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Synthesis, Characterization, Antimicrobial Assessments, Potential Antioxidant And Molecular Docking Studies Of Polyfunctional Substituted Azoles, Azines, And Their Analogs Linked Sulfamethoxazole Moiety

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Abstract:
A new series of substituted azoles, azines, and their analogs linked sulfamethoxazole moiety was designed and synthesized. The antioxidant activity study of our synthesized compounds and molecular docking enhancement were achieved. Moreover, the antimicrobial assay indicated that compound **1** is the more active compound against all the stains compared to the reference drugs. So, reactions of β-oxobutanamide derivative 1 with different reagents afforded compounds **2a,b, 5, 7** and **8 12** and **14.** Coupling of **1** with diazonium salt yield aryl hydrazones **10a,b** which reacts with ethyl chloroformate to yield the triazinediones **11a,b**. Reactions of enaminone **12** with some electrophilic and nucleophilic reagents to yield compounds **16, 21, 22, 23**, **24** and **25** respectively. The structures of newly synthesized compounds were characterized by spectral and elemental analyses.

Keywords: Azoles, azines; antioxidants; antibacterial agents; molecular docking.

1. Introduction

Sulfamethoxazole (SMZ or SMX) is used as an antibiotic agent, for bacterial infections such as urinary tract infections, bronchitis, and prostatitis. Also, it is effective against both Gram-negative and Gram-positive bacteria [1]. It inhibits the synthesis of bacterial dihydrofolic acid due to it's the similarity structural to an endogenous substrate, para-aminobenzoic acid (PABA) [2, 3]. Most bacteria find their needs for folic acid by making it from PABA, as opposed to Animalia that require exogenous folic acid sources. SMX inhibits dihydropteroate synthase, this is the enzyme in which responsible for the conversion of bacteria of PABA to dihydrofolic acid. [4]. The Inhibition of this pathway stops the formation of tetrahydrofolate and, ultimately, the formayion of bacterial purines and DNA. Azole antifungal agents have greatly added to the therapeutic options for the treatment of systemic fungal infections [5]. Pyrimidine is one of the most important heterocyclic moieties. Because it is essential to DNA and RNA, it is widely transferred in living organisms [6]. Pyrimidines are also used as an antitumor [7], anti-inflammatory [8], analgesic [8], antiviral [9, 10,11], anti-neoplastic [12], antitubercular [13,14], and diuretic agents [15]. Also, pyridines, triazines, thiophenes, pyrazoles, and their analogues have many important applications [16-18]. The importance of such compounds lies in their diverse pharmaceutical activities, namely antimicrobial [19], antidiabetic [20], antiviral [21], anti-inflammatory and analgesic [22], activities. Owing to their interesting biological activities and medicinal properties, these compounds have been the targets of investigations by several researchers. So, we report here the use different reagents in several heterocyclic transformations to obtain pyrazole, pyridine, thiophene, and

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triazine derivatives of biological activity, with an estimation of the antioxidant and antimicrobial activity of our synthesized compounds

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2. Methods and materials:

2. 1 Chemistry

All spectral analyses were carried out at South Valley, Mansoura, Sohag, Cairo Universities, and Nawah Scientific Research Center. Uncorrected melting points were measured on an electrothermal melting apparatus. For IR spectra the prepared compounds were mixed with potassium bromide and examined as disks by a Shimadzu FT-IR 8101 PC spectrometer. The FT-IR spectra were recorded in the wavenumber range of 4000-400 cm^{-1}. The ^{1}H NMR and ^{13}C NMR spectra were recorded by Bruker (400MHz) and (100MHz) spectrometers using TMS as an internal reference. The chemical shifts were measured in ppm as a unit of measurement. Mansoura, Sohag, and Cairo Universities. The MS spectra were determined using a Davion Compact Mass Spectrometer (CMS) (Probe/TLC-MS) at Nawah Scientific Research Center, Cairo, Egypt. Elemental analyses were determined at the microanalytical unit. Cairo University and was calc. as; C = 12.011, H = 1.008, and N = 14.007. Spectrophotometric apparatus (Spekol 11 spectrophotometer, analytic Jena AG, Jena, Germany). UV lamp (Vilber Lourmat-6.LC, VILBER Smart Imaging, Marne-la-Vallée, France). 1,1-Diphenyl-2-picrylhydrazyl (DPPH•), methanol, and DMSO were purchased from Sigma Aldrich (St. Louis, USA).

Praparation of compounds 2 a,b.

General procedure: A mixture of **1** (0.01 mol), malononitrile (0.01 mol) or ethyl cyanoacetate, and (0.01 mol) of elemental sulfur was refluxed in ethanol with a catalytic amount of TEA for 6 hours, left to cool. The reaction mixture was poured onto crushed ice and acidified with a few drops of HCl. The solid product so formed was filtered off and recrystallized from the proper solvent.

5-amino-4-cyano-3-methyl-N-(4-(N-(5-methylisoxazol-3-yl) sulfamoyl)phenyl)thiophene-2-carboxamide (2a)

It was collected as brown crystals, yield = 70%, m.p = 225-227 ºC. (FTIR; KBr, υ, cm^{-1}) = 3371 (NH_2), 3317-3205 (NH), 3083 (Ar-CH), 2212 (CN), 1636 (CONH); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) = 2.30 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 6.14 (s, 1H, isoxazole proton), 7.79-7.87 (m, 4H, aromatic protons), 9.99 (s, NH), 11.35 (s, NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) = 12.44 (q), 15.45(q), 66.80(d), 88.94(s), 95.86(d), 112.78(s), 115,69(q, CN), 120.44(d), 120.44(d), 128.28(d), 128.28(d), 133.72(s), 142.42(s), 143.79(s), 157.98(s), 161.04(s), 170.79(s). MS (Atmospheric-pressure chemical ionization) [APCI]): m/z (%) = 416.2 $[M^{-1}]^{-}$ *Anal. calcd* (%) for $C_{17}H_{15}N_5O_4S_2$: C, 48.91; H, 3.62; N, 16.78, S, 15.36; found: C, 48.92; H, 3.63; N, 16.78, S, 15.36.

Ethyl 2-amino-4-methyl-5-((4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)-carbamoyl)thiophene-3-carboxylate (2b)

It was collected as brown crystals, yield = 77%, m.p = 221-223 ºC**.** (FTIR; KBr, υ, cm^{-1}) = 3076 (CH aromatic), 2978 (CH aliphatic), 1711 (CO_2Et), 1649 (CONH); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) = 1.28-1.34 (t, 3H, CH_3, J = 6 Hz)), 2.30 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 4.24-4.26 (q, 2H, CH2, J = 2.7 Hz), 6.14 (s, 1H, isoxazole proton),7.8-7.9 (m, 4H, aromatic protons), 11.03 (s, NH), 11.27 (s, NH); MS (Atmospheric-pressure chemical ionization) [APCI]): m/z (%) = 465.1 $[M^{+1}]^{+}$ *Anal. calcd* (%) for $C_{19}H_{20}N_4O_6S_2$: C, 49.13; H, 4.34; N, 12.06, S, 13.81; found: C, 49.14; H, 4.35; N, 12.10, S, 13.82.

5-cyano-4-(4-methoxyphenyl)-1,3-dimethyl-N-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)-6-thioxo-1,6-dihydropyridine-2-carboxamide (5)

A mixture of β- oxobutanilide **1** (0.01 mol) and 2-cyano-2-(4-methoxy-phenyl)-thioacetamide (0.01 mol) was refluxed in dioxane with a few drops of catalytic pip. The reaction followed using TLC and was completed after 15 min. The product formed as yellow oily drops, was let to be cooled, then formed as a solid product, and was recrystallized in benzene as an off-white powder. Yield = 65%, m.p. = 208-210 ºC. (FTIR; KBr, υ, cm^{-1}) = 3539-3300 (2 NH), 3092 (CH aromatic), 2966 (CH

aliphatic.), 2230(CN), 1677(CONH); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) = 2.26 (s, 3H, CH_3), 2.44 (s,3H, CH_3), 3.70 (s, 3H, CH_3), 6.11 (s, 1H, isoxazole proton), 7.00-7.90 (m, 8H, aromatic protons), 10.90 (s, NH), 11.20 (s, NH), 14.40 (s, NH); *Anal. calcd* for Chemical Formula: $C_{26}H_{23}N_5O_5S_2$; C, 56.82; H, 4.22; N, 12.74; S, 11.67; found: C, 56.87; H, 4.18; N, 12.67, S, 11.65.

4-methyl-N-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)-3-phenyl-2-thioxo-2,3-dihydrothiazole-5-carboxamide (7)

A mixture of (0.015 mol) of **1,** (0.01 mol) of phenyl isothiocyanate and (0.01 mol) of elemental sulfur, was refluxed in DMF with a catalytic amount of TEA for 2 hours. The reaction mixture was followed by TLC when all amounts of phenyl isothiocyanate were consumed, the mixture let to cool then poured onto acidified ice with HCl. The PPT recrystallized from methanol as a pure product with a deep black color. Yield = 55%, m.p = 169-171 ºC. (FTIR; KBr, υ, cm^{-1}) = 3475-3348 (2 NH), 3063 (CH aromatic.), 2966 (CH aliphatic.), 1693(CONH); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) =2.30 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 6.14 (s, 1H, isoxazole proton), 7.70-7.87 (m, 4H, aromatic protons); MS (Atmospheric-pressure chemical ionization) [APCI]): m/z (%) = 486; *Anal. calcd* (%) for $C_{21}H_{18}N_4O_4S_3$: C, 51.84; H, 3.73; N, 11.51, S, 19.77, found: C, 51.85; H, 3.75; N, 11.51, S, 19.78.

(E)-N-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)-3-(2-phenyl-hydrazono)butanamide (8)

A mixture of (0.01 mol) of **1** and (0.02 mol) of phenylhydrazine in 20 ml MeOH was refluxed for 10 min. ppt formed on hot, filtered off then washed by MeOH, and recrystallized from methanol as an off-white powder. Yield = 70%, m.p = 170-172 ºC. (FTIR; KBr, υ, cm^{-1}) = 3264, 3235 and 3104 (3 NH), 1650 (CONH); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) = 1.95 (s, 3H, CH_3), 2.30 (s,3H, CH_3), 3.35 (s, 2H, CH2), 6.13 (s, 1H, isoxazole proton), 7.05-7.13 (m, 4H, aromatic protons), 8.85 (s, 1H, NH), 10.7 (s, 1H, NH), 11.60 (s, 1H, NH); MS (Atmospheric pressure chemical ionization [APCI]): m/z (%) = 427.6 $[M-H]^-$; *Anal. calcd* for $C_{20}H_{21}N_5O_4S$ (%): C, 56.19; H, 4.95; N, 16.38; S, 7.50; found: C, 56.21; H, 4.97; N, 16.40; S, 7.52.

General procedures for the synthesis of (11a,b)

Aryl hydrazone **10a** or **10b** (0.01 mol) dissolved in a mixture of EtOH/EtONa, (0.01 mol) of ethyl chloroformate was added gradually. The mixture was refluxed for 1 hour. ppt Formed on hot during the reaction was filtered off, washed with water and collected as off-white ppt, yield= 60-70 %,

4-(6-acetyl-3,5-dioxo-2-(p-tolyl)-2,3-dihydro-1,2,4-triazin-4(5H)-yl)-N-(5-methylisoxazol-3-yl)benzenesulfonamide (11a)

Compound **11a** was collected as white crystals, m.p = 280-282 ºC (FTIR; KBr, υ, cm^{-1}) = 3445 (NH), 3080 (CH aromatic), 1755 ($COCH_3$), 1660 (CONH); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) = 2.30 (s, 3H, CH_3), 2.49 (s, 3H, CH3), 2.55 (s, 3H, CH3), 6.67 (s, 1H, isoxazole proton), 7.48-7.55 (d, 1H, aromatic proton, *J* = 28.6Hz), 7.57-7.60 (d, 1H, aromatic proton, *J* = 8.6Hz), 7.88-7.90 (d, 1H, aromatic proton, *J* = 10.4Hz), 7.99-8.01 (d, 1H, aromatic proton, *J* = 9.6Hz), 11.51 (s, NH); ^{13}C NMR spectrum (100 MHz, DMSO-d_6, δ, ppm) revealed signals at 12.95 (q), 21.00(q), 26.20(q), 64.74(d), 95.92(s), 103.34(s), 116.42(s), 120.47(d), 120.62(s), 127.52(d), 128.63(d), 130.52(d), 132.40(d), 135.00(s), 140.04(d), 143.56(s), 150.86(s), 169.80(s), 170.69(s, CO), 172.96(s, CO), 198.45(s, CO); MS (Electron ionization) [EI]): m/z (%) = 482 $[M]^+$ *Anal. Calcd*. (%) for $C_{22}H_{19}N_5O_6S$: C, 54.88; H, 3.98; N, 14.55; S, 6.66, found: C, 54.90; H, 3.98; N, 14.57; S, 6.67.

4-(6-acetyl-2-(4-chlorophenyl)-3,5-dioxo-2,3-dihydro-1,2,4-triazin-4(5H)-yl)-N-(5-methylisoxazol-3 yl)benzenesulfonamide (11b)

Compound **11b** was collected as white crystals, m.p = 190-192 ºC, (FTIR; KBr, υ, cm^{-1}) = 3425 (NH), 1744 ($COCH_3$), 1666 (CONH); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm) = 2.29 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 6.67 (s, 1H, isoxazole proton), 7.24-7.26 (d, 1H, aromatic proton, *J* = 8.4Hz), 7.47-7.49 (d, 1H, aromatic proton, *J* = 8.4Hz), 7.97-8.00 (d, 1H, aromatic proton, *J* = 9.2Hz), 8.01-8.03 (d, 1H, aromatic proton, *J* = 9.2Hz), 11.50 (s, NH); ^{13}C-NMR spectrum (100 MHz, DMSO-d_6, δ, ppm) revealed signals at 12.96 (q), 26.09(q), 64.74(d), 117.85 (s), 117.89(s),128.60(d), 129.88(d), 130.59(d), 132.47(d), 141.61(d), 142.21(s), 143.66(s), 150.41(s), 156.86(s), 162.45(s), 170.64(s, CO), 172.95(s, CO), 197.97(s, CO);MS (Electron ionization) [EI]): m/z (%) = 504 $[M]^{2+}$ *Anal. calcd.* (%) for $C_{21}H_{16}ClN_5O_6S$: C, 50.25; H, 3.21; N, 13.95, S, 6.39; found: C, 50.25; H, 3.22; N, 13.97, S, 6.40.

(E)-2-((dimethylamino)methylene)-N-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)-3-oxobutanamide (12)

A mixture of **1** (0.01 mol) and DMF-DMA (0.01 mol) in dry benzene was refluxed for 1 hour. The reaction mixture started colorless and then turned orange, and when the mixture was poured into a flask containing 20 ml of *n*-hexane, yellow crystals gradually formed. The formed product was filtered off and recrystallized from methanol. Yield = 98%, m.p. 163-165 ºC. (FTIR; KBr, υ, cm^{-1}) 3258-3122 (2 NH), 3076 (CH arom.), 2975 (CH aliph.), 1659 (CONH); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm) = 2.32 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 3.24 (s, H, CH), 3.27 (s, 6H, $2CH_3$) 6.50 (s, 1H, isoxazole proton), 7.64-7.66 (d, 2H, aromatic protons), 7.76-7.81(d, 2H, aromatic protons), 8.10 (s, NH), 11.43 (s, NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) = 12.56, 12.56, 31.24, and 35.50 (q) assigned to $4CH_3$ groups, signals at 97.349(d), 107.87(d), 121.68(d), 121.68(d), 128.77(d), 128.77(d), for 6CH carbons, signals at 129.85(s), 144.85(s), 155.25(s), 158.39(s), 16.73(s) assigned to 5 quaternary carbons, in addition to signals at 165.66(s, CO), 186.80(s, COCH3); MS (electron spray ionization) [ESI]): m/z (%) = 391 $[M-H]^-$; *Anal. calcd* for $C_{17}H_{20}N_4O_5S$: C, 52.03; H, 5.14; N, 14.28, S, 8.17; found: C, 52.05; H, 5.16; N, 14.30, S, 8.18.

(E)-2-(ethoxymethylene)-N-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)-3-oxobutanamide (14)

A mixture of **1** (0.01 mol) and triethyl orthoformate (0.03 mol) in acetic acid was refluxed for 6 hours, The ppt formed was filtered off and then washed by ethanol, collected as a white powder and recrystallized from ethanol. Yield = 45%, m.p. = 210-212 ºC. (FTIR; KBr, υ, cm^{-1}) = 3077 (CH aromatic), 2987 (CH aliphatic), 1709 ($COCH_3$), 1652 (CONH); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) =1.06 (t, 3H, CH_3), 1.91 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 3.36 (q, 2H, CH_2), 6.15 (s, 1H, isoxazole proton), 7.74-7.89 (m, 4H, aromatic protons), 8.65 (s, H, olefinic CH), 12.11 (s, NH), 12.37 (s, NH)); MS (Atmospheric pressure chemical ionization) [APCI]): m/z (%) = 392.3 $[M^{-1}]^-$ *Anal. calcd* (%) for $C_{17}H_{19}N_3O_6S$: C, 51.90; H, 4.87; N, 10.68; S, 8.15, found: C, 51.90; H, 4.88; N, 10.69; S, 8.17.

4-(3-acetyl-6-amino-5-cyano-2-oxopyridin-1(2H)-yl)-N-(5-methylisoxazol-3-yl)benzenesulfonamide (16)

For a (0.01 mol) of **12** in 20 ml dioxane with a catalytic amount of piperidine, (0.01 mol) of malononitrile was added. The reaction mixture started with an orange color and then converted to red. The reaction was completed after 20 minutes. The reaction mixture was poured into a flask containing crushed ice acidified with a few drops of HCl. Orange ppt was collected then washed and recrystallized from ethanol to yield = 80%, m.p = 269-270 ºC. (FTIR; KBr, υ, cm^{-1}) = 3383 (NH_2), 3274 (NH), 3003 (CH aromatic), 2213 (CN), 1679 ($COCH_3$), 1647 (CONH); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) = 2.37 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 6.61 (s, 1H, isoxazole proton), 7.63-7.65 (d, 2H, aromatic protons, *J*= 8Hz), 7.99-8.01 (d, 2H, aromatic protons, *J* = 12Hz); MS (Atmospheric pressure chemical ionization [APCI]): m/z (%) = 414 $[M^{+1}]$; *Anal. calcd* for $C_{18}H_{15}N_5O_5S$: C, 52.30; H, 3.66; N, 16.94, S, 7.76; found: C, 52.20; H, 3.76; N, 16.84, S, 7.81.

4-(3,5-diacetyl-6-hydroxy-2-oxopyridin-1(2H)-yl)-N-(5-methylisoxazol-3-yl)benzenesulfonamide (21)

A mixture of **12** (0.01 mol) and EAA (0.02 mol) was refluxed in 20 ml methanol with a catalytic amount of piperidine for 1 hour, after reaction completion the mixture was poured into a flask containing crushed ice with a few drops of HCl. A pale yellow PPT was collected and recrystallized from benzene. Yield = 45%, m.p. 140-142 ºC. (FTIR; KBr, υ, cm^{-1}) = 3437 (NH), 3091 (CH arom), 1679 ($COCH_3$), 1645 (CONH); ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm) = 1.27 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 3.28 (s, 3H, CH_3), 6.48 (s, 1H, isoxazole proton), 7.5-8.80 (m, 4H, aromatic protons), 11.60(s, NH), 11.70(s, OH); MS (Atmospheric-pressure chemical ionization) [APCI]): m/z (%) = 414 $[M^{+1}]$; *Anal. calcd* for $C_{19}H_{17}N_3O_7S$: C, 52.90; H, 3.97; N, 9.74, S, 7.43; found: C, 52.92; H, 3.95; N, 9.76, S, 7.44.

4-Methyl-*N*-(4-(*N*-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)-1-phenyl-*H*-pyrazole-5-crboxamide (22)

A mixture of (0.01 mol) of **12** and (0.01mole) of phenylhydrazine was refluxed in 20 ml of methanol with a catalytic amount of piperidine, the reaction was completed after 30 minutes, and the solvent was left to volatile. The collected ppt dissolved in ethanol and poured into a flask containing crushed ice with a few drops of HCl. The product formed immediately and was isolated as a pure product. Yield = 65%, m.p. 82 ºC. (FTIR; KBr, υ, cm^{-1}) = 3367-3322 (2 NH), 3072 (CH arom.), 1676 ($COCH_3$); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm) = 2.39 (s, 3H, CH_3), 3.27 (s, 3H, CH_3), 6.51 (s, 1H, isoxazole proton), 7.42-

7.82 (m, 4H, aromatic protons), 8.30 and 8.5 (s, NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) = 12.04 (q), 12.56(q), 97.51(d), 119.30(d), 120.32(d), 120.32(d), 125.78(d), 125.78(d), 128.66(d), 128.66(d), 129.04(d), 129.04(d), 129.76(d), 129.76(d), 139.94(s), 140.00(s), 144.00(s), 147.00 (s), 161.00(s), 163.00(s), 172.00(s); *Anal. calcd* (%) for $C_{21}H_{19}N_5O_4S$: C, 57.66; H, 4.38; N, 16.01, S, 7.33; found: C, 57.64; H, 4.38; N, 16.04, S, 7.40.

4-methyl-N-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)-2-oxo-1,2-dihydropyrimidine-5-carboxamide (23)

Refluxing of compound **12**(0.01 mol) and urea (0.01 mol) in 20 ml methanol with a catalytic amount of sodium bicarbonate, the reaction took 4 hours to complete. After the reaction complete was poured in acidified crushed ice with HCl, and pale yellow ppt was collected and recrystallized from ethanol. Yield = 55%, m.p. 175-177 ºC. (FTIR; KBr, υ, cm^{-1}) = 3255-3210 (2 NH), 1642 (CONH); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm) = 2.40 (s, 3H, CH_3), 2.52 (s, 3H, CH_3), 6.50 (s, 1H, isoxazole proton), 7.73-7.90 (m, 4H, aromatic protons), 8.67 (d, CH-pyrimidinone), 9.56 (s, NH), 11.74 (d, pyrimidine-NH); MS (Atmospheric-pressure chemical ionization) [APCI]: m/z (%) = 388 [M^{-1}]$^-$; *Anal. Calcd.* for $C_{16}H_{15}N_5O_5S$: C, 49.35; H, 3.88; N, 17.99, S, 8.23; found: C, 49.37; H, 3.88; N, 17.98, S, 8.26.

1-carbamothioyl-3-methyl-N-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)-1H-pyrazole-4-carboxamide (24)

A mixture of (0.01 mol) of **12** and (0.01 mol) of thiosemicarbazide was refluxed in 20 ml methanol with a catalytic amount of sodium bicarbonate, the reaction took 30 minutes to complete. The reaction mixture was poured into acidified crushed ice with HCl. Yellow ppt was collected and recrystallized from methanol as a pure product. Yield = 45%, m.p. 177-179 ºC. (FTIR; KBr, υ, cm^{-1}) = 3434 (NH_2), 3371-3227 (2 NH), 1647 (CONH); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm) = 2.29 (s, 3H, CH_3), 3.19(s, 6H, $2CH_3$), 6.55 (s, 1H, isoxazole proton), 7.71-7.73 (d, 2H, aromatic protons), 7.79-7.81 (d, 2H, aromatic protons); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) 12.45 (q), 26.37(q), 97.41(d), 97.41(d), 101.49(d), 119.93(s), 119.93(s), 128.84(d), 128.84(d), 129.56(s), 144.17(s), 161.00(s), 162.33(s), 162.33(s), 167.72(s), 197.11(s, CS); *Anal. calcd* (%) for $C_{16}H_{16}N_6O_4S_2$: C, 45.70; H, 3.84; N, 19.99, S, 15.25; found: C, 45.71; H, 3.86; N, 20.00, S, 15.27.

2-methyl-N-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)-benzo[4,5]imidazo[1,2-a]pyrimidine-3-carboxamide (25)

To a solution of compound **12** (0.01 mol), (0.01 mol) of 2-aminobenzimidazole was added and the mixture was refluxed in ethanolic piperidine. The ppt formed on hot, isolated after reaction completion after 6 hours. The product washed with ethanol, dried, pale yellow ppt was collected and recrystallized from ethanol with a yield of 60% and m.p = 270-272 ºC. (FTIR; KBr, υ, cm^{-1}) = 3369-3293 (2 NH), 3090 (CH aromatic.), 1690 (CONH);^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm) = 2.39 (s, 3H, CH_3), 3.27 (s, 6H, $2CH_3$), 6.53 (s, 1H, isoxazole proton), 7.50-8.02 (m, 8H, aromatic protons), 8.98 and 11.30 (s, 2NH); MS (Atmospheric-pressure chemical ionization) [APCI]): m/z (%) = 461 [M-H]$^-$ *Anal. calcd* (%) for $C_{22}H_{18}N_6O_4S$: C, 57.13; H, 3.92; N, 18.17, S, 6.93; found: C, 57.14; H, 3.95; N, 18.18, S, 6.94.

2. Antioxidant activity

The antioxidant capacity of the tested samples was investigated following the DPPH• colorimetric method using ascorbic acid as a standard by way of the assay reported by Kitts *et al* [23]. The serial dilution of each sample was prepared by mixing the sample solution with methanol in an equivalent amount. DPPH• solution was prepared in a concentration of 0.135 mM and mixed with each prepared concentration in the serial dilution with an equivalent amount. After the addition of DPPH• solution, the samples were kept in the dark for 30 minutes at room temperature. The absorbance of each sample was measured at 517 nm in the next step. The % DPPH• remaining was calculated by applying the subsequent equation (Eq. (1)):

$$\% \text{ DPPH}^\bullet \text{ remaining} = [\text{DPPH}^\bullet]_T / [\text{DPPH}^\bullet]_{T=0} \times 100 \qquad \text{Eq. (1)}$$

The values of % DPPH• remaining were plotted versus sample concentration mg/mL using an exponential curve to identify the effective concentration "IC_{50}". IC_{50} specified the amounts of antioxidants needed to decrease the initial concentration of DPPH• solution by 50%. The values of IC_{50} point out the inverse relationship with the antioxidant capacity of the tested sample [24].

3. Antimicrobial activity

Microorganisms

Test organisms used in this study including Gram-positive bacteria (*Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 6538) and Gram-negative bacteria (*Escherichia coli* ATCC 8739 and *Klebsiella pneumonia* ATCC 43816), in addition to one fungal strain *Candida albicans* ATCC 10231 were obtained from available culture collection at Botany and Microbiology Department, Faculty of Science, Al-Azhar University (Assiut Branch), Egypt. These common isolates and strains are usually used in our laboratories as standard pathogenic microbes for various biological activities [25,26]. Bacterial strains were grown in Mueller Hinton broth at 37 ºC for 24 h, while *C. albicans* was grown in Sabouraud dextrose broth, and incubated for 2 days at 25 ºC.

Agar well diffusion method

The antimicrobial assay was performed as described [27], by using the well-diffusion method. Well-diameter was 8 mm filled with 100 µL of the 1% (w/v), well dissolved in DMSO, test samples. Chloramphenicol (1 mg/mL) was used as a positive control for bacteria and clotrimazole (1 mg/mL) for *C. albicans*, while sterilized DMSO was used as a negative control. Muller-Hinton plates previously inoculated with 24-hour-old broth cultures of the bacterial strains were used for antibacterial activity. Sabouraud dextrose plates previously inoculated with a spore suspension of *C. albicans* were used for antifungal activity. The diameter of the inhibition zone around the well, measured in millimeters, is used as positive bioactivity.

Statistical analysis

All the experiments were conducted in triplicate and analysis of variance was made using SPSS, program version 16. The mean of the data was calculated by analysis of variance (ANOVA), and statistical analysis was observed (at $P < 0.05$) to establish significant differences.

4. Molecular Docking Study

Beginning with the X-ray structure, a molecular docking simulation was performed to investigate the bindings of the ligand structures of the sulfamethoxazole analogs **1, 12, 16, 21, 22, 23, 24** and **25** with PDB (1AJ0) file, a demonstrative protein of DHPS (dihydropteroate synthase enzyme of *E. Coli*) used in this study.

3. **Results and discussion**

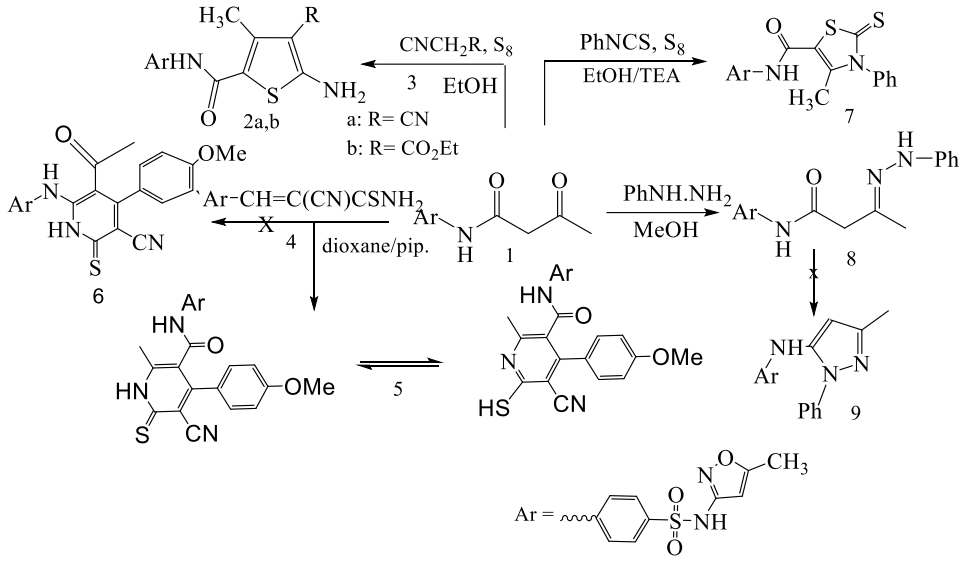
3.1. Chemistry

The multicomponent reaction of *β*-oxobutanamide derivative **1** with a mixture of malononitrile or ethylcyanoacetate, elemental sulfur was reported. So, the reaction of the three components, *β*-oxobutanamide derivative **1,** malononitrile or ethyl cyanoacetate, and elemental sulfur in ethanolic triethylamine under conventional heating afforded the expected thiophene derivatives **2a, b** (Scheme 1). The structure of **2a** was elucidated as an example on compatible spectroscopic data and its correct elemental analysis. The IR spectrum of **2a** exhibits the presence ofabsorption bands of NH_2, NH groups at υ = 3371, 3317, and 3205cm^{-1} and CN group at υ = 2212 cm^{-1}. ^{1}H NMR of structure **2a** revealed singlet signals at δ = 2.31 and 2.38 ppm corresponding to $2CH_3$ protons, singlet signal at δ = 6.15 ppm assigned to isoxazole -CH, singlet signal at δ = 9.99 and 11.35 ppm assigned to 2 NH protons beside the multiplet signals of 6 protons corresponding to aromatic and NH_2 protons at δ = 7.80-7.87 ppm. ^{13}C NMR Spectrum of compound **2a** showed a signal at δ =115.69 (CN) beside all carbon signals assigned to the structure. Also, the mass spectrum of **2a** displayed a molecular ion peak at m/z = 418[M^+] corresponding to the molecular formula $C_{17}H_{15}N_5O_4S_2$.

The behavior of β-oxoanilide **1** with electrophilic reagents under alkaline conditions was studied. Thus, reactions of **1** with *p*-methoxybenzylidine cyanothioacetamide is an example of arylidine cyanothioacetamide yields the acceptable structure **5** rather than the structure **6**. Spectral and chemical evidence didn't fit structure **6**. So, structure **5** is the sole reaction product based on spectroscopic data. IR spectrum of compound **5** shows presence bands of an amidic carbonyl group at υ = 1677 cm^{-1}.

While the ^{1}H NMR spectrum detected a singlet signal for 6H at δ = 2.26 ppm for two CH_3 protons, and 3.70 ppm for CH_3 protons, 3 singlet signals at δ = 10.9, 11.2 and 14.4ppm assigned to 2NH and SH protons respectively.

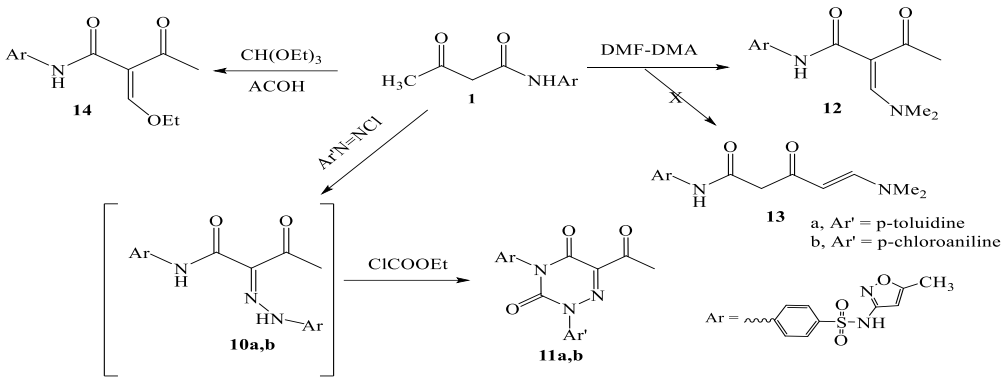
In view of the growing biological importance of thiazole derivatives, it has encouraged us to synthesize new derivatives of thiazole. Thus, the reaction of **1** with phenyl isothiocyanate and elemental sulfur gave the thiazole-2-thione derivative **7**. Analytical and spectral data of the product agree with the proposed structure. On the other hand, the reaction of **1** with nucleophilic reagents was also investigated. So, treatment of **1** with phenyl hydrazine afforded the condensation product **8**. Compound **8** was established based on its spectral data. Converting hydrazine derivative **8** to pyrazole **9** failed under all reaction conditions.



Scheme 1

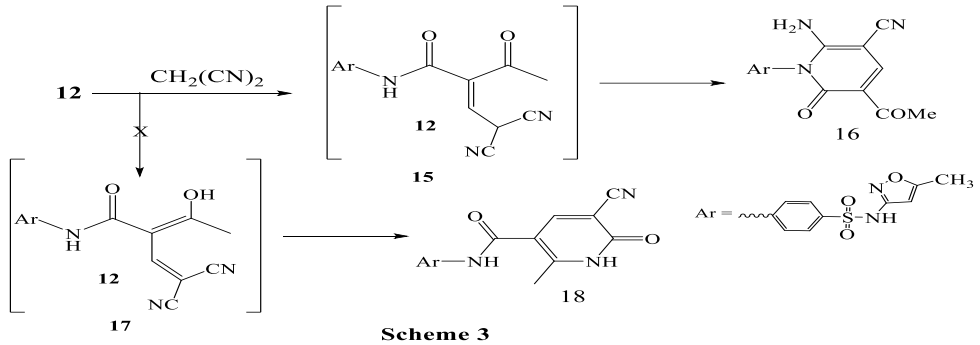
Aryl hydrazones **10a,b**. [28] were achieved by coupling **1** with diazotized aromatic amines in ethanol buffered with sodium acetate at 0-5 ºC. Aryl hydrazones **10a,b** were treated with ethyl chloroformate in refluxing ethanol to yield the triazinedione derivative **11a,b.** Structures **11a,b** were established on its compatible spectral data and elemental analysis. ^{1}H NMR spectrum of **11a** as example display a singlet signal at δ = 2.30, 2.49 and 2.55 ppm assigned to 3CH_3 protons, singlet signal (1H) at δ = 6.67 ppm assigned to isoxazole-4H, doublet signals at δ = 7.52 ppm assigned to Ar-H, *J* = 28.6Hz, doublet signals at δ = 7.58 ppm assigned to Ar-H, *J* = 8.6Hz, doublet signals at δ = 7.89 ppm assigned to Ar-H, *J* = 10.4Hz, doublet signals at δ = 8.00 ppm assigned to Ar-H, *J* = 9.6Hz, singlet signal at δ = 11.51 ppm assigned to NH group and the multiplet signals at δ = 7.45-8.01 ppm assigned to aromatic protons. ^{13}C NMR spectrum revealed signals at δC =12.96 (q), 26.09(q), 64.74(d), 117.89(s), 128.60(d), 129.88(d), 130.59(d), 132.47(d), 141.61(d), 142.21(s), 143.66(s), 150.41(s), 156.86(s), 162.45(s), 170.64(s, CO), 172.95(s, CO), 197.97(s, CO). The mass spectrum of **11a** revealed a molecular ion peak at m/z = 482[M^+] corresponding to the molecular formula $C_{22}H_{19}N_5O_6S$. Treatment of **1** with *N, N*-dimethylformamide-dimethylacetal (DMF-DMA) in refluxing dry benzene yield a product that may be either structure **12** or its isomeric **13**. Establishing the exact structure of the reaction product as structure **12** rather than **13** was based on spectral data. The ^{1}H NMR spectrum indicated the presence of a singlet signal at δ = 1.66 ppm assigned for CH_3 protons, and a singlet signal at δ = 2.32 and 2.39 ppm for the $N(CH_3)_2$ protons. Singlet signal at δ = 3.27 ppm assigned to CH_3 protons, singlet signal at δ = 3.27 ppm assigned to olefinic proton, singlet signal at δ = 6.50 ppm attributed to isoxazole-H, multiplet signals at δ = 7.64-7.78 ppm assigned to aromatic and NH protons and singlet signals at δ = 11.43 ppm assigned to NH groups. ^{13}C NMR spectrum revealed signals at δ C = 12.56, 12.56, 31.24, and 35.50 (q) assigned to four CH_3 groups, 97.49(d), 107.87(d), 121.68(d), 121.68(d), 128.77(d), 128.77(d) for 6CH carbons, signals at 129.85(s), 144.85(s), 155.25(s), 158.39(s), 160.73(s) assigned to five quaternary carbons, in addition to signals at 165.66(s, CO), 186.80(s, $COCH_3$). Also, the mass spectrum revealed a molecular ion peak at m/z = 391[M^-] for the molecular formula $C_{17}H_{20}N_4O_5S$. Similarly, when **1** was condensed with triethylorthoformate in refluxing acetic acid, afforded the ethoxymethelene derivative **14,** and establishing the structure **14** was based on the spectral data (IR, ^{1}H NMR, MS). The ^{1}H NMR spectrum showed, the presence of triplet signals at δ = 1.06 ppm assigned to ethoxy CH_3 protons, singlet signal at δ = 1.91 ppm for CH_3, singlet signal at δ = 2.30 ppm for $COCH_3$, quartet signals at δ = 3.36 ppm for ethoxy CH_2, singlet signal at δ = 6.15 ppm for isoxazole-H, multiplet signals at δ = 7.74-7.89 ppm assigned to aromatic

protons, singlet signal at δ = 8.65 ppm assigned to olefinic CH, singlet signals at δ = 12.11 and 12.37 ppm assigned to 2 NH. Its mass spectrum revealed the molecular ion peak at m/z = 392[M$^-$] for the molecular formula $C_{17}H_{18}N_3O_6S$.



Scheme 2

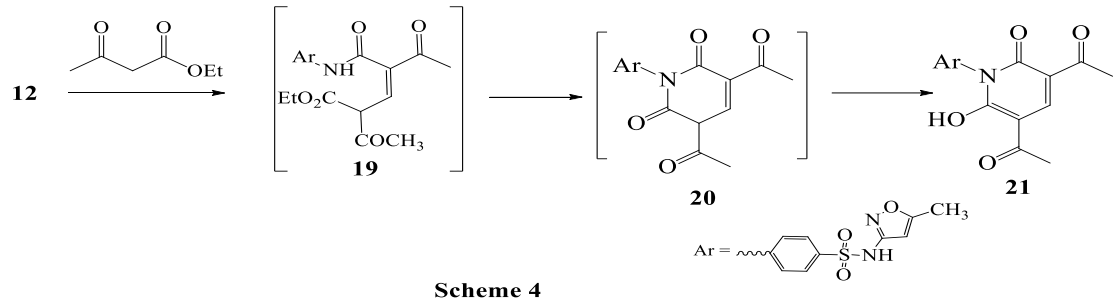
Enaminone derivative **12** is interesting for further chemical transformations. So, enaminone **12** was reacted with malononitrile in ethanolic piperidine to yield a product that may be formulated as structures **16** or **18**. Structure **18** was ruled out, and structure **16** was only considered to be the reaction product based on its spectral analysis. IR spectrum of compound **16** shows the presence bands of acetyl CO at υ = 1679 cm^{-1}, ring CO at υ = 1647 cm^{-1}, CN group at υ = 2213 cm^{-1}, and NH_2 at υ = 3202 and 3384 cm^{-1}. The ^{1}H NMR spectrum revealed a singlet signal at δ = 2.37 and 2.51ppm assigned for two CH_3,



Scheme 3

singlet signal at δ = 6.61 ppm confirmed to isoxazole-H, multiplet signals at δ = 6.63-8.18 ppm assigned for aromatic protons and NH_2 protons. Also, the mass spectrum revealed the molecular ion peak at m/z = 414[M$^+$] for the molecular formula $C_{18}H_{15}N_5O_5S$.

Formations of **16** *via* initial addition of active methylene in malononitrile to yield Michael adduct *via* elimination of dimethylamine, which is cyclized and aromatized to form **16**. Also, the behavior of enaminone **12** towards active methylene diketones was investigated. So, ethyl acetoacetate was reacted with enaminone **12** in an ethanolic piperidine solution to yield the expected pyridone derivative **21**. The assignment of structure **21** for the reaction product was based on its correct elemental analysis and spectroscopic data. The ^{1}H NMR spectrum showed a singlet signal at δ = 1.27, 2.39 and 3.28 ppm for one CH_3 and two $COCH_3$ protons, respectively. Singlet signal at δ 6.48 ppm for isoxazole-H, multiplet at δ = 7.50-8.80 ppm for aromatic protons, singlet signal at δ = 9.90 for pyridine-4*H*, singlet signal at δ = 11.60 for NH, and singlet signal at δ = 11.70 ppm for OH. Also, the MS spectrum revealed the molecular ion peak at m/z = 432[M]$^+$ corresponding to the molecular formula $C_{19}H_{17}N_3O_7S$. Compound **21** is assumed to be formed by condensing ethyl acetoacetate with enaminone **12** by elimination of dimethyl amine, which gives intermediate **19**, then cyclized *via* ethanol elimination to yield **20**, and rearrangement to aromatize to form pyridone derivative **21**.

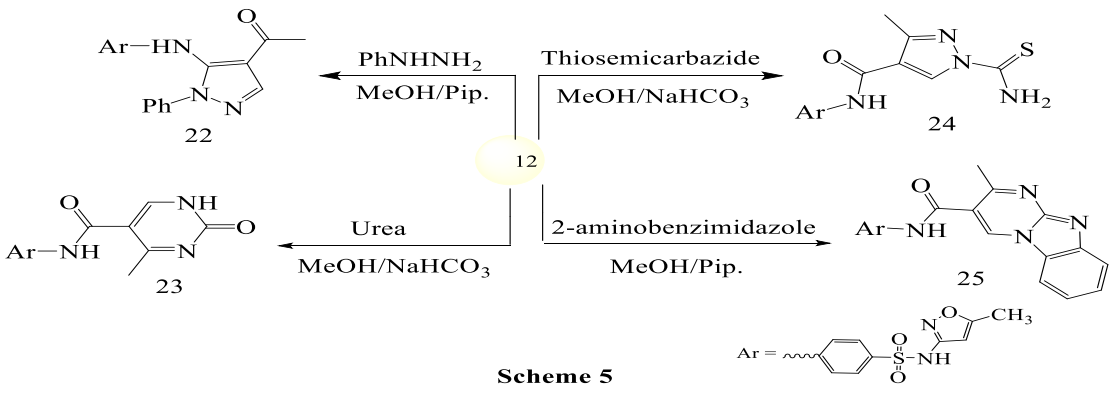


Scheme 4

Furthermore, the reaction between enaminone **12** and binucleophilic reagents was also investigated. Thus, enaminone **12** was reacted with phenyl hydrazine in an equimolar ratio to yield compound **22**. Establishing the exact structure of the reaction product based on its elemental and spectroscopic data. Thus, The IR spectrum of this reaction product showed among its absorption bands those corresponding to the presence amidic carbonyl at υ =1676 cm^{-1} and. The ^1H NMR spectrum showed a singlet signal at δ = 2.39 and 2.61 ppm for two CH_3 protons, singlet signal at δ = 6.48 ppm assigned to isoxazole-H, singlet signals at δ = 8.30 and 8.50 ppm assigned for two NH protons, beside the multiplet signals δ = 7.42-7.82 ppm corresponding to aromatic protons. ^{13}C NMR spectrum displayed signals at δ C = 12.04 (q), 12.56(q), 97.51(d), 119.30(d), 120.32(d), 125.78(d), 128.66(d), 129.04(d), 129.04(d), 129.76(d), 139.94(s), 140.00(s), 144.00(s), 147.00 (s), 161.00(s), 163.00(s), 172.00(s).

Also, enaminone **12** was treated with urea to afford pyrimidinone **23**. Establishing the structure of the product based on its spectroscopic data (IR, ^1H NMR, and elemental analysis). Thus, IR spectrum showed the presence of absorption bands of amidic carbonyl at υ =1642 cm^{-1}, besides all other function group assignments to the structure. The ^1H NMR exhibited the presence of singlet signal at δ = 2.40 and 2.52 ppm for two methyl groups, singlet signal at δ = 6.50 ppm assigned to isoxazole-H, multiplet signals at δ = 7.73 ppm for aromatic protons, doublet signals at δ = 8.67 ppm assigned to CH-pyrimidine, singlet signal at δ = 9.56 assigned to two NH groups and doublet signals at δ = 11.74 ppm assigned to pyrimidin-NH. MS spectrum showeded the molecular ion peak at m/z = 388[M^-] corresponding to the molecular formula $C_{16}H_{15}N_5O_5S$. However, a large no. of reports deals with the reactions of thiosemicarbazide with α-halo ketones [29] depending on the nature of the substituents both in the α-halo ketones and the thiosemicarbazide. Similarly, the reaction of enaminone **12** with thiosemicarbazide afforded the thiosemicarbazone which *in-situ* transferred under the reaction conditions to the pyrazole derivative **24.** Compound **24** established based on its correct spectral data. IR Spectrum showed a peak at υ = 1647.72 cm^{-1} for amidic carbonyl, NH_2 at υ= 3371.64, 3227.71 cm^{-1} beside the other function groups. The ^1H NMR spectrum showed singlet signals at δ 2.29 and 3.19 ppm for two CH_3 protons, singlet signals at δ = 6.55 ppm for isoxazole-H, multiplet signals at δ = 7.71-7.81 ppm for aromatic protons, singlet signal at δ = 8.98 ppm for pyrazole-H, broad signal at δ = 9.75-9.79 ppm for NH_2 and singlet signals at δ = 12.50 and 12.35 ppm for two NH groups. The ^{13}C NMR revealed δC = 12.45 (q), 26.37(q), 97.41(d), 97.41(d), 101.49(d), 119.93(s), 119.93(s), 128.84(d), 128.84(d), 129.56(s), 144.17(s), 161.00(s), 162.33(s), 162.33(s), 167.72(s), 197.11(s, CS).

On the other hand, the reaction of enaminone **12** with 2-aminobenzimidazole afforded the expected benzoimidazopyrimidine derivative **25.** Establishing the structure **25** based on spectral data, ^1H NMR showed the presence of singlet signals at δ 2.39 and 3.24 ppm for two CH_3 protons, singlet signal at δ 6.53 ppm for isoxazole-H, multiplet signals at δ 7.50-8.02 ppm for aromatic protons and pyrimidine-H, singlet signals at δ 8.97 and δ 11.30 ppm assigned to two NH groups. Also, the MS spectrum showed a molecular ion peak at m/z = 461[M^-] for the molecular formula $C_{22}H_{18}N_6O_4S$.



Scheme 5

3.2. Antioxidant activity

DPPH Antioxidant Activity

The antioxidant activity of the newly synthesized *N*-(5-methylisoxazol-3-yl)aryl-sulfonamide derivatives was assessed by a DPPH• free radical assay. Ascorbic acid was selected as a reference standard for comparison of results. The results shown in **Table 1** demonstrated that the estimated compounds, in general, revealed good antioxidant potency for the DPPH• free radical scavenging in the solution. In particular, compounds **1** (82.55% at 1.259 mg/mL), **2b** (71.11% at 0.266 mg/mL), **11a** (70.23% at 1.015 mg/mL), **11b** (86.07% at 0.37 mg/mL), **23** (71.11% at 1.167 mg/mL) and **24** (80.79% at 0.35 mg/mL) relative to the result of ascorbic acid (85.19% at 0.062 mg/mL). To facilitate the comparison of the antioxidant potency between the assessed compounds, IC_{50} values (mg/mL) were calculated by plotting the exponential curves for the sample concentrations versus the remaining DPPH•. It is worth mentioning that compounds **2a** (IC_{50}= 0.0373 mg/mL), and **2b** (IC_{50}= 0.0971 mg/mL) revealed the most potent antioxidant activities in comparison to ascorbic acid (IC_{50}= 0.0222 mg/mL) (**Figure 1**). Also, compounds **24** (IC_{50}= 0.1378 mg/mL), and **11b** (IC_{50}= 0.1807 mg/mL) presented good activities. Moderate activities were recorded by compounds **1**, **11a**, **12**, and **23,** with IC_{50} values ranging from 0.4083 to 0.6565 mg/mL. The lowest activity was noticed for compound **14** with IC_{50} values at 1.2833 mg/mL.

To study the mechanism of action for the reactions of the tested compounds with DPPH• radicals in the solution, the most active compound should produce stable free radicals to terminate the free radical reaction. Thus, the tested *N*-(5-methylisoxazol-3-yl)aryl-sulfonamide derivatives are rich sources of reactive atom species, for instance, sulfur, oxygen, and nitrogen, that provide stable free radicals. Based on the structure-activity relationships, the following points were documented:

(1) Incorporation of thiophene rings (integrated enaminonitrile, and enaminoester moieties) is crucial for potent antioxidant activities (compounds **2a**, and **2b**). (2) The introduction of six-membered rings such as pyrimidine, and triazine having thione, and dione groups (compounds **24**, and **11b**) improved the activities. (3) The incorporation of triazine rings, such as compounds **11a**, and **11b** is good for potent results, while the methyl group substituted at the phenyl ring, is preferred over the chlorine substituent on the phenyl ring which diminishes the activity.

(4) The introduction of dimethyl-aminomethylene, and ethoxymethylene scaffolds reduces the antioxidant activity (compounds **12**, and **14**). (5)

The transformation of *β*-diketone moiety (compound **1**) into the investigated five- and six-membered heterocycles is critical for enhanced antioxidant activities.

Table 1. The antioxidant results (% remaining DPPH, % scavenging activity, and IC_{50} (mg/mL)) of the investigated samples.

Sample	Concentrations (mg/ml)	% Remaining DPPH	% Scavenging activity	IC_{50} (mg/mL)
1	1.259	17.45	82.55	0.4083
	0.63	40.47	59.53	
	0.315	54.25	45.75	
	0.157	67.45	32.55	
2a	0.061	42.38	57.62	0.0373
	0.031	46.63	53.37	
	0.015	59.24	40.76	
	0.008	74.05	25.95	
2b	0.266	28.89	71.11	0.0971
	0.133	35.19	64.81	
	0.066	57.04	42.96	
	0.033	71.11	28.89	
11a	1.015	29.77	70.23	0.5455
	0.507	49.27	50.73	
	0.254	69.5	30.5	
	0.127	84.9	15.1	
11b	0.37	13.93	86.07	0.1807
	0.185	67.89	32.11	
	0.092	87.39	12.61	
	0.046	90.18	9.824	
12	0.983	42.82	57.18	0.6565
	0.492	48.24	51.76	

	0.246	63.49	36.51	
	0.123	81.67	18.33	
14	2.9	46.19	53.81	1.2833
	1.45	65.98	34.02	
	0.725	77.71	22.29	
	0.363	89.44	10.56	
23	1.167	28.89	71.11	0.4951
	0.583	44.72	55.28	
	0.292	58.8	41.2	
	0.146	69.06	30.94	
24	0.35	19.21	80.79	0.1378
	0.175	43.4	56.6	
	0.088	59.24	40.76	
	0.044	78.89	21.11	
Ascorbic acid	0.062	15.267	85.19	0.0222
	0.031	39.084	62.07	
	0.016	61.069	40.74	
	0.008	74.809	27.41	

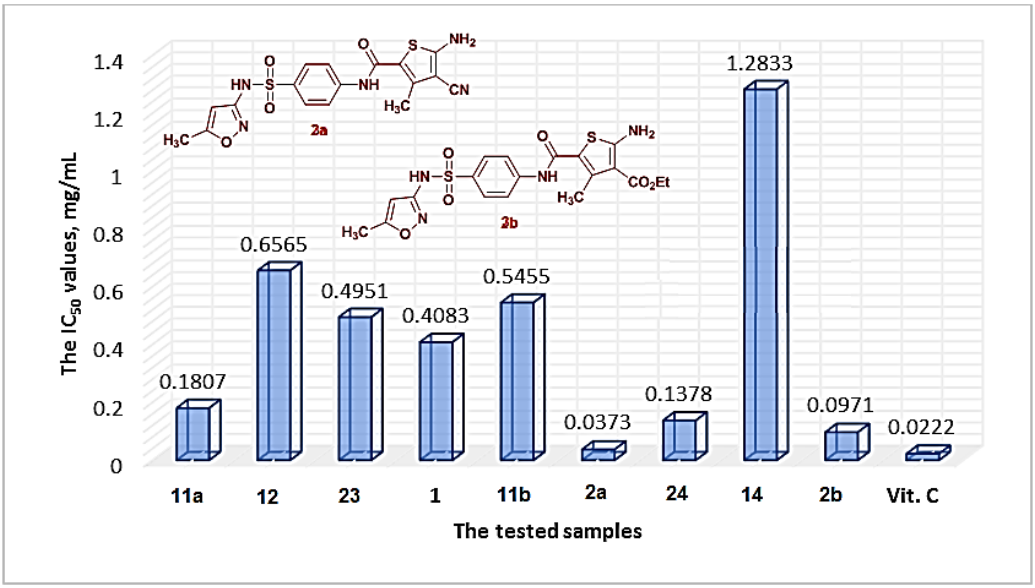


Fig. 1. Comparison of the antioxidant results expressed as IC_{50} in mg/mL of the tested samples relative to the antioxidant standard.

3.3. Antimicrobial activity

Antimicrobial activity was performed at the Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Assiut, Egypt. Test samples and reference standards (positive controls) were evaluated *in vitro* for their antibacterial activity by the well diffusion method, against two Gram-positive bacteria (*B. subtilis* and *S. aureus*) and two Gram-negative bacteria (*E. coli* and *K. pneumonia*), while antifungal activity was performed against *C. albicans*.

According to the results in **Table 2**, compounds **1**, **12**, and **16** showed a wide spectrum of highly antibacterial activity against *E. coli, K. pneumonia*, and *C. albicans*, while compounds **12**, **25**, and **22** had no antibacterial activity against *B. subtilis.* Compound **1** displayed the highest activity against *B. subtilis* with an inhibition zone of 32.67 mm as compared to the control. Moreover, compound **1** was the only inhibitor of *S. aureus* with a 12.67 mm inhibition zone, while the rest of the tested compounds showed no activity against *S. aureus*. In general, the investigated compounds that presented antimicrobial activity showed fluctuating results in comparison to positive control.

Table 2. Antimicrobial activity of the tested sample (1% w/v) against tested bacteria and *Candida* expressed as inhibition diameter zones in millimeters (mm) using the well-diffusion method.

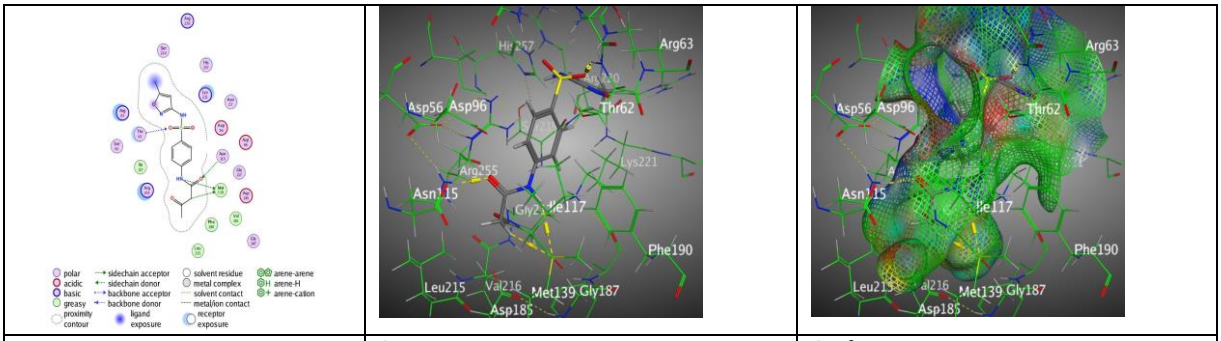
Sample	The mean diameter of the inhibition zone (Mean±SD) (mm)				
	B. subtilis	***S. aureus***	***E. coli***	***K. pneumonia***	***C. albicans***
1	32.67±2.52^{a}	12.67±0.58^{b}	12.00±0.00^{bc}	13.67±0.58^{b}	9.67±0.58^{e}
12	0.00±0.00	0.00±0.00	13.00±1.00^{b}	13.33±0.58^{bc}	9.00±0.00^{e}
16	12.67±0.58^{d}	0.00±0.00	11.67±0.58^{cd}	13.00±0.00^{bc}	12.33±0.58^{cd}
21	16.00±1.00^{c}	0.00±0.00	11.33±0.58^{cd}	12.33±0.58^{bc}	13.00±0.00^{bc}
22	0.00±0.00	0.00±0.00	9.33±0.58^{e}	11.00±0.00^{de}	14.00±0.00^{b}
23	15.33±0.58^{c}	0.00±0.00	12.33±0.58^{bc}	10.67±0.58^{e}	12.33±0.58^{cd}
24	11.67±058^{d}	0.00±0.00	11.33±0.58^{cd}	13.67±1.15^{b}	11.67±0.58^{d}
25	0.00±0.00	0.00±0.00	10.67±0.58^{d}	12.00±1.00^{cd}	10.00±1.00^{e}
Chloramphenicol	18.33±0.58^{b}	14.33±0.58^{a}	15.33±0.58^{a}	16.00±1.00^{a}	-
Clotrimazole	-	-	-	-	18.67±0.58^{a}

(-): Not detected.

The data given as averages of three replicates (Mean±S*D*). Values followed by the different letters are significantly different at $p < 0.05$.

3.4. Molecular docking

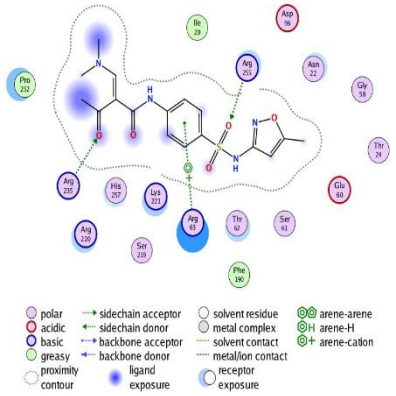
Our newly synthesized sulfamethoxazole analogs displayed potent activity against *E. coli*. The findings of molecular docking using the M.O.E. "v10.2015.10" program on the sulfamethoxazole analogs **1, 12, 16, 21, 22, 23, 24** and **25** are shown in Table 2. Developing hypotheses on the sulphonamide derivatives demonstrated good binding effects on the PDB: 1AJ0 protein of DHPS (dihydropteroate synthase enzyme of *E. coli*) [30]. Sulfamethoxazole analog **1** has a binding energy of -6.7788 Kcal/mol and forms multiple interactions with the protein. Specifically, it involves two H-donor interactions with N 17 of the amide moiety C19 of the methylene group and Met 139 of the 1AJ0 protein. Additionally, **1** participates in two H-acceptor interactions with O 9 of the sulphonamide group O 21 of the amide moiety, and Thr 62 and Asn 115 of the protein. These interactions are within proximity, with distances ranging from 3.02 to 4.04 Å. The strong binding energy and the presence of both H-donor and H-acceptor interactions suggest that **1** forms a stable complex, as seen in (figure 2).

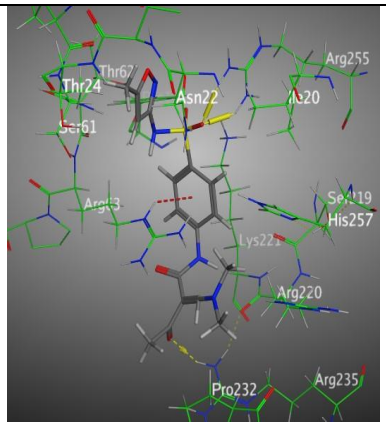


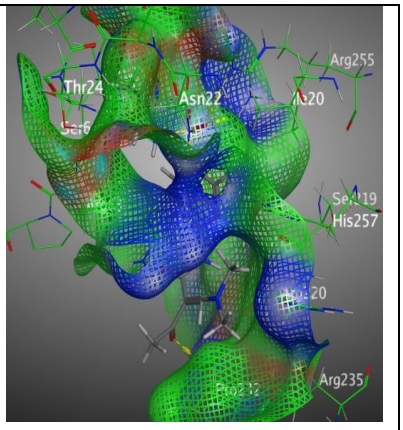
2D	3D	Surface Map

Fig. 2. Images of interactions for 1 with (PDB: 1aj0).

Meanwhile, sulfamethoxazole analog **12** has a binding energy of -6.0713 Kcal/mol and forms interactions with the protein that include two H-acceptor interactions with O 10 of the sulphonamide moiety, and O 23 of the ketone group, and Arg 255 and Arg 235, respectively. It also forms a π-cation interaction with a phenyl ring on the ligand and Asn 11 of the PDB: 1aj0 protein. These interactions are relatively close, with distances ranging from 2.89 to 3.99 Å. (Figure 3).



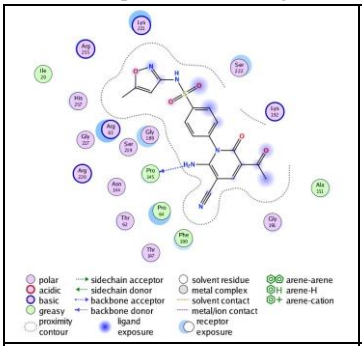


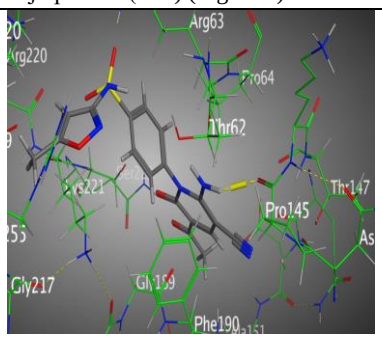


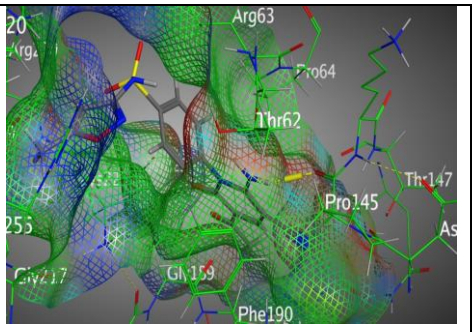
2D	3D	Surface Map

Fig. 3. Images of interactions for **12** with (PDB: 1aj0).

Similarly, Sulfamethoxazole analog **16** presented a binding energy of -7.3789 Kcal/mol and formed a single H-donor interaction with N 23 of the amino group and Pro 145 of the protein. This interaction has a distance of 3.20 Å. While 16 exhibits a lower binding energy compared to some other compounds, the specific H-donor interaction suggests that it forms a stable complex with the target PDB: 1aj0 protein (π-H) (Figure 4).



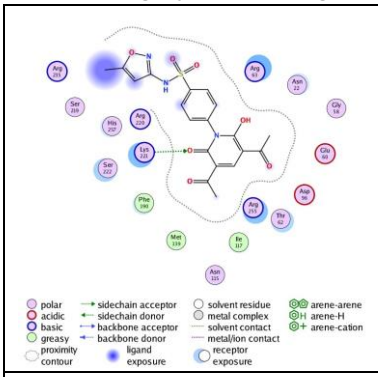


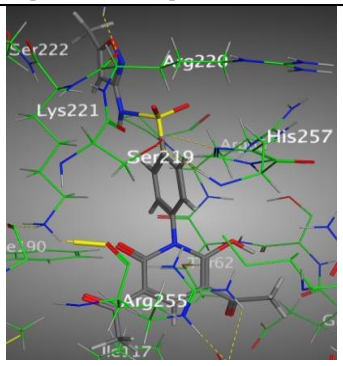


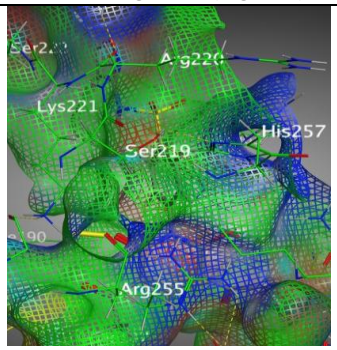
2D	3D	Surface Map

Fig. 4. Images of interactions for **16** with (PDB: 1aj0).

Also, Sulfamethoxazole analog **21** revealed a binding energy of -7.9464 Kcal/mol and formed a single H-acceptor interaction with O 24 of the pyridone ring and Lys 221 of the protein. The distance of this interaction is 3.00 Å. While **21** exhibits a slightly lower binding energy, the specific H-acceptor interaction suggests a stable binding mode (Figure 5).



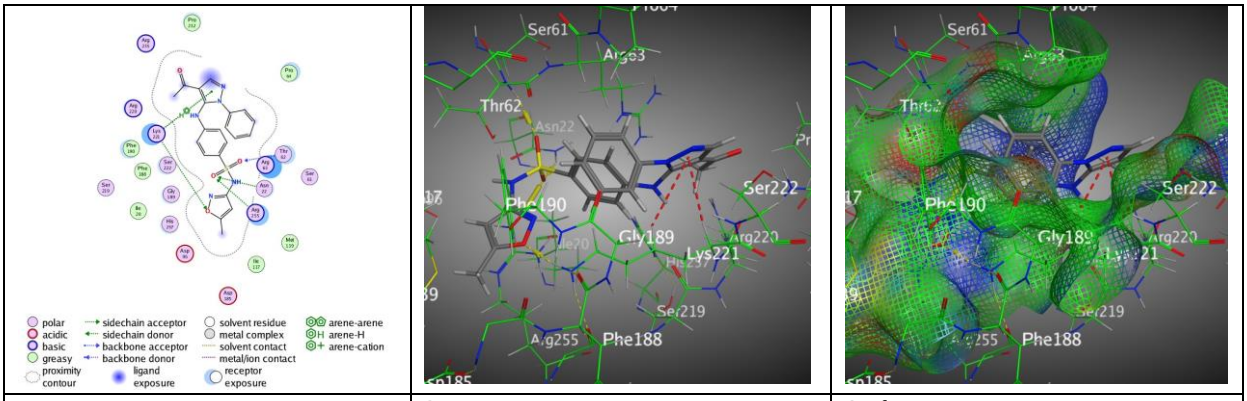




2D	3D	Surface Map

Fig. 5. Images of interactions for **21** with (PDB: 1aj0).

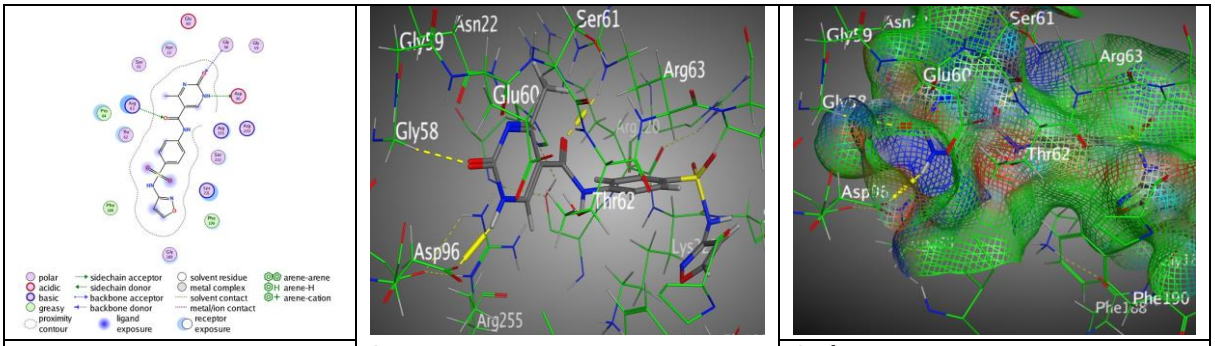
Moreover, Sulfamethoxazole analog **22** exhibited a binding energy of -7.8084 Kcal/mol and formed various interactions, including H-acceptor interactions with O 8 and O 9 of the sulphonamide moiety and isoxazole ring with Asn 22, Arg 255, Thr 62, and Lys 221 of the protein. It also forms a π-H interaction with a pyrazole rings and Lys 221 of the protein. These interactions have distances ranging from 2.87 to 4.37 Å. **22**'s binding energy and the diversity of interactions suggest it has a strong binding affinity for the target protein. (Figure 6).



2D	3D	Surface Map

Fig. 6. Images of interactions for **22** with (PDB: 1aj0).

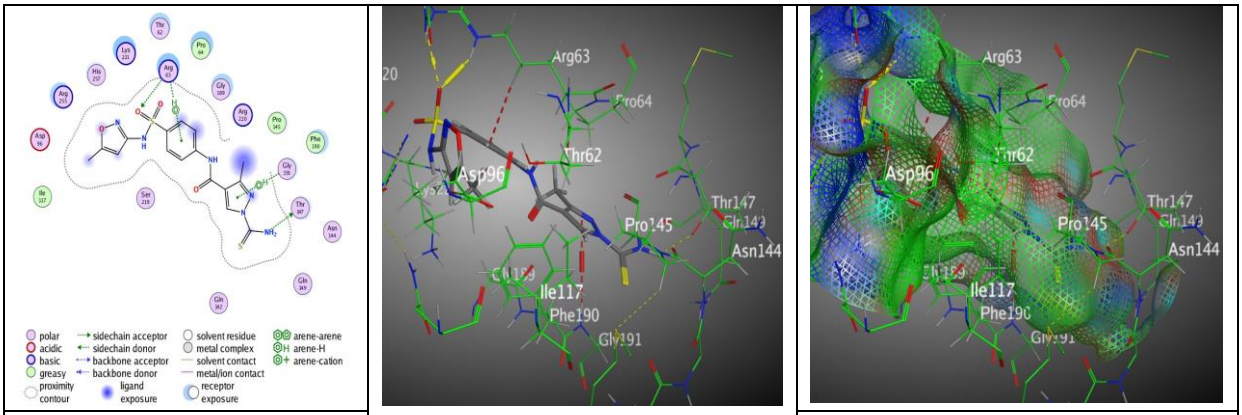
Additionally, Sulfamethoxazole analog **23** showed a binding energy of -7.3108 Kcal/mol and formed multiple interactions, including an H-donor interaction with N 23 of the pyrimidine ring and Asp 96, as well as H-acceptor interactions with O 19 of the amide moiety and O 25 of the pyrimidine ring and Arg 63 and Gly 58, respectively. These interactions have distances ranging from 3.06 to 3.46 Å (Figure 7). The moderate binding energy and the presence of H-donor and H-acceptor interactions suggest that **23** may have a stable binding mode.



2D	3D	Surface Map

Fig. 7. Images of interactions for **23** with (PDB: 1aj0).

Furthermore, Sulfamethoxazole analog **24** exhibited a binding energy of -7.3832 Kcal/mol and formed multiple interactions, including an H-donor interaction with N 27 of the thioamide end and Thr 147 of the protein, an H-acceptor interaction with O 8 of the sulphonamide moiety and Arg 63 of the protein, and π-H interactions with a pyrazole ring and benzene ring on the ligand and Arg 63 and Gly 191 of the PDB: 1aj0 protein. These interactions have distances ranging from 3.15 to 4.51 Å. **24** exhibiting strong binding energy and a variety of interactions, suggesting a stable binding mode (Figure 8).

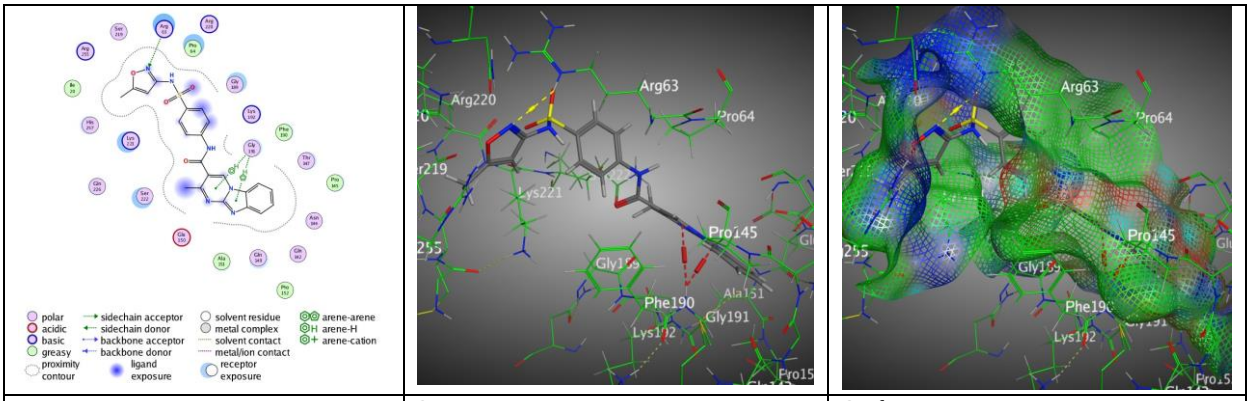


2D	3D	Surface Map

Fig. 8 Images of interactions for **24** with (PDB: 1aj0).

Likewise, Sulfamethoxazole analog **25** demonstrated the highest binding energy in the dataset, with a value of -8.0039 Kcal/mol. It forms multiple interactions, including an H-acceptor interaction with N 15 of the isoxazole ring and Arg 63 of the

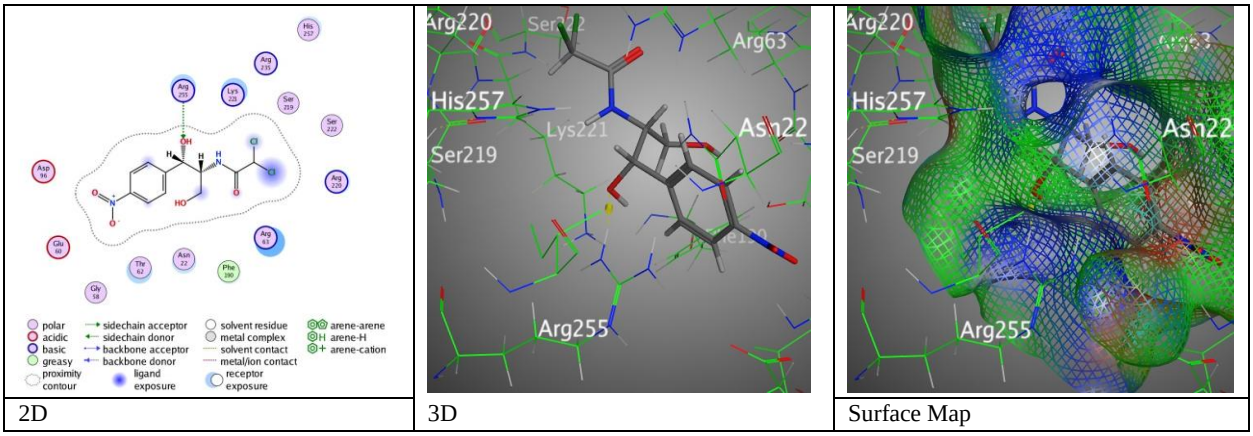
protein, as well as π-H interactions with the pyrazolpyridine ring on the ligand and Gly 191 of the protein. These interactions have distances ranging from 3.56 to 4.76 Å (Figure 9).



2D	3D	Surface Map

Fig. 9. Images of interactions for **25** with (PDB: 1aj0).

Finally, Chloramphenicol demonstrated a binding energy of -6.0759 Kcal/mol and formed a single H-acceptor interaction with O 20 of the hydroxyl group and Arg 255 of the protein. The distance of this interaction is 3.00 Å. While Chloramphenicol exhibits a reasonable binding energy, it forms only one type of interaction (Figure 10).



2D	3D	Surface Map

Fig. 10. Images of interactions for **Chloramphenicol** with (PDB: 1aj0).

4. Conflicts of interest

The authors declare no conflict of interest.

5. Acknowledgments

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6. Disclosure statement

No potential conflict of interest was reported by the author(s).

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