RESEARCH ARTICLE

Design, Molecular Docking, Synthesis and Preliminary Evaluation of the Antimicrobial Activity and Stability against β-lactamases of Dipeptides Linked to Cephalexin

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ABSTRACT

Bacterial infections have a large effect on public health. Some bacteria are innately resistant to certain classes of antibiotics, either because they lack the target or are impermeable to the drug. Others are innately susceptible but develop resistance by one of a growing variety of mechanisms. Synthesis of new derivatives of cephalexin that may have improved antibacterial, broader spectrum of activities, resistance against certain β-lactamases and/or pharmacokinetic properties. These derivatives include the incorporation of certain dipeptides at the amino group of cephalexin molecule. The dipeptides to be used are: valine-boc-tryptophan, alanine-boc-tryptophan, valine- boc-histidine, alanine-boc-histidine. The incorporation of dipeptide moiety on the acyl side chain very close to β-lactam ring may widen the spectrum of activity. This chemical addition act as isosteric group to the alkoximino that protect Beta lactam ring from bacterial beta lactamase enzyme. These new derivatives were successfully synthesized with reasonable yields show some resistance against β-lactamases and improve the pharmacokinetic properties. This approach may give new life for old drugs namely, cephalosporins and penicillins that contain amino group at the acyl side chain close to β-lactam ring. The chemical structures of these derivatives were confirmed by infrared (IR), proton nuclear magnetic resonance (HNMR) spectroscopy and elemental analyses, molecular docking, swiss ADME software. All the synthesized compounds were subjected for preliminary evaluation of antimicrobial activity using well diffusion method, against certain microbes.

Keywords: ADME studies, Antimicrobial assay, Cephalexin, Dipeptides, Docking studies.

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INTRODUCTION

Throughout the world, infectious diseases were the leading cause of death. Since then, the antibiotic's selective action on pathogenic bacteria has ushered in a significant period in human history.[1] Antibiotic resistance in microorganisms, on the other hand, jeopardizes antibiotic effectiveness, and antibiotic use promotes resistance.[2] Antibiotics are low-molecular-weight compounds that are typically produced by microbes and are active against other microorganisms at low quantities. Fluoroquinolones, for example, are derived from natural materials (the bark of the Cincona tree), whereas sulfa and oxazolidinones are synthetic compounds.[2,3] Antibiotic resistance is a global health crisis, decreasing the efficacy of antibiotics, which keep millions of patients alive.[4-10] Bacterial infections pose a life-threatening concern once more.[11] The misuse and abuse of antibiotics, as well as the lack of a new antibiotic synthesis, are all contributing to the antibiotic resistance problem. To deal with such a problem, coordinated actions are essential.[12,13]

Bacterial strains are becoming increasingly resistant to currently available treatments. There appears to be a relationship between the structure of the complexes and their antibacterial action.[14] Antimicrobial resistance has become one of the most serious health issues, particularly in hospitals.[15] Despite the continual development and development of new antibiotics, resistance in a variety of microorganisms remains high.[16] One of the most effective techniques has been the preparation of several semisynthetic derivatives of cephalosporins based on the structure-activity relationship. There is a lot of interest in finding new cephalosporins with a broader antibacterial spectrum and resistance to -lactamase–

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producing bacteria that can be taken orally. Preparation of several cephalosporin semisynthetic derivatives.[17]

Cephalexin is a β-lactam antibiotic with bactericidal properties that works similarly to benzyl penicillin by blocking bacterial cell wall formation. It is particularly effective against G (+) cocci and has a moderate effect on G (-) bacilli. Both penicillinase- and non-penicillinase-producing staphylococci are included in sensitive G (+) cocci, whereas methicillin-resistant staphylococci are not. Most streptococci are also susceptible to penicillin, enterococci, which are usually resistant. Some G (+) anaerobes can also be affected. In most cases, cephalexin is ineffective against Listeria monocytogenes. Cephalexin is active against some Enterobacteriaceae strains, including *E. coli, K. pneumoniae, P. mirabilis, Salmonella*, and *Shigella* spp., but not against Enterobacter, indole-positive Proteus, and Serratia spp. It also kills Moraxella catarrhalis (*B. catarrhalis*) and *N. meningitidis, H. influenzae*, on the other hand, is resistant. Mycobacteria, mycoplasma, and fungi are not sensitive, nor are Bacteroides fragilis and *P. aeruginosa*.[18] The inclusion of favored chemical moieties, such as amino acids, has been discovered to have significant antibacterial potential. Amino acids connected to cephalexin via an amide bond can have a lot of advantages, such as increased activity, resistance to -lactamases, and/or better pharmacokinetic features. When prepared properly, the proposed chemicals could be used as injectables, when prepared as sodium salt.

EXPERIMENTAL WORK

Materials and Methods

The commercial sources provided all the reagents and solvents. (Ethyl chloroformate (ECF) was purchased from Sigma Aldrich/ Germany, Boc-tryptophan; Boc-histidine were purchased from Shanghai World Yang Chemical/China, Valine (CDH India), Alanine (PDH) England, tri ethyl amine from Thomas baker, India, Cephalexin monohydrate was from SDI Samarra, Iraq.

Staurt Electric melting point device was used to determine melting points (England). Compound identification was accomplished utilizing an IR spectra acquired on a Shimadzu FTIR infrared spectrometer with KBr disks. Varian, Agilant 500 MHz, determined ^{1}H-NMR (USA). Elementar Analysen systeme, VarioEL, Germany did elemental microanalyses (CHN).

Chemical Synthesis

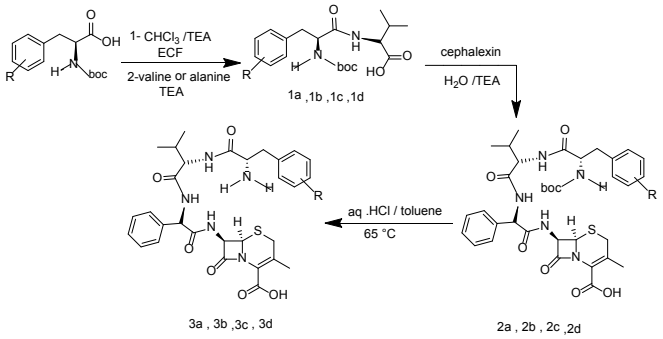
The methodologies outlined in were used to synthesize intermediates and final products (Scheme 1).

General Procedure for Synthesis of the Intermediates of Cephalexin 1(a-d)

Boc-amino acid 10 mmol (Boc-tryptophan 3.04 gm/Boc-histidine 2.55 gm) was dissolved in dry chloroform 50 mL containing TEA (1.39 mL) and then cooled in an ice bath at (-2 to -4°C). Ethyl chloroformate, ECF (0.94 mL) was added drop by drop over a 10 minutes interval, and the mixture was agitated constantly for another 60 minutes a suitable amino acid 10 mmol (valine 1.17 gm; alanine 0.89 gm) in distilled water (10 mL) containing TEA (2*1.39 mL was cooled to 0°C and immediately added to the above-mentioned solution, stirring for 4 hours at –2 to –4.[19] Then there are two layers. They were separated using a separatory funnel into an organic (chloroform) lower layer containing (Boc-aromatic amino acids unreacted, TEA excess, product). TEA excess, product). aqueous (water) upper layer containing (TEA-HCl, unreacted cephalexin), washing aqueous layer with 15 mL dry chloroform to draw out some product during separation using a separatory funnel, layer the organic layer on top of the main organic layer of the initial separation. To remove TEA as TEA-HCl salt, wash the main organic layer with 15 mL aq. HCl, separate them using a separatory funnel into organic and aqueous layers, and take the organic layer. Using a rotary evaporator, evaporate chloroform to obtain a combination of (Boc-aromatic amino acid unreacted, product) Boc-aromatic amino acids are soluble in ether, therefore wash it with 15 mL ether then filtering, while the product settles on the filter paper as a precipitate Overnight drying at ambient temperature system. as described and shown in Scheme 1.

General Procedure for Synthesis of the Intermediates 2 (a–d)

Each of the Dipeptide 1(a–d) (3 gm) was dissolved in 50 cc dry chloroform containing 1.39 mL TEA and frozen in an ice bath at (-2 to -4°C). Ethyl chloroformate, ECF (0.94 mL) was added drop by drop over a 10-minute period, and the mixture was agitated constantly for another 30 minutes. Cephalexin (3.65 gm) was frozen to 0°C in distilled water (10 mL) adding TEA (2*1.39 mL). added to the abovementioned solution, stirring for 4 hours at –2 to –4°C and 2 hours at room temperature.[9] Then there are two layers. They were separated using a separatory funnel into an organic (chloroform) lower layer that included (Boc-Dipeptide 1 (a–d) unreacted TEA; product). upper aqueous (water) layer containing (TEA-HCl; cephalexin un)), washing aqueous layer with 15 mL dry chloroform to pull out some product may be pass to it during separation. separation technique of them by using separatory funnel, add the organic layer on the main organic layer of first separation.washing the main organic layer with 15 mL of aq. HCl to get rid of TEA as TEA-HCl salt, separation of them by using separatory funnel into organic and aqueous layer, take the organic layer



Scheme 1: Synthesis of intermediates and target compounds

evaporate chloroform using rotary evaporator to get mixture of (Boc-Dipeptide 1 (a–d) un reacted, product) washing it with 15 mL ethyl acetae then filter, Boc-Dipeptide are soluble in ethyl acetae while the product till as precipitate on filter paper. drying at room temperature overnight. described and as shown in Scheme 1.

General Procedure for Synthesis of the Target Derivatives of Cephalexin 3 (a–d):

Added adequate amount of each protected carbamate intermediate of cephalexin 2 (a-d) in toluene 15 cc as a solvent, then added 10 mL of aqueous hydrochloric acid (1.5 M) to toluene, then heated at 65°C to obtain two layers. The tert-butyl cation is present as a polymer or oligomer in the organic toluene layer. The product was obtained as HCl- salt in aqueous layer form, so it was dissolved in 15 mL of ethanol, then added (1.39 mL) of TEA, then refrigerated, then ppt.formed and filtered, evaporate of ethanol to get the product with free NH_2. The reaction was confirmed for completeness by removing all CO_2 gas bubbles generated as a result of the carbamate bond being completely broken.[20] These compounds were obtained by deprotection of the amino group of compounds boc-dipeptide –cephalexin to afford the new derivatives of cephalexin described and as shown in. Scheme 1.

R in Aromatic Amino Acids: pyrrole: tryptophan, imidazole: Histidine (Tert-butoxycarbonyl)-L-tryptophyl-D-valine) 1a):

Yellowish brown grease (74% of Yield); m.p 182.5–185°C; IR (KBr) v (cm^{-1}): 3610 (NH sec- amide), 3402 (NH of indole ring), 3288 (O-H Carboxylic acid), 3040 (C-H Aromatic), 2931 (C-H Alkyl), 1750 (C=O tert-butoxycarbonyl), 1710 C=O carboxylic acid, 1672 (C=O sec.amide), 1605 (C=C alkene), 1394(C-N).[21] ^{1}H-NMR (DMSO-d6, 500 MHz): δ 10.88 (d, 1H, NH indole ring), δ 8.32(d, 1H, NH sec-amide), δ 7.39 (d, 1H, NH of carbamate), δ 6.98-7.51 (m, 4H, aromatic ring protons), δ 7.17 (d, 1H, CH of indole ring), δ 4.6(m, 1H, CH alpha to sec-amide & carbamate), δ 2.98, 3.38 (d, 2H, diastereomeric CH_2 adjacent to CH alpha between sec-amide&carbamate), δ1.9 (m, 1H, CH of valine), δ 1.10, 1.10 (d, 6H, CH_3 of valine), δ 1.34 (s, 9H, for CH_3 of carbamate), δ 4.34 (t, 1H, CH of valine).[22]

(6R, 7R)-7-((6S, 9R, 12R)-6-((1H-indol-3-yl)methyl)-9-isopropyl-2, 2-dimethyl-4, 7, 10-trioxo-12-phenyl-3-oxa-5, 8, 11-triazatridecan-13-amido)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (2a):

Dark Brown crystal (80% of Yield); m.p 206–208°C; IR (KBr) v (cm^{-1}): 3610 (NH sec- amide), 3402 (NH of indole ring), 3265 (O-H Carboxylic acid), 3049 (C-H Aromatic), 2947 (C-H Alkyl), 1764 (C=O β-lactam), 1750 (C=O tert-butoxycarbonyl), 1720 (C=O carboxylic acid), 1668 (C=O sec.amide), 1620 (C=C alkene), 1410 (C-N), 702 (C-S-C).[21]

(6R, 7R)-7-((R)-2-((S)-2-((S)-2-amino-3-(1H-indol-3-yl) propanamido)-3-methylbutanamido)-2-phenylacetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (3a)

Brown crystal (83% of Yield); m.p 130–132°C; IR (KBr) v (cm^{-1}): 3610 (NH sec - amide), 3402 (NH of indole ring), 3363 (NH 1°-amine) 3236 (O-H Carboxylic acid), 3030 (C-H Aromatic), 2972 (C-H Alkyl), 1763 (C=O β-lactam), 1700 C=O carboxylic acid, 1672 (C=O sec.amide), 1630 (C=C alkene), 1410 (C-N), 692 (C-S-C).[21] ^{1}H-NMR (DMSO-d6, 500 MHz): δ 10.88 (d, 1H, NH indole ring), δ 8.18, 8.32 (d, 3 H, NH sec-amide), δ 9.1 (d, 2H, NH 1°-amine), δ 6.94-7.46 (m, 9H, aromatic ring protons), δ 7.27 (d, 1H, CH of indole ring), δ 3.95 (m, 1H, CH alpha to sec-amide&1-amine), δ 3.00–3.36 (d, 2H, diastereomeric CH_2, t, 2H, diastereomeric), δ 4.44 (m, 1H, CH of alanine), δ 5.5 (t, 1H, H alpha to C=O beta lactam ring), δ 4.98 (d, 1H, H alpha to N beta lactam ring, δ 1.10, 1.10 (d, 6 H, CH_3 of valine), δ 1.88 (d, 3H allylic CH_3), 5.82 (d, 1H, CH alpha to sec- amide and benzene ring), δ 2.70 (m, 1H, CH of valine).[22]

Chemical Formula: $C_{21}H_{29}N_3O_5$.

Elemental Analysis: Calculated C 60, 74:, H: 5.77, N: 13.28 Found C: 59.818, H: 5.569, N: 13.42.

(tert-butoxycarbonyl)-L-tryptophyl-L-alanine (1b): Bright yellow grease (76.9% of Yield); mp 97.8°C; IR (KBr) v (cm^{-1}): 3580 (NH sec- amide), 3419 (NH of indole ring), 3275 (O-H Carboxylic acid), 3040 (C-H Aromatic), 2980 (C-H Alkyl), 1750 (C=O tert-butoxycarbonyl), 1705 C=O carboxylic acid, 1680 (C=O sec.amide), 1600 (C=C alkene), 1390 (C-N).[21; ^{1}H-NMR (DMSO-d6, 500 MHz): δ 10.84 (d, 1H, NH indole ring), δ 8.32(d, 1H, NH sec-amide), δ 7.35 (d, 1H, NH of carbamate), δ 6.98-7.55 (m, 4H, aromatic ring protons), δ 7.14 (d, 1H, CH of indole ring), δ 4.91 (m, 1H, CH alpha to sec-amide & carbamate), δ 3.03, 3.43 (d, 2H, diastereomeric CH_2 adjacent to CH alpha between sec-amide & carbamate), , δ 1.28 (d, 6H, CH_3 of alanine), δ 1.41 (s, 9H, for CH_3 of carbamate), δ 4.44 (m, 1H, CH of alanine).[22]

(6R, 7R)-7-((6S, 9S, 12R)-6-((1H-indol-3-yl)methyl)-2, 2, 9-trimethyl-4, 7, 10-trioxo-12-phenyl-3-oxa-5, 8, 11-triazatridecan-H, NH sec-amide), δ 9.2(d, 2H, NH of 1-amine), δ 6.96-7.43 (m, 9H, aromatic ring protons), δ 7.32 (d, 1H, CH of indole ring), δ 4.4 4 (m, 1H, CH alpha to sec-amide & 1-amine), δ2.95-3.43(d, 2H, diastereomeric CH_2, t, 2H, diastereomeric), δ δ 4.44 (m, 1H, CH of alanine), δ 5.57 (t, 1H, H alpha to C=O beta lactam ring), δ 4.91 (d, 1H, H alpha to N beta lactam ring, δ 1.23(d, 3H, CH_3), 1.97 (d, 3H allylic CH_3).[22]

Chemical Formula: $C_{21}H_{29}N_3O_5$,

Elemental Analysis: Calculated C: 59.59 H: 5.33, N: 13.965 Found C: 57.79, H:5.163, N: 13.711.

(tert-butoxycarbonyl)-L-histidyl-D-valine (1c): Yellowish brown grease (80.6% of Yield); m.p 130°C dec.; IR (KBr) v (cm^{-1}): 3560 (NH sec- amide), 3439 (NH imidazole ring), 3250 (O-H Carboxylic acid), 3026 (C-H Aromatic), 2955 (C-H Alkyl), 1735 (C=O tert-butoxycarbonyl), 1710 C=O carboxylic acid, 1691 (C=O sec.amide), 1620 (C=C alkene), 1400(C-N). [(21)], ^{1}H-NMR (DMSO-d6, 500 MHz): δ 7.40 (d, 2H, NH sec-amide), δ 8.32, 8.82 (d, 2H, CH imidazole ring), δ 4.94(m, 1H, CH alpha to sec-amide & carbamate), δ (m, 2H, diastereomeric CH_2 alpha imidazole ring, 3.99 (t, 1H, CH of alanine), δ 1.23 (d, 3H, CH_3), δ 1.90(m, 1H, CH of alanine), δ 0.99, 1.03 (d, 6H, CH_3 of valine), δ 1.42 (s, 9H, CH_3 of carbamate), δ 7.39 (d, 1H, for NH of carbamate).[22]

(6R, 7R)-7-((6S, 9R, 12S)-6-((1H-imidazol-4-yl)methyl)-9-isopropyl-2, 2-dimethyl-4, 7, 10-trioxo-12-phenyl-3-oxa-5, 8, 11-triazatridecan-13-amido)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (2c):

Dark yellow crystal (85% of Yield); mp 199.5–202°C; IR (KBr) ν (cm^{-1}): 3610 (NH sec- amide), 3423 (NH of indole ring), 3259 (O-H Carboxylic acid), 3049 (C-H Aromatic), 2980 (C-H Alkyl), 1766 (C=O β-lactam), 1740 (C=O tert-butoxycarbonyl), 1700 (C=O carboxylic acid), 1680 (C=O sec.amide), 1653 (C=C alkene), 1400 (C-N), 698 (C-S-C).[21]

(6R, 7R)-7-((S)-2-((R)-2-((S)-2-amino-3-(1H-imidazol-4-yl) propanamido)-3-methylbutanamido)-2-phenylacetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (3c):

Light yellow crystal (89% of Yield); mp 185 –187.9°C; IR (KBr) ν (cm^{-1}): 3610 (NH sec- amide), 3423 (NH imidazole ring), 3259 (O-H Carboxylic acid), 3049 (C-H Aromatic), 2980 (C-H Alkyl), 1766 (C=O β-lactam), 1700 (C=O carboxylic acid), 1680 (C=O sec.amide), 1653 (C=C alkene), 1400 (C-N), 698 (C-S-C).[21] ^{1}H-NMR (DMSO-d6, 500 MHz): δ 9.12, 9.14 (d, 2H, NH of 1-amine), 8.21, 8.21 (d, 3 H, NH sec-amide), δ 7.40, 8.82 (d, 2H, CH of imidazole ring), δ 7.27-7.37 (m, 5H, aromatic ring protons), δ 4.01 (m, 1H, CH alpha to sec-amide&1-amine), δ2.89-3.21 (d, 4 H, diastereomeric CH_2, t, 2H, diastereomeric), δ 4.44 (m, 1H, CH of alanine), δ 5.56 (t, 1H, H alpha to C=O beta lactam ring), δ 5.03 (d, 1H, H alpha to N beta lactam ring, δ 1.23(d, 3H, CH_3), 1.97(d, 3H allylic CH_3), δ 0.99, 1.03 (d, 6H, CH_3 of valine), δ 4.34 (m, 1H, CH alpha to sec-amide&1-amine), δ 5.72(d, 1H, CH alpha to sec- amide and benzene ring), δ 2.70 (m, 1H, CH of valine).[22]
Chemical Formula: $C_{27}H_{33}N_7O_6S$
Elemental analysis: Calculated C: 55.56 H: 5.70, N: 16.80 Found C: 53.761, H: 4.437, N: 17.107

(Tert-butoxycarbonyl)-L-histidyl-D-alanine (1d): Yellowish brown grease (87.7% of Yield); m.p 121°C dec.; IR (KBr) ν (cm^{-1}):

3600 (NH sec- amide), 3406(NH imidazole ring), 3257 (O-H Carboxylic acid), 3028 (C-H Aromatic), 2982 (C-H Alkyl), 1757 (C=O tert-butoxycarbonyl), 1705 C=O carboxylic acid, 1680 (C=O sec.amide), 1650 (C=C alkene), 1400 (C-N). [(21)]; ^{1}H-NMR (DMSO-d6, 500 MHz): δ7.40, 8.30 (d, 2H, NH sec-amide), δ 7.59, 8.72 (d, 2H, CH imidazole ring), δ 4.79 (m, 1H, CH alpha to sec-amide& carbamate), δ 2.93, 3.27 (m, 2H, CH_2 diasteremeric alpha to imidazole ring, 4.30(m, 1H, CH of alanine), δ 1.23(d, 3H, CH_3 of alanine), 1.90 (m, 1H, CH of alanine), δ 0.99, 1.03 (d, 6H, CH_3 of valine), δ 1.37 -1.42 (s, 9H, CH_3 of carbamate), δ 7.39 (d, 1H, for NH of carbamate).[22]

(6R, 7R)-7-((6S, 9R, 12R)-6-((1H-imidazol-4-yl)methyl)-2, 2, 9-trimethyl-4, 7, 10-trioxo-12-phenyl-3-oxa-5, 8, 11-triazatridecan-13-amido)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (2d):

Dark yellow crystal (84.6% of Yield); mp 222–225°C; IR (KBr) ν (cm^{-1}): 3516 (NH sec- amide), 3456 (NH imidazole ring), 3286 (O-H Carboxylic acid), 3041 (C-H Aromatic), 2918 (C-H Alkyl), 1772 (C=O β-lactam), 1755 (C=O tert-butoxycarbonyl), 1720 (C=O carboxylic acid), 1680 (C=O sec.amide), 1658 (C=C alkene), 1400 (C-N), 709 (C-S-C).[22]

(6R, 7R)-7-((R)-2-((R)-2-((S)-2-amino-3-(1H-imidazol-4-yl) propanamido)propanamido)-2-phenylacetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (3d):

Yellow crystal (83% of Yield); m.p 222-225 °C dec.; IR (KBr) ν (cm^{-1}): 3620 (NH sec- amide), 3406 (NH imidazole ring), 3380 (NH 1°-amine), 3290 (O-H Carboxylic acid), 3057 (C-H Aromatic), 2916 (C-H Alkyl), 1768(C=O β-lactam), 1705 (C=O carboxylic acid), 1680 (C=O sec.amide), 1658 (C=C alkene), 1400 (C-N), 690 (C-S-C).[21] ^{1}H-NMR (DMSO-d6, 500 MHz): δ 8.10, 8.30 (d, 2H, NH sec-amide), δ 7.59, 8.72 (d, 2H, CH imidazole ring), δ 9.11, 9.13(d, 2H, NH of 1-amine), δ 5.57 (t, 1H, H alpha to C=O beta lactam ring), δ 5.09 (d, 1H, H alpha to N beta lactam ring, δ 2.82-3.22 (d, 4 H, diastereomeric CH_2, t, 2H, diastereomeric), 4.39 (m, 1H, CH of alanine), δ 1.23 (d, 3H, CH_3 of alanine), δ 2.00(d, 3H allylic CH_3), δ 4.02 (m, 1H, CH alpha to sec-amide&1-amine), δ 5.73 (d, 1H, CH alpha to sec- amide and benzene ring), δ 7.28-7.34 (m, 5H, aromatic ring protons).[22]
Chemical Formula: $C_{22}H_{29}N_7O_6S$
Elemental Analysis: Calculated C: 54.04 H: 5.26, N: 17.65 Found C: 52.138, H:4.878, N:18.316.

COMPUTATIONAL METHODS

ADME Procedures

The SwissADME server was used to determine the physicochemical and pharmacokinetic parameters of all ligands 3 (a–d). Chem. Sketch (v. 12) was used to create the chemical structure of the intended compounds, which was then translated to SMILE names using the Swiss ADME program.[23]

Docking Studies

The use of molecular docking studies in the development of novel drugs, as well as the prediction of ligand-receptor interactions and the biological activity of proposed compounds, is advantageous. Hermes visualizer program (v. 1.10.1) is included in the CCDC GOLD Suite (v. 5.7.1) and is used to visualize receptors, ligands, type of interaction (H-bond, short contact, etc.), active site, and bond length computation.[24]

Ligand and Receptor Preparation

The crystal structure of the beta lactamase enzyme from *K. pneumoniae* was taken from the Protein Data Bank (PDB ID: 4R3B)(25), and missing atoms were added using SwissPDB Viewer (SPDBV) (v. 3.7). The crystal structures of proteins are prepared by removing all water molecules and adding hydrogen atoms to achieve the proper ionization and tautomeric state of amino acids. CheBio3D (v. 17.1) was used to minimize the energy of the produced ligands using the MM2 force field.

Molecular Docking Protocol

The receptors were setup for the docking process using GOLD Suite's HERMES –Structure visualization software. The radius (10 Å) of the active site was calculated using the protein's reference ligand. As a configuration template,

ChemScore kinase was employed. The scoring function was Chem Piecewise linear potential (CHEMPLP). All parameters used in the docking procedure were remained at their default levels, and all solutions were scored using the CHEMPLP fitness function. The ligands' interaction with the protein residues of the enzyme beta lactamase was assessed using docking outcomes such as docked posture, binding mode, and binding free energy.

RESULTS AND DISCUSSION

Antimicrobial Assay

The antibacterial activity of the studied substances was assessed by an agar well diffusion assay, which involves the use of pure culture for all bacterial species, at the College of Pharmacy, Mustansiriyah University in Iraq. Using the number 0.5 McFarland turbidity standard, a concentration of 1.5108 CFU/mL was created for each bacteria that was inoculated using a glass spreader on the surface Mueller Hinton agar plates that have already been prepared (MHA). Then, after allowing them to dry, they were punched into agar in five 6 mm diameter wells. As a result, each agar plate was divided into five wells. (100l) of bacteria and (500, 250, 125, and 62.5) g of chemical dilutions were placed into wells on MHA plate. Dimethylsulfoxide (DMSO) was used as a negative controller. The plates were maintained at 37°C for one day. The antibacterial activity was determined by measuring the distance from the inhibition zone. The antimicrobial evaluation was based on the diameter of the inhibition zone that developed across the well. These chemicals were tested for antibacterial activity against six different microorganisms. (*Methicillin- resistance S. aureus, S. aureus, E. coli, K.pneumoniae, P. aeruginosa, C. famata* in different concentration. In antibacterial activity, cephalexin was employed as a reference medication, whereas in antifungal activity, itraconazole was used, as shown in Table 1.

Absorption, Distribution, Metabolism and Excretion (ADME) Results Interpretation

Swiss ADME server analyzed the ADME properties of the final synthesized substances.[23] The pharmacokinetic properties of all produced substances were evaluated (absorption, distribution, metabolism, excretion). The drug-like characteristics of all target compounds were determined using Lipinski's rule of five in this study.[26] This technique, often known as Pfizer's rule of five (RO5), has been widely used as

Table 1: Antimicrobial evaluation of the target synthesized compounds

		Inhibition zone (mm)					
		Gram positive		*Gram negative*			*Fungus*
Compounds	*Conc. µg/mL*	*S. aureus*	*MRSA*	*K. pneumoniae*	*E. coli*	*P. aeruginosa*	*Candida famata*
Cephalexin disc	30	14	-	12	10	-	-
Itraconazole disc	10	-	-	-	-	-	8
Dmso	pure	-	-	-	-	-	-
3a	500	16	6	4	-	4	9
	250	10	-	-	-	-	8
	125	8	-	2	6	-	6
	62.5	6	-	4	-	2	3
3b	500	12	5	6	8	4	8
	250	8	4	6	-	-	8
	125	6	4	4	4	3	4
	62.5	-	-	-	-	2	2
3c	500	16	-	8	10	-	-
	250	9	-	-	-	5	8
	125	8	-	6	6	2	6
	62.5	6	-	4	8	2	8
3d	500	10	4	6	8	6	6
	250	6	-	6	8	6	4
	125	4	-	2	4	6	4
	62.5	-	-	-	-	4	4

a filter for compounds that could potentially be used as a lead for drug design. In a summary, Lipinski's rule of five relates to the orally administration of medications that must possess the following features in order to be administered orally: a) ≤ 5 hydrogen bonds donor b) ≤10 hydrogen bond acceptor c) Log P ≤ 5 d) molecular weight (M.Wt.) ≤500. In addition, the topological polar surface area (TPSA) was calculated, as this is a key characteristic in determining drug bioavailability.[27] Thus, compounds that are passively absorbed and have a TPSA >140 A^o are regarded to have a low oral bioavailability. These new cephalexin derivatives do not follow the Lipinski rule of five and require a zwitter ion carrier such as amino acids or peptides for transport.[17] Some compounds had no effect on the liver enzyme cyt –p450, whereas 3a and 3b block subtype 3A4. The outcomes of all of our produced substances revealed that, the TPSA was above 140, which is in the range (212–224.9), and the bioavailability for all ligands was 0.17, indicating that none of the ligands reached the systemic circulation as indicated in Table 2.

Interpretation of Docking Results

All freshly synthesized compounds were successfully docked using GOLD Suite software 3 (a, b, c, d). GOLD is a "genetic technique for docking flexible ligands into protein binding sites." (28) GOLD has been thoroughly tested and has shown great posture prediction rendering and virtual screening results.[29] This is included with the GOLD Suite, which also includes several software. Hermes, Mercury, Isostar, and Conquest, as well as GoldMine, etc. To rectify distorted geometries by relocating atoms and releasing internal constraints, energy minimization for ligands and proteins is required. After lowering the energy, the geometry is mended, implying that just a small amount of energy was gained. The selectivity and binding energies of t must be predicted of the ligands for the target, In the modeled complexes, the interactions between our ligands 3(a, b, c, d) and the target were investigated, and the fitness function ability of this complex was observed by all desired molecules. Compounds 3(a, b, c, d) and cephalexin were ranked for their inhibitory efficacy based on their PLP fitness implicated in complex formation at the active sites. The docked compounds' PLP fitness on the target beta lactamase enzyme was found to be in the range of (68.39–83.02) Docking study revealed amino acids Table 3, demonstrating the interaction with our ligands through hydrogen bonds and short contacts. The length of these bonds established between individual protein atoms, as measured by GOLD, was less than 3A^o in our manufactured molecules.

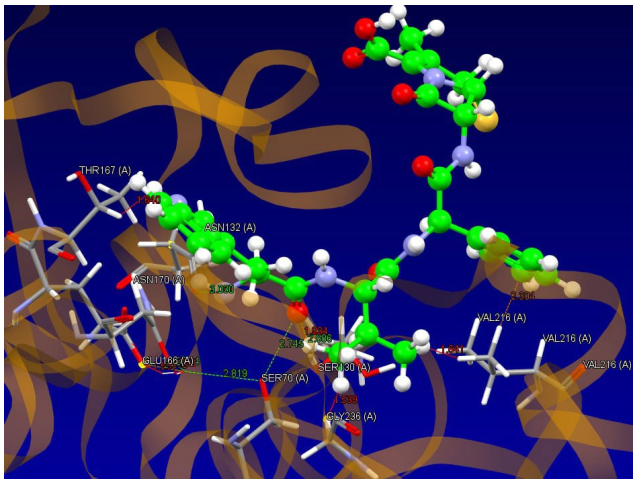


Figure 1: H-bond and short contact interaction profile for cephalexin binding to the beta lactamase enzyme in the conventional medication cephalexin (PDB code: 4R3B). H-bond interaction between cephalexin and amino acid residues [cephalexin is shaped like a ball and stick, whereas amino acids are capped sticks].

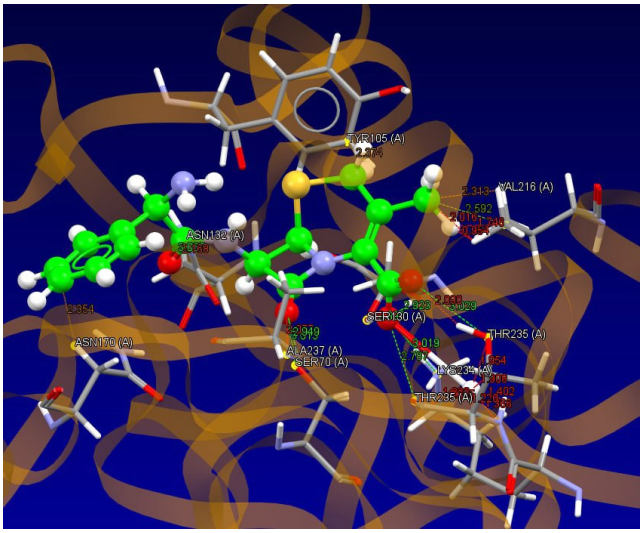


Figure 2: H-bond and short-contact interaction profile of chemical 3a with beta lactamase enzyme (PDB code: 4R3B). H-bond interaction between compound 3a and amino acid residues [molecule 3a has a ball and stick style, whereas amino acids have capped sticks].

Table 2: ADME results of the target synthesized compounds

Comp.	*H-bond acceptor*	*H-bond donor*	*MR*	*TPSA (A^o)*	*GI Abs*	*BBB permeant*	*Bioavailability*	*Lipiniski violation*
3a	7	6	172.85	212.02	low	NO	0.17	3 violation MW > 500, nor O>10, NH or OH > 5
3b	7	6	163.24	212.02	low	NO	0.17	3 violation MW > 500, nor O>10, NH or OH > 5
3c	8	6	153.15	224.91	low	NO	0.17	3 violation MW > 500, nor O>10, NH or OH > 5
3d	8	6	143.53	24.91	low	NO	0.17	3 violation MW > 500, nor O>10, NH or OH > 5

Molecular binding pattern of cephalexin with, *K. pneumoniae* beta lactamase enzyme, It is clear from Table 3 and Figure 4 that compound 3c has no H-bond interaction with the specific amino acid residue ARG 241, which may give the reason of it's in vivo lower antibacterial activity against *K. pneumoniae* In this work, the synthesized ligands 3a, 3d gave promising docking results with *K. pneumoniae* beta lactamase enzyme complex in comparison to cephalexin, The best docking PLP fitness was found in Compound 3b, which was 83.02. Finally, our docking analysis and the experimental data have a strong relationship.[25] The primary hydrogen bond in beta lactamase enzyme (PDB ID: 4R3B) is shown in the following figures.

Figure 1 shows cephalexin binding through the amino acids ASN 132, SER 70, ALA 237, SER 130, THR 235, LYS 234; Figure 2 shows compound 3a binding with ASN 132, SER 70, SER 130 amino acids; Figure 3 shows compound 3b binding with ASN 132, GLY 238, ARG 241 (3); Figure 4 shows compound 3c binding with ASN 276 (2), GLU 240, THR 167, ASP 104, Figure 5 the compound shows 3d binding with ASP 104, ALA 237, SER 70 (2), ASN 170, GLU 166.

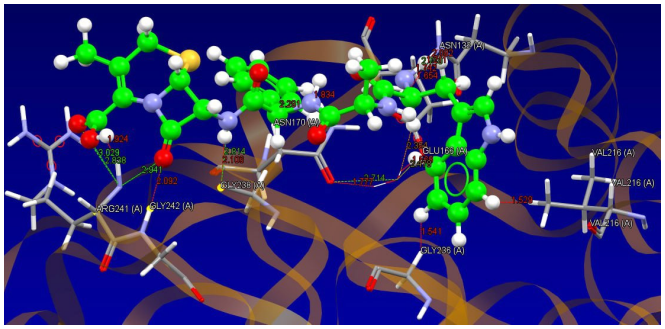


Figure 3: H-bond and short-contact interaction profile of chemical 3b with beta lactamase enzyme (PDB code: 4R3B). H-bond interaction between compound 3b and amino acid residues [molecule 3b has a ball and stick style, whereas amino acids have capped sticks].

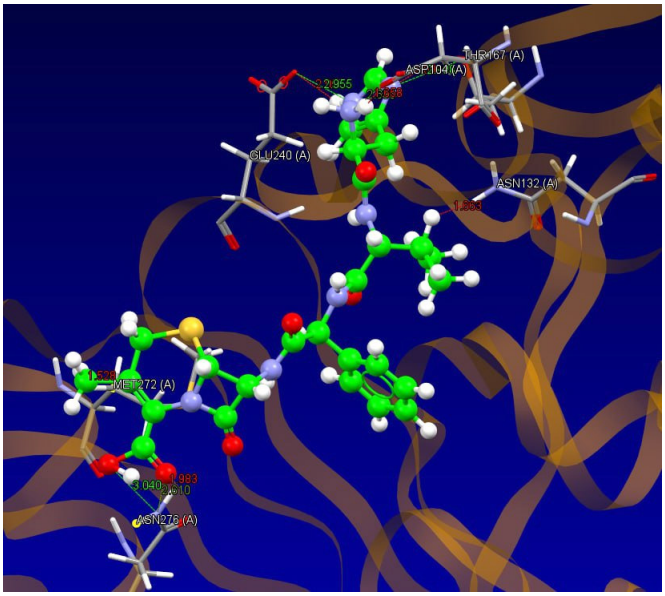


Figure 4: H-bond and short-contact interaction profile of chemical 3c with beta lactamase enzyme (PDB code: 4R3B). H-bond interaction between compound 3c and amino acid residues [molecule 3c has a ball and stick style, whereas amino acids have capped sticks].

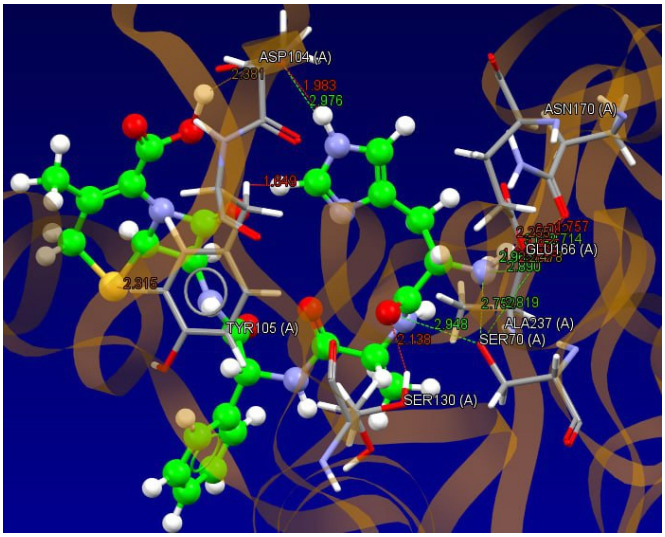


Figure 5: H-bond and short-contact interaction profile of chemical 3d with beta lactamase enzyme (PDB code: 4R3B). H-bond interaction between compound 3d and amino acid residues [molecule 3dhas a ball and stick style, whereas amino acids have capped sticks].

Table 3: The binding energies for target compounds and the reference drug cephalexin docked with *K. pneumoniae* beta lactamase complex.

Docking score with amino acid (PDB: 4R3B) BETA-LATAMASE			
Ligand	*plp fitness*	*A.A (H-bond)*	*A.A (other interaction)*
Cephalexin	65.05	ASN 132, SER 70, ALA 237, SER 130, THR 235, LYS 234	TYR 105, ASN 132, ASN 170 (2), ALA 237, VAL 216 (3), THR 235 (3)
3a	74.24	ASN 132, SER 70, SER 130	VAL 216 (2), ASN 170, THR 167, GLU 166, ASN 132, SER 130, GLY 236
3b	83.02	ASN 132, GLY 238, ARG 241 (3)	GLY 236, VAL 216, ASN 170 (2), GLY 238, ASN 132 (3), GLU 166, (2), GLY 242(2)
3c	68.39	ASN 276 (2), GLU 240, THR 167, ASP 104	MET 272, ASN 276, GLU 240, THR 167, ASP 104, ASN 132
3d	72.19	ASP 104, ALA 237, SER 70 (2), ASN 170, GLU 166	TYR 105 (2), ASP 104, SER 130, ASN 170, ALA 237, GLU 166

CONCLUSION

The synthetic technique for the designed target compounds was completed successfully, and FTIR, ^{1}H-NMR, and Elemental Microanalysis were used to establish the characterization and identification of the synthesized compounds.

The anti-bacterial assessment of the synthesized products reveal that the (6R, 7R)-7-((R)-2-((S)-2-((S)-2-amino-3-(1H-indol-3-yl)propanamido)propanamido)-2- phenylacetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid 3 b pharmacophores give significant antibacterial action.

ADME investigations revealed that the synthesized compounds 3a, 3b, 3c, and 3d did not agree with the Lipinski rule, necessitating the use of a particular carrier for absorption and transport.

The Preliminary study of antibacterial activity indicated that compounds 3a, 3b showed good activity against *S. aureus* and *MRSA* bacteria, and compound 3c, 3d gave the highest activity against *E. coli, K. pneumoniae, P. aeruginosa* in comparison with the reference drug cephalexin, some derivatives give antifungal activitiy against *Candida famata* in different concentration.

The Prescence of indole ring in tryptophan derivatives and imidazole ring in histidine derivatives essential for antimicrobial activity especially in MRSA, *P. aeuroginosa* and *C. famata*.

Histidine derivatives' antifungal activity is attributed to the imidazole ring, which acts as a bioisostere to itraconazole's triazole.

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