Egypt. J. Chem. **Vol. 65**, No. 6. pp. 159 - 169 (2022)

Egyptian Journal of Chemistry
http://ejchem.journals.ekb.eg/

Synthesis, Characterization, Molecular Docking and Biological Activity Studies of Hydrazones with 3,4,5-Trimethoxyphenyl Moiety

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Abstract

In this work a series of hydrazone Schiff base compounds have been synthesized and characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry. The structures of the target compounds designed to have 3,4,5-trimethoxy in acid hydrazide part and different substituents in the imine part. The antibacterial activity of the synthesized compounds has been studied against two gram positive bacteria (*Staphylococcus aureus and Staphylococcus epidermidis*) and two gram negative bacteria (*Escherichia coli and Klebsiella sp.*), while the antifungal activity was studied against candida albicans fungi. The results revealed that most of the synthesized hydrazone derivatives exhibit a moderate antimicrobial activity when compared with the standard drug ampicillin. Molecular docking studies were carried out on the bacteria strain *Staphylococcus aureus* with target protein DHFR and its complex with trimethoprim (PDB ID: 2W9H).

Keywords: 3,4,5-trimethoxyphenyl; hydrazone; Schiff base; Antimicrobial; Molecular Docking.

1. Introduction

Aromatic Schiff bases which are synthesized be the condensation of amino compounds with aromatic aldehydes and amino stable compounds that have azo-methine groups in their structures, these kind of compounds are reported have a broad range of biological activities [1]. Drug resistance by pathogenic microorganisms is a serious problem that makes the approved drugs inactive and useless in the treatment of many diseases. Therefore, the searching of new antimicrobial agents is still continuous to overcome this problem with active compounds that have new structures which can't be resisted by the pathogenic microorganisms [2]. Among such compounds, hydrazone with azomethine formed by the reaction of aromatic aldehydes and acid hydrazides[3]. This class of organic compounds attracted many researchers to involve variety of their derivatives in drug discovery and development to find potential treatment for the multi drug resistance microorganisms [4]. Hydrazones were evaluated for their ability to remove free radicals (acting as antioxidants) which relate to deferent diseases accompanied with oxidative stress phenomenon including cancers, Alzheimer′s disease (AD) and cardiovascular disease, in addition to theinvolvement of these compounds in pharmaceutical applications and drugdesign, they are used as additives inthe

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Receive Date: 29 September 2021, Revise Date: 31 October 2021, Accept Date: 25 November 2021
DOI: 10.21608/EJCHEM.2021.98746.4593

protective formulas such as sun screens[5]. Molecular docking studies play a crucial role in drug design and discovery through the explanation of how the synthetic or natural molecules interacts with target proteins (protein ligand interaction) [6]. Dihydrofolatereductase (DHFR) is an enzyme that reduces dihydrofolic acid to tetrahydrofolic by using NADPH as a cofactor in the biosynthetic pathways of purines, thymidylate, methionine, glycine, pantothenic acid and N-formyl-methionyltRNA[7,8]. Further, studies have focused on molecules that can act as DHFR inhibitor to achieve antimicrobial and anticancer activities [9]. *S. aureus* is one of pathogenic bacteria that have multi-drug-resistant strains and a member of Firmicutes[10]. It can cause infection of the nose, skin, vagina, urethra, and gastrointestinal tract [11,12]. There are many hydrazone derivatives that have pharmacophore for a wide range of pharmacological and biological activities [13] such as: anti-inflammatory [14], anti-fungal [15], antioxidant [16], antimicrobial [17], anticancer [18], and anticonvulsant activities [19]. In the view of the above remarkable consideration, here we report the synthesis of series of hydrazones derived from gallic acid to have 3,4,5-trimethoxyphenyl ring and their evaluation as promising antibacterial and antifungal agents.

2. Experimental

2.1 General

All the used chemicals were obtained from commercial sources, with a purity range of 95-98%, that were used as received (without further purification). Melting points of all synthesized compounds were measured in open capillary tubes in a Gallen-Kamp MFB-600 melting point apparatus. FT-IR spectra measurements were recorded using FT-IR-8400S-Shimadizu spectrophotometer. Mass spectra were recorded on Shimadzu model GCMSQP 1000 EX spectrometer (Japan). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on VARIAN-INOVA 500MHZ spectrophotometer (Germany), $CDCl_3$ and DMSO-d_6 were used as solvents, and tetramethylsilane TMS as internal standard.

2.2: Synthesis of 3,4,5-Trimethoxy benzoic acid (2)

In 3-neck round bottom flask, sodium hydroxide (8.0 g) was dissolved in water (50 ml), the solution was cooled down, gallic acid mono hydrate (**1**: 5.136 gm, 0.03 mol) was added and the flask was sealed with stopper. The mixture was stirred until turned clear. Dimethyl sulfate (6.70 ml, 0.07 mol) was added drop wise to solution and with stirring for 20 min at (30-35) Cº, 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) Cº. The mixture was heated up for 2 h after second addition. A solution of sodium hydroxide (2gm 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 re-crystallized from ethanol to give 3,4,5-trimethoxy benzoic (**2**) acid as a very light brown crystalline material [20]. Yield: 85%; M.P: 167-170 Cº; *Rf* = 0.53 (hexane : ethyl acetate; 3:2), M.F: $C_{10}H_{12}O_5$; M.W; 212.20; FT-IR (cm^{-1}): 3250-2522 (COOH), 3074-3018 (C-H, aromatic), 2931-2837 (C-H, aliphatic), 1681 (C=O), 1585 (C=C, aromatic), 1465 (CH3, bending), 1267-1182 (C-O). ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 12.96 (s, 1H, COOH), 7.23 (s, 2H, aromatic), 3.83 (s, 6H, 2 *meta*-OCH3), 3.73 (s, 3H, para-OCH3). MS-EI (m/z, %): 211 ([M^+-1], 30), 196 (100), 182 (75), 166 (18), 138 (23).

2.3: Synthesis of methyl 3,4,5-trimethoxybenzoate (3)

To the solution of the compound **2** (8.48 gm, 0.04 mol in 50.0 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 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 filtered then the solvent was evaporated the solid precipitate washed with cold water and dried to give white crystalline solid of desired ester [21]. Yield: 80%; M.P: 83-85 Cº; *Rf* = 0.81 (hexane : ethyl acetate; 3:2); M.F: $C_{11}H_{14}O_5$; M.W; 226.23; FT-IR (cm^{-1}): 1712 (C=O, ester), 3082-3012 (C-H, aromatic), 2915-2841 (C-H, aliphatic), 1589 (C=C, aromatic).

2.4: Synthesis of 3,4,5-trimethoxybenzohydrazide (4)

Compounds (**3**: 4.52, 0.02 mol) were dissolved in absolute ethanol (30 ml), hydrazine hydrate (80%, 6 ml) was added and heated to reflux for 8 h. 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 the desired of acids hydrazide[22]. Yield: 67%; M.P: 160-162 Cº; *Rf* = 0.32 (hexane : ethyl acetate; 4:1); M.F: $C_{10}H_{14}N_2O_4$; M.W; 226.23; FT-IR (cm^{-1}): 3363-3336 (NH2), 3194 (NH), 3086-3014 (C-H, aromatic), 2968-2841 (C-H, aliphatic), 1651 (C=O, amide), 1610 (NH2, bending), 1581 (C=C, aromatic).

2.5: General procedure for the synthesis of compounds (5-12)

Substituted of acids hydrazide (**5-12**:4 mmol) was dissolved in 40 ml of absolute ethanol. The appropriate aromatic aldehyde (4.4 mmol) was added with 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, 24].

2.5.1:*N′*-benzylidene-3,4,5-trimethoxybenzohydrazide (5)

Product, white solid, yield :84%; M.P: 133-136; M.F: $C_{17}H_{18}N_2O_4$; M.W; 314.34; FT-IR (cm^{-1}): 3142 (NH), 3057-3003 (C-H, aromatic), 2964-2823 (C-H, aliphatic), 1639 (C=O, amide), 1585 (N=CH, imine), 1562 (C=C, aromatic); ^{1}HNMR (500 MHZ, $CDCl_3$, δ ppm): 10.50 (s, 1H, NH), 8.40 (s, 1H, N=CH), 7.62-7.20 (m, 7H, aromatic), 3.89 (s, 6H, 2 *meta*-OCH3), 3.85 (s, 3H, *para*-OCH3), ^{13}C NMR (126 MHz, $CDCl_3$, δ ppm): 153.20 (C=O, amide), 141.30 (N=CH), 149.16 (2C), 133.64 (1C), 130.46 (2C), 128.67- (1C), 127.61 (2C), 117.44 (2C), 104.91 (2C), (12C, aromatic), 60.92 (*para*-OCH3), 56.27 (2 *meta*-OCH3). MS-EI (m/z ,%): 314 (M^+, 84), 211 (100).

2.5.2: 3,4,5-trimethoxy-*N′*-(4-methoxybenzylidene)benzohydrazide (6)

Product, white solid, yield :67%; M.P: 150-153; M.F: $C_{18}H_{20}N_2O_5$; M.W; 344.37; FT-IR(cm^{-1}) : 3165 (NH), 3066 (C-H, aromatic), 2968-2837 (C-H, aliphatic), 1635 (C=O, amide), 1604 (N=CH, imine), 1579 (C=C, aromatic), 1336 (CH3, bending); ^{1}H NMR (500 MHz, DMSO d_6 ,δ ppm): 11.60 (S, 1H, NH), 8.42 (s, 1H, N=CH), 7.69 (d, *J* = 8.3 Hz, 2H,aromatic), 7.24 (s, 2H, aromatic), 7.04 (d, *J* = 8.4 Hz, 2H, aromatic), 3.84 (s, 6H, 2 *meta*-OCH3), 3.74-3.35 (s, 6H, 2 *para*-OCH3). ^{13}C NMR (126 MHz, DMSO-d_6, δ ppm): 162.84 (C=O, amide), 148.15 (C=N), 161.34 (1C), 153.16 (2C), 140.80 (1C), 129.16 (2C), 127.34 (2C), 114.85 (2C), 105.61 (2C), (12C, aromatic), 60.60 (2 *para*-OCH3), 56.57-55.79 (2 *meta*-OCH3). MS-EI (m/z,%): 344 (M^+, 38), 210 (100).

2.5.3: N'-(4-ethoxybenzylidene)-3,4,5-trimethoxybenzohydrazide (7)

Product, white solid, yield: 65%; M.P: 148-151; M.F: $C_{19}H_{22}N_2O_5$; M.W; 358.39; FT-IR (cm^{-1}): 3165 (NH), 3070 (C-H, aromatic), 2970 (C-H, aliphatic), 1635 (C=O, amide), 1606 (N=CH, imine), 1579 (C=C), 1411 (CH2, bending), 1336 (CH3, bending). ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 11.59 (s, 1H, NH), 8.42 (s, 1H, N=CH), 7.68 (d, *J* = 8.4 Hz, 2H, aromatic), 7.24 (s, 2H, aromatic), 7.24 (d, *J* = 8.4 Hz, 2H, aromatic), 4.11-4.07 (q, *J* = 7.0 Hz, 2H, OCH2), 3.74 (s, 6H, 2 *meta*-OCH3), 3.34 (s, 3H, *para*-OCH3), 1.37-1.34 (t, *J* = 7.0 Hz, 3H, CH3). ^{13}C NMR (126 MHz, $CDCl_3$, δ ppm): 162.81 (C=O, amide), 148.17 (N=CH), 160.63 (1C), 153.16 (2C), 140.75 (1C), 129.16 (2C), 127.19 2C), 115.25 (2C), 105.60 (2C), (12C, aromatic), 63.73 (OCH2), 60.60 (*para*-OCH3), 56.56 (2*meta*OCH3), 15.06 (CH3). MS-EI (m/z, %): 358 (M^+, 74), 348 (32), 246 (20), 210(100).

2.5.4: 3,4,5-trimethoxy-*N′*-(3,4,5-trimethoxybenzylidene)benzohydrazide (8)

Product, white solid, yield: 79%; M.P: 240-243; M.F: $C_{20}H_{24}N_2O_7$; M.W; 404.42; FT-IR (cm^{-1}): 3215 (NH), 3016 (C-H, aromatic), 2939-2831 (C-H, aliphatic), 1645 (C=O, amide), 1639 (N=CH, imine), 1575 (C=C, aromatic), 1498-1411 (CH3, bending); ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 10.01 (s, 1H, NH), 8.28 (s, 1H, N=CH), 7.13 (s, 2H, aromatic), 6.90 (s, 2H, aromatic), 3.92-3.86 (s, 12H, 4 *meta*-OCH3), 3.85-3.83 (s, 6H, 2 *para*- OCH3). ^{13}C NMR (126 MHz, $CDCl_3$,δ ppm): 159.93 (C=O, amide), 138.81 (N=CH), 153.49 (2C), 148.77 (2C), 141.43 (1C), 140.37 (1C), 129.03 (1C), 128.43 (1C), 108.10 (2C), 104.88 -103.28 (2C), (12C, aromatic), 60.91- 60.88 (2 *para*-OCH3), 56.32-56.29 (4 *meta*-OCH3). MS-EI (m/z, %): 404 (M^+, 28), 390 (10), 210 (100).

2.5.5: 3,4,5-trimethoxy-*N′*-(4-nitrobenzylidene)benzohydrazide (9)

Product, yellow solid, yield: 64%; M.P: 215-217; M.F: $C_{17}H_{17}N_3O_6$; M.W; 359.34; FT-IR(cm^{-1}) : 3190 (NH), 3047 (C-H, aromatic), 2980-2839 (C-H, aliphatic), 1639 (C=O, amide), 1581 (N=CH, imine), 1556 (C=C, aromatic), 1516-1332 (NO2), 850 (*para*-NO2); ^{1}H NMR (500 MHz, $CDCl_3$,δ ppm): 9.57 (s, 1H, NH), 8.50 (s, 1H, N=CH), 8.25 (d, , *J* = 8.2 Hz, 2H, aromatic), 7.87 (d, *J* = 8.4 Hz, 2H, aromatic), 7.13 (s, 2H, aromatic), 3.92 (s, 6H, 2 *meta*-OCH3), 3.91 (s, 3H, *para*-OCH3). ^{13}C NMR (126 MHz, $CDCl_3$, δ ppm): 164.13 (C=O, amide), 145.35 (N=CH), 152.88 -152.83 (2C), 148.11 (1C), 141.18 (1C), 140.52 (1C), 128.21 (1C), 127.80 (2C), 123.68 (2C), 105.56 (2C), (12C, aromatic), 60.65 (*para*-OCH3), 56.23 (2 *meta*-OCH3). MS-EI, (m/z, %): 359 (M^+, 100), 298 (16),210 (22), 148 (96), 118 (18).

2.5.6: *N′*-(4-bromobenzylidene)-3,4,5-trimethoxy benzohydrazide (10)

Product, white solid, yield: 63%; M.P: 210-213; M.F: $C_{17}H_{17}BrN_2O_4$; M.W: 393.24; FT-IR (cm^{-1}): 3257 (NH), 3086-3034 (C-H, aromatic), 2937-2837 (C-H, aliphatic), 1666 (C=O, amide), 1633 (N=CH, imine), 1589 (C=C, aromatic), 831 (*para*-Br). ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 10.09 (s, 1H, NH), 8.32 (s, 1H, N=CH), 7.50 (d, *J* = 8.3 Hz, 2H, aromatic), 7.46 (d, *J* = 8.4 Hz, 2H, aromatic), 7.12 (s, 2H, aromatic), 3.88 (s, 6H, 2 *meta*-OCH3), 3.85 (s, 3H, *para*- OCH3). ^{13}C NMR (126 MHz, $CDCl_3$, δ ppm): 163.94 (C=O, amide), 147.11 (N=CH), 152.84 (2C), 133.22 (2C), 131.64 (3C), 128.77 (3C), 105.42 (2C), (12C, aromatic), 60.68 (*para*-OCH3), 56.23 (2 *meta*-OCH3). MS-EI, (m/z,%): 394 ([$M^{81}Br]^+$,98), 392 ([$M^{79}Br]^+$,100), 79 (92).

2.5.7: *N′*-(4-chlorobenzylidene)-3,4,5-trimethoxybenzohydrazide (11)

Product, white solid, yield (69%), M.P: 180-184; M.F: $C_{17}H_{17}ClN_2O_4$; M.W: 348.78; FT-IR (cm^{-1}): 3201 (NH), 3064-3014 (C-H, aromatic), 2943-2835 (C-H, aliphatic), 1639 (C=O, amide), 1602 (N=CH, imine), 1581(C=C, aromatic), 831 (*para*-Cl). ^{1}H NMR (500 MHz, DMSO-d $_6$, δ ppm): 11.79 (s, 1H, NH), 8.47 (s, 1H, N=CH), 7.77 (d, *J* = 8.1 Hz, 2H), 7.54 (d, *J*= 8.1 Hz, 2H, aromatic), 7.25 (s, 2H, aromatic), 3.74 (s, 6H, 2 *meta*-OCH3), 3.34 (s, 3H, *para*-OCH3). ^{13}C NMR (126 MHz, DMSO-d $_6$, δ ppm): 163.07 (C=O, amide), 146.85 (N=CH), 153.19 (2C), 134.99 (1C), 133.76 (1C), 129.45 (2C), 129.18 (2C), 128.88 (2C) 105.72 (2C), (12C, aromatic), 60.61 (*para*-OCH3), 56.58 (2 *meta*-OCH3). MS-EI, (m/z, %): .350 ([$M^{37}Cl$] ,18), 348 ([$M^{35}Cl$] , 63), 246 (56), 210 (100).

2.5.8: *N′*-(4-hydroxybenzylidene)-3,4,5-trimethoxy benzohydrazide (12)

Product, light green solid, yield: 81%; M.P: 136-140; M.F: $C_{17}H_{18}N_2O_5$; M.W: 330.34; FT-IR (cm^{-1}): 3520 (OH), 3360 (NH), 3088 (C-H, aromatic), 2945-2839 (C-H, aliphatic), 1647 (C=O, amide), 1627 (N=CH, imine), 1599 (C=C, aromatic). ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 10.53 (s, 1H, NH), 9.01 (s, 1H, OH), 8.26 (s, 1H, N=CH), 7.58 (d, *J* = 8.2 Hz, 2H, aromatic), 7.13 (s, 2H, aromatic), 6.81 (d, *J* = 8.2 Hz, 2H, aromatic), 3.86 (s, 6H, 2 *meta*-OCH3), 3.82 (s, 3H, *para*-OCH3). ^{13}C NMR (126 MHz, $CDCl_3$, δ ppm): 159.54 (C=O, amide), 148.94 (N=CH), 152.78 (2C), 143.18 (1C), 133.09 (2C), 129.14 (2C), 125.33 (1C), 115.67 (2C), 105.20 (2C), 60.65 (*para*-OCH3), 56.19 (2 *meta*-OCH3). MS-EI (m/z, %): 330 (M^+, 100), 211 (14), 119 (58).

2.6: In vitro antimicrobial activity

The antimicrobial activity of the synthesized compounds (**5-12**) was carried out by the disc diffusion method. In this work, the antibacterial activity was screened in vitro against two gram positive bacteria (*Staphylococcus aureus and Staphylococcus epidermidis*) and two gram negative bacteria (*Escherichia coli and Klebsiella sp.*), while antifungal activity was screened against fungi candida albicans by the measuring on agar plates [25]. The bacterial strains were sub-cultured by using Nutrient agar medium. They were incubated at 37 °C for 24 hours. In sterile Petri dish, 20 ml of disinfected Nutrient agar were placed. The bacterial strain cultures were modified to 0.5 McFarland standard. The dishes were swabbed with the inocula of the bacterial strains and left for 15 minutes to be adsorbed into the gel. Wells were made in the gel by a sterile cork borer of 6 mm diameter. The wells were filled with the solutions of the test compounds (1000 µg/ml in DMSO), ampicillin (1000 µg/ml) was employed as standard drug and dimethyl sulfoxide (1µl, DMSO) was used as blank (solvent). The zones of inhibition were then measured after incubation at 37 °C for 24 hours [26, 27].

2.7: Preparation of ligands and protein receptor

In this study, we have used the crystal structure of the enzyme saDHFR [PDB ID: 2W9H] retrieved from Protein Data Bank (PDB). Whereas, the missing atoms were added with the assist of Swiss PDB Viewer (v.3.7), all water molecules have been removed when prepared the protein through addition of hydrogen atoms to acquire a right ionization and tautomeric states. The energy of synthesized ligands were minimized by Chem 3D (v. 18.0) of applying the MM2 force field [28].

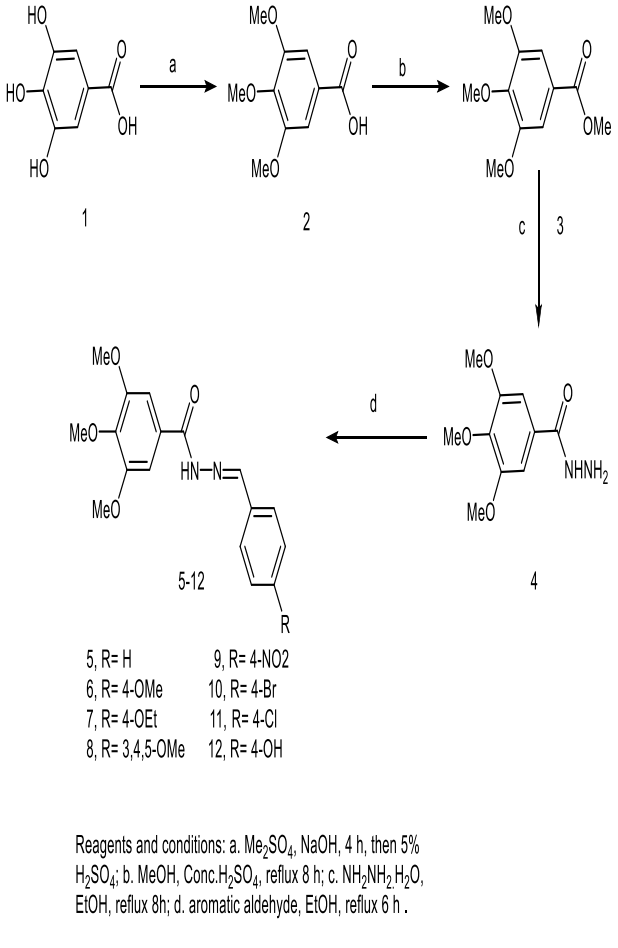
2.8: Molecular docking

The molecular docking calculation were carried out of the full license version of GOLD (v.2021.1.0) [29]. The receptors for the docking process were installed from Herms visualizer software in the GOLD suite. Furthermore, the bonding site was identified with 10A° radius around the ligands inside of crystal structures. saDHFR protein was downloaded from the PDB website (2W9H) which selected to dock the compounds. Cambridge Crystallographic Data Center (CCDC) superstar was used to value the hole and the binding site. ChemScore Kinase was used as template for the configuration. In contrast, Chem Piecewise linear potential (CHEMPLP) has been utilized for the scoring all parameters, and all solutions that used during the docking process while represent steric adjunct, distance ,and angle-depended hydrogen between protein and ligand were determined with according to the fitness function of CHEMPLP [30]. The results of interaction between the amino acids residues of the protein saDHFR and synthesized ligands were evaluated by docking studies of the binding mode, docked pose, and binding free energy [31].

3: Results and discussion

3.1 Chemistry

The synthesis of the intermediates and target compounds (**1-12**) is outlined in Scheme (**1**). 3,4,5-Trimethoxy benzoic acid **2** wasprepared by methylation of gallic acid **1** with dimethyl sulfate in presence of NaOH[32]. Preparation of 3,4,5-trimethoxy benzohydrazide**4** was achieved by two steps, esterification of 3,4,5-trimethoxy benzoic acid **2** with methanol in the presence of sulfuric acid as catalyst to result methyl-3,4,5-trimethoxy benzoate **3** , followed by reaction with hydrazine hydrate in ethanol. The target hydrazones (**5-12**) were synthesized by the reaction of **4** with the appropriate aromatic aldehyde (benzaldehyde, 4-methoxy benzaldehyde, 4-ethoxy benzaldehyde, 3,4,5-trimethoxy benzaldehyde, 4-nitro benzaldehyde, 4-bromo benzaldehyde, 4-chloro benzaldehyde, 4-hydroxy benzaldehyde), in ethanol and heated under reflux to results 3,4,5-trimethoxy-N'-(substituted benzylidene) benzohydrazides (**5-12**) [33]. The FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectral data confirmed the structures of the target compounds (**5-12**), ^{1}H NMR spectra showed the presence of the singlet signals at (11.59-9.57), (8.50-8.26), and (7.88-6.80) correspond to –CO-NH- , -CH=N , and aromatic protons respectively, FT-IR spectra showed the characteristic absorption bands at (3360-3142, NH), (1666-1635, C=O), (1633-1585, CH=N), ^{13}C NMR spectra showed the presence of C=O at (164.13-153.20), and CH=N at (148.17-138.81) beside signals for all of the other carbon atoms. Mass spectra confirmed the molecular formula by showing $[M+1]^{+}$ of all hydrazone derivatives (**5-12**).The detailed data are shown in the experimental section.



Scheme 1. Pathway of synthesis of hydrazone derivatives (**5-12**).

3.2: In vitro antimicrobial assays

The variations in the structures of **5-12** is only at the *para* position of the aldehyde part, while the acidhydrazide part consist 3,4,5-trimethoxyphenyl for all of them. To study the biological activity and the effect of the different substituents on that, the synthesized compounds were subjected to antibacterial and antifungal tests against two gram positive bacteria (*Staphylococcus aureus* and *Staphylococcus epidermidis*), two gram negative bacteria (*Escherichia coli* and *Klebsiellasp*) and candida albicans fungi. For *S. aureus* (G^+), compounds **6-11** showed a moderate antibacterial activity with inhibition zone diameter 10-14 mm, while compounds **5** and **12** did not show any effect. For *S. epidermidis* (G^+), compounds **5-8** and **1**0 showed a moderate antibacterial activity with inhibition zone diameter 11-15 mm, while compounds **9**, **11** and **12** did not show any effect. For *E. coli* (G^-) compounds **5-7**, **10** and **11** showed a moderate antibacterial activity with inhibition zone diameter 10-13 mm, while compounds **8, 9** and **12** did not show any effect. For *Klebsiella* (G^-), compounds **5** and **7-11** showed a moderate antibacterial activity with inhibition zone diameter 9-14 mm, while compounds **6** and **12** did not show any effect. The results of antifungal activity of the test compounds was tested against *Candida albicans*, the results reveled that compounds **6**,**7** and **10** have moderate antifungal activity with inhibition zone 12 mm, while others have no effect. It is clearly observed that compound **10** which has -OH group in its structure did not show any kind if biological activities within the employed studies, on the other hand the variation in the other substituents does not make significant changes in the activities except some cases in which test compounds showed moderate activity against certain microorganisms and no effect on others. Table 1 shows the obtained results.

Table (1): The antimicrobial activity of the compounds 5-12.

Compound	Inhibition zone diameter (mm)				
	***S. aureus* (G^+)**	***S. epidermidis* (G^+)**	***E. coli* (G^-)**	***Klebsiella* (G^-)**	***Candida albicans***
DMSO	-	-	-	-	-
5	-	12	12	9	-
6	12	12	10	-	12
7	14	15	12	13	12
8	10	12	-	14	-
9	10	-	-	12	-
10	12	11	13	12	12
11	13	-	11	14	-
12	-	-	-	-	-
Ampicillin	27	20	29	30	30

3.3: Molecular studies

To study the interactions between molecules (ligands) and certain sites in the protein structure (binding sites), GOLD (Genetic Optimization for Ligand Docking) is used, it is a genetic algorithm for docking flexible ligands into protein binding sites. It is reported that the results obtained from GOLD for virtual screening studies show high level of accuracy close to the experimental results. The energy minimization for both ligand and protein repair the distorted geometries by transferring atoms to release internal restrictions [34]. According to the resembles between trimethoprim and the general structure of **5-12**,the binding site of the wild-type *Staphylococcus aureus* DHFR in complex with trimethoprim is the target site in this study, the molecular docking study of compounds **5-12** (ligands) with this target is performed to investigate the hydrogen bonding, van der waals, electrostatic, steric, π- π stacking,

dipole-dipole, and other interactions by using GOLD with bond length below of 3Aº [35]. The binding energy values, numbers of amino acids involved in the interaction, numbers and lengths of interaction bonds are shown in Table 2. Figure 2 show the predicted complexes of 5-12 with the binding site in *Staphylococcus aureus* DHFR. In comparison with 2W9H which have binding energy value 96.09, the tested compounds showed good levels of binding energies (74.99 to 83.87). The better interactions are shown with amino acids residues -SER49, THR121, GLN95, and ASN18. Thus, the biological activity of the tested compound could be attributed to the deactivation of the enzyme dihydrofolatereductase.

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	No. of bonds	Lengths of bonds	
2W9H	96.09	4	ASP27	2	3.054	2.971
			LEU5	1	3.030	
			PHE92	1	2.577	
5	76.70	1	SER49	1	2.839	
6	80.69	2	GLN95	1	2.990	
			SER49	1	2.931	
7	83.87	3	SER49	1	3.064	
			THR121	1	3.031	
			GLN95	1	2.874	
8	74.99	2	ASN18	1	2.869	
			THR46	1	2.507	
9	82.65	2	THR121	1	2.938	
			SER49	1	2.819	
10	77.42	2	SER49	2	3.007	2.966
11	80.04	1	SER49	1	2.577	
12	79.15	3	SER49	1	3.050	
			THR121	1	3.033	
			GLN95	1	2.817	

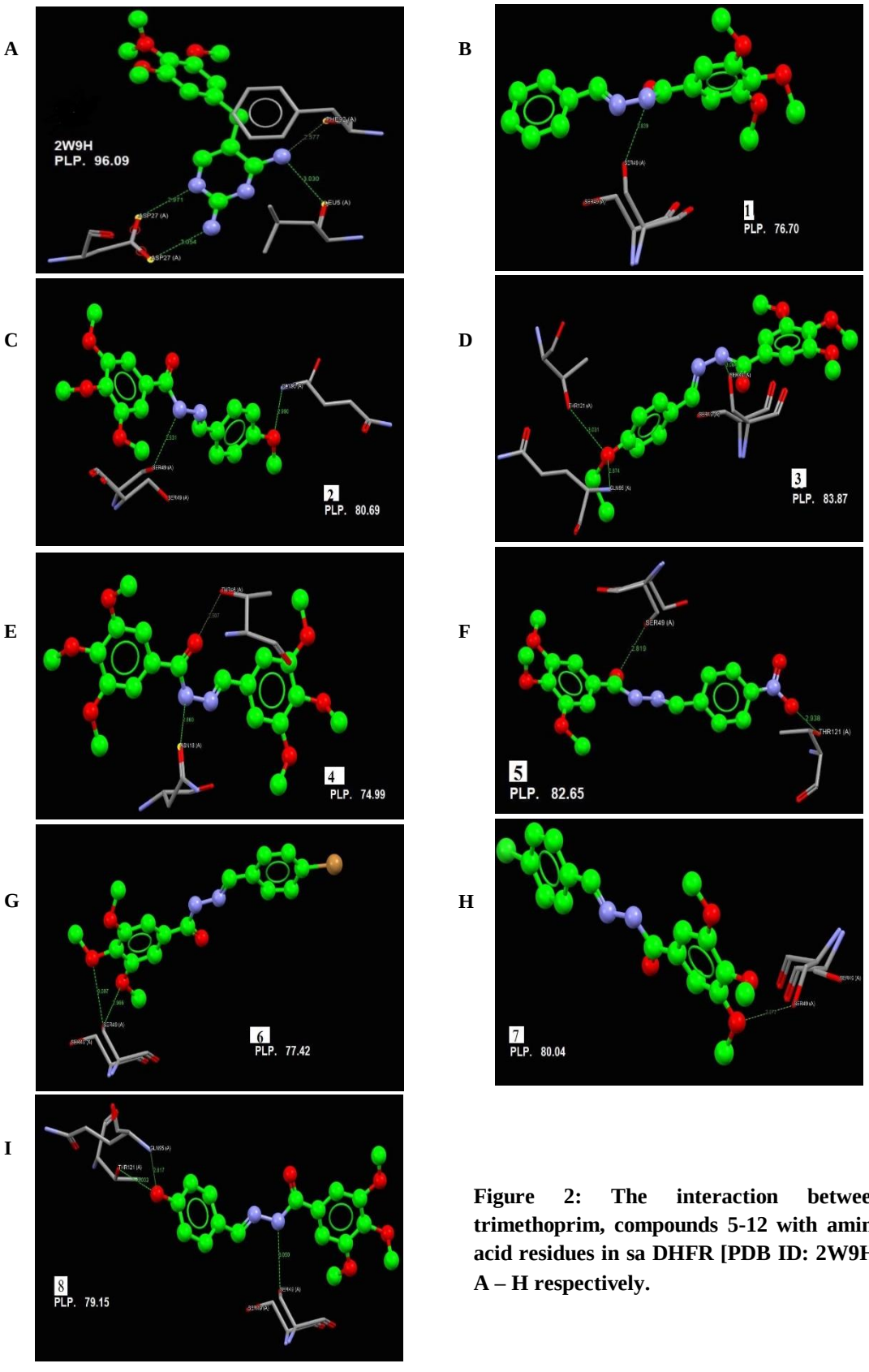


Figure 2: The interaction between trimethoprim, compounds 5-12 with amino acid residues in sa DHFR [PDB ID: 2W9H] A – H respectively.

Conclusions

We have synthesized a series of hydrazones with 3,4,5-trimethoxyphenyl group (**5-12**), their structures were confirmed by IR, ^{1}H NMR, ^{13}C NMR, and Mass analysis. The synthesized compounds can be considered as promising antibacterial and antifungal agents as the results of the biological activity studies indicated. The molecular docking studies showed that the possible mechanism of action is the binding of these compounds with a specific site in the enzyme dihydrofolatereductase (DHFR) causing significant inhibition of its activity.

Acknowledgements

The authors thank the the Chemistry Department at the College of Science, Al-Mustansiriyah University for the assistance in providing all the necessary 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.

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