Ministry of Higher Education

and Scientific Research

Journal of Kufa for Chemical Sciences

A refereed

Research Journal Chemical Sciences

Vol.2 No.9 Year 2022

ISSN 2077-2351

Synthesis, Biological Activity, and Molecular Docking Studied of New Substituted Hydrazones

Nabel B. Ayrim[*a], Asim A. Balakit[b] and Souad J. Lafta[a]

[a]Department of Chemistry, College of Science, Mustansiriyah University, Baghdad-Iraq.

[b]College of Pharmacy, University of Babylon, Babylon, Iraq.

***Corresponding author, e-mail: nabelbunyan@uomustansiriyah.edu.iq.; (Nabel Bunyan Ayrim)**

Abstract

Series of new hydrazones derived from different carboxylic acid hydrazides and aromatic aldehydes (**7-10**, **20-25**) have been synthesized and characterized by spectral techniques (FT-IR, ^{1}H NMR, ^{13}C NMR, and Mass spectrometry). The target compounds are designed to have the different substituents at the para position for both parts of the structures (hydrazide and aldehyde parts. The synthesized compounds have been evaluated as antimicrobial agents by studying their biological activity against two bacteria strains *Staphylococcus aureus* and *Staphylococcus epidermidis* (as gram-positive bacteria), *Escherichia coli* and *Klebsiella sp* (as gram-negative bacteria), the antifungal activity was studied against *Candida albicans* fungi. The obtained results showed a moderate antimicrobial activity for the test compounds when compared with ampicillin. The synthesized compounds were also subjected to molecular docking studies which were carried out against the bacteria strain *Staphylococcus aureus* DHFR.

Keyword: hydrazones; antibacterial; antifungal; molecular docking.

1. Introduction

Hydrazones are a class of organic compounds that have azomethine –NHN=CH group in their structures, this group is formed by the condensation reaction between aldehyde or ketone with carboxylic acid hydrazide. [1]. This class of compounds has attracted the attention of researchers, especially in the field of medicinal chemistry. It is found in many drugs like Thiacetazone and Nifuroxazide (INN) due to its potential biological activities. [2]. Different

hydrazones are reported as effective drugs with a low level of toxicity [3]. The reported hydrazones show a broad spectrum of biological activities such as anticonvulsant, analgesic, antidepressant, anti-inflammatory, antimycobacterial, antimalarial, antiplatelet,
antimicrobial, anticancer, anti-HIV, anthelmintic, vasodilator, antiviral, antidiabetic, and trypanocidal activities [4-12]. Hydrazones are also used as precursors for the synthesis of important heterocyclic compounds [13]. Drug resistance is a phenomenon in which microorganisms resist the influence of the drug molecules especially antibiotics and antifungal agents as a consequence of abuse and extensive use of medications [14]. To tackle these serious problems new organic compounds have been developed to act as new antibacterial and antifungal agents [15], Hydrazones are among these compounds due to their potential activities and easy synthesis protocols [16]. Hydrazone derivatives have also been reported as potent antioxidants due to their ability to interact with the free radicals and prevent their damaging effect on the cells [17], based on that hydrazone derivatives can act as effective molecules for the treatment of diseases which are related to the oxidative stress conditions such as cardiovascular disorders, cancer, atherosclerosis, proteins and DNA damage caused by reactive oxygen species [18,19].
In the present study, we report the synthesis of a series of hydrazones derived from different benzoic acid hydrazides and aromatic aldehydes. The synthesized molecules were subjected to molecular docking studies to predict the ability to bind with a specific site in the enzyme dihydrofolate reductase (DHFR) to evaluate its biological activity based on that. The antimicrobial activity of the target compounds was also studied against different bacterial strains of bacteria and fungi.

2. Experimental

2.1 General

The used chemicals were obtained from Sigma Aldrich. Melting points were recorded by using the Gallen-Kamp MFB-600 melting point apparatus. The FT-IR spectra were recorded on an FT-IR-8400S-Shimadzu spectrophotometer. Mass spectra were recorded on Shimadzu model GCMSQP 1000 EX spectrometer (Japan). NMR spectra were recorded on VARIAN-INOVA 500 MHZ spectrophotometer (Germany), deuterated solvents (DMSO-d_6 and $CDCl_3$) were used for sample preparation, and tetramethylsilane TMS was used as an internal standard.

2.2: Synthesis of 4-methoxy benzoic acid (2)

In 3-neck round bottom flask, sodium hydroxide 8.0 gm was dissolved in water 50 ml, the solution was cooled down, 4-hydroxy benzoic acid (**1**: 5.33 gm, 0.035 mol) was added and the flask was sealed with a stopper. The mixture was stirred until turned clear. Dimethyl sulfate (6.70 ml, 0.07 mol) was added drop wise to the solution and with stirring for 20 min at (30-35) ºC, the stopper was removed occasionally to release the generated gas. Another portion of dimethyl sulfate (6.70 ml, 0.07 mol) was added gradually to the solution and stirred for 10 min, the temperature was then raised and kept at (30-35) ºC. The mixture was heated up for 2 h after the second addition. A solution of sodium hydroxide (2 gm in 3 ml of water) was added to the mixture and refluxed for 2 h. Then, the reaction mixture was cooled to room temperature and acidified with 5% dilute sulfuric acid, the precipitated solid was filtrated, washed with cold water, and recrystallized from ethanol to give 4-methoxy benzoic acid (**2**) as a white crystalline material [20]. Yield (72%); M.P: 180-183 ºC; *Rf* = 0.83 (hexane : ethyl acetate ; 3:1); M.F: $C_8H_8O_3$; M.W: 152.15; FT-IR (cm^{-1}): 3208-2555 (-OH), 3028 (C-H, aromatic), 2982-2839 (C-H, aliphatic), 1681 (C=O), 1602 (C=C), 1427 (CH_3, bending), 1259 (C-O).

2.3: General procedure for the synthesis of methyl benzoate derivatives (3-4, 14-16)

To the solution of the compounds (**3-4, 14-16**: 0.04 mol in 50 ml methanol) a catalytic amount of sulfuric acid was added, the mixture was refluxed for 8 h and then cooled down to room temperature. Methanol was evaporated under reduced pressure. The crude product was poured into an aqueous solution of $NaHCO_3$ (5%, 40 ml) and extracted with ethyl acetate (2×20 ml). The organic layers were combined, the excess of solvent was dried with anhydrous magnesium sulfate and the mixture was filtrated, then the solvent was evaporated the solid precipitate was washed with cold water and dried to give desired ester [21].

2.3.1: Methyl 4-methoxybenzoate (3):

Product, white crystalline solid; yield: 85%; M.P: 49-52 ºC; *Rf* = 0.93 (hexane : ethyl acetate; 3:2),; M.F: $C_9H_{10}O_3$; M.W: 166.18; FT-IR (cm^{-1}): 1705 (C=O, ester), 3080-3009 (C-H, aromatic), 2915-2843 (C-H, aliphatic), 1606 (C=C, aromatic).

2.3.2: Methyl 4-hydroxybenzoate (4):

Product, yellow crystalline solid; yield: 79%; M.P: 127-130 ºC; *Rf* = 0.35 (hexane : ethyl acetate; 4:1); M.F: $C_8H_8O_3$; M.W: 152.15; FT-IR (cm^{-1}): 1678 (C=O, ester), 3296 (OH, phenolic), 3047-3030 (C-H, aromatic), 2962 (C-H, aliphatic), 1604 (C=C, aromatic).

2.3.3: Methyl benzoate (14):

Product, light liquid; yield: 75%; *Rf* = 0.63 (hexane : ethyl acetate; 3:2); M.F: $C_8H_8O_2$; M.W: 136.15; FT-IR (cm^{-1}): 1718 (C=O, ester), 3063-3034 (C-H, aromatic), 2951-2843 (C-H, aliphatic), 1600 (C=C, aromatic).

2.3.4: Methyl 4-methylbenzoate (15):

Product, light liquid; yield: 85; *Rf* = 0.72 (hexane : ethyl acetate; 2:3); M.F: $C_9H_{10}O_2$; M.W: 150.18; FT-IR (cm^{-1}): 1716 (C=O, ester), 3032 (C-H, aromatic), 2951-2843 (C-H, aliphatic), 1614 (C=C, aromatic), 1435 (CH_3, bending).

2.3.5: Methyl 4-chlorobenzoate (16):

Product, light-yellow crystalline solid; yield: 78%; M.P: 41-44 ºC; *Rf* = 0.86 (hexane : ethyl acetate; 3:2); M.F: $C_8H_7ClO_2$; M.W: 170.59; FT-IR (cm^{-1}): 1718 (C=O, ester), 3059 (C-H, aromatic), 2953-2852 (C-H, aliphatic), 1595 (C=C, aromatic), 852 (C-Cl).

2.4: General procedure for the synthesis of acid hydrazide derivatives of (5-6,17-19):

Compounds (**5-6,17-19**: 0.02 mol) were dissolved in absolute ethanol 30 ml, hydrazine hydrate (80%, 6 ml) was added and heated to reflux for 8 hr. The mixture was left to cool down; the crude product was collected by filtration, washed with cold water, dried, and recrystallized from ethanol to give a white crystalline of acids hydrazide [22].

2.4.1: 4-Methoxybenzohydrazide (5):

Yield: (79%), M.P: 138-140 ºC; *Rf* = 0.28 (hexane : ethyl acetate; 3:2); M.F: $C_8H_{10}N_2O_2$; M.W: 166.18; FT-IR (cm^{-1}): 3323 (NH_2), 3205 (NH), 3057-3003 (C-H, aromatic), 2960-2839 (C-H, aliphatic), 1618 (C=O, amide), 1606 (NH_2, bending), 1575 (C=C, aromatic).

2.4.2: 4-hydroxybenzohydrazide (6):

Yield (79%), M.P: 274-277 ºC; *Rf* = 0.65 (hexane : ethyl acetate; 3:2); M.F: $C_7H_8N_2O_2$; M.W: 152.15; FT-IR(cm^{-1}) : 3271 (NH_2), 3196 (NH), 3315 (OH, phenolic), 3003 (C-H, aromatic), 2958-2812 (C-H, aliphatic), 1616 (C=O, amide), 1589 (NH_2, bending), 1535 (C=C, aromatic).

2.4.3: Benzohydrazide (17):

Yield: (65%), M.P: 113-116 ºC; *Rf* = 0.63 (hexane : ethyl acetate; 3:2); M.F: $C_7H_8N_2O$; M.W: 136.15; FT-IR (cm^{-1}): 3298-3250 (NH_2), 3194 (NH), 3049 (C-H, aromatic), 2978-2870 (C-H, aliphatic), 1658 (C=O, amide), 1612 (NH_2, bending), 1556 (C=C, aromatic).

2.4.4: 4-Methylbenzohydrazide (18):

Yield (85%), M.P: 110-113 ºC; *Rf* = 0.31 (hexane : ethyl acetate; 3:2); M.F: $C_8H_{10}N_2O$; M.W: 150.18; FT-IR (cm^{-1}): 3304-3223 (NH_2), 3188 (NH), 3022 (C-H, aromatic), 2918-2850 (C-H, aliphatic), 1660 (C=O, amide), 1612 (NH_2, bending), 1575 (C=C, aromatic), 1344 (CH_3, bending).

2.4.5: 4-Chlorobenzohydrazide (19):

Yield (82%), M.P: 166-169 ºC; *Rf* = 0.43 (hexane : ethyl acetate; 4:1); M.F: $C_7H_7ClN_2O$; M.W: 170.60; FT-IR(cm^{-1}): 3309-3205 (NH_2), 3196 (NH), 3009 (C-H, aromatic), 2874 (C-H, aliphatic), 1660 (C=O, amide), 1614 (NH_2, bending), 1565 (C=C, aromatic), 839 (CH-Cl).

2.5: General procedure for the synthesis of hydrazone derivatives (7-10, 20-25)

Substituted acids hydrazide (**7-10**,**20-25**: 4 mmol) was dissolved in 40 ml of absolute ethanol. The appropriate aromatic aldehyde (4.4 mmol) was added with a few drops of glacial acetic acid, the mixture was heated under reflux for 6 h, after that the mixture was cooled down and the precipitated product was filtrated, washed with cold water, dried, and recrystallized from ethanol [23].

2.5.1: 4-Methoxy-*N'*-(4-methoxybenzylidene)benzohydrazide (7):

Product, white solid, yield: 81%, M.P: 178-181 ºC; *Rf* = 0.58 (hexane : ethyl acetate; 3:2) M.F: $C_{16}H_{16}N_2O_3$; M.W: 284.32; FT-IR(cm^{-1}) : 3211 (NH), 3066-3016 (C-H, aromatic), 2970-2816 (C-H, aliphatic), 1651 (C=O, amide), 1604 (N=CH, imine), 1575 (C=C, aromatic), 1350 (CH_3, bending). ^{1}H NMR (500 MHz, DMSO-d_6 ,δ ppm): 11.60 (s, 1H, NH), 8.40 (s, 1H, N=CH), 7.92 (d, *J* = 7.1 Hz, 2H, aromatic), 7.68 (d, *J* = 8.2 Hz, 2H, aromatic), 7.05 (d, *J* = 8.5 Hz, 2H, aromatic), 7.03 (d, *J* = 8.3 Hz, 2H, aromatic), 3.89–3.78 (s, 6H, 2 *para*-OCH_3). ^{13}C NMR (126 MHz, DMSO-d_6 ,δ ppm): 162.84 (C=O, amide), 147.56 (C=N), 162.40-161.21 (2C), 129.92 (2C), 129.06 (2C), 127.50 (1C), 126.09 (1C), 114.81 (2C), 114.16 (2C), (12C, aromatic), 55.89-55.77 (2 *para*-OCH_3). MS-EI (m/z, %): 284 (M^+, 100), 269 (18), 150 (75).

2.5.2: 4-Methoxy-*N'*-(3,4,5-trimethoxybenzylidene)benzohydrazide (8):

Product, light yellow, yield: 80%, M.P: 200-202 ºC; *Rf* = 0.3 (hexane : ethyl acetate; 3:2); M.F: $C_{18}H_{20}N_2O_5$; M.W: 344.37; FT-IR (cm^{-1}): 3223 (NH), 3070-3003 (C-H, aromatic), 2974-2839 (C-H, aliphatic), 1639 (C=O, amide), 1604 (N=CH, imine), 1575 (C=C, aromatic), 1463-1332 (CH_3, bending). ^{1}H NMR (500 MHz, DMSO-d_6 ,δ ppm): 11.73 (s, 1H, NH), 8.40 (s, 1H, N=CH), 7.92 (d, *J* = 2.2 Hz, 2H, aromatic), 7.07 (d, *J* = 8.4 Hz, 2H, aromatic), 7.02 (s, 2H, aromatic), 3.85-3.81 (s, 6H, 2 *meta*-OCH_3), 3.72 (s, 3H, *para*-OCH_3), 3.35 (s, 3H, *para*-OCH_3). ^{13}C NMR (126 MHz, DMSO-d_6 ,δ ppm): 163.04 (C=O, amide) , 147.72 (C=N), 162.47 (1C), 153.67 (2C), 139.61 (1C), 130.47 (1C), 130.01 (2C), 125.98 (1C), 114.20 (2C), 104.69 (2C), (12C, aromatic), 60.59 (*para*-OCH_3), 56.42 (2 *meta*-OCH_3) , 55.90 (*para*-OCH_3). MS-EI (m/z, %): 344 (M^+, 41), 329 (22), 150 (100).

2.5.3: 4-Hydroxy-*N′*-(4-methoxybenzylidene) benzohydrazide (9):

Product, white solid, yield: 65%, M.P: 235-238 ºC; *Rf* = 0.4 (hexane : ethyl acetate; 3:2); M.F: $C_{15}H_{14}N_2O_3$; M.W: 270.29; FT-IR (cm^{-1}): 3352 (OH), 3217 (NH), 3012 (C-H, aromatic), 2960-2837 (C-H, aliphatic), 1647 (C=O, amide), 1604 (N=CH, imine), 1587 (C=C, aromatic), 1365 (CH_3, bending). ^{1}H NMR (500 MHz, DMSO-d_6 ,δ ppm): 11.53 (s, 1H, NH), 10.11 (s, 1H, OH), 8.39 (s, 1H, N=CH), 7.82 (d, *J* = 8.6 Hz, 2H, aromatic), 7.66 (d, *J* = 8.1 Hz, 2H, aromatic), 7.02 (d, *J* = 8.3 Hz, 2H, aromatic), 6.88 (d, *J* = 3.0 Hz, 2H), 3.81(s, 3H, *para*-OCH_3). ^{13}C NMR (126 MHz, DMSO-d_6 ,δ ppm): 163.11 (C=O, amide), 147.26 (C=N), 161.16 – 161.07 (2C), 130.06 (2C), 129.01 (2C), 127.57 (1C), 124.52 (1C), 115.47 (2C), 114.79 (2C), (12C, aromatic), 55.75 (*para*-OCH_3). MS-EI (m/z, %): 270 (M^+, 100), 254 (15), 154 (45), 136 (80), 120 (20).

2.5.4: 4-Hydroxy-*N′*-(3,4,5-trimethoxybenzylidene) benzohydrazide (10):

Product, white solid, yield: 76%, M.P: 268-271 ºC; *Rf* = 0.33 (hexane : ethyl acetate; 4:1); M.F: $C_{17}H_{18}N_2O_5$; M.W: 330.34; FT-IR (cm^{-1}): 3279 (OH), 3107 (NH), 3030 (C-H, aromatic), 2949-2839 (C-H, aliphatic), 1639 (C=O, amide), 1604 (N=CH, imine), 1575 (C=C, aromatic), 1415-1321 (CH_3, bending). ^{1}H NMR (500 MHz, DMSO-d_6 ,δ ppm): 11.65 (s, 1H, NH), 10.13 (s, 1H, OH), 8.38 (s, 1H, N=CH), 7.82 (d, *J* = 8.2 Hz, 2H, aromatic), 7.01 (s, 2H, aromatic), 6.88 (d, *J* = 8.3 Hz, 2H, aromatic), 3.72 (s, 6H, 2 *meta*-OCH_3), 3.37 (s, 3H, *para*-OCH_3). ^{13}C NMR (126 MHz, DMSO-d_6 ,δ ppm): 163.28 (C=O, amide), 147.40 (C=N), 161.15 (1C), 153.66 (2C), 139.55 (1C), 130.53 (1C), 130.15-130.13 (2C), 124.40 (1C), 115.49 (2C), 104.64 (2C), (12C, aromatic), 60.59 (*para*-OCH_3), 56.40 (2 *meta*-OCH_3). MS-EI (m/z, %): 330 (M^+, 45), 270 (25), 154 (32), 136 (100), 93 (15).

2.5.5: *N′*-(4-Methoxybenzylidene)benzohydrazide (20):

Product, white solid, yield: 79%, M.P: 155-158 ºC; *Rf* = 0.72 (hexane : ethyl acetate; 2:3); M.F: $C_{15}H_{14}N_2O_2$; M.W: 254.29; FT-IR (cm^{-1}): 3209 (NH), 3024-3001 (C-H, aromatic), 2976-2839 (C-H, aliphatic), 1639 (C=O, amide), 1600 (N=CH, imine), 1573 (C=C, aromatic), 1359 (CH_3, bending); ^{1}H NMR (500 MHz, $CDCl_3$,δ ppm): 9.91 (s, 1H, NH), 8.34 (s, 1H, N=CH), 7.90 (d, *J* = 7.6 Hz, 2H, aromatic), 7.67 (d, *J* = 8.2 Hz, 2H, aromatic), 7.52 (t, *J* = 7.5 Hz, 1H, aromatic), 7.43 (t, *J* = 7.7 Hz, 2H, aromatic), 6.87 (d, *J* = 8.3 Hz, 2H, aromatic), 3.83 (s, 3H, OCH_3). ^{13}C NMR (126 MHz, $CDCl_3$,δ ppm): 164.31 (C=O, amide), 148.70 (C=N), 161.55 (1C), 133.41 (1C), 131.88 (1C), 129.44 – 129.40 (2C), 128.68 (2C), 127.39 (2C), 126.34 (1C), 114.15 (2C), (12C, aromatic), 55.36 (OCH_3). MS-EI (m/z, %): 254 (M^+, 100), 239 (15), 154 (35), 120 (83).

2.5.6: *N'*-(3,4,5-Trimethoxybenzylidene)benzohydrazide (21):

Product, white solid, yield: 80%, M.P: 205-207 ºC; *Rf* = 0.45 (hexane : ethyl acetate; 3:2); M.F: $C_{17}H_{18}N_2O_4$; M.W: 314.34; FT-IR (cm^{-1}): 3223 (NH), 3061-3026 (C-H, aromatic), 2962-2841 (C-H, aliphatic), 1643 (C=O, amide), 1573 (N=CH, imine), 1546 (C=C, aromatic), 1456-1330 (CH_3, bending). ^{1} H NMR (500 MHz, $CDCl_3$,δ ppm): 10.32 (s, 1H, NH), 8.37 (s, 1H, N=CH), 7.95 (d, *J* = 7.6 Hz, 2H, aromatic), 7.53 (t, *J* = 7.3 Hz, 1H, aromatic), 7.43 (t, *J* = 7.6 Hz, 2H, aromatic), 6.92 (s, 2H, aromatic), 3.86 (s, 6H, 2 *meta*-OCH_3), 3.80 (s, 3H, *para*-OCH_3). ^{13}C NMR (126 MHz, $CDCl_3$,δ ppm): 164.49 (C=O, amide), 148.95 (C=N), 153.45 (2C), 142.0 (1C), 140.21 (1C), 133.16-132.08 (2C), 129.16 (1C), 128.70-128.67 (2C), 127.46 (1C), 104.83-104.81 (2C), (12C, aromatic), 60.93 (*para*-OCH_3), 56.18 (2 *meta*-OCH_3). MS-EI (m/z, %): 314 (M^+, 85), 299 (18), 154 (23), 120 (100).

2.5.7: *N'*-(4-methoxybenzylidene)-4-methylbenzohydrazide (22):

Product, white solid, yield: 75%, M.P: 216-220 ºC; *Rf* = 0.58 (hexane : ethyl acetate; 3:2); M.F: $C_{16}H_{16}N_2O_2$; M.W: 268.32; FT-IR (cm^{-1}): 3167 (NH), 3049-3003 (C-H, aromatic), 2974-2839 (C-H, aliphatic), 1633 (C=O, amide), 1602 (N=CH, imine), 1552 (C=C, aromatic), 1420-1367 (CH_3, bending). ^{1}H NMR (500 MHz, DMSO-d_6 ,δ ppm): 11.67 (s, 1H, NH), 8.41 (s, 1H, N=CH), 7.83 (d, *J* = 7.8 Hz, 2H, aromatic), 7.68 (d, *J* = 8.3 Hz, 2H, aromatic), 7.34 (d, *J* = 7.8 Hz, 2H, aromatic), 7.04 (d, *J* = 8.3 Hz, 2H, aromatic), 3.82 (s, 3H, *para*-OCH_3), 2.39 (s, 3H, *para*-CH_3). ^{13}C NMR (126 MHz, DMSO-d_6 ,δ ppm): 163.24 (C=O, amide), 147.88 (C=N), 161.28 (1C), 142.12 (1C), 131.16 (1C), 129.45 (2C), 129.13 (2C), 128.06 (2C), 127.44 (1C), 114.82 (2C), (12C, aromatic), 55.77 (*para*-OCH_3), 21.50 (*para*-CH_3). MS-EI (m/z, %): 268 (M^+, 100), 134 (65).

2.5.8: 4-Methyl-*N'*-(3,4,5-trimethoxybenzylidene)benzohydrazide (23):

Product, white solid, yield: 77%, M.P: 200-204 ºC; *Rf* = 0.66 (hexane : ethyl acetate; 2:3); M.F: $C_{18}H_{20}N_2O_4$; M.W: 328.37; FT-IR (cm^{-1}): 3221 (NH), 3045 (C-H, aromatic), 2995-2818 (C-H, aliphatic), 1647 (C=O, amide), 1575 (N=CH, imine), 1502 (C=C, aromatic), 1452-1329 (CH_3, bending). ^{1}H NMR (500 MHz, DMSO-d_6 ,δ ppm): 11.78 (s, 1H, NH), 8.40 (s, 1H, N=CH), 7.83 (d, *J* = 7.8 Hz, 2H, aromatic), 7.35 (d, *J* = 7.8 Hz, 2H, aromatic), 7.03 (s, 2H, aromatic), 3.72 (s, 6H, 2 *meta*-OCH_3), 3.34 (s, 3H, *para*-OCH_3), 2.51 (s, 3H, *para*-CH_3). ^{13}C NMR (126 MHz, DMSO-d_6 ,δ ppm): 163.43 (C=O, amide), 148.02 (C=N), 153.67 (2C), 139.63 (1C), 137.64 (1C), 130.40 (2C), 129.47 (2C), 128.11 (2C), 104.71 (2C), (12C, aromatic), 60.60 (*para*-OCH_3), 56.43(2 *meta*-OCH_3). MS-EI (m/z, %): 328 (M^+, 35), 134 (100).

2.5.9: 4-Chloro-*N′*-(4-methoxybenzylidene)benzohydrazide (24):

Product, white solid, yield: 70%, M.P: 195-198 ºC; *Rf* = 0.73 (hexane : ethyl acetate; 2:3); M.F: $C_{15}H_{13}ClN_2O_2$; M.W: 288.37; FT-IR (cm^{-1}): 3244 (NH), 3070-3045 (C-H, aromatic), 2972-2845 (C-H, aliphatic), 1658 (C=O, amide), 1604 (N=CH, imine), 1570 (C=C, aromatic), 1371 (CH_3, bending), 839 (*para*-Cl). ^{1}H NMR (500 MHz, $CDCl_3$,δ ppm): 10.71(s, 1H, NH), 8.39 (s, 1H, N=CH), 7.81 (d, *J* = 5.1 Hz, 2H, aromatic), 7.75 (d, *J* = 5.0 Hz, 2H, aromatic), 7.61 (d, *J* = 8.3 Hz, 2H, aromatic), 6.84 (d, *J* = 8.3 Hz, 2H, aromatic), 3.81 (s, 3H, *para*-OCH_3). ^{13}C NMR (126 MHz, $CDCl_3$,δ ppm): 162.63-161.91 (C=O, amide), 149.71 (C=N), 150.46 (1C), 145.59 (1C), 140.57 (1C), 129.52-129.01 (2C), 125.86 (1C), 123.73 (2C), 121.33 (2C), 114.37-114.25 (2C), (12C, aromatic), 55.38 (*para*-OCH_3). MS-EI (m/z, %): 290 ([M^{37}Cl], 24), 288 ([M^{35} Cl, 73), 154 (100), 121 (13).

2.5.10: 4-Chloro-*N′*-(3,4,5-trimethoxybenzylidene)benzohydrazide (25):

Product, white solid, yield: 72%, M.P: 180-182 ºC; *Rf* = 0.27 (hexane : ethyl acetate; 2:3) M.F: $C_{17}H_{17}ClN_2O_4$; M.W: 348.78; FT-IR (cm^{-1}): 3205 (NH), 3061 (C-H, aromatic), 2947-2841 (C-H, aliphatic), 1637 (C=O, amide), 1602 (N=CH, imine), 1573 (C=C, aromatic), 1415-1329 (CH_3, bending), 842 (*para*-Cl). ^{1}H NMR (500 MHz, $CDCl_3$,δ ppm): 10.40 (s, 1H, NH), 8.35 (s, 1H, N=CH), 7.88 (d, *J* = 8.0 Hz, 2H, aromatic), 7.39 (d, *J* = 8.1 Hz, 2H, aromatic), 6.90 (s, 2H, aromatic), 3.87 (s, 6H, 2 *meta*-OCH_3), 3.82 (s, 3H, *para*-OCH_3). ^{13}C NMR (126 MHz, $CDCl_3$,δ ppm): 176.86 (C=O, amide), 149.36 (C=N), 153.49 (2C), 140.41 (1C), 138.26 (1C), 131.48 (2C), 128.95 (2C), 128.87 (2C), 104.86 (2C), (12C, aromatic), 60.95 (*para*-OCH_3), 56.19 (2 *meta*-OCH_3). MS-EI (m/z, %): 350 ([M^{37}Cl], 20), 348 ([M^{35} Cl], 58), 154 (100), 121 (15).

2.6: In vitro antimicrobial activity

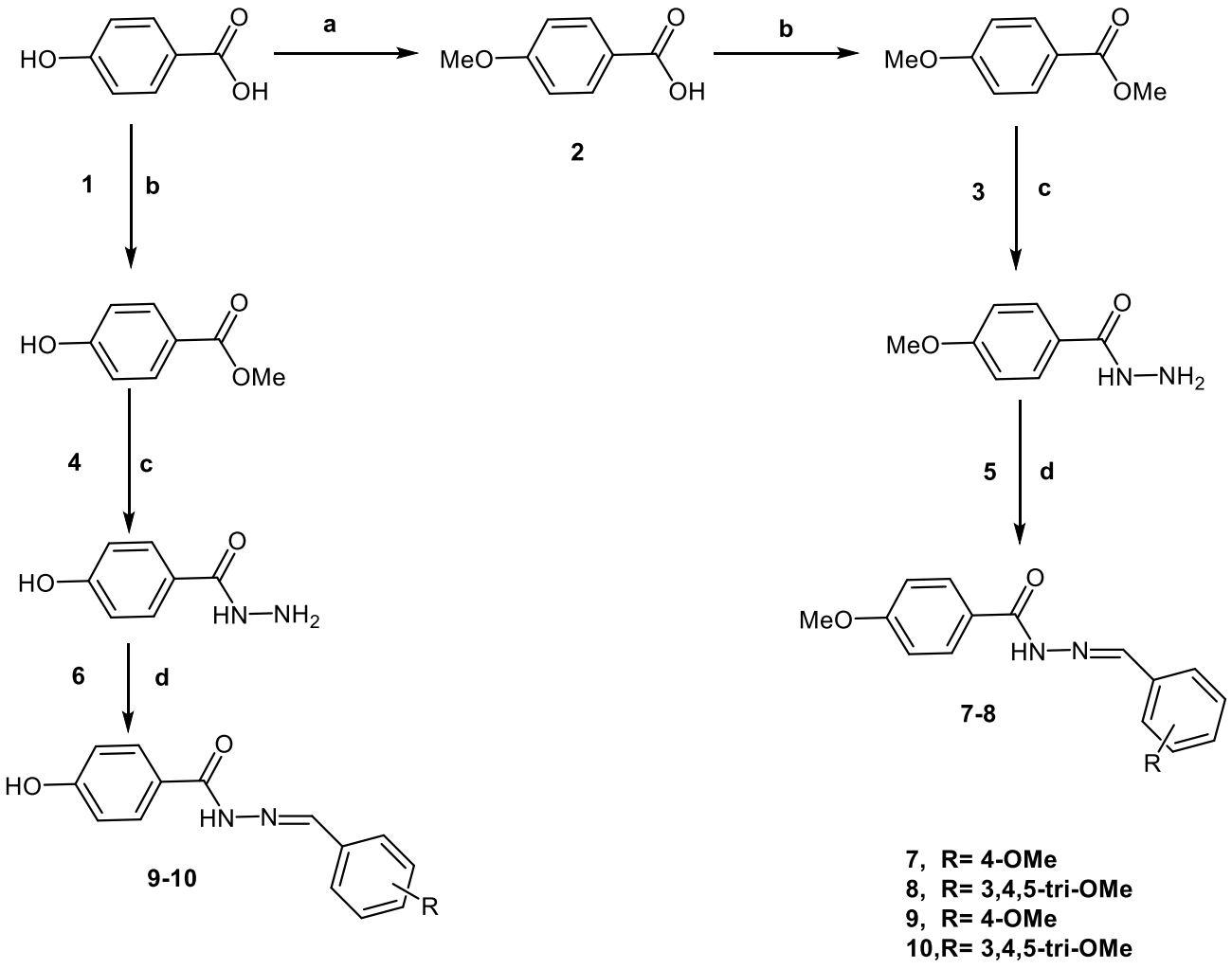
The target compounds (**7-10, 20-25**) were subjected to antimicrobial activities studies, disc diffusion method was used to study the antibacterial and antifungal activities. The in vitro antibacterial activity was measured against the bacterial strains of two gram-positive bacteria (*Staphylococcus aureus and Staphylococcus epidermidis*) and two gram-negative bacteria (*Escherichia coli and Klebsiella sp.*), while antifungal activity was evaluated against fungi *Candida albicans* [24]. The bacterial strains were sub-cultured by using a Nutrient agar medium. The cultured media were incubated at 37 ºC for 24 hours. Disinfected Nutrient agar (20 ml) was placed in sterile Petri dishes. The bacterial strain cultures were modified to 0.5 McFarland standards. The dishes were swabbed with the inoculant of the bacterial strains and left for 15 minutes to be absorbed into the gel. Wells with a diameter of 6 mm was made in the gel by using sterile cork. The wells were filled with the solutions of the test compounds (1000 µg/ml in DMSO), ampicillin (1000 µg/ml) was used as a standard drug, and dimethyl sulfoxide (1µl, DMSO) was used as blank (solvent). The zones of inhibition were then measured after incubation at 37 ºC for 24 hours [25, 26].

3: Results and discussion

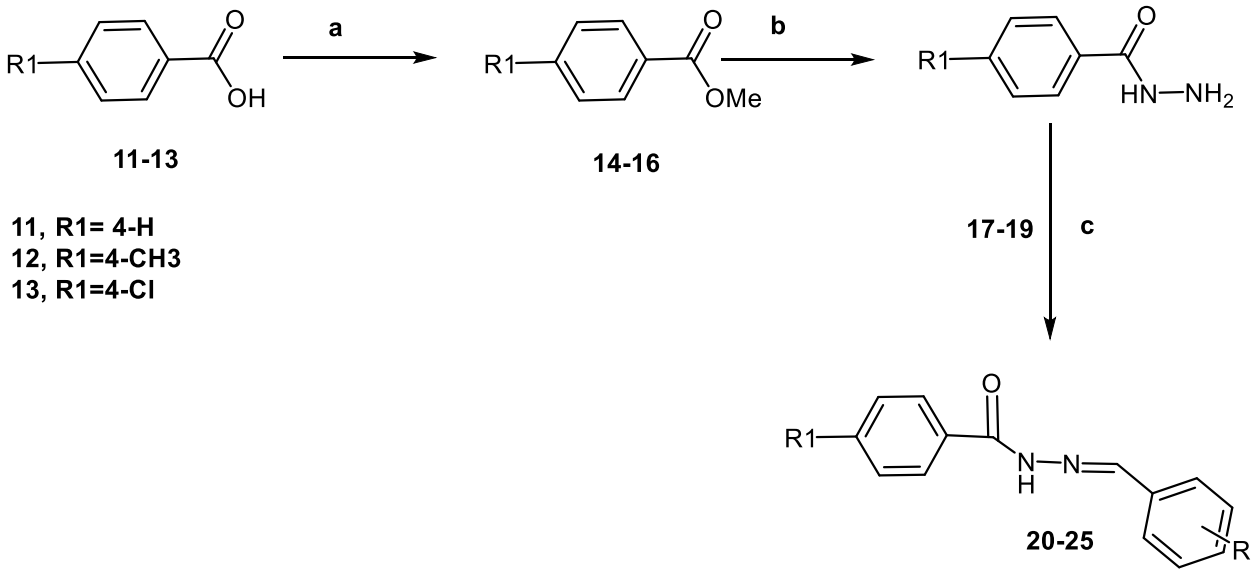
3.1 Chemistry

The steps of the applied synthetic routs for the synthesis of the hydrazone derivatives (**7-10, 20-25**) are illustrated in Schemes 1 and 2. Methylation of 4-hdroxy benzoic acid (**1**) with dimethyl sulfate in the presence of aq. 10% NaOH afforded 4-methoxy benzoic acid (**2**). Esterification of benzoic acids derivatives (**1-2, 11-13**) in presence methanol and sulfuric acid resulted the desired derivatives of methyl benzoate (**3-4, 14-16**). Benzohydrazide derivatives (**5-6, 17-19**) were synthesized by the reaction of esters (**1-2, 11-13**) with hydrazine hydrate in ethanol. The target compounds (**7-10, 20-25**) were synthesized by the reaction of benzohydrazide derivatives (**5-6, 17-19**) with various aromatic aldehyde in ethanol under reflux conditions [27,28]. The FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectral analysis confirmed the structures of (**7-10, 20-25**). The FT-IR spectra showed the characteristic absorption bands at (3244-3107 cm^{-1}, N-H), (1658-1633 cm^{-1}, C=O), (1604-1573 cm^{-1}, CH=N), Figures.1 and 2 shows the FT-IR spectra of compound (**7, 22**). The ^{1}H NMR spectra showed the presence of the singlet signals at (11.78-9.91 ppm), (8.41-8.34 ppm), and (7.95-6.83 ppm) corresponding to –CO-NH- , -CH=N , and phenyl ring protons respectively, Figures. 3 and 4 shows the ^{1}H NMR spectra of compound (**7, 22**). The ^{13}C NMR spectra showed the signals that correspond to C=O at (176.86-161.91

ppm), and CH=N at (149.71-147.26 ppm) beside the other signals correspond to carbon atom in the structures of the target compounds, Figures. 5 and 6 shows the ^{13}C NMR spectra of compound (**7, 22**) .The mass spectra confirmed the molecular weights of the synthesized compounds by showing the peaks of the molecular ions at the calculated molecular weight values of the target compounds (the details are shown in the experimental section), Figures. 7 and 8 shows the mass spectra of compound (**7, 22**).



Scheme 1. Pathway of synthesis hydrazones (**7-10**): Reagents and conditions: **a**. Me_2SO_4, NaOH, 4 h, then 5% H_2SO_4; **b**. MeOH, Conc.H_2SO_4, 8 h; **c**. $NH_2NH_2.H_2O$, EtOH, 8h; **d**. aromatic aldehyde, EtOH, 6 h.



Scheme 2. Pathway of synthesis hydrazones (**20**-**25**): Reagent and condition: **a**. MeOH, Conc.H_2SO_4, 8 h; **b**. $NH_2NH_2.H_2O$, EtOH, 8h; **c**. aromatic aldehyde, EtOH, 6 h.

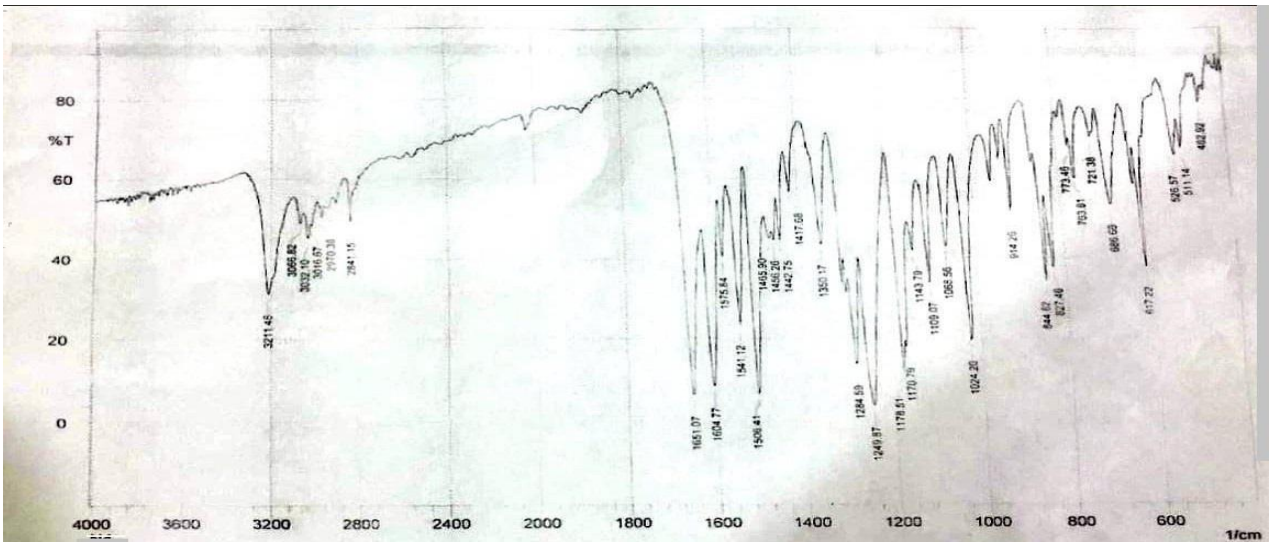


Figure. 1. FT-IR spectra of compounds **7**.

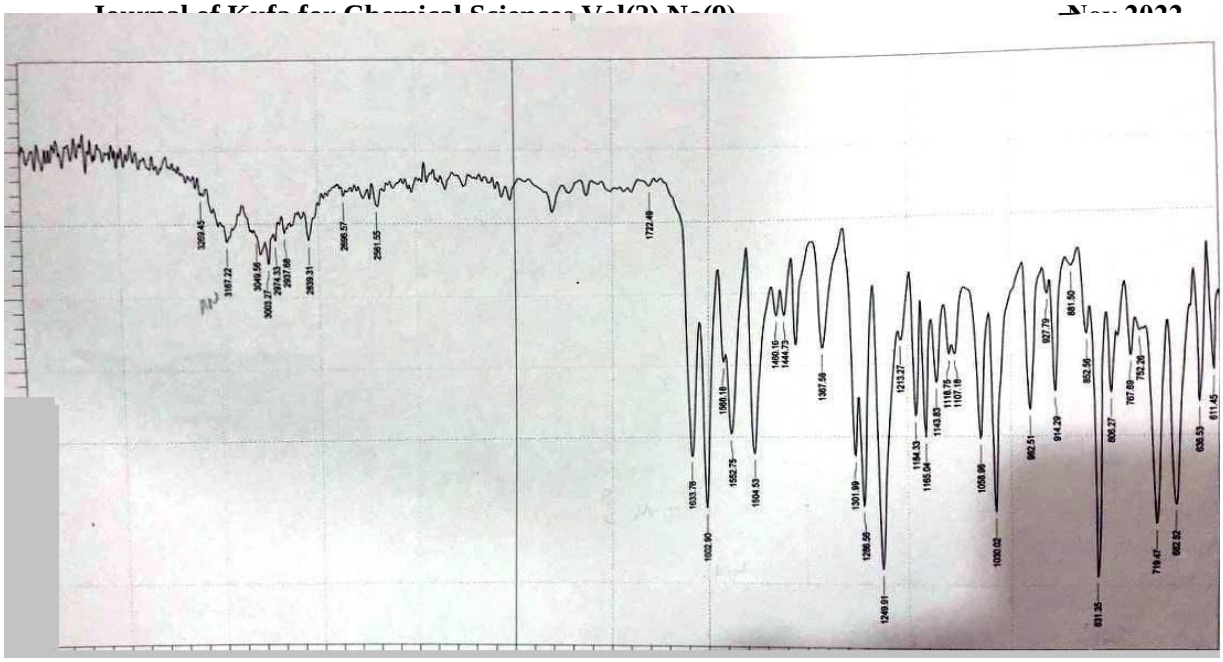


Figure. 2. FT-IR spectra of compounds **22**.

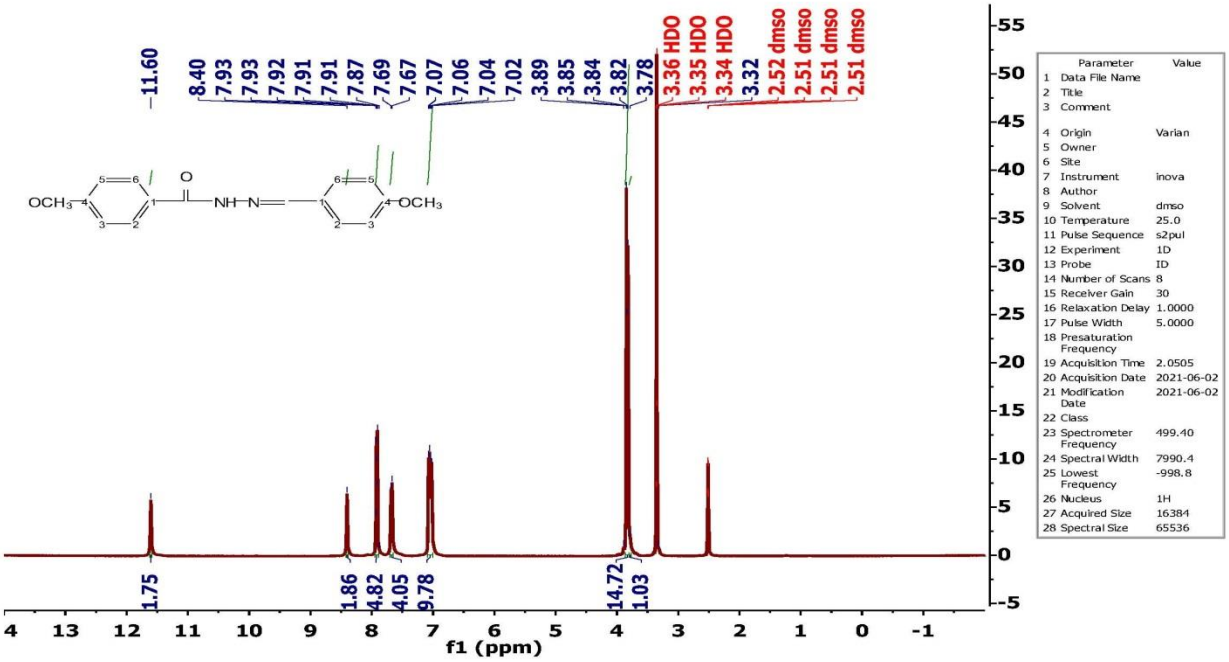


Figure. 3. ^{1}H NMR spectra of compounds **7**

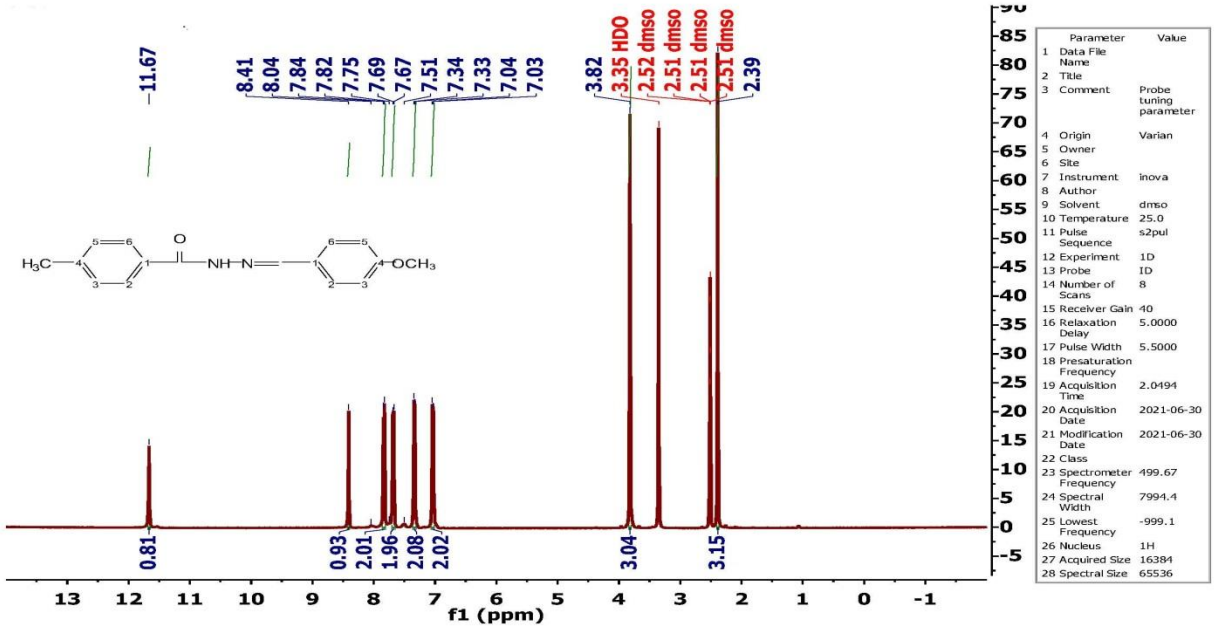


Figure. 4. ^{1}H NMR spectra of compounds **22**

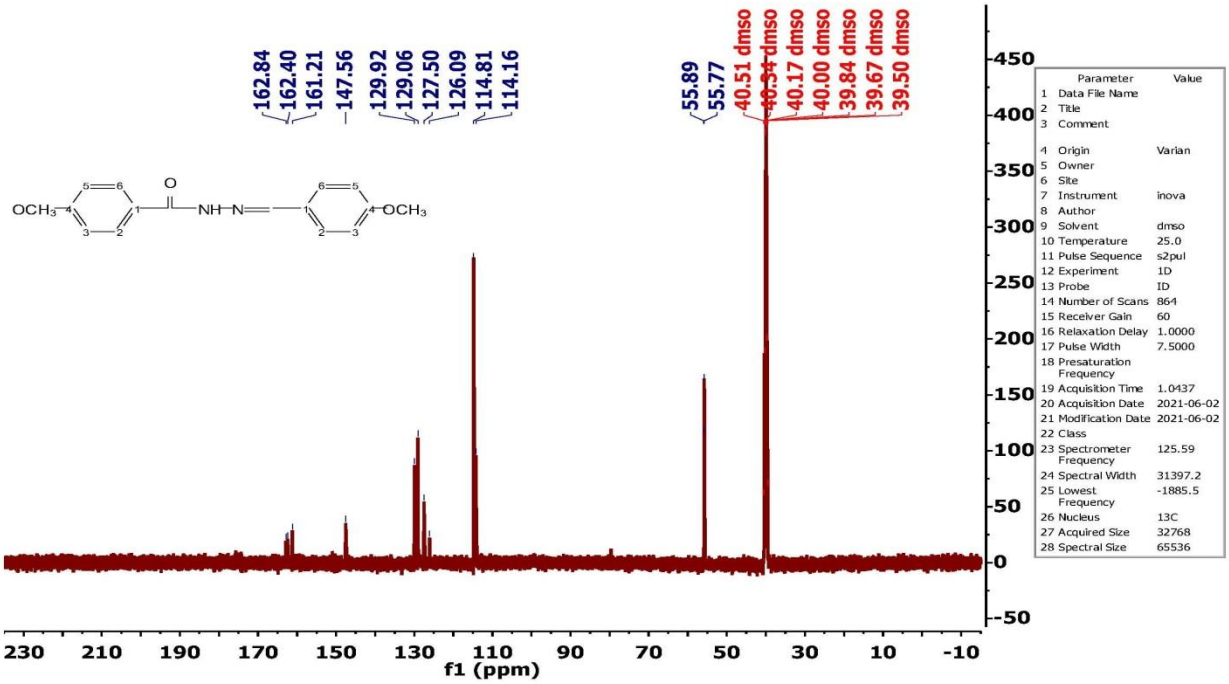


Figure. 5. ^{13}C NMR spectra of compounds **7**

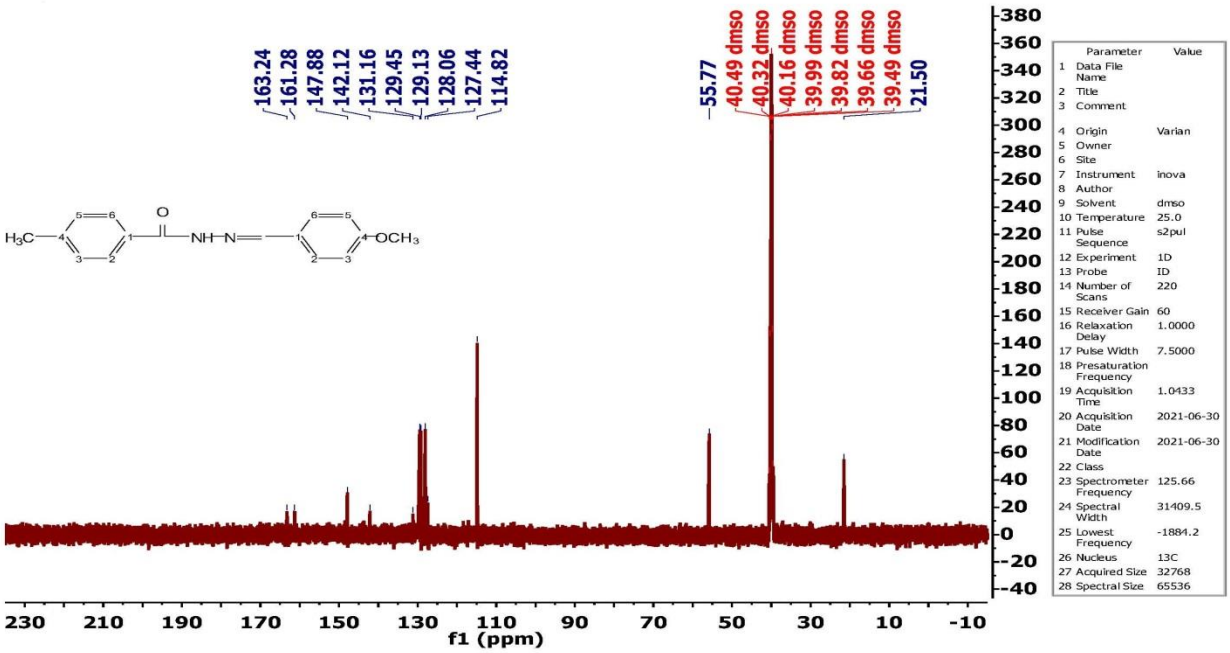


Figure. 6. ^{13}C NMR spectra of compounds **22**

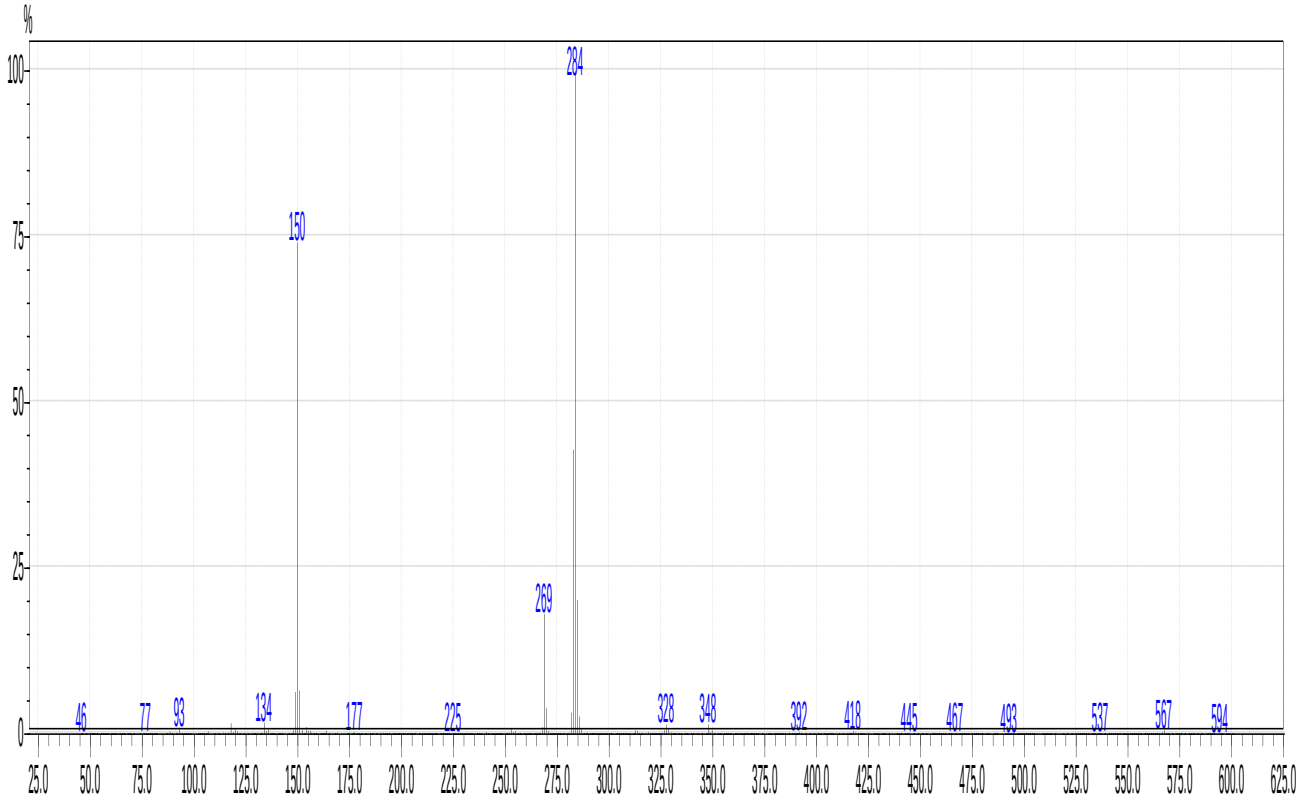


Figure. 7. Mass spectra of compounds **7**

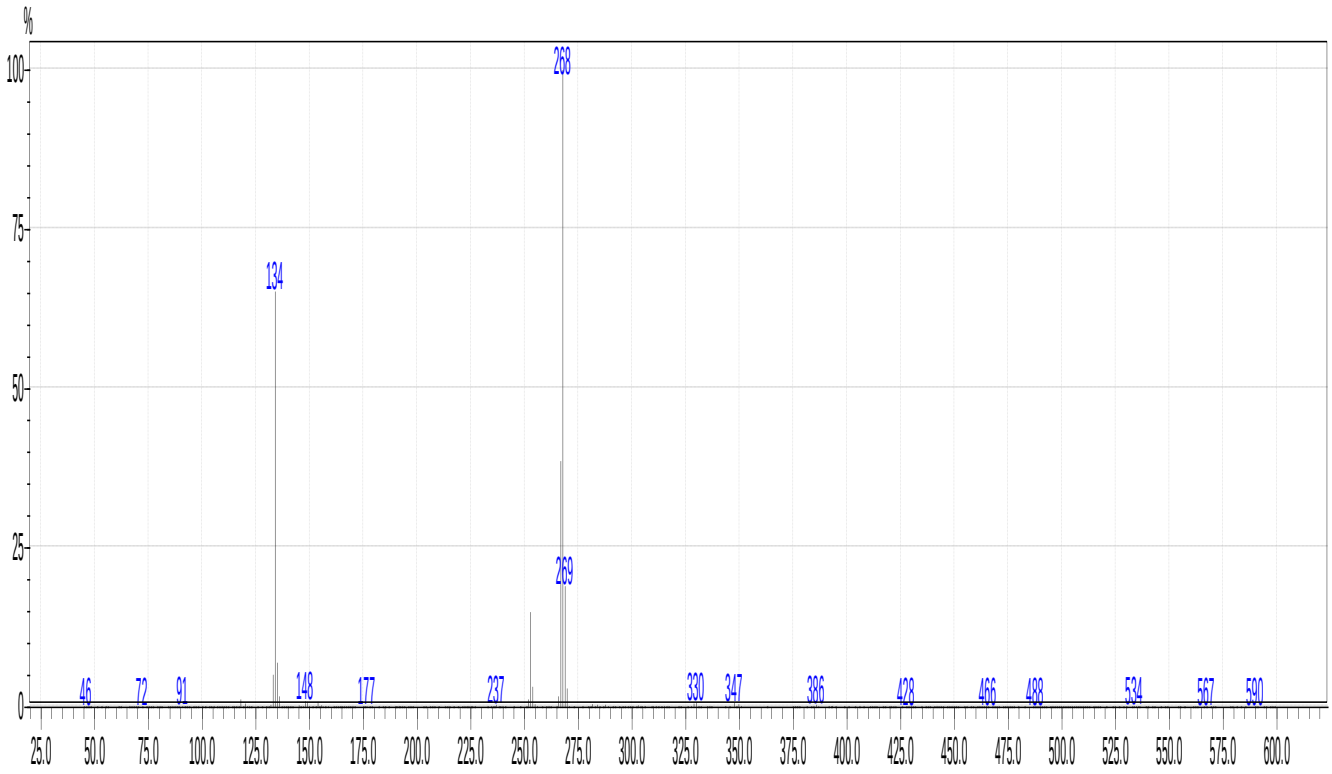


Fig. 8. Mass spectra of compounds **22**

3.2: In vitro antimicrobial assays

The antimicrobial screening studies for compounds **(7-10**) and **(20-25)** were achieved by the disk diffusion method against two strains of gram-positive bacteria (*Staphylococcus aureus* and *Staphylococcus epidermidis*), two strains of gram-negative bacteria (*Escherichia coli* and *Klebsiella sp*) and *Candida albicans* fungi. The target compounds were designed to have different substituents at both aromatic sides of the hydrazone structure to determine whether the differences in the electronic properties of the molecule according to the differences like the substituents influence the antimicrobial activity. Table 1 shows the obtained results. For *S. aureus (G+),* compounds **(8-10)** and **(20-25**) showed a moderate antibacterial activity with inhibition zone diameter (12-19) mm, while compound **7** did not show any effect, the highest activity was recorded for compound **(8)**. For *S. epidermidis (G+),* compounds **(8**,**10**,**22)** and **(25)** showed a moderate antibacterial activity with inhibition zone diameter 11-14 mm, while other compounds did not show any effect. For *E. coli (G-)* compounds **(7**,**8**,**10**,**20)** and (**22-25)** showed a moderate

antibacterial activity with inhibition zone diameter 10-12 mm, while compounds **(9)** and **(21)** did not show any effect. For *Klebsiella (G-),* compounds **(8-10)** and **(22-25)** showed a moderate antibacterial activity with inhibition zone diameter (9-12) mm, while compounds **(7, 20)** and **(21)** did not show any effect. The results of antifungal activity of the test compounds were tested against *Candida albicans*, the results revealed that compounds **(8-10)** and **(23, 25)** have moderate antifungal activity with inhibition zone (11-12) mm, while others have no effect

Table 1: The antimicrobial activity of the compounds (7-10, 20-25)

Compound	Inhibition zone diameter (mm)				
	S. aureus (G^+)	***S. epidermidis*** (G^+)	***E. coli*** (G^-)	***Klebsiella*** (G^-)	***Candida albicans***
DMSO	-	-	-	-	-
7	-	-	11	-	-
8	19	11	12	10	12
9	12	-	-	11	11
10	14	12	12	12	12
20	13	-	10	-	-
21	12	-	-	-	-
22	13	14	12	12	-
23	12	-	11	12	12
24	12	-	12	9	-
25	13	11	12	12	12
Ampicillin	27	20	29	30	30

3.3: Molecular docking study

Docking studies were carried out to set up the mechanism of interaction between Schiff bases compounds (**7-10)** and **(20-25**) and target protein for emerging virtual screening to prevent bacterial growth [29]. In this study, we have used the crystal structure Sa.DHFR [PDB ID: 2W9H] was downloaded from the Protein Data Bank (PDB). Following, Swiss PDB viewer (v.3.7) is a benefit to add their missing atoms of the crystal structure of download protein were prepared by releasing all water molecules and addition of hydrogen atoms to the target protein to afford a right ionization and tautomeric states of amino acid residues [30]. The perfect license version of Gold (v.2021.1.0) was used for molecular docking and it uses genetic optimization for docking ligands into protein binding sites to find the complete range of ligand adjustable flexibility of protein [31]. The active site was identified with a 10 Aº radius around the ligands present in the complex of the download protein structure, the binding of any synthesis

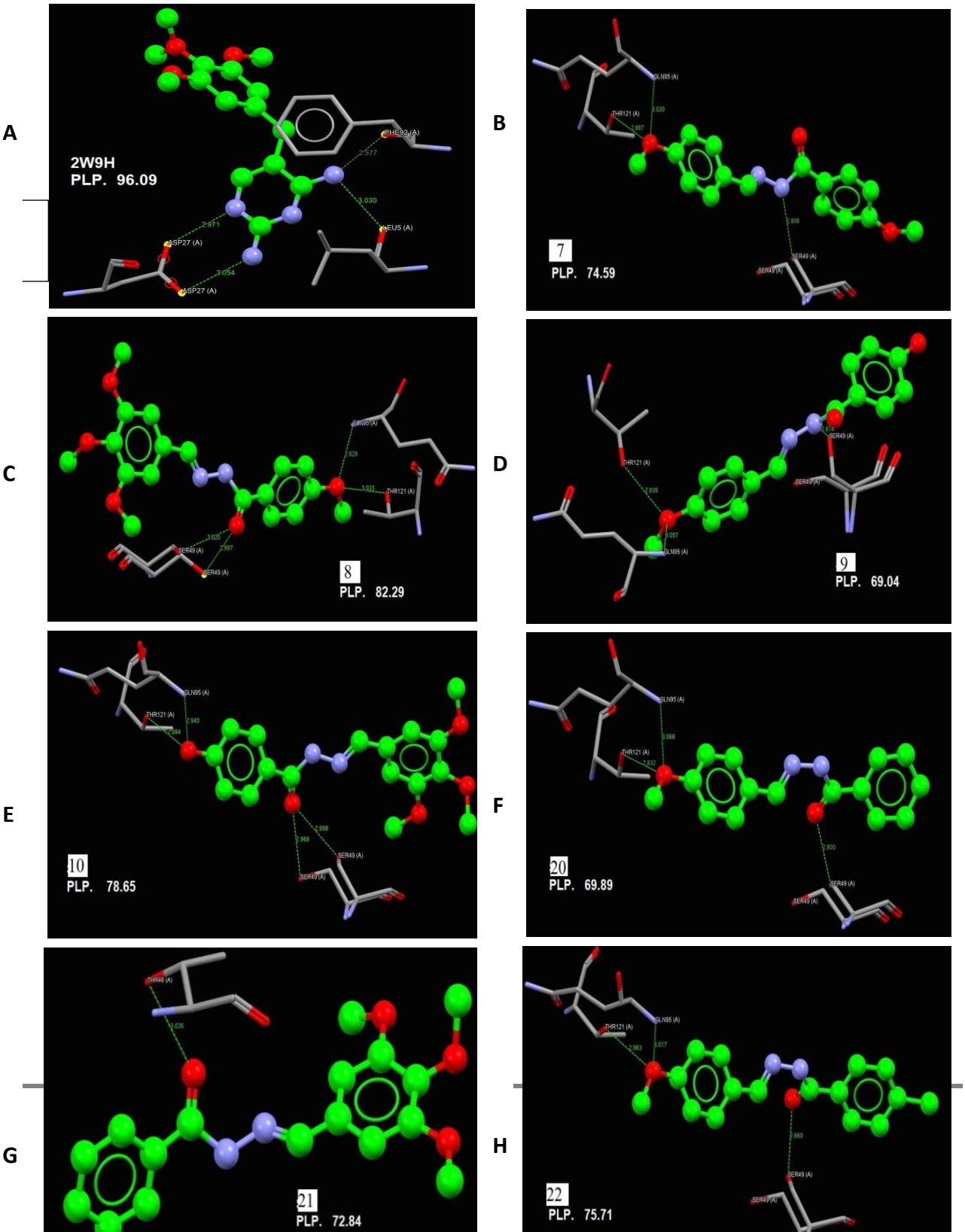
compounds (ligands) in the active site of target protein (Sa.DHFR) revealed the relationship between them via inhibits or affects its catalytic functions [32] and the results are illustrated in Table. 2.

Table 2: The binding energies for hydrazone derivatives and reference docked.

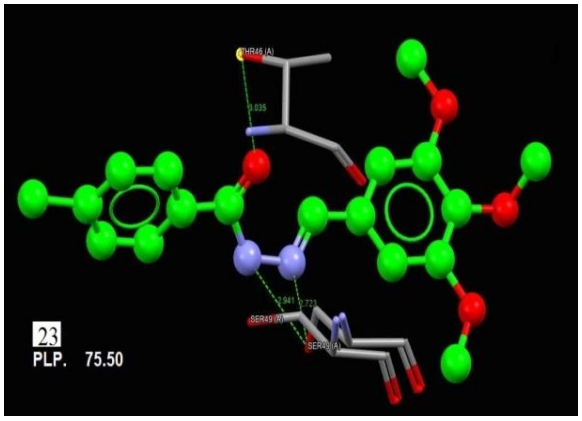
Compounds	Binding energy (PLP Fitness)	No. of Amino acids included in H-bonding	Amino acids included in H-bonding	Number of bonds	Lengths of bonds	
2W9H	96.09	4	ASP27	2	3.054	2.971
			LEU5	1	3.030	
			PHE92	1	2.577	
7	74.59	3	GLN95	1	3.020	
			THR121	1	2.887	
			SER49	1	2.806	
8	82.29	4	THR121	1	3.033	
			SER49	2	3.020	2.997
			GLN95	1	2.828	
9	69.04	3	GLN95	1	3.057	
			THR121	1	2.939	
			SER49	1	2.874	
10	78.65	4	SER49	2	2.998	2.968
			THR121	1	2.984	
			GLN95	1	2.940	
20	69.89	3	GLN95	1	3.068	
			THR121	1	2.832	
			SER49	1	2.800	
21	72.84	1	THR46	1	3.035	
22	75.71	3	GLN95	1	3.017	
			THR121	1	2.963	
			SER49	1	2.869	
23	75.50	3	THR46	1	3.035	
			SER49	2	2.941	2.723
24	69.87	1	SER49	1	2.997	
25	79.40	1	SER49	1	2.727	

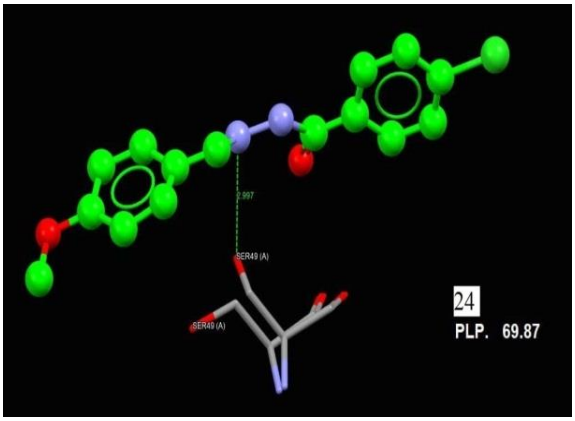
Hydrogen bond interactions such as Vander Waals, electrostatic, steric, p-p stacking, dipole-dipole, and others are calculated by GOLD, and all bond lengths below 3Aº [33]. These compounds **(7-10)** and **(20-25)** are shown in Figure. **1** have donor or receptor groups as (3,4,5-tri-OCH_3, OCH_3, OH, NH, C=O, C=N, Cl) on phenyl ring were revealed good binding with the target protein (PDB ID: 2W9H) and better interaction (H-bond) with amino acids-SER49,

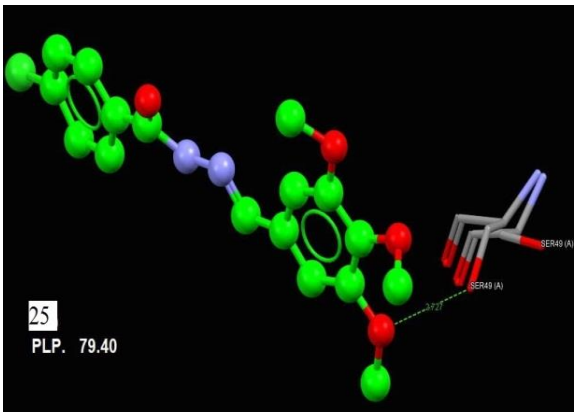
THR121, GLN95, and THR 46 which are bending site to growth-inhibitory power against S.aureus through deactivation of the enzyme dihydrofolate reductase.



I







K

Figure 2: The interaction between trimethoprim, compounds 7-10 and 20-25 with amino acid residues in Sa.DHFR [PDB ID: 2W9H] A–K respectively.

Conclusion

We have synthesized a series of hydrazone derivatives **(7-10)** and **(20-25**), their structures were confirmed by FT-IR, ^{1}H NMR, ^{13}C NMR, and Mass analysis. In vitro antimicrobial activity studies revealed that compounds (**7-10)** and **(20-25**) showed lower effect when compared with ampicillin against the studied pathogenic bacteria which included two-gram positive bacteria (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and two gram-negative bacteria (*Escherichia coli and Klebsiella sp),* and *Candida albicans* fungi. A molecular docking study is used to explain the binding mode between the studied compounds and the target protein (PDB ID: 2W9H). The results showed these compounds have a good binding affinity with enzyme

dihydrofolate reductase (Sa.DHFR), the better interactions with amino acids-SER49, THR121, GLN95, and THR46.

Acknowledgements

The authors thank the Chemistry Department at the College of Science, Al-Mustansiriyah University for the assistance in providing all the requirements for the completion of this research. We also thank Lec. Yasir M. Kadhim, Department of Pharmaceutical Chemistry, College of pharmacy, Al-Nahrain University, Baghdad, Iraq, for the advice given to carry out the docking studies.

References

[1] I. Amine Khodja and H. Boulebd, "Synthesis, biological evaluation, theoretical investigations, docking study and ADME parameters of some 1,4-bisphenylhydrazone derivatives as potent antioxidant agents and acetylcholinesterase inhibitors," *Mol. Divers.*, vol. 25, no. 1, pp. 279–290, 2021, doi: 10.1007/s11030-020-10064-8.

[2] R. Article, "CHEMISTRY Hydrazide – hydrazones as potential antimicrobial agents : overview of the literature since 2010," 2016, doi: 10.1007/s00044-016-1756-y.

[3] M. Asif and A. Husain, "Analgesic , Anti-Inflammatory , and Antiplatelet Profile of Hydrazones Containing Synthetic Molecules," vol. 2013, 2013.

[4] H. S. Seleem, G. A. El-inany, B. A. El-shetary, and M. A. Mousa, "The ligational behavior of a phenolic quinolyl hydrazone towards copper (II) - ions," no. Ii, pp. 1–9, 2011, doi: 10.1186/1752-153X-5-2.

[5] B. F. Abdel-wahab, G. E. A. Awad, and F. A. Badria, "European Journal of Medicinal Chemistry of new imidazole-based heterocycles," *Eur. J. Med. Chem.*, vol. 46, no. 5, pp. 1505–1511, 2011, doi: 10.1016/j.ejmech.2011.01.062.

[6] A. S. Abu-surrah *et al.*, "European Journal of Medicinal Chemistry New palladium (II) complexes bearing pyrazole-based Schiff base ligands : Synthesis , characterization and cytotoxicity," *Eur. J. Med. Chem.*, vol. 45, no. 2, pp. 471–475, 2010, doi: 10.1016/j.ejmech.2009.10.029.

[7] O. O. Ajani, C. A. Obafemi, O. C. Nwinyi, and D. A. Akinpelu, "Bioorganic & Medicinal Chemistry Microwave assisted synthesis and antimicrobial activity of 2-quinoxalinone-3-

hydrazone derivatives," *Bioorg. Med. Chem.*, vol. 18, no. 1, pp. 214–221, 2010, doi: 10.1016/j.bmc.2009.10.064.

[8] M. S. Al-said, M. S. Bashandy, S. I. Al-qasoumi, and M. M. Ghorab, "European Journal of Medicinal Chemistry Anti-breast cancer activity of some novel 1 , 2-dihydropyridine , thiophene and thiazole derivatives," *Eur. J. Med. Chem.*, vol. 46, no. 1, pp. 137–141, 2011, doi: 10.1016/j.ejmech.2010.10.024.

[9] V. Kumar *et al.*, "Rapid ‘One-pot’ Synthesis of a Novel Benzimidazole-5-carboxylate and its Hydrazone Derivatives as Potential Anti-inflammatory and Antimicrobial Agents," *Bioorg. Med. Chem. Lett.*, 2015, doi: 10.1016/j.bmcl.2015.02.043.

[10] N. Kaushik-basu, D. Manvar, and A. Basu, "European Journal of Medicinal Chemistry Synthesis of novel di fl unisal hydrazide e hydrazones as anti-hepatitis C virus agents and hepatocellular carcinoma inhibitors *," vol. 108, pp. 301–308, 2016, doi: 10.1016/j.ejmech.2015.10.041.

[11] Y. Dizdaroglu *et al.*, "Design , synthesis and molecular modelling studies of some pyrazole derivatives as carbonic anhydrase inhibitors," *J. Enzyme Inhib. Med. Chem.*, vol. 35, no. 1, pp. 289–297, 2020, doi: 10.1080/14756366.2019.1695791.

[12] S. A. Moustafa, M. M. Ali, and A. A. El-rashedy, "Synthesis , anticancer activity and molecular docking study of Schiff base complexes containing thiazole moiety," vol. 5, pp. 85–96, 2016, doi: 10.1016/j.bjbas.2016.01.001.

[13] A. Munir *et al.*, "Synthesis, in-vitro, in-vivo anti-inflammatory activities and molecular docking studies of acyl and salicylic acid hydrazide derivatives," *Bioorg. Chem.*, vol. 104, no. July, p. 104168, 2020, doi: 10.1016/j.bioorg.2020.104168.

[14] Ł. Popiołek, A. Biernasiuk, A. Berecka, A. Gumieniczek, A. Malm, and M. Wujec, "New hydrazide–hydrazones of isonicotinic acid: synthesis, lipophilicity and in vitro antimicrobial screening," *Chem. Biol. Drug Des.*, vol. 91, no. 4, pp. 915–923, 2018, doi: 10.1111/cbdd.13158.

[15] B. S. H, S. Veeramanikandan, and B. Sherine, "CODEN (USA): PCHHAX Design , Synthesis , Structural Elucidation and Biological Applications of Benzohydrazide Derivatives," vol. 9, no. 18, pp. 44–50, 2017.

[16] A. M. Abu-dief and I. M. A. Mohamed, "ScienceDirect A review on versatile applications

of transition metal complexes incorporating Schiff bases," *Beni-Suef Univ. J. Basic Appl. Sci.*, vol. 4, no. 2, pp. 119–133, 2015, doi: 10.1016/j.bjbas.2015.05.004.

[17] I. O. Alisi, A. Uzairu, and S. E. Abechi, "In silico design of hydrazone antioxidants and analysis of their free radical-scavenging mechanism by thermodynamic studies," 2019.

[18] V. I. Tzankova, N. G. Hristova-avakumova, and A. Vera, "AC SC," *J. Mol. Struct.*, 2018, doi: 10.1016/j.molstruc.2018.03.119.

[19] H. S. Kareem *et al.*, "Correlation of antioxidant activities with theoretical studies for new hydrazone compounds bearing a 3,4,5-trimethoxy benzyl moiety," *Eur. J. Med. Chem.*, vol. 103, pp. 497–505, 2015, doi: 10.1016/j.ejmech.2015.09.016.

[20] I. H. R. Tomi, G. Q. Ali, A. H. Jawad, and E. Yousif, "Synthesis and characterization of gallic acid derivatives and their utilized as organic photo-stabilizers for poly (vinyl chloride)," *J. Polym. Res.*, vol. 24, no. 8, 2017, doi: 10.1007/s10965-017-1283-7.

[21] O. Tapanyiğit, O. Demirkol, E. Güler, M. Erşatır, M. E. Çam, and E. S. Giray, "Synthesis and investigation of anti-inflammatory and anticonvulsant activities of novel coumarin-diacylated hydrazide derivatives," *Arab. J. Chem.*, vol. 13, no. 12, pp. 9105–9117, 2020, doi: 10.1016/j.arabjc.2020.10.034.

[22] C. V. Maridevarmath, L. Naik, V. S. Negalurmath, M. Basanagouda, and G. H. Malimath, "Synthesis, characterization and photophysical studies on novel benzofuran-3-acetic acid hydrazide derivatives by solvatochromic and computational methods," *J. Mol. Struct.*, vol. 1188, pp. 142–152, 2019, doi: 10.1016/j.molstruc.2019.03.063.

[23] Y. U. Cebeci, H. Bayrak, and Y. Şirin, "Synthesis of novel Schiff bases and azol-β-lactam derivatives starting from morpholine and thiomorpholine and investigation of their antitubercular, antiurease activity, acethylcolinesterase inhibition effect and antioxidant capacity," *Bioorg. Chem.*, vol. 88, no. February, 2019, doi: 10.1016/j.bioorg.2019.102928.

[24] R. Kayarmar, G. K. Nagaraja, P. Naik, H. Manjunatha, B. C. Revanasiddappa, and T. Arulmoli, "Synthesis and characterization of novel imidazoquinoline based 2-azetidinones as potent antimicrobial and anticancer agents," *J. Saudi Chem. Soc.*, vol. 21, pp. S434–S444, 2017, doi: 10.1016/j.jscs.2014.07.003.

[25] N. B. Patel and J. C. Patel, "Synthesis and antimicrobial activity of Schiff bases and 2-azetidinones derived from quinazolin-4(3H)-one," *Arab. J. Chem.*, vol. 4, no. 4, pp. 403–411, 2011, doi: 10.1016/j.arabjc.2010.07.005.

[26] N. Rambabu, B. Ram, and P. K. Dubey, "Synthesis and Biological Activity of Novel (E) -N ' - (Substituted) -3 , 4 , 5-trimethoxybenzohydrazide Analogs," no. c, 2017.

[27] J. E. De Carvalho, C. Vieira, A. Samara, and N. Formagio, "Synthesis, Antiproliferative Activity and Molecular Properties Predictions of Galloyl Derivatives," pp. 5360–5373, 2015, doi: 10.3390/molecules20045360.

[28] X. F. Cao, Y. S. Wang, S. W. Li, C. S. Chen, and S. Y. Ke, "Synthesis and biological activity of a series of novel N-substituted β-lactams derived from natural gallic acid," *J. Chinese Chem. Soc.*, vol. 58, no. 1, pp. 35–40, 2011, doi: 10.1002/jccs.201190055.

[29] R. K, J. T. Kakkassery, V. P. Raphael, R. Johnson, and V. T. K, "In vitro antibacterial and in silico docking studies of two Schiff bases on Staphylococcus aureus and its target proteins," *Futur. J. Pharm. Sci.*, vol. 7, no. 1, pp. 3–11, 2021, doi: 10.1186/s43094-021-00225-3.

[30] Y. M. Kadhim, S. J. Lafta, and M. F. Mahdi, "Synthesis in microwave , pharmacological evaluation , molecular docking and ADME studies of Schiff bases of Diclofenac targeting COX-2 Synthesis in microwave , pharmacological evaluation , molecular docking and ADME studies of Schiff bases of Diclofenac t," no. June, 2020.

[31] M. L. Verdonk, J. C. Cole, M. J. Hartshorn, C. W. Murray, and R. D. Taylor, "Giftgas over Byen. Civilbefolkningens Beskyttelse Under Den Næste krig.," *Proteins*, vol. 52, no. January, pp. 609–623, 2003.

[32] L. Chaput and L. Mouawad, "Efficient conformational sampling and weak scoring in docking programs? Strategy of the wisdom of crowds," *J. Cheminform.*, vol. 9, no. 1, pp. 1–18, 2017, doi: 10.1186/s13321-017-0227-x.

[33] L. H. Al-Wahaibi *et al.*, "Quantitative analysis of hydrogen and chalcogen bonds in two pyrimidine-5-carbonitrile derivatives, potential DHFR inhibitors: An integrated crystallographic and theoretical study," *RSC Adv.*, vol. 10, no. 60, pp. 36806–36817, 2020, doi: 10.1039/d0ra07215j.