hydrazide (2) was treated with different anhydrides , like succinic, malice and phthalic, anhydrides using acetic acid, to furnish compounds (5),(6) and (7), respectively.
In another reaction of hydrazide (2) with tetrahydrofuran in acetic acid afforded compound (9). **(scheme 1)**.

The reaction of hydrazide (2) with phenyl thioisocyanate, provided (10), which was treated with 5% NaOH solution, to afford mercapto triazole derivative (11). (Cyclized compound), **(scheme 2)**.

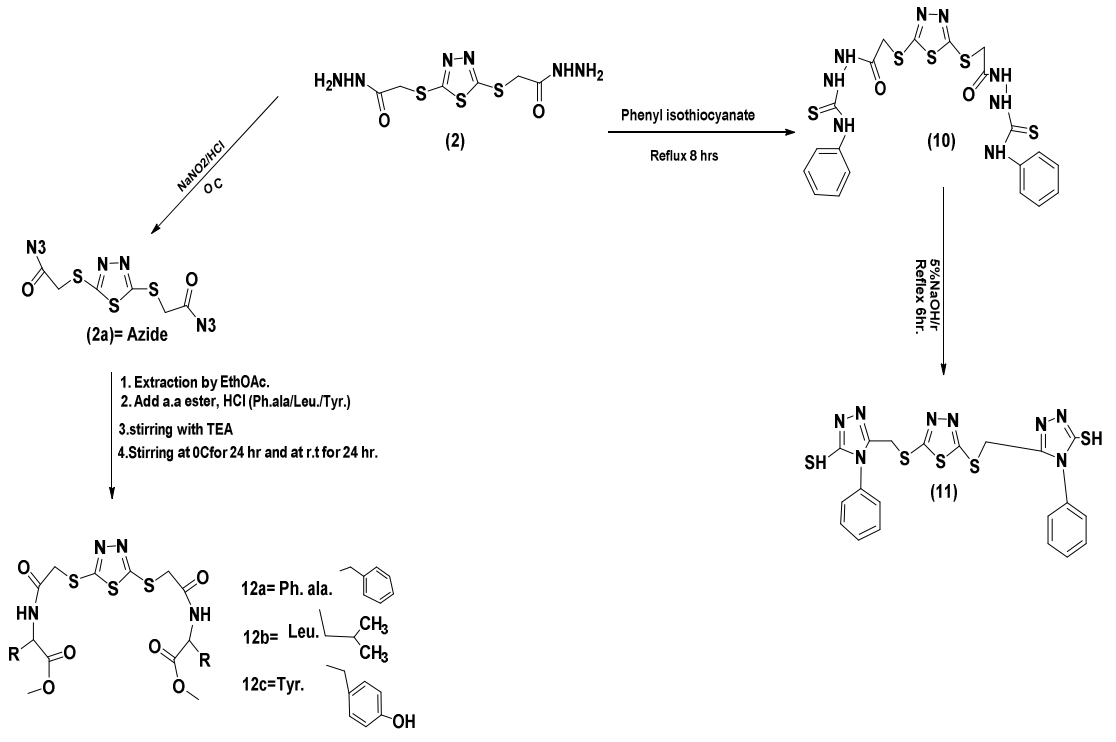
The synthesis of new amino acid derivatives coupled with biologically active heterocyclic rings attracted our attentions, and the acyl azide pathway is one of the first method developed for peptide coupling by Curtius [24]. Synthesis of the target amino acid series (12a-c) were successfully produced via the azide coupling method [22, 25] which was reported to reduce the degree of racemization in the amino acid coupling. The *in situ* generated azide (2a) solution in ethyl acetate, reacted with an amino acid methyl ester hydrochloride, (**ph.ala**, **leuc**. and **tyr**.) in the presence of triethylamine (TEA) to afford (12a-c) in a moderate yield, **(scheme 2)**.

Further development of azide coupling was obtained by the synthesis of N-substituted dipeptide series (14a-c).Thus, boiling the amino acid ester derivative (12c), (Tyr. methyl ester) with hydrazine hydrate afforded the hydrazide (13), **(scheme 3).**

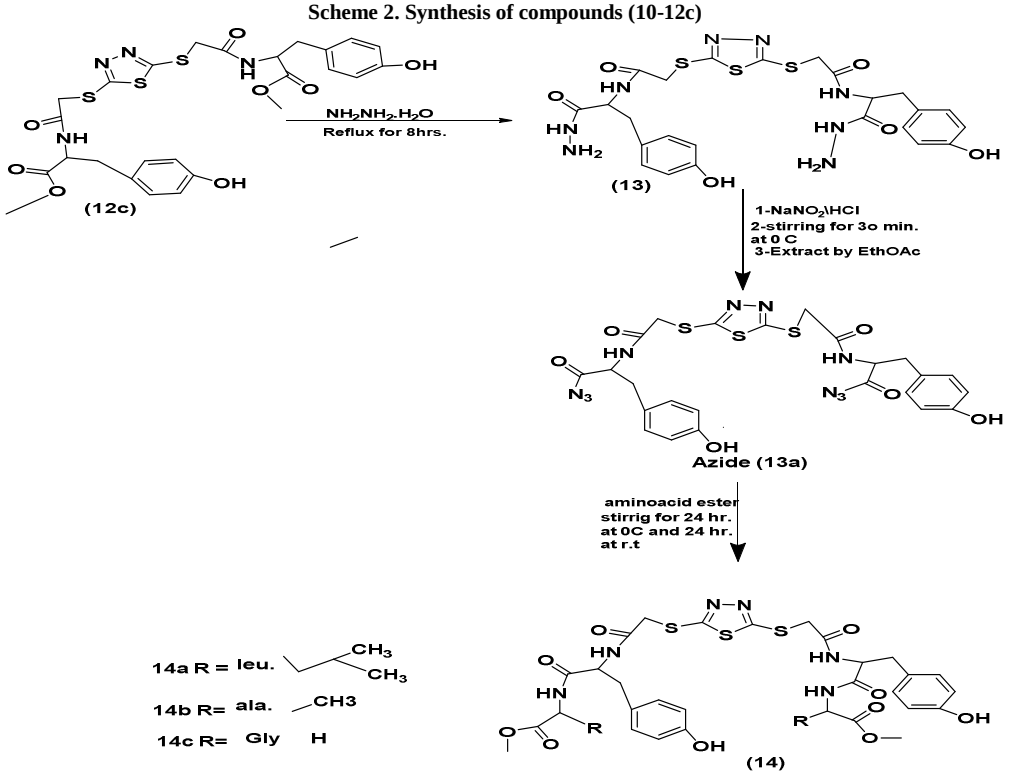
The nitrosation of hydrazide (13) finally produced the azide (13a), by treatment with $NaNO_2$ and HCl mixture. The *in situ* generated azide (13a) in ethyl acetate reacted with amino acid methyl ester hydrochloride (**leu**.,**ala**., and **gly**.) in the presence of triethylamine (TEA), produced dipeptide derivatives (14a-c), in a reasonable yield, **(scheme 3)**.
The IR spectrum of compound (3) showed strong absorption bands at1732, 1684 cm^{-1} that accounted for carbonyl (C=O) group of pyrazolone ring and acyclic amide, respectively. Compound (4) showed absorption band at 1728 cm^{-1} due to carbonyl, (C=O) group of the amide. Reaction of anhydrides with the hydrazide (2) displayed characteristic bands in the range of 3211-3210 cm^{-1} and assigned to the (NH) stretching of amide,compounds (5-7), also the anhydride derivatives exhibited characteristic absorptions, at 1691 cm^{-1} due to cyclic (C=O) stretching of amide for compound (5), other absorption bands at 1735-1734, 1794-1785 cm^{-1} for compound (6) and (7), respectively, that accounted for symmetric and asymmetric stretching of cyclic carbonyl (C=O) group of amide. Compound (8), displayed characteristic absorption band at 2845 cm^{-1} due to symmetric (CH_3) stretching,1730 cm^{-1} due to carbonyl (C=O) of cyclic ester and 1701 cm^{-1}, stretching of acyclic (C=O) group.



Scheme1. Synthesis of compounds (3-9)



Scheme 2. Synthesis of compounds (10-12c)



Scheme 3. Synthesis of compounds (13-14c)

Compound (9) showed a band at, 2920 cm^{-1} due to aliphatic (CH$_2$) Stretching , 1674 cm^{-1} assigned to (C=O) stretching of amide and other important band at 1610 cm^{-1} due to stretching of (C=N) group present in the heterocyclic ring. The IR spectrum of compound (10) demonstrated a characteristic peak at 3188 cm^{-1} that assigned to (NH) stretching of amide, other peaks at1685 ,1252 cm^{-1} that are attributed to stretching of (C=O) and (C=S) groups, respectively. Compound (11) demonstrated peaks at 3119 cm^{-1} due to (NH) stretching and 1610 cm^{-1} due to (C=N) stretching of the triazole ring.

The IR spectra for amino acid derivatives (12a-c) showed distinguished peaks in the range of 3330-3261 cm^{-1} that assigned to (NH) stretching of the amide, 1755-1728 cm^{-1} attributed to stretching of (C=O) ester groups, and other important absorption bands in the range of 1676-1645 cm^{-1} that accounted for (C=O) stretching of the amide.
Compound (13) exhibited peaks at 3321 cm^{-1} due to phenolic (OH) stretching , two peaks at 3269 and 3292 cm^{-1} that assigned to (NH_2) stretching and 3086 cm^{-1} which is attributed to the aromatic (CH) stretching. The disappearance of (C=O) ester group at 1755cm^{-1}, and the appearance of bands at 1689, 1633 cm^{-1} that assigned to (C=O) stretching of amides, and confirm the formation of compound (13).

The IR spectra for the dipeptide derivatives (14a-c) showed the following bands, 3419-3340 cm^{-1} due to the stretching of phenolic (OH) group, 3277- 3255 cm^{-1} That accounted for *sec*- (NH) stretching of amide, other important peaks are in the range of 1734 -1712-cm^{-1} due to (C=O) stretching of ester group, and 1649-1637 cm^{-1}, assigned to (C=O) stretching of amide. The ^{1}HNMR spectrum of compound (3) displayed the following characteristic signals: Two singlets, one at 4.43 ppm due to CH_2 of pyrazolone ring and another at 2.14 ppm, attributed to CH_3 group attached to the pyrazolone ring.

Compound (4) showed two singlets at 3.35 and 2.15 ppm, attributed to the six protons, ($2xCH_3$) of 2 and 5-dimethyl pyrazolone ring, respectively.

Two triplets observed at 2.45 and 2.36 ppm attributed to the CH_2 of succinic anhydride, compound (5). While compound, (7) showed the aromatic protons as multiplet, in the range of 7.99-7.93 ppm, assigned to phthalic anhydride.

The ^{1}HNMR spectrum of compound (8) exhibited a singlet peak at 5.82 ppm assigned to one proton of (CH) pyran ring. A triplet is observed in the ^{1}HNMR spectrum of compound (9), attributed to (CH_2) of pyrrolidine ring.

Compound (10) displayed a characteristic, singlet peak at 10.42 ppm, assigned to two protons, one for the thioamide (S=C-NH-Ph.) and another for (S=C-NH-NH), also, another singlet peak appeared at 9.76 ppm due to the proton of secondary amide (NH-C=O). While the aromatic protons displayed in the range of 7.44 -7.17 ppm as a multiplet.

The ^{1}HNMR spectrum of compound (11) demonstrated the following signals, one at 13.95 ppm, as a singlet, assigned to NH-thione, due to tautomerism, and another singlet at 13.08 ppm, due to thiol group (SH). While the aromatic protons observed in the range of 7.65-7.26 ppm as a multiplet.

The amino acid derivatives (12a-c) were prepared from hydrazide (2) [25] via the racemization-free azide coupling method [22, 26].

Treatment of hydrazide (2) with nitrous acid ($NaNO_2$ /HCl) in a strongly acidic medium at low temperature. The *in situ* resulting azide (2a) is unstable at a higher temperature, so it was extracted with cold ethyl acetate, neutralized and also washed at low temperature.

The azide solution in ethyl acetate reacted with different amino acid methyl esters hydrochloride, (**ph.ala**, **leu**., **tyr**), previously treated with triethylamine in ethyl acetate at low temperature to afford 2,5-di mercapto-1,3,4 thiadiazole amino acid derivatives (12a-c), in a reasonable yield. The ^{1}HNMR data showed doublet signal in the range of 8.78-8.69 ppm attributed to the NH-proton of the peptide bond.

Multiplet signals in the range of 7.29-7.19 ppm assigned to the ten aromatic protons of compound (12a), multiplet signal at 4.52-4.23 ppm for α-CH proton of the amino acids and singlet peak at 3.62-3.59 ppm for the six protons, (2x OCH_3) of the ester groups. Also, the germinal coupling between the two protons of the thioacetyl group SCH_2C=O displayed between 4.42-4.02 ppm as two doublet signals, the other signals for the amino acid residues are reported in the experimental part.

The dipeptide derivatives (14a-c), (**tyr-leu**.), (**tyr.-ala**) and (**tyr.-gly**) respectively, were prepared from their corresponding amino acid methyl ester derivative (12c) after conversion to hydrazide (13) by refluxing with an excess of hydrazine hydrate in methanol. Then nitrosation with a mixture of ($NaNO_2$/ HCl) gave the azide (13a). The dipeptide methyl ester derivatives (14a-c) were obtained by the azide coupling method in (30-49) % yields. The ^{1}HNMR spectra revealed two doublets for the four NH protons of the peptide bonds, other two multiplets in the range of 4.5- 4.28 ppm for the α-CH protons of the four amino acids. In addition to other several peaks corresponding to protons of individual side chains and aromatic protons are listed in the experimental part.

Table1. *In vitro* antibacterial activity of the synthesized compounds (3-14c)

Compound Conc. µg /ml		Zone of Inhibition in mm	Zone of Inhibition in mm	Zone of Inhibition in mm	Zone of Inhibition in mm	Zone of Inhibition in mm	Zone of Inhibition in mm
		S.aureus	***S.epidermidis***	***S.pyogenes***	***E.coli***	***P.aeruginosa***	***K.pneumonia***
3	50	-	-	-	-	-	-
	100	-	-	-	-	-	-
4	50	-	-	-	-	-	-
	100	-	-	-	2	-	-
5	50	4	-	-	-	-	-
	100	11	-	-	-	-	-
6	50	-	-	-	-	-	-
	100	-	-	-	-	11	3
7	50	-	-	-	-	-	-
	100	-	-	-	-	-	-
8	50	-	-	-	-	-	-
	100	-	-	-	6	-	-
9	50	-	-	-	-	-	-
	100	-	-	-	-	-	-
10	50	-	-	-	-	-	-
	100	-	-	-	-	-	-
11	50	-	-	3	-	6	-
	100	-	11	9	-	11	11
12a	50	-	-	5	-	-	4
	100	10	-	9	12	-	13
12b	50	-	-	-	-	-	-
	100	-	-	-	11	-	-
12c	50	-	-	5	-	8	-
	100	-	-	-	-	-	-
14a	50	-	-	-	-	-	-
	100	-	-	-	-	-	-
14b	50	-	-	6	-	-	-
	100	-	-	8	-	-	-
14c	50	-	-	3	-	-	-
	100	-	7	8	-	-	-
Netilmicin	30	25	-	24	20	13	8
DMSO(control)		-	-	-	-	-	-

Highly active = +++ (inhibition zone > 16 mm)
Moderately active = ++ (inhibition zone 10-15 mm)
Slightly active = + (inhibition zone < 10 mm)

Table2. *In vitro* antifungal activity of the synthesized compounds (3-14 c)

Compound Conc. 250µg/ml	Zone of inhibition in mm	Zone of inhibition in mm	Zone of inhibition in mm
	Aspergillus niger	***Aspergillus terreus***	***Candida albicans***
3	**5**	**6**	-
4	**10**	**13**	**15**
5	**7.5**	-	-
6	-	-	-
7	**2**	-	-
8	-	-	-
9	**8**	**16**	-
10	**7**	-	-
11	**4**	**3**	-
12a	**9**	**6**	-
12b	**8**	**7**	-
12c	-	-	-
14a	-	-	-
14b	**5**	**3**	**7**
14c	-	**22**	-
Fluconazole	**20**	-	**17**
DMSO	-	-	-

Antimicrobial Evaluation:
The antimicrobial activities of the synthesized derivatives attached to 2,5-dimercapto-1,3,4thiadiazole were measured by using well diffusion technique with a comparison to netilmicin and fluconazole, as standard antibacterial and antifungal agents, respectively. The recorded data,**(Tables 1 and 2)** lead to the following conclusion:

- All the tested compounds exhibited slightly to moderate antibacterial activity, and the compound (12a) showed the greatest antibacterial activity against Gram-negative *K.pneumonia* and *E.coli* at 100µg/ml concentrations, respectively.
-It is evident that compound (14c) has the most potent antifungal activity against *Aspergillus terreus* at a concentration of 250 µg/ml while fluconazole showed no antifungal activity.
- Compounds (9) exhibited high antifungal activity against *Aspergillus terreus* at 250 µg/ml concentration.
- Compound (4) showed the most potent antifungal activity against *C.albicans* and with moderate activity against

Aspergillus niger and *Aspergillus terreus*.
Docking studies are under progress, to identify the chemical structures in the molecule responsible for the antimicrobial activities of the synthesized compounds, and the results will be published later.

CONCLUSION

A new series of 2,5-di-mercapto-1,3,4-thiadiazole derivatives were successfully prepared and tested for their antimicrobial activities. Amino acids attached to the heterocyclic ring displayed remarkable antifungal activities, especially compound (14c), which exhibited the most potent activity against *Aspergillus terreus*. While, the comparison of the antimicrobial activities of other synthesized compounds, with that of standard antimicrobial drugs, reveals that the produced derivatives show moderate to good activity against the bacterial and fungal strains used in this study.

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