Original
ISSN 2413-0516

Molecular Docking, ADME Study, Synthesis, Characterization and Preliminary Antimicrobial Activity Evaluation of New Phenylalanine Derivatives of Sulfonamide

Haider Mohsen Naeem*[1], Zainab Abdelhadi Dakhel[2]

[1]Iraqi Ministry of Health, Dhi-Qar Directorate, Dhi-Qar, Iraq.
[2]Department of Pharmaceutical Chemistry, College of Pharmacy, University of Baghdad, Baghdad, Iraq.
*Correspondence to: Haider Mohsen Naeem (E-mail: haideralkabi7737@gmail.com)
(Submitted: 10 October 2024 – Revised version received: 24 October 2024 – Accepted: 23 November 2024 – Published online: 26 December 2024)

Abstract
Objective: The main objective for this study is the synthesis of a series of new phenylalanine derivatives of sulfonamide.
Methods: The final compounds P1-P4 were synthesized starting with the synthesis of two intermediate compounds I and II, which were reacted with each other in to get compound III. Then, the later was reacted with hydrazine hydrate led to the compound IV that was eventually reacted with different aldehydes to produce the target compounds. Characterization of their structure was performed using ATR-FTIR and HNMR spectroscopies. The assessment of the antimicrobial activity was done for all of them. The final compounds were subjected to molecular docking and ADME study, which were conducted in comparison to standard drugs (sulfamethoxazole and sulfadiazine).
Results: The synthesized final compounds exerted a broad inhibitory activity on certain microbes, including Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pneumonia*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and one fungus (*Candida albicans*). However, compounds P3 and P4 had the highest activity. The molecular docking findings represented compounds P1, P2 and P3 with high docking scores compared with standard drugs, while the ADME study showed a desired drug-likeness and accepted pharmacokinetic properties.
Conclusions: The target compounds were successfully synthesized with good antimicrobial activity. The compounds P1, P2 and P3 recorded the highest docking scores, while all the final compounds had good ADME findings.
Keywords: Antimicrobial, molecular docking, schiff base, sulfonamide, zone inhibition (ZI)

Introduction

The primary sulfonamide moiety ($R\text{-}SO_2NH_2$) is involved in numerous prescribed drugs, including cyclooxygenase-2 (COX-2) inhibitors, diuretics, and carbonic anhydrase inhibitors.[1,2] Initially discovered in the 1900s, sulfonamides rose to popularity as the first potent chemotherapeutic medicines for a variety of bacterial diseases in the late 1930s.[3–5] Their primary mode of action is the competitive inhibition of dihydropteroate synthetase (DHPS), an enzyme essential to the bacterial synthesis of folic acid. This mechanism confers bacteriostatic qualities because they emulate para-aminobenzoic acid (PABA), which prevents the creation of folate, which is necessary for the synthesis of nucleic acids and the growth of bacteria.[6,7]

Bacterial resistance and the development of stronger antibiotics have compromised the medicinal effectiveness of sulfonamides. These resistance mechanisms include enzymatic breakdown and target modification[8] or through their acquisition of extra genetic material from resistant strains via conjugation, transformation, or transduction to antimicrobial-susceptible bacteria.[9] Today, sulfonamides are primarily indicated for the treatment of particular infections like urinary tract infections and infections in immunocompromised patients as an alternative therapeutic option.[10]

Sulfonamides are categorized into antibacterial and non-antibacterial types.[11–13] Hypersensitivity responses can range from mild symptoms such as fever and rash, potentially accompanied by hepatitis, lymphadenopathy, and/or haemolytic anaemia, to severe conditions including Stevens-Johnson syndrome and toxic epidermal necrolysis.[14,15]

Materials and Methods

Materials and Instruments

All starting materials, solvents, and reagents utilized were received from a chemical store of a college of pharmacy, University of Baghdad; unless an unavailable one, chlorosulfonic acid was bought from the commercial chemical supplier of high quality. Melting points were measured utilizing a digital melting point apparatus (Stuart SMP30). The assessment of product purity and reaction monitoring was conducted through thin layer chromatography (TLC). ^{1}H-NMR (proton-nuclear magnetic resonance) spectra were obtained with a BRUKER Avance III 400 MHz spectrometer, employing dimethyl sulfoxide (DMSO) as the solvent. FTIR (Fourier transform infrared) spectra were obtained utilizing an FTIR spectrometer (Shimadzu, Japan) at the College of Pharmacy, University of Baghdad.

Molecular docking: The docking investigation utilized the Glide program, integrated with Schrodinger's licensed Maestro software version 13.0135. The final products were evaluated comparing with the reference drugs sulfamethoxazole and sulfadiazine. The crystal structure of Yersinia pestis DHPS complexed with pterine-sulfa conjugate compound 16 was obtained from the Protein Data Bank (PDB ID: 5JQ9).[16] The ligands were evaluated through the assessment of ligand binding geometries and potential energy calculations using DHPS (PDB ID: 5JQ9). This study employs molecular docking techniques utilizing grid-based Ligand Docking with Energetics (Glide) to rank ligands based on

the glide scoring function (G score) and to analyze receptor-ligand interactions.[17]

ADME studies: Ligand-based ADME prediction was conducted to evaluate pharmacokinetic characteristics, including intestinal absorption, systemic distribution, metabolism and excretion (ADME), employing Qikprobe software inside Schrodinger Maestro.[18]

Chemical Synthesis

The synthetic procedures for the synthesized final compounds and their intermediates illustrated in the Scheme 1 steps:

Synthesis of L-phenylalanine methyl ester HCL ((S)-1-methoxy-1-oxo-3-phenylpropan-2-aminium) (compound I)

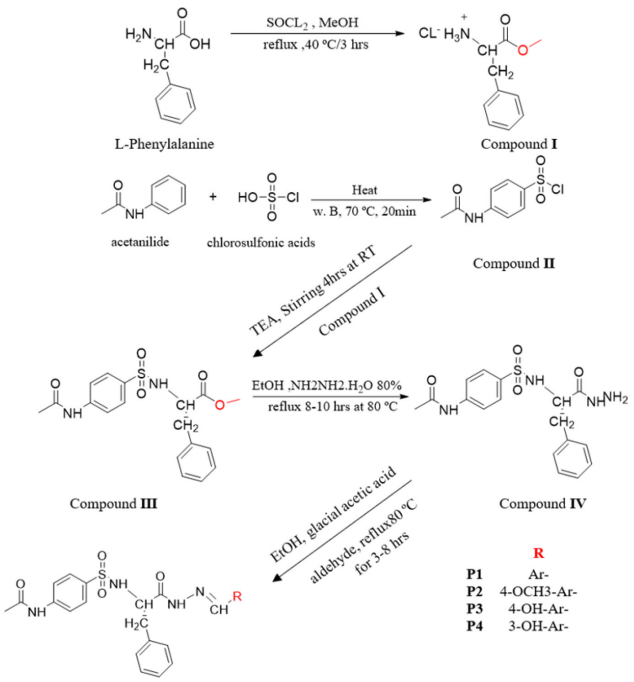
Methanolic suspension of L-phenylalanine (30.26 mmol, 5 gm in 50 ml of methanol) was cool down to 0°C, then adding thionyl chloride 1.2 equivalent (79.98 mmol, 5.758 ml) dropwise for 5 min. After the entire addition, the mixture then refluxed at 40°C for 3 hours. Subsequently, it was allowed to cool to ambient temperature, and the solvent was evaporated multiple times to eliminate excess thionyl chloride, yielding the solid substance (crude product). 25 ml of methanol was added and cooled to 0°C, followed by the addition of 100 ml of diethyl ether with gentle stirring using a glass rod. The resulting white crystalline precipitate was vacuum filtered to obtain the pure product, which was subsequently washed with a diethyl ether: methanol (5:1) mixture and dried to yield L-phenylalanine methyl ester HCL.[19]

Chemical formula, ($C_{10}H_{14}NO^{+}$), White crystals, melting point(m.p.) = 152–155°C, yield = 76%, Rf = 0.65 (solvent system, Ethanol: Water, 9:1), FT-IR (v = cm-1): 3086–2800 (broad band, N–H stretching of ammonium salt), 1732 (C=O str. of ester), 1604 (N–H bending of ammonium salt), 1207, 1134 (asym. and sym. str. of C–O–C mixed with C–N str.).

Synthesis of 4-acetamidobenzenesulfonyl chloride (compound II)

To a 250-ml Erlenmeyer flask, 37.03 mmol (5 g) of acetanilide was introduced along with a magnetic stir bar. The flask was secured on a water bath kept at 10–15 °C, fitted with a magnetic stirrer. Subsequently, 224.88 mmol (15 ml) of chlorosulfonic acid was measured into a 100-ml separatory funnel. The acid was added in a single pour into the flask while the mixture was stirred, and it was allowed to stand for 10–15 minutes to eliminate additional fumes while maintaining the temperature below 20°C to expedite the full dissolution of the solid. Subsequent to the first reaction, the cooling water was withdrawn, and the reaction mixture was stirred until it reached room temperature. The mixture was heated for 10 to 20 minutes at a temperature ranging from 70 to 80°C while continuously stirring until the rate of gas evolution ceased to increase. The mixture was then cooled below room temperature and poured over 300 cc of crushed ice while being mixed with a glass rod. The precipitate obtained is a crude product of 4-acetamidobenzenesulfonyl chloride. The crude product was recovered using vacuum filtration and rinsed with cold water. The filtered cake was then pressed with clean cork for immediate use in the subsequent step.[19]

Chemical formula, ($C_8H_8ClNO_3S$), Off white powder, m.p. = 143–146°C, yield = 64%, Rf = 0.50 (solvent system, Chloroform: Ethyl acetate, 1:1), FT-IR (v = cm-1): 3302 (N–H str. of 2° amide), 3051 and 3005 (C–H str. of aromatic ring), 2939 and 2866 (C–H asym. and sym. str. of CH3 group), 1678 (C=O str. of 2° amide), 1581–1492 (C=C str. of aromatic ring overlap with N–H ben. of 2° amide) and 1365 and 1161 (asym. and sym. str. of O=S=O group).



Scheme 1. **The synthetic steps of final products, compounds P1-P4.**

Synthesis methyl ((4-acetamidophenyl) sulfonyl)-L-phenylalaninate (compound III)

Triethyl amine (TEA) (18.604 mmol, 2.58 ml) was added gradually to the round flask contain the suspension of (9.302 mmol, 2 gm) from the compound **I** in 20 ml of DCM that already put on ice bath with 0°C, the mixture stirred for 20 min. Then (9.302 mmol, 2.173 gm) from the compound **II** that was suspended with 20 ml of DCM added to the reaction mixture and stirred for 40 min. Subsequently, the ice bath was removed, and the mixture was stirred for 4 hours at room temperature. The reaction solution was extracted three times with 20 mL of distilled water. The organic layer was evaporated, followed by the decantation of the gummy result using diethyl ether. The solid crude product was washed with water and dried using vacuum filtering to get compound III.[20]

Chemical formula, ($C_{18}H_{20}N_2O_5S$), Pale yellow powder, m.p. = 192–196°C, yield = 87%, Rf = 0.3 (solvent system, Ethyl acetate: Chloroform, 6:4), FT-IR (v = cm-1): 3344 (N–H str. of 2° sulfonamide), 3167 (N–H str. of 2° amide), 3032 (C–H str. of aromatic ring), 1716 (C=O str. of ester), 1678 (C=O str. of 2° amide), 1593–1492 (C=C str. of aromatic ring overlap with N–H ben. of 2° amide) and 1334 and 1161 (asym. and sym. str. of O=S=O group).

Synthesis of (S)-N-(4-(N-(1-hydrazineyl-1-oxo-3-phenylpropan-2-yl) sulfamoyl) phenyl) acetamide (compound IV)

The compound **III** (5.138 mmol, 2 gm) and hydrazine hydrate 80% (51.38 mmol, 2.46 ml) in 20 ml of ethanol were mixed in 100 ml round flask. Then the mixture stirred for 10 hr. at 80°C. The reaction mixture was allowed to cool to room

temperature, after which the filtered precipitate was washed with distilled water and recrystallized using ethanol.[21]

Chemical formula, ($C_{17}H_{20}N_4O_4S$), White powder, m.p. = 225–228°C, yield = 95%, Rf = 0.55 (solvent system, Ethyl acetate: Chloroform: Methanol, 4:4:2), FT-IR (v = cm-1): 3300 and 3294 (N–H asym. and sym. str. of 1° amine of hydrazide), 3240 (N–H str. of 2° sulfonamide), 3190 (N–H str. of 2° amide), 3105 and 3032 (C–H str. of aromatic ring), 1670 (C=O str. of 2° amide), 1589 and 1539 (C=C str. of aromatic ring overlap with N–H ben. of 2° amide) and 1315 and 1153 (asym. and sym. str. of O=S=O group).

Synthesis of Schiff bases, the compounds P1-P4 (The final compounds)

Few drops of glacial acetic acid were added to ethanolic solution of (1.328 mmol) of every aldehyde that follows: benzaldehyde (0.134 ml), 4-methoxy benzaldehyde (0.2 ml), 4-hydroxybenzaldehyde and 3-hydroxy benzaldehyde (0.162 gm) for each one. Then each one of that solutions was added to the compound **IV** (1.328 mmol, 0.5 gm) dissolved in 10 ml of ethanol contained in 100 ml round flask separately. The reaction mixtures were stirred at 80°C for 3–6 hr. After that, the mixtures cool down to room temperature, the products were filtered and washed by cold distilled water.[22]

(S, E)-N-(4-(N-(1-(2-benzylidenehydrazineyl)-1-oxo-3-phenylpropan-2-yl) sulfamoyl) phenyl) acetamide (Compound P1)

Chemical formula, ($C_{24}H_{24}N_4O_4S$), White powder, m.p. = 210–215°C, yield = 75%, Rf = 0.69 (solvent system Ethyl acetate: Chloroform: Methanol, 4:4:2), FT-IR (v = cm-1): 3302 (N–H str. of 2° sulfonamide), 3240 (N–H str. of 2° amide), 3109 and 3066 (C–H str. of aromatic ring), 1676 (C=O str. of 2° amide), 1658 (C=N str. of imine), 1593 and 1543 (C=C str. of aromatic ring) and 1334 and 1157 (asym. and sym. str. of O=S=O group). ^{1}HNMR : (400 MHz, DMSO) δ 11.35 (s, 1H, -NH- of -NH-CO-CH3), 10.25 (s, 1H, -NH-C=O), 10.17 (s, 1H, -NH-SO2-), 8.34 – 8.03(m, 2H, Ar), 8.00 (s, 1H, -CH=N-), 7.66 – 7.55 (m, 4H, Ar-SO2), 7.56 – 7.47 (m, 2H, Ar), 7.46 – 7.38 (m, 3H, Ar), 7.21 – 7.17 (m, 3H, Ar), 5.02 (dd, 1H, -CH-), 2.81 (dd, 2H, -CH2-), 2.07 (s, 3H, CH3-C=O).

(S,E)-N-(4-(N-(1-(2-(4-methoxybenzylidene) hydrazineyl)-1-oxo-3-phenylpropan-2-yl) sulfamoyl) phenyl) acetamide (compound P2)

Chemical formula, ($C_{25}H_{26}N_4O_5S$), White powder, m.p. = 225–229°C, yield = 76%, Rf = 0.42 (solvent system, Methanol: Ethyl acetate, 6:4), FT-IR (v = cm-1): 3313 (N–H str. of 2° sulfonamide), 3251 (N–H str. of 2° amide), 3066 and 3039 (C–H str. of aromatic ring), 1662 (C=O str. of 2° amide), 1606 (C=N str. of imine), 1593–1512 (C=C str. of aromatic ring), 1334 and 1157 (asym. and sym. str. of O=S=O group) and 1253 and 1018 (C-O str. of methoxy group). ^{1}HNMR : (400 MHz, DMSO) δ 11.22 (s, 1H, -NH- of -NH-CO-CH3), 10.25 (s, 1H, -NH-C=O), 10.18 (s, 1H, -NH-SO2-), 7.95 (s, 1H, -CH=N-), 7.84 – 7.54 (m, 4H, Ar-SO2), 7.54 – 7.35 (m, 2H, Ar), 7.27 – 7.11 (m, 4H, Ar), 7.10 – 6.94 (m, 3H, Ar), 5.00 (dd, 1H,-CH-), 3.82 (s, 3H, -OCH3), 2.80 (dd, 2H, -CH2-), 2.08 (s, 3H, CH3-C=O).

(S,E)-N-(4-(N-(1-(2-(4-hydroxybenzylidene) hydrazineyl)-1-oxo-3-phenylpropan-2-yl) sulfamoyl) phenyl) acetamide(compound P3)

Chemical formula, ($C_{24}H_{24}N_4O_5S$), White powder, m.p. = 163–168°C, yield = 73%, Rf = 0.57 (solvent system, Methanol: Ethyl acetate, 6:4), FT-IR (v = cm-1): 3549 and 3487 (O–H str. of -Ar-OH group) 3309 (N–H str. of 2° sulfonamide), 3248 (N–H str. of 2° amide), 3097 (C–H str. of aromatic ring), 1623 (C=O str. of 2° amide), 1612 (C=N str. of imine), 1589–516 (C=C str. of aromatic ring), 1315 and 1157 (asym. and sym. str. of O=S=O group). ^{1}HNMR : (400 MHz, DMSO) δ 11.26 (s, 1H, -NH- of -NH-CO-CH3), 10.23 (s, 1H, -NH-C=O), 9.72 (s, 1H, -NH-SO2-),9.61 (s, 1H, -OH), 7.91 (s, 1H, -CH=N-), 7.68 – 7.53 (m, 4H, Ar-SO2), 7.51 – 7.42 (m, 2H, Ar), 7.29 – 7.14 (m, 3H, Ar), 7.11 – 6.78 (m, 4H, Ar), 4.99 (dd, 1H, -CH-), 2.82 (dd, 2H, -CH2-), 2.07 (s, 3H, CH3-C=O).

(S,E)-N-(4-(N-(1-(2-(3-hydroxybenzylidene) hydrazineyl)-1-oxo-3-phenylpropan-2-yl) sulfamoyl) phenyl) acetamide (compound P4)

Chemical formula, ($C_{24}H_{24}N_4O_5S$), White powder, m.p. = 260–263°C, yield = 52%, Rf = 0.51 (solvent system, Methanol: Ethyl acetate, 6:4), FT-IR (v = cm-1): 3379 (N–H str. of 2° sulfonamide), 3232 (N–H str. of 2° amide), 3379–3100 (O–H str. of -Ar-OH group broad band), 3066 and 3028 (C–H str. of aromatic ring), 1662 (C=O str. of 2° amide), 1589 (C=N str. of imine), 1527–1496 (C=C str. of aromatic ring), 1319 and 1157 (asym. and sym. str. of O=S=O group). ^{1}HNMR : (400 MHz, DMSO) δ 11.13 (s, 1H, -NH- of -NH-CO-CH3), 10.24 (s, 1H, -NH-C=O), 10.17 (s, 1H, -NH-SO2-),9.95 (s, 1H, -OH), 7.88 (s, 1H, -CH=N-), 7.59 – 7.54 (m, 4H, Ar-SO2), 7.51 (s, 1H, Ar), 7.21 – 7.18 (m, 3H, Ar), 7.16 – 7.12 (m, 2H, Ar), 6.86 – 6.79 (m, 3H, Ar), 4.97 (dd, 1H,-CH-), 2.77 (dd, 2H, -CH2-), 1.99 (s, 3H, CH3-C=O).

All IR spectra of final compounds and their intermediates arranged in appendix from Figures 2–9 and HNMR spectra of final compounds listed in Figures 10–13.

Antimicrobial assessment: Using the broth dilution and agar diffusion methods, the antimicrobial efficacy (displayed as the minimum inhibitory concentration (MIC) and zone of inhibition (ZI)) of target compounds was evaluated against four bacteria (*S. aureus, S. pneumoniae, P. aeruginosa*, and *E. coli*) and one fungus (*C. albicans*). A stock solution of the finished products and standard medications (sulfamethoxazole, sulfadiazine, and fluconazole) at 10 mg/ml was utilized to generate a range of concentrations from 10 to 1000 mcg/ml for MAC and MIC testing. The negative control, DMSO, was used to solubilize the final compounds.[23]

***Sensitivity assay*:** The diluted samples, using Muller-Hinton broth as the diluent, were prepared on a microtiter plate. Each well of the plate was inoculated at 37°C for 18–20 hours with 20 µl of a bacterial suspension equivalent to McFarland standard no. 0.5 (1.5 x 10^8 CFU/ml), excluding the negative control well. Following this, 20 µl of resazurin, prepared by dissolving 0.015 g of resazurin in 100 ml of distilled water and stored at 4°C, was added to each well, and a 2-hour incubation was conducted to assess color changes. The sub-MIC was determined in the broth microdilutions as the lowest concentration at which the color of resazurin changed from blue to pink.[24-26]

Statistical analysis: A two-way ANOVA was used to evaluate the significant differences between the data gathered from the final compounds and the reference controls. The IBM SPSS Statistics 25 software was used to analyze differences.

Results

The docking results, including the docked ligands, binding mode, and binding free energy, were summarized in Table 1. while the two dimensions (2D) docking structures of final compounds and reference drugs with DHPS enzyme are presented in Figure 1 (Appendix section).

The ADME study found target compounds as potential drug-like molecules, with favourable oral bioavailability and less CNS penetration. The QPPCaco model for the gut-blood barrier showed acceptable to good permeability values. However, all target compounds lack structural liability to participate in further metabolic processes like aromatic hydroxyl oxidation and enol oxidation, with permeability values ranging from acceptable to good (values >500 nm/sec are considered excellent, while values <25 are deemed bad).[27,28] These findings listed in Table 2.

- Lipinski Rule of Five include HB donor ≤ 5, HB acceptor ≤ 10, molecular weight < 500, log p < 5, (Limit 0–3 obey this rule).
- Oral Absorption (1 = Low, 2 = Medium, or 3 = high).
- CNS Predicted central nervous system activity scale ranging from a –2 (inactive) to +2 (active).
- Primary Metabolites < 7 (Limit 0–3 accepted).
- Gut-blood barrier QPPCaco with scale of <25 poor permeability to >500 great.
- % oral absorption with scale of <25% poor to >80% high.

The antimicrobial evaluation of the final synthesized compounds (P1-P4) were clarified in Table 3–5 as the findings of MIC, Zone inhibition and Two-way ANOVA test respectively. While antimicrobial for final compounds compared with standard drugs were shown in Figure 14 (in appendix).

The tested compounds with inhibitory zones greater than 15 mm are considered highly active, with inhibitory zones from 15–10 mm moderately active, with zones from 5–10 slightly active, and with zones less than 5 mm inactive).[29]

Table 1. **The docking score and the interactions of final compounds and standard drugs with DHPS (PDB ID: 5JQ9)**

Compound	Docking score Kcal/mol	Type of interaction
P1	−6.494	H-bonding: THR 62, GLY 189, SER 222, ARG 255 Pi-cation: LYS 221 Pi-Pi stacking: PHE 190
P2	−6.466	H-bonding: THR 62, GLY 189, SER 222, ARG 255 Pi-cation: LYS 221 Pi-Pi stacking: PHE 190
P3	−6.5	H-bonding: THR 62, GLY 189, SER 222, ARG 255 Pi-cation: LYS 221 Pi-Pi stacking: PHE 190
P4	−4.803	H-bonding: GLN 142, GLY 189, SER 222, ARG 255 Pi-cation: LYS 221
Sulfamethoxazole	−5.895	H-bonding: GLU 60, ARG 63, ASP 96, ARG 255, HIE 256 Salt bridge: ARG 63
Sulfadiazine	−5.712	H-bonding: GLU 60, ARG 63, ASP 96, ARG 255, HIE 257 Pi-cation: LYS 221 Pi-Pi stacking: PHE 190 Salt bridge: ARG 63

Discussion

From the molecular docking results of the synthesized final compounds and reference drugs, the reference compounds sulfamethoxazole and sulfadiazine had docking score −5.895 and −5.712 respectively. The docking score of synthesized compounds ranging from strong affinity to low or poor affinity in compare with reference compounds and these are clarifying as follow: The compounds with strong affinity, P3, P1 and P2 were scored −6.5, −6.494 and −6.466 respectively and the compound P4 with low affinity was scored −4.803.

Also, from the above docking results, The H-bonding with GLY 189, SER 222, ARG 255 and Pi-cation bond with LYS 221 essential for the affinity of the final compounds to the target protein.

Interpretation of synthetic results can summarize as follow: Compound **I** was indicated by appearance of a broad band for N-H bond stretching, clarifying the formation of the salt of ammonium ion 3217 to 2800 cm^{-1} and sharp band for C=O stretching of the ester at 1732 cm^{-1}. The formation of compound **II** was demonstrated by the appearance of a characteristic band at 1365 and 1161 cm^{-1} for O=S=O asymmetric and symmetric stretching of the sulfonyl group. The formation of compound **III** was illustrated by the appearance of a band at 3344 cm^{-1} for N–H stretching of secondary sulfonamide and a band at 1334 cm^{-1} and 1161 cm^{-1} for O=S=O asymmetric and symmetric stretching of sulfonyl group and band at 1716 cm^{-1} for C=O stretching of ester, The formation of compound **IV** was demonstrated by the appearance of double bands at 3300 and 3294 cm^{-1} for N–H stretching of asymmetric and symmetric primary amine of the hydrazide and the disappearance of the ester band.

All of the IR spectra of the final compounds showed the disappearance of bands related to N–H stretching of the primary amine of hydrazide and the appearance of bands of N–H stretching for secondary sulfonamide and N–H stretching for secondary amide in range at 3379 cm^{-1} to 3232 cm^{-1}. All final compounds exhibit nearly the same signals of the ^1H NMR spectrum for protons of these functional groups with variable chemical shifts as listed below:

Singlet for 1 proton of -NH of the acetamido group, singlet for 1 proton of -NH of amide group adjacent to imine group, singlet for 1 proton of -NH of sulfonamide, singlet 1 proton of -CH=N- of imine group, singlet 1 proton of -CH- of methine group, singlet for 2 protons of the -CH2- of benzylic carbon atom, singlet for 3 protons of $-CH_3$ of the acetamido group for compounds **P1-P4,** respectively.

In addition to differentiate signals, for compound **P2** singlet for 3 protons of the -OCH3 group at 3.82, for compound **P3** and **P4**, singlet for 1 proton of the -OH group at 9.61 ppm and 9.95 ppm, respectively.

Table 2. **Drug likeness characteristics for final compounds and reference drugsa**

Compound	Mol. MW	Rule of 5	Human oral absorption	CNS	#Metab	Donor HB	Accpt HB	QPP Caco	% Oral absorp.
P1	464.538	0	3	−2	2	2.25	8.75	464.53	91.811
P2	494.564	0	3	−2	3	2.25	9.5	494.56	91.922
P3	480.537	0	3	−2	3	3.25	9.5	480.53	77.873
P4	480.537	0	3	−2	3	3.25	9.5	480.53	78.124
Sulfadiazine	250.27	0	3	−2	3	2.5	7.5	250.27	68.642
Sulfamethoxazole	253.27	0	3	−2	2	2.5	7	253.27	70.212

Table 3. **The MIC results for final products and compared standard drugs**

Compounds	MIC (mm)				
	Gram positive bacteria		Gram negative bacteria		Fungi
	S. aureus	*S. pneumonia*	*E. coli*	*P. aeruginosa*	*Candida albicans*
	Conc. (mcg/ml)				
Sulfamethoxazole	250	500	500	500	–
Sulfadiazine	125	500	1000	1000	–
Fluconazole	–	–	–	–	250
DMSO	Solvent and control				
P1	500	500	500	500	500
P2	500	1000	1000	1000	500
P3	500	500	500	1000	500
P4	250	500	500	500	500

Table 4. **Zone inhibition of final compounds and their compared standards in mm**

Isolate / Compound	*S. aureus*	*S. pneumonia*	*E. coli*	*P. aeruginosa*	*C. albicans*	Mean	S. E.M	SD	*P*-value
P1	7	19	11	8	8	10.60	2.20	4.93	0.157
P2	10	20	15	10	10	13.00	2.00	4.47	0.046
P3	17	23	20	12	15	17.40	1.91	4.27	0.2
P4	20	23	20	17	20	20.00	0.94	2.12	0.161
Sulfamethoxazole	34	35	35	31	_	27	3.44	15.18	0.008
Sulfadiazine	30	30	30	30	_	24	3.44	13.41	0.001
Fluconazole	_	_	_	_	32	6.4	3.44	14.31	0.001
DMSO	Solvent and control								

Table 5. **Two-way ANOVA test for the inhibitory zones of the tested compounds**

Tests of between-subjects effects

Dependent Variable: YI

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1977.314[a]	10	197.731	1.986	.082
Intercept	10013.257	1	10013.257	100.576	.000
T	1637.143	6	272.857	2.741	.036
B	340.171	4	85.043	.854	.505
Error	2389.429	24	99.560		
Total	14380.000	35			
Corrected Total	4366.743	34			

[a]R Squared = .453 (Adjusted R Squared = .225).

All synthesized final compounds had antimicrobial activity displayed as the zone inhibition values in Table 4 (low, moderate or higher antimicrobial activity that variable for each microbial species), and the T variable that was mentioned in Table 5 (it was one of the two independent variables that represent the test compounds, along with the other one, B, representing the microbial species) had a *P* value of 0,036 (that is, mean $P < 0.05$). There is a significance difference related to the effect on the dependent variable that represents the zone inhibition values.

All synthesized final compounds, P1-P4 were shown the broad antimicrobial activity (including their antifungal activity) with higher activity against S. pneumonia(the more sensitive organism to antimicrobial activity of all these compounds), the compounds P3 and P4 were exhibited the higher antibacterial activity with inhibitions of zone ranging from 12 to 23 mm, however the compound P2 approximately was the moderate findings ranging from 10 to 20 mm of the zone inhibition and compound P1 was the lowest one with inhibition zones ranging from 7 to 19 mm. Also, that was clarified that the *S. pneumonia* and E. coli were the most sensitive bacteria for activity of the synthesized compounds. The antimicrobial activities of the target compounds are explained in the Figure 2.

Conclusion

The successful chemical synthesis of novel phenylalanine-based sulfonamide compounds P1-P4 had been accomplished. The compounds were developed by a molecular docking study targeting the Yersinia pestis DHPS protein (PDB ID: 5JQ9). They also demonstrated acceptable pharmacokinetic characteristics using simulated ADME tests.

The physical characteristics, including melting point and description, together with FT-IR and 1H-NMR spectra, have been analyzed for the identification and characterization of the synthesized compounds, and the findings corroborate their chemical structure.

The investigation on antimicrobial activity demonstrated that all synthesized final compounds exhibited inhibitory action based on their MIC values and inhibition zones with broad antimicrobial activity. The compounds P3 and P4 had higher antimicrobial activity while compounds P1 and P2 with low to moderate activity.

Conflict of Interest

None.

References

1. Hassan OM, Sarsam SW. Synthesis, characterization and preliminary anti-inflammatory evaluation of new etodolac derivatives. Iraqi Journal of Pharmaceutical Sciences (P-ISSN 1683-3597 E-ISSN 2521-3512). 2019 Jun 9;28(1):106–12. https://doi.org/10.31351/vol28iss1pp106-112
2. Azzam RA, Elsayed RE, Elgemeie GH. Design, synthesis, and antimicrobial evaluation of a new series of N-sulfonamide 2-pyridones as dual inhibitors of DHPS and DHFR enzymes. ACS Omega. 2020 Apr 28;5(18):10401–14. https://doi.org/10.1021/acsomega.0c00280
3. Mondal S, Malakar S. Synthesis of sulfonamide and their synthetic and therapeutic applications: Recent Advances. Tetrahedron. 2020 Nov 27;76(48):131662. https://doi.org/10.1016/j.tet.2020.131662
4. Carta F, Scozzafava A, Supuran CT. Sulfonamides: a patent review (2008–2012). Expert opinion on therapeutic patents. 2012 Jul 1;22(7):747–58. https://doi.org/10.1517/13543776.2012.698264
5. Wainwright M, Kristiansen JE. On the 75th anniversary of Prontosil. Dyes and Pigments. 2011 Mar 1;88(3):231–4. https://doi.org/10.1016/j.dyepig.2010.08.012
6. Norman Hodges. Antibiotics and synthetic antimicrobial agent. in: Gilmore BF, Denyer SP, editors. Hugo and Russell's pharmaceutical microbiology. John Wiley & Sons; 2011.p 183–184.
7. Nunes OC, Manaia CM, Kolvenbach BA, Corvini PF. Living with sulfonamides: a diverse range of mechanisms observed in bacteria. Applied Microbiology and Biotechnology. 2020 Dec;104(24):10389–408. https://doi.org/10.1007/s00253-020-10982-5
8. Tenover FC. Mechanisms of antimicrobial resistance in bacteria. The American journal of medicine. 2006 Jun1; 119(6): S3–10. https://doi.org/10.1016/j.amjmed.2006.03.011
9. Levy SB, Marshall B. Antibacterial resistance worldwide: causes, challenges and responses. Nature medicine. 2004 Dec;10(Suppl 12): S122–9. https://doi.org/10.1038/nm1145
10. John M. Beale, JR. Anti-infective Agents. in: Beale JM, Block J, Hill R. Organic medicinal and pharmaceutical chemistry. Philadelphia: Lippincott Williams & Wilkins; 2010.p228–229.
11. Wulf NR, Matuszewski KA. Sulfonamide cross-reactivity: is there evidence to support broad cross-allergenicity? American Journal of Health-System Pharmacy. 2013 Sep 1;70(17):1483–94. https://doi.org/10.2146/ajhp120291
12. Sadler BM, Stein DS. Clinical pharmacology and pharmacokinetics of amprenavir. Annals of Pharmacotherapy. 2002 Jan;36(1):102–18. https://doi.org/10.1345/aph.10423
13. Wire MB, Shelton MJ, Studenberg S. Fosamprenavir: clinical pharmacokinetics and drug interactions of the amprenavir prodrug. Clinical Pharmacokinetics. 2006 Feb; 45:137–68. https://doi.org/10.2165/00003088-200645020-00002
14. Choquet-Kastylevsky G, Vial T, Descotes J. Allergic adverse reactions to sulfonamides. Current Allergy and Asthma Reports. 2002 Jan;2(1):16–25. https://doi.org/10.1007/s11882-002-0033-y
15. Dorn JM, Alpern M, McNulty C, Volcheck GW. Sulfonamide drug allergy. Current Allergy and Asthma Reports. 2018 Jul; 18:1–0. https://doi.org/10.1007/s11882-018-0791-9
16. Ali RM, Al-Hamashi AA. Molecular docking, ADMET, molecular dynamic simulation, synthesis, and preliminary antiproliferative study of 1, 2, 4-thiadiazole derivatives as possible histone deacetylase inhibitors. Tropical Journal of Pharmaceutical Research. 2024 Sep 6;23(7):1069–76. https://doi.org/10.4314/tjpr.v23i7.4
17. Mosleh AA, Dakhel ZA. Molecular docking, microwave-assisted synthesis, characterization, and preliminary evaluation of the anti-microbial activity of new sulfonamide derivatives of the 1, 2, 4-triazole-3-thiol ring system. Pharmacia. 2024 Sep 3; 71:1-9. https://doi.org/10.3897/pharmacia.71.e132720
18. Saeed AM, Al-Hamashi AA. Molecular Docking, ADMET Study, Synthesis, Characterization and Preliminary Antiproliferative Activity of Potential Histone Deacetylase Inhibitors with Isoxazole as New Zinc Binding Group. Iraqi Journal of Pharmaceutical Sciences (P-ISSN 1683-3597 E-ISSN 2521-3512). 2023 Nov 3;32(Suppl.):188-203. https://doi.org/10.31351/vol32issSuppl.pp188-203
19. Ovung A, Bhattacharyya J. Sulfonamide drugs: Structure, antibacterial property, toxicity, and biophysical interactions. Biophysical Reviews. 2021 Apr;13(2):259–72. https://doi.org/10.1007/s12551-021-00795-9
20. Gavernet L, Elvira JE, Samaja GA, Pastore V, Sella Cravero M, Enrique A, *et al*, Synthesis and anticonvulsant activity of amino acid-derived sulfamides. Journal of Medicinal Chemistry. 2009 Mar 26;52(6):1592–601. https://doi.org/10.1021/jm800764p
21. Kamms ZD, Hadi MK. Synthesis, Characterization and preliminary Anti-Microbial Evaluation of New Ibuprofen Hydrazide Derivatives. Pakistan Journal of Medical & Health Sciences. 2022 Apr 27;16(03):693-.
22. Abdulrahman HS, Hassan Mohammed M, Al-Ani LA, Ahmad MH, Hashim NM, Yehye WA. Synthesis of phthalimide imine derivatives as a potential anticancer agent. Journal of Chemistry. 2020;2020(1):3928204. https://doi.org/10.1155/2020/3928204
23. Kanaan S, Omar TN. Synthesis and Preliminary Anti-Inflammatory and Anti-Microbial Evaluation of New 4, 5-Dihydro-1H-Pyrazole Derivatives. Iraqi Journal of Pharmaceutical Sciences (P-ISSN 1683-3597 E-ISSN

2521-3512). 2023 Nov 4;32(Suppl.):262-70. https://doi.org/10.31351/vol32issSuppl.pp262–270
24. Franconi I, Lupetti A. In Vitro Susceptibility Tests in the Context of Antifungal Resistance: Beyond Minimum Inhibitory Concentration in Candida spp. Journal of Fungi. 2023 Dec 12;9(12):1188. https://doi.org/10.3390/jof9121188
25. Abduljabbar TT, Hadi MK. Synthesis, characterization and antibacterial evaluation of some coumarin derivatives. Iraqi Journal of Pharmaceutical Sciences (P-ISSN 1683-3597 E-ISSN 2521-3512). 2021 Jun 19;30(1):249–57. https://doi.org/10.31351/vol30iss1pp249-257
26. Mahdi L, Al Mathkhury HJ, Sana'a AK, Rasool KH, Zwain L, Salman IM, *et al,* Antibacterial activity of lactobacillus buchneri bacteriocin against vibrio parahaemolyticus. Current Applied Science and Technology. 2017;17(1):81–6. https://li01.tci-thaijo.org/index.php/cast/article/view/129081
27. Tang C, Ye Y, Feng Y, Quinn RJ. TCM, brain function and drug space. Nat Prod Rep. 2015;33(1):6–25. https://doi.org/10.1039/C5NP00049A
28. Ntie-Kang F. An in-silico evaluation of the ADMET profile of the Streptome DB database. Springer Plus. 2013;2(1):353. https://doi.org/10.1186/2193-1801-2-353
29. EL KOLLI ME. Mechanism of antimicrobial activity and antioxidant activities of the essential oil and the methanolic extract of carum montanum from Algeria. Annals of Agricultural Science, Moshtohor. 2018 Apr 1;56(4th ICBAA):253–62. https://dx.doi.org/10.21608/assjm.2018.18553

Appendix

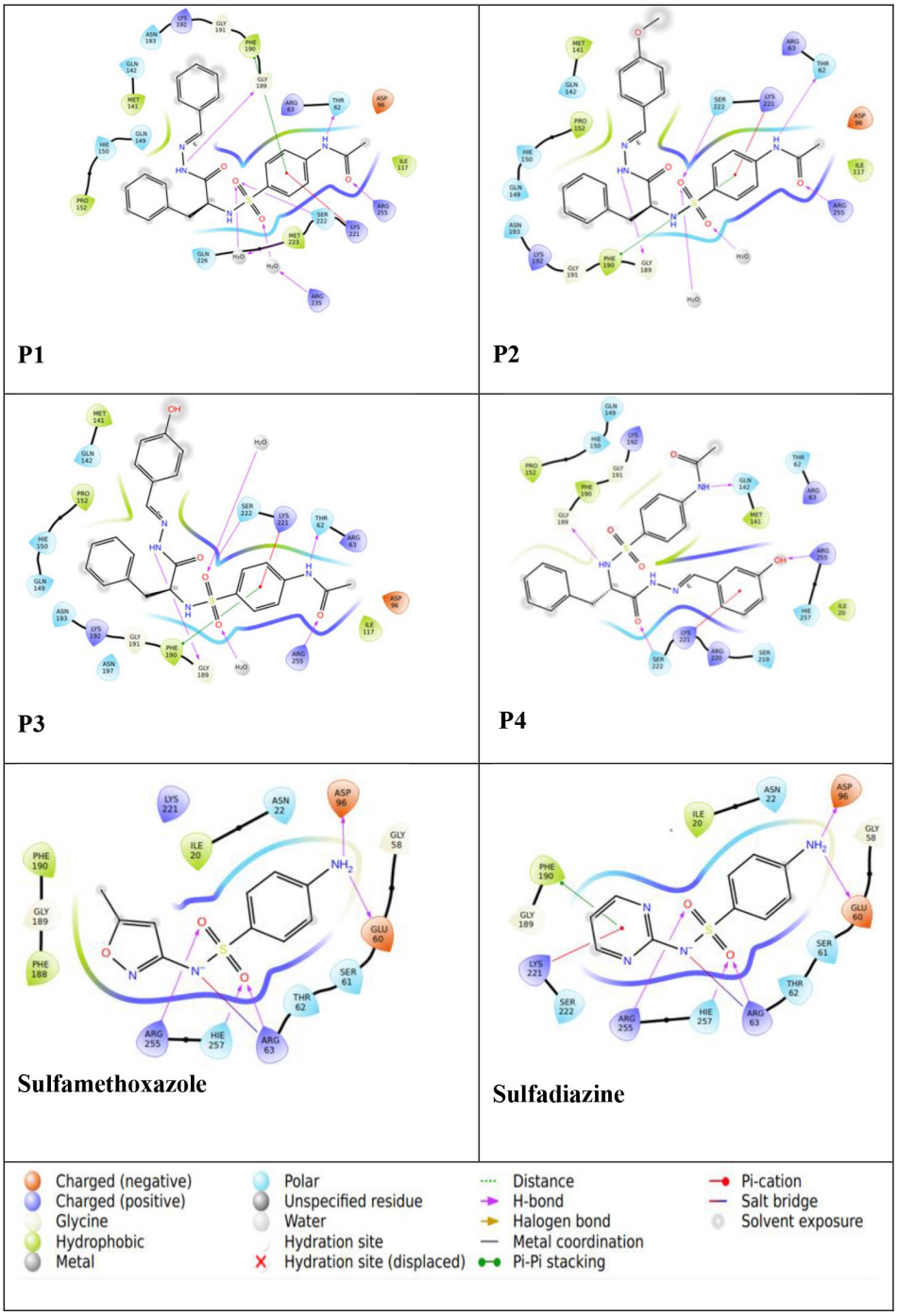


Fig. 1 **The 2D docking structures of final compounds and reference drugs with DHPS enzyme.**

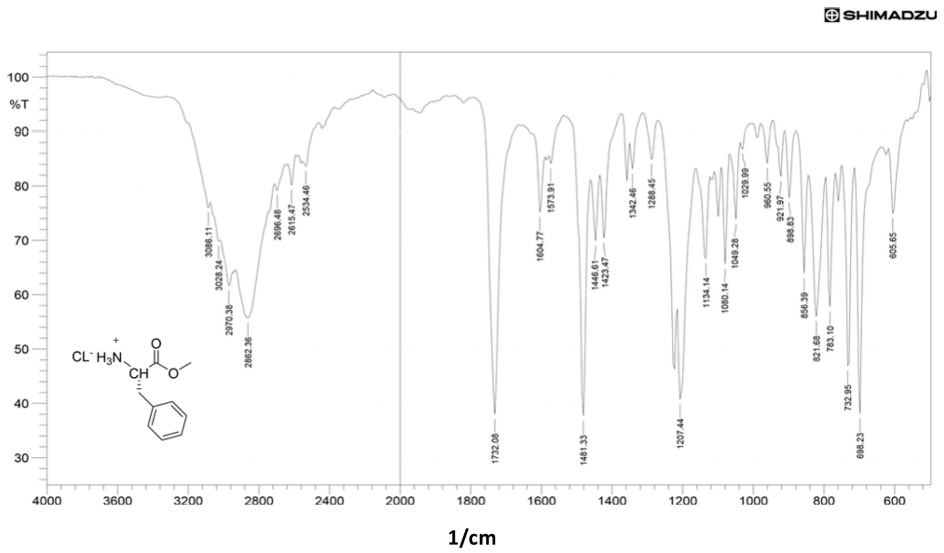


Fig. 2 **IR spectrum of compound I.**

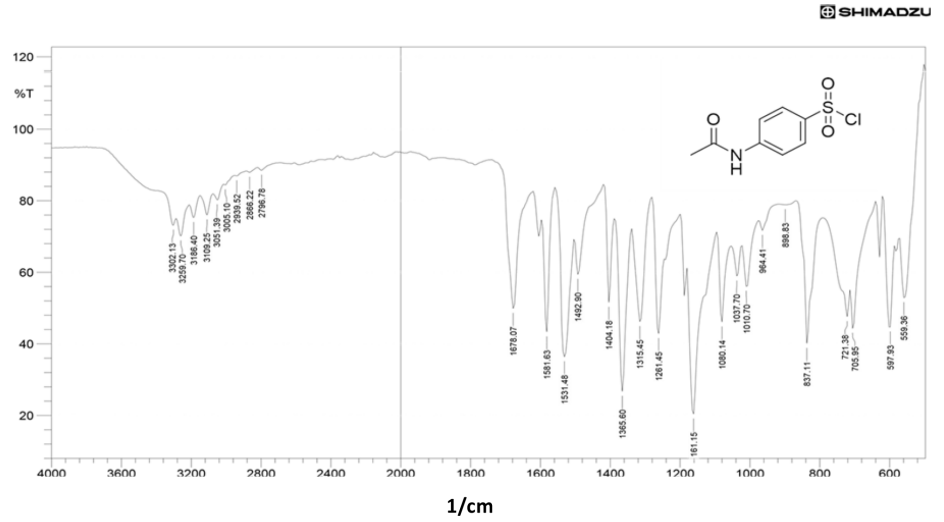


Fig. 3 **IR spectrum of compound II.**

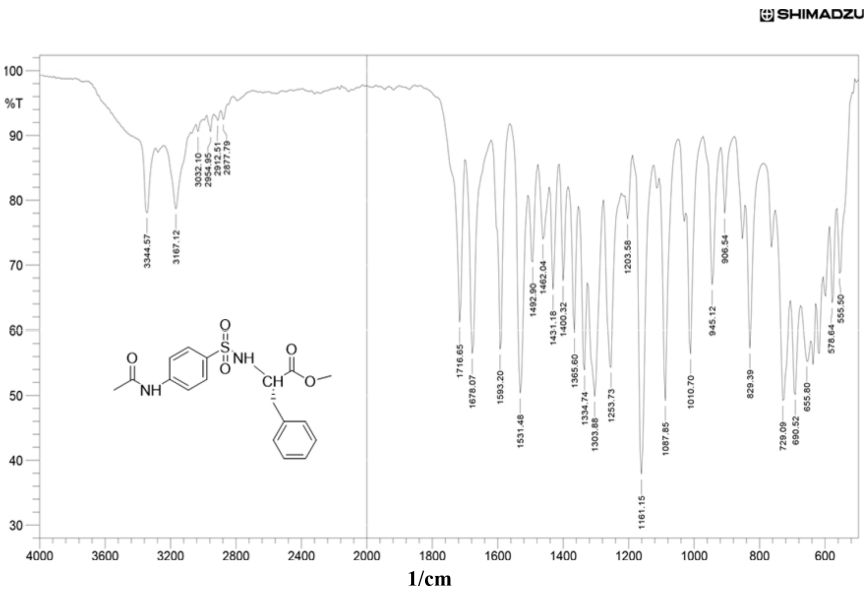


Fig. 4 **IR spectrum of compound III.**

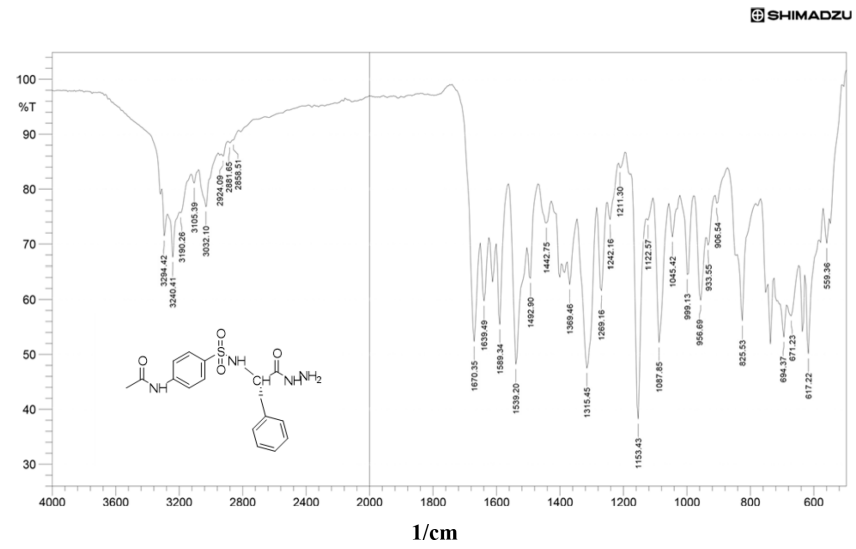


Fig. 5 **IR spectrum of compound IV.**

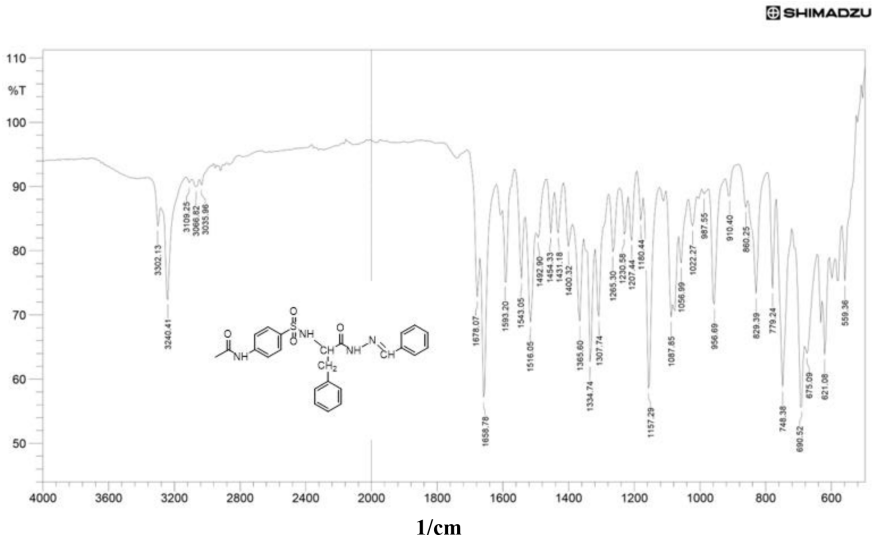


Fig. 6 **IR spectrum of compound P1.**

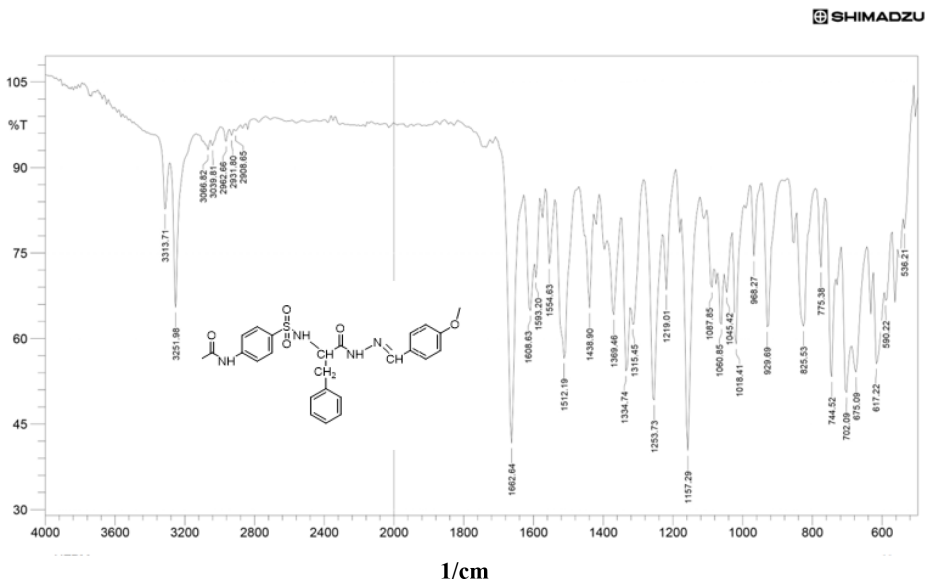


Fig. 7 **IR spectrum of compound P2.**

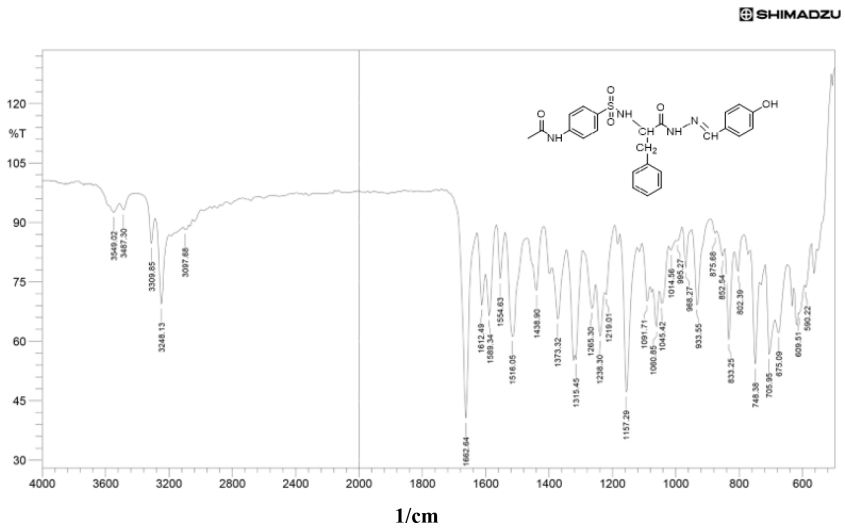


Fig. 8 **IR spectrum of compound P3.**

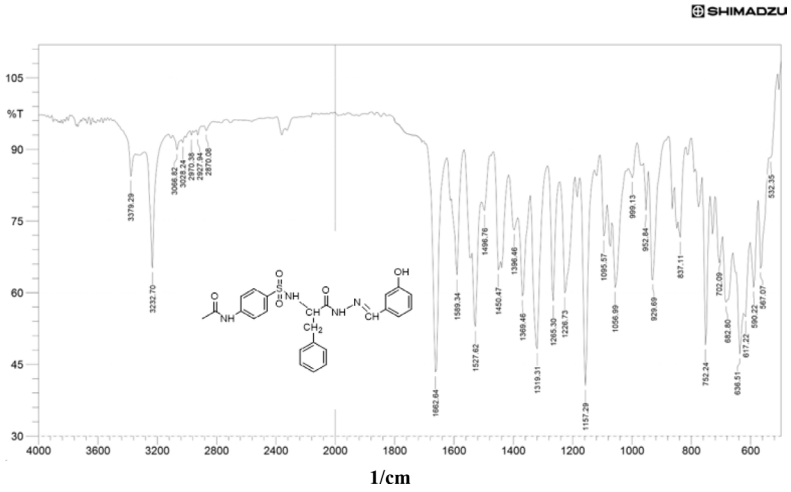


Fig. 9 **IR spectrum of compound P4.**

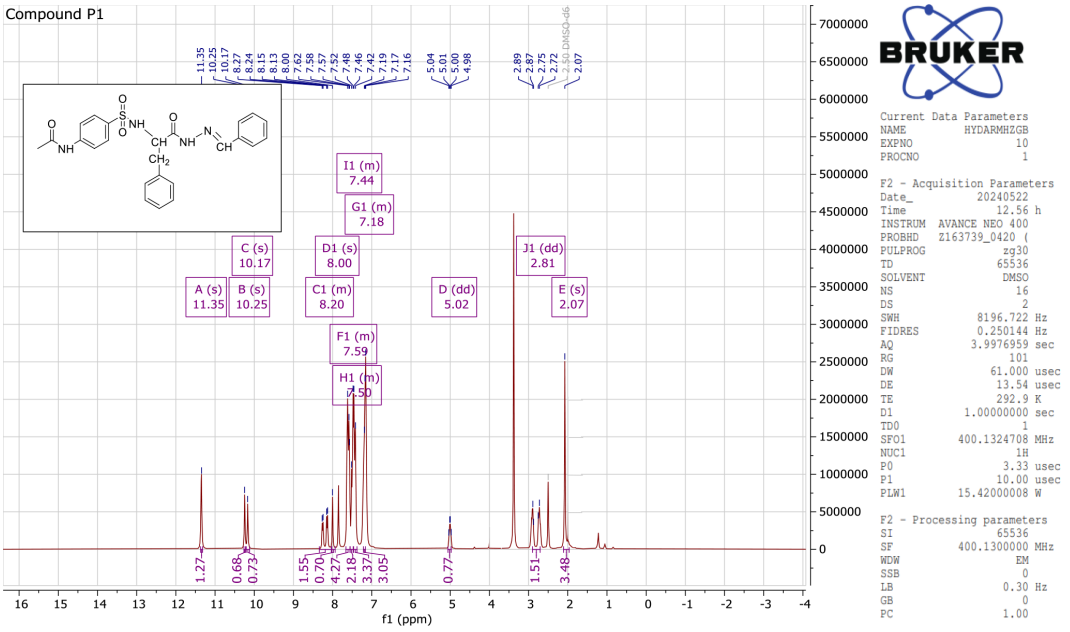


Fig. 10 **^{1}HNMR spectrum of compound P1 (molecular formula $C_{24}H_{24}N_4O_4S$).**

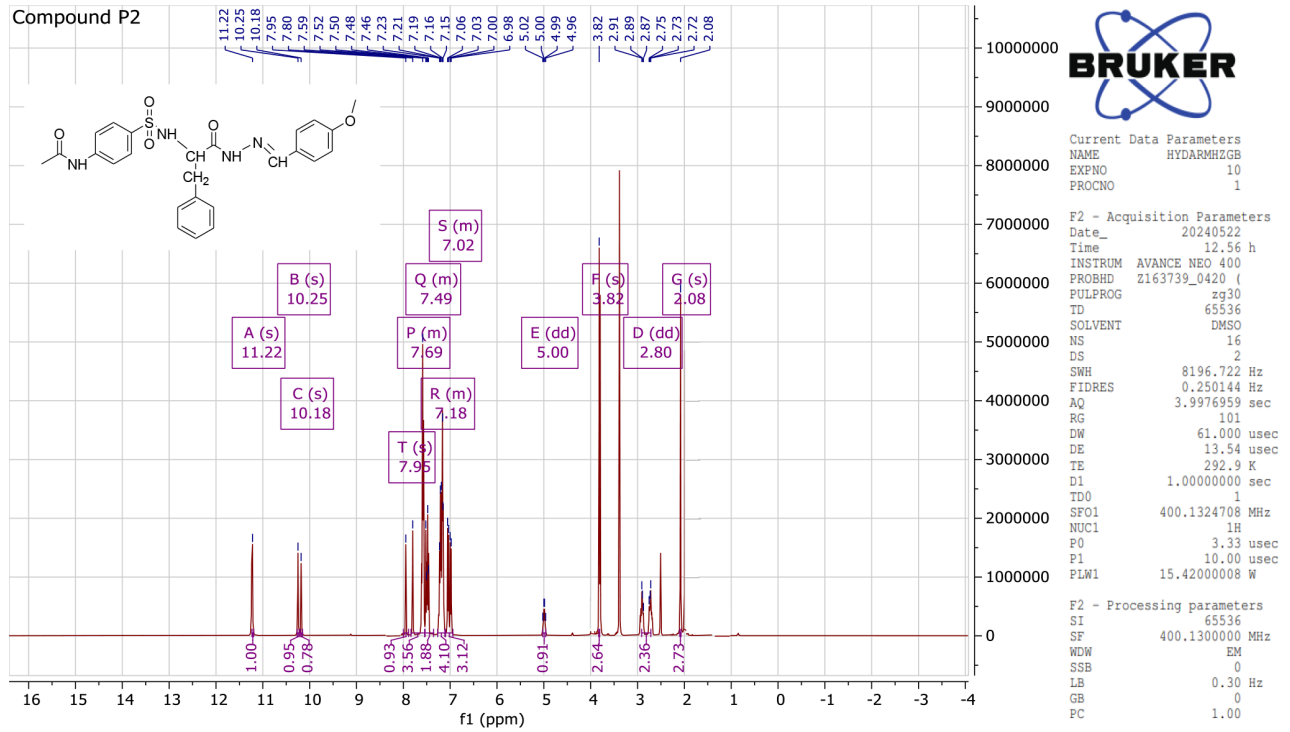


Fig. 11 **^{1}HNMR spectrum of compound P2 (molecular formula $C_{25}H_{26}N_4O_5S$).**

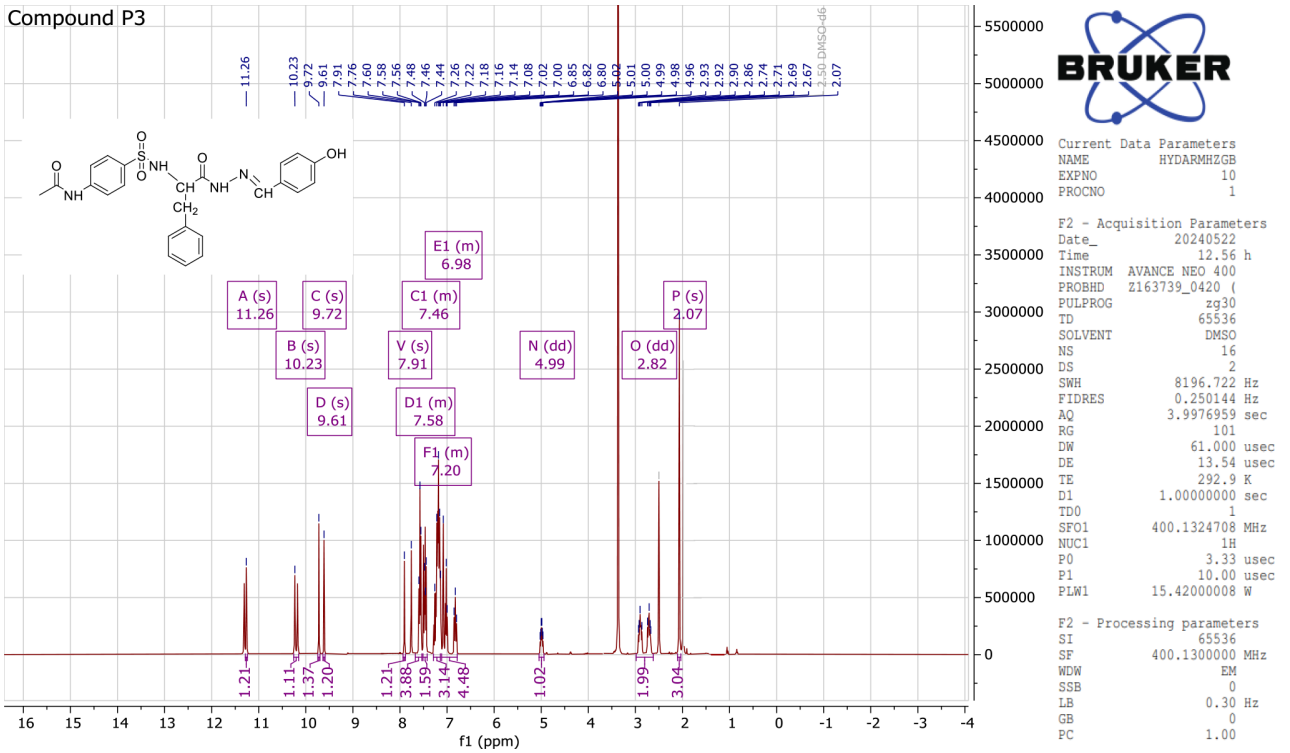


Fig. 12 **^{1}HNMR spectrum of compound P3 (molecular formula $C_{24}H_{24}N_4O_5S$).**

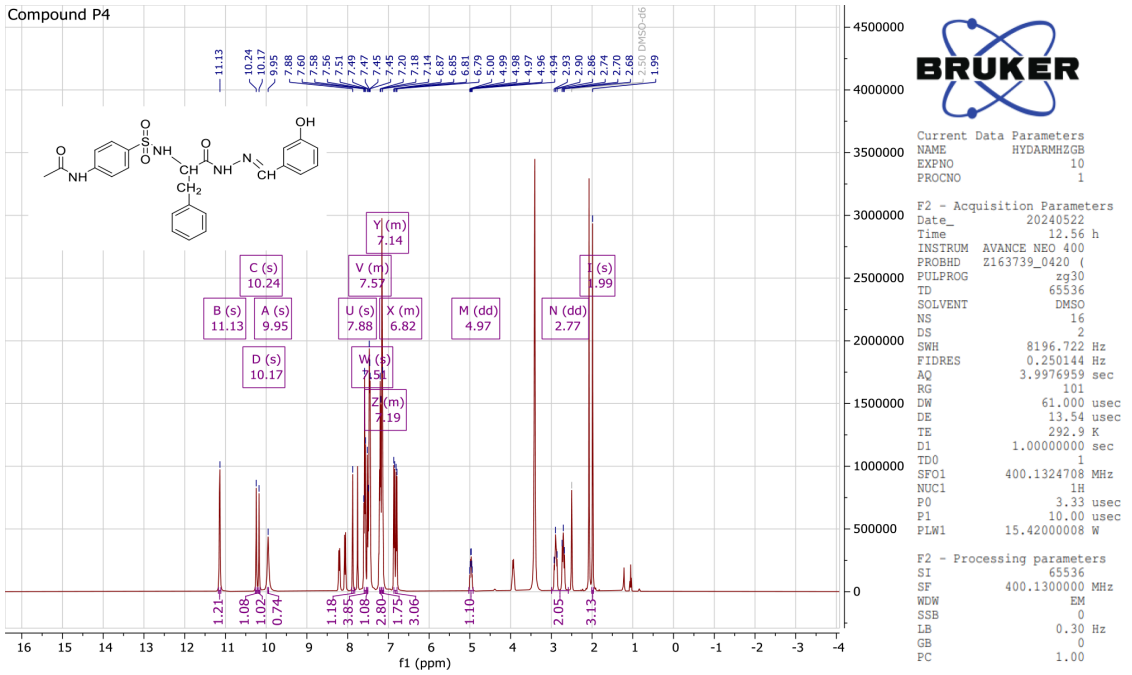


Fig. 13 **^{1}HNMR spectrum of compound P4 (molecular formula $C_{24}H_{24}N_4O_5S$).**

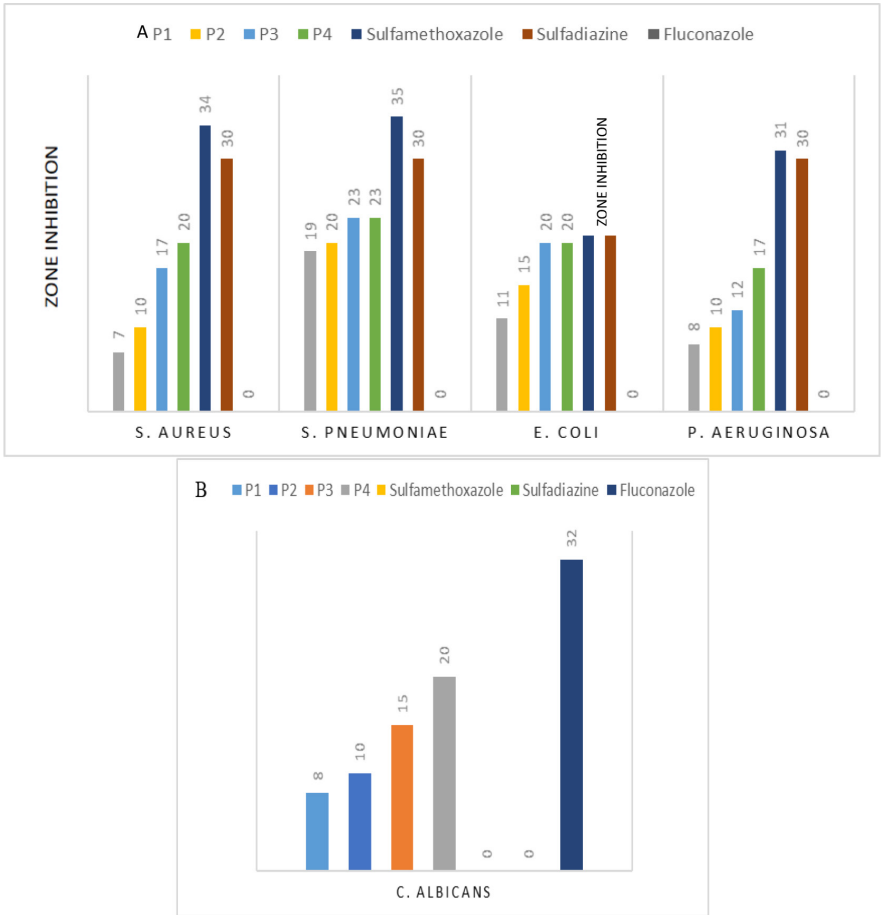


Fig. 14 **Antimicrobial for final compounds compared with standards drugs A) Antibacterial activity, B) antifungal activity.**