

Design, Molecular Docking, Synthesis of Aromatic Amino Acids Linked to Cephalexin.

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Abstract:

Infections caused by bacteria have a significant impact on public health. Chemical synthesis of new derivatives of cephalosporin linked to amino acid (tryptophan or histidine) through an amide bond at the acyl side chain is achieved. This is a new

approach of incorporating, tryptophan and histidine into the the primary amino group of cephalosporin, in order to provide a bulky group very close to the β -lactam ring. This chemical addition act as isosteric group to the alkoximino that protect beta lactam ring from bacterial beta lactamase enzyme. The new derivatives may show resistance to β -lactamases, improve activity and pharmacokinetic properties and may give new life for old drugs that are susceptible to hydrolysis by most β -lactamases. The chemical structures of these derivatives were confirmed by: FTIR, $^1\text{H-NMR}$ spectroscopy, elemental micro analysis and some physical properties. Molecular docking on serine beta lactamase and prediction of ADME parameters were recorded using GOLD suite and Swiss ADME software respectively. Docking scores of the new derivatives of Cephalosporin on β -lactamases were higher than those of Cephalosporin, which may indicate better activity.

Key words: Cephalosporin, Tryptophan, Histidine, Beta lactamase, Molecular Docking, ADME Studies.

تصميم ونمذجة جزيئية وتخليق لحوامض امينية اروماتيه مرتبطة بالسيفالكسين.

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الخلاصة:

ان الاصابة التي تسببها البكتريا لها تاثير مهم على الصحة العامة. تم تصنيع مشتقات جديدة للسيفالكسين مرتبطة بالاحماض الامينية (التربتوفان والهستيدين). هذه المشتقات تتضمن ربط احماض امينية اروماتيه معينة بمجموعة الامين الموجودة بجزيئة السيفالكسين عبر الأصرة الأمايدية. هذه طريقة جديدة لدمج التربتوفان والهستيدين في المجموعة الأمينية الأولية من سيفالكسين، من أجل توفير مجموعة ضخمة قريبة جداً من حلقة بيتا لاكتام. تعمل هذه الإضافة الكيميائية كمجموعة ايزوستيرية إلى الكوكس امينو التي تحمي حلقة بيتا لاكتام من إنزيم بيتا لاكتاميز البكتيري. هذه الفرضية تتضمن اعطاء حيوية جديدة للأدوية القديمة السيفالوسبورينات والبنيسيلينات الحاوية على مجموعة أمين ضمن جزء الاسيل القريب الى حلقة البيتا لاكتام. تم تخليق هذه المشتقات الجديدة بنجاح بكميات مقبولة وتأكيد التركيب الكيميائي عن طريق اطياف الاشعة تحت الحمراء الدقيق والرنين النووي المغناطيسي البروتوني وتحليل العناصر وبعض الخواص الفيزيائية. اجريت دراسة فحص

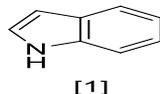
الالتحام الجزيئي للمركبات المصنعه كمثبطات لأنزيم السيرين بيتا لاكتاميز بالمقارنة مع السيفالكسين عن طريق برنامج GOLD [v. 5.6.2] suite واجري فحص خصائص حركية الدواء للمركبات عن طريق البرنامج واطهرت فعالية مهمة عالية مقارنة مع السيفالكسين كمثبط للأنزيم واستخدم كذلك Swiss ADME للتنبؤ والتعرف على اي من المركبات لها درجة امتصاص عن طريق الجهاز الهضمي.

الكلمات المفتاحية: سيفالكسين, تربتوفان, هستدين, انزيم البيتا لاكتاميز, الالتحام الجزيئي, فحص خصائص حركية الدواء.

Introduction:

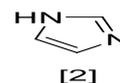
Resistant bacteria have developed as the most pressing public health concerns. [1]

Bacteria are naturally resistant to certain antibiotic classes, either because they lack the target or because the drug is impermeable to them. Others are predisposed to infection but build resistance through one of a growing number of methods. Some strains are carried on plasmids, which reproduce in an extracellular matrix in a circular pattern. extrachromosomal DNA molecules, which can be passed on to bacteria of different species. [2] Cephalixin is an antibacterial medication that belongs to the β -lactam family. It has a bactericidal effect and acts in a similar way to benzyl penicillin by inhibiting bacterial cell wall formation. [3] Indole [1] is a significant heterocyclic system because it is incorporated into proteins as an amino acid. Tryptophan is one of the important aromatic amino acids because it is the building block for many pharmaceuticals like indomethacin and serves as the skeleton for indole alkaloids, which include strychnine and LSD. [4]



The indole derivatives have been emerged as the drugs of immense importance in the recent times and well known for their significant biological activities such as cytotoxic, antimicrobial, antidiabetic, and anti-inflammatory activities. [5,6] The high therapeutic properties of the imidazole [2] related drugs have encouraged the medicinal chemists to synthesize a large number of novel chemotherapeutic agents. Imidazole drugs have broadened scope in

remedying various dispositions in clinical medicines. [7]



Indole derivatives have recently emerged as important medications with significant pharmacological effects. [8-10]

The antimicrobial properties of cephalosporin derivatives have been described. Different heterocyclic nuclei's azetidinone congeners, on the other hand, had significant bactericidal and bacteriostatic properties, Incorporation of β -lactam (azetidinone) moieties, is advantageous for antibacterial and antifungal activity. [11,12] Because of the presence of a primary amine group at the acyl side chain's -carbon, the proposed compounds are suitable for oral usage due to their expected stability in aqueous acidic conditions. Despite the continuous improvement and development of new antibiotics, resistance in a variety of microorganisms continues to rise. [13] The most effective synthesis of several semisynthetic cephalosporin derivatives is based on the structure-activity relationship. [14] In the light of facts and explore to developed the good antimicrobial activities of Cephalixin derivatives are designed and synthesized in the current research. with a broader antimicrobial spectrum and resistance against β -lactamase producing bacteria.

Experimental Work:

Materials and Methods:

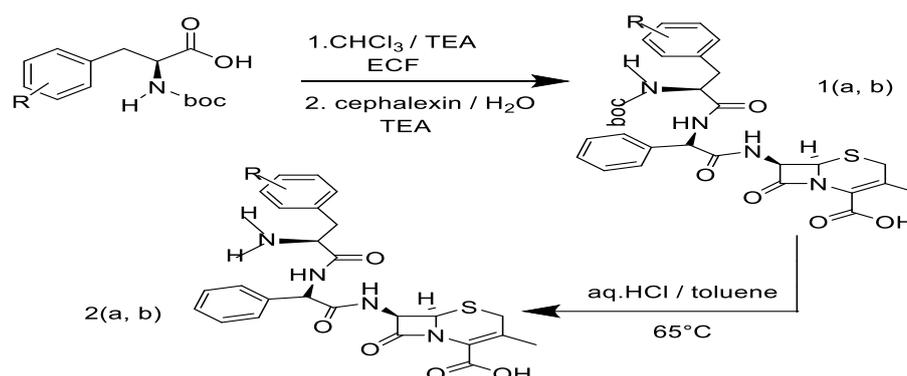
All reagents and solvents were purchased from commercial sources. Ethyl chloroformate (ECF) was purchased from Sigma Aldrich in Germany, Boc-tryptophan and Boc-histidine were purchased from

Shanghai World Yang Chemical in China, triethylamine (Thomas Baker) in India, Cephalexin monohydrate was purchased from SDI Samarra-Iraq. Stuart Electric melting point device was used to determine melting points (England). Compound identification was accomplished utilizing an IR spectrum acquired on a Shimadzu FT-IR infrared spectrometer with KBr disks.

Varian, Agilent 500MHz, determined $^1\text{H-NMR}$ (USA). Elemental microanalysis (C, H, N) was carried out. VarioEL, Germany, Elementar Analysen Systeme.

Chemical synthesis:

The synthesis of intermediates and target compounds was done by using the procedures illustrated in scheme 1.



R in aromatic amino acids = Tryptophan (a), Histidine (b).

Scheme (1): Synthesis of intermediates and target compounds.

General procedure for synthesis of the intermediates 1(a,b):

Suitable Boc-amino acid 10 mmol (Boc-tryptophan (a) 3.04gm or Boc-histidine (b) 2.55gm) was dissolved in 50ml dry chloroform containing (10 mmol, 1.39ml) TEA and frozen in an ice bath (-2 to -4°C). Ethyl chloroformate (10 mmol, 0.94ml) was added drop by drop over a 10min. interval. Then the mixture was continuously mixed by stirring for 60 min. Cephalexin (10mmol, 3.65gm) in distilled water (10ml) including TEA (10mmol, 1.39ml) was cooled to 0 °C and immediately added to the mixture solution, stirring for 4 hours at (-2 to -4). Two layers were formed which separated later by using a separatory funnel into an organic (chloroform) lower layer that contains (Boc-aromatic amino acid unreacted, excess TEA, product). Upper aqueous (water) layer containing (TEA-HCl, unreacted cephalexin), washing aqueous layer with (15ml) chloroform. Using a separatory funnel, to remove TEA as TEA-HCl salt, wash the primary organic layer with (15ml) aq. HCl (0.1M) and

separate them. By separating the organic and aqueous layers, get a mixture of (Boc-aromatic amino acid unreacted, product) by washing it with (15ml) ether and filtering it. Boc-aromatic amino acids are soluble in ether; therefore, the product will settle as a precipitate on filter paper. Overnight drying at temperature (40°C).⁽¹⁵⁾

(6S,7S)-7-((R)-2-((R)-2-((tert-butoxycarbonyl)amino)-3-(1H-indol-3-yl)propanamido)-phenylacetamido)-3-methyl-8-oxo-5-thia-1-

azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (1a): Light yellow powder (Yield=72%); m.p. (°C)193.5-196; IR (KBr) $\nu(\text{cm}^{-1})$: 3410 (NH 2°amide) , 3402 (NH of indole ring), 3188 (O-H carboxylic acid) , 3041 (C-H Aromatic), 2978 (C-H Aliphatic), 1772 (C=O β -lactam), 1750 (C=O *tert*-butoxycarbonyl), 1700 (C=O carboxylic acid), 1687 (C=O 2° amide), 1654 (C=C), 1425 (C-N), 698 (C-S-C)⁽¹⁶⁾ ; $^1\text{H-NMR}$ (DMSO- d_6 , 500MHz): δ 10.79 (s,1H, NH indole ring), δ 8.31(s, 2H, NH

2° amide), δ 7.39 (s, 1H, NH of carbamate), δ 6.98-7.58 (m, 5H, aromatic ring protons and 1H, CH indole ring), δ 5.82 (d, 1H, CH alpha to 2° amide and benzene ring), δ 4.92 (m, 1H, CH alpha to 2° amide & carbamate), δ 2.01 (s, 3H, CH₃ allylic), δ 1.34 (s, 9H, CH₃ of carbamate).^[17]

(6R,7R)-7-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-(1H-imidazol-4-yl)propanamido)-2-phenylacetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (1b): Light yellow powder (Yield=71%); m.p. (°C) 198-201; IR (KBr) $\nu(\text{cm}^{-1})$: 3420 (NH 2° amide), 3405 (NH imidazole ring), 3200 (O-H carboxylic acid), 3030 (C-H aromatic), 2931 (C-H aliphatic), 1768 (C=O β -lactam), 1740 (C=O tert-butoxycarbonyl), 1710 (C=O carboxylic acid), 1649 (C=O 2° amide), 1620 (C=C), 1400 (C-N), 700 (C-S-C)^[16]; ¹H-NMR (DMSO-d₆, 500MHz): δ 8.15 (s, 2H, NH 2° amide), δ 7.66, 8.70 (d, 2H, CH imidazole ring), δ 7.37 (d, 1H, NH carbamate), δ 7.26-7.32 (m, 5H, aromatic ring protons), δ 5.72 (d, 1H, CH alpha to 2° amide and benzene ring), δ 5.58 (t, 1H, alpha to C=O β -lactam), δ 4.96 (d, 1H, H alpha to N β -lactam ring), δ 4.92 (m, 1H, CH alpha to 2° amide & carbamate), δ 1.97 (s, 3H, CH₃ allylic), δ 1.36 (s, 9H, CH₃ of carbamate).^[17]

General procedure for synthesis of the target derivatives of cephalixin 2(a,b) :

Added a sufficient amount of each protected carbamate intermediate of cephalixin 1(a,b) (10mmol) in toluene (15ml) as a solvent, then added aqueous hydrochloric acid (10 ml) 1.5M, then heated at 65°C to obtain two layers organic toluene layer containing tert-butyl cation as the tert-butyl cation Whether it's a polymer or an oligomer, The product was obtained as HCl- salt in aqueous layer form, so it was added to (15ml) of ethanol, then added (10mmol, 1.39 ml) of TEA, the mixture then cooled and the formed precipitate was filtered and taken filtrate, ethanol evaporated to yield the product with

free amino group. The reaction was confirmed for completeness by removing all CO₂ gas bubbles that developed as a result of the carbamate bond breaking entirely. Deprotection of the amino group of compounds was used to make these compounds. To make the new compounds, boc-aromatic amino acid-cephalexin was used to be afford the new cephalixin compounds.^[16]

(6S,7S)-7-((R)-2-((R)-2-amino-3-(1H-indol-3-yl)propanamido)-2-phenylacetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (2a): Bright yellow crystals (Yield=80 %) ; m.p. (°C) 150.5-153; IR (KBr) $\nu(\text{cm}^{-1})$: 3410 (NH 2° amide), 3402 (NH of indole ring), 3381 (NH 1° amine), 3186 (O-H carboxylic acid), 3041 (C-H aromatic), 2982 (C-H aliphatic), 1770 (C=O β -lactam), 1710 (C=O carboxylic acid), 1672 (C=O sec.amide), 1610 (C=C), 1400 (C-N), 702 (C-S-C)^[16]; ¹H-NMR (DMSO-d₆, 500MHz): δ 10.81 (s, 1H, NH indole ring), δ 9.12 (s, 2H, NH 1° amine), δ 8.18 (s, 2H, NH 2° amide), δ 6.98-7.58 (m, 5H, aromatic ring protons), δ 7.12 (d, 1H, CH indole ring), δ 5.82 (d, 1H, CH alpha to 2° amide and benzene ring), δ 5.6 (t, 1H, H alpha to C=O β -lactam ring), δ 5.1 (d, 1H, H alpha to N of β -lactam ring), δ 4.92 (m, 1H, CH alpha to 2° amide & 1° amine), δ 2.01 (s, 3H, CH₃ allylic).^[17]

Chemical Formula: C₂₇H₂₇N₅O₅S.
Elemental analysis: (Calculated) C:60.77, H:5, N:13.12 (Found) C:58.352, H:4.884, N:13.369.

(6R,7R)-7-((S)-2-((S)-2-amino-3-(1H-imidazol-yl)propanamido)-2-phenylacetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid (2b): Dark Brown crystals (88% of Yield) ; m.p. (°C) 108-111; IR (KBr) $\nu(\text{cm}^{-1})$: 3420 (NH 2° amide), 3410 (NH imidazole ring), 3383 (NH 1° amine), 3150 (O-H Carboxylic acid), 3030 (C-H aromatic), 2930 (C-H aliphatic), 1780 (C=O

β -lactam), 1700 (C=O carboxylic acid), 1650(C=O 2^oamide), 1631 (C=C), 1410(C-N), 698 (C-S-C)^[16] ; ¹H-NMR (DMSO-d⁶, 500MHz): δ 9.12 (s, 2H, NH₂ of 1^oamine), δ 8.32 (s, 2H, NH 2^oamide), δ 7.66, 8.70 (d, 2H, CH imidazole ring), δ 7.37(s,1H, NH carbamate), δ 6.92-7.62 (m, 5H, aromatic ring protons), δ 5.72 (d, 1H, CH alpha to 2^oamide and benzene ring), δ 5.08 (t, 1H, alpha to C=O β -lactam), δ 5.04 (d, 1H, H alpha to N of β -lactam ring), δ 4.01 (m,1H, CH alpha to 2^oamide & 1^oamine), δ 2.5 (s,3H, CH₃ allylic) .^[17]

Chemical Formula: C₂₂H₂₄N₆O₅S.
Elemental analysis: (Calculated) C:54.45, H:4.99, N:17.35 (Found) C:52.87, H:3.786, N:17.684.

Computational Methods:

Procedures for ADME.

The SwissADME server was used to assess the physicochemical and pharmacokinetic parameters of derivatives 2(a,b). Chem.Biodraw (v.17) was used to create the chemical structure of the intended compounds, which was then transformed to SMILE names using the Swiss ADME program.^[18]

Docking Technology.

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.^[19]

Preparation of the ligand and receptor.

The crystal structure of the beta lactamase enzyme from K.pneumoniae was taken from the Protein Data Bank (PDB ID: 4R3B) which is serine beta lactamase type^[20], and missing atoms were added using SwissPDB Viewer (SPDBV) (v.3.7). Processes are used to prepare the crystal structures of proteins. To acquire the proper

ionization and tautomeric state of amino acid residues, remove all water molecules and introduce hydrogen atoms. ChemBio3D (v.17.1) was used to minimize the energy of the produced ligands using the MM2 force field.

Protocol for Molecular Docking.

The receptors were put up for the docking process using GOLD Suite's HERMES – Structure visualization software. The radius (10^oA) 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 left 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:

Interpretation of ADME Results.

Swiss ADME server analyzed the ADME properties of the final synthesized substances^[18]. The pharmacokinetic properties of all produced substances were evaluated (absorption, distribution, metabolism, excretion). The drug-like characteristics of all target compounds were investigated in this study. Lipinski's rule of five was used to calculate the results.^[21] This rule, often known as Pfizer's rule of five (RO5), is extensively employed as a filter for compounds that are likely to be used as a lead for drug discovery 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.W) ≤ 500 . In addition, the topological polar surface area (TPSA) was calculated, as this is a key characteristic in

determining drug bioavailability. [22] The lipinski rule of five is used to these new cephalixin derivatives. In 2b, there was no effect on the liver enzyme CYP-450 Only the subtype CYP 2C9 is inhibited by derivative and 2a. Oral bioavailability is expected to be low for passively absorbed

compounds with a TPSA >140 Å². Our all-produced compounds had a TPSA over 140 Å², which is in the range (182.92-195.81 Å²) and a bioavailability of 0.55, indicating that all ligands reach the systemic circulation, as shown in the table (1).

Table (1): ADME results of the target synthesized compounds

Comp.	H-bond acceptor	H-bond donor	MR	TPSA (Å ²)	GI Abs	BBB permeant	Bioavailability	Lipinski violation
2a	6	5	146.82	182.92	low	NO	0.55	1 violation MW > 500
2b	7	5	126.11	195.81	low	NO	0.55	1 violation nor O >10

Interpretation of Docking Results.

All freshly synthesized compounds were successfully docked using GOLD Suite software 2(a,b). GOLD is a "genetic approach for docking flexible ligands into protein binding sites." [23] GOLD has been extensively tested and has shown great posture prediction rendering and virtual screening outcomes. [24] This is available as part of the GOLD Suite, which also contains Hermes, Mercury, Isostar, and Conquest, as well as GoldMine.... 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 repaired, implying that the minimum level of energy has been achieved. The selectivity and binding energies of the ligands for the target must be predicted. In the modeled complexes, the interactions between our ligands 2(a,b) and

the target were investigated, and the fitness function ability of this complex was observed by all desired molecules. The inhibitory effect The PLP fitness involved in the complex formation at the active sites was used to rate the activity of compounds 2(a,b) and cephalixin. The docked compounds' PLP fitness on the target beta lactamase enzyme was found to be in the range of (71.9-74.13) Amino acids were discovered by docking analysis as shown in table (2). Using hydrogen bonds and brief interactions with our ligands, we demonstrated the interaction.

The length of these bonds established between individual protein atoms, as measured by GOLD, was below that of our manufactured molecules 3Å². [25] Other binding forces, such as van der Waals, electrostatic, steric, pi-pi stacking, dipole-dipole, and others, are present in the short contacts.

Table (2): The binding energies for target compounds and the reference drug cephalixin 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, A237, SER 130, THR 235, LYS 234	TYR 105, ASN 132, ASN 170 (2), ALA 237, VAL 216 (3), THR 235 (3)
2a	74.13	ASN 132, ASP 104 VAL 216	THR 167, ASP 104, GLU 240, VAL 216 (2), TYR 105, SER 130
2b	71.9	ARG 241, GLY 242, THR 235, ALA 237, SER 70	GLY 242, ARG 241 (2), GLU 166, THR 167, ALA 237 (2), SER 130, THR 235

Cephalexin's Molecular Binding Pattern with the Beta Lactamase Enzyme from K.pneumoniae. In comparison to cephalixin, the synthesized ligands 2a,2b showed promising docking results with k.pneumoniae beta lactamase enzyme complex, with compound 2a showing the best results. PLP fitness, which was 74.13, was docked. Finally, our docking analysis and the experimental data have a strong

relationship. [20] The hydrogen bond in the beta lactamase enzyme is shown in the following figures (PDB ID: 4R3B) in figure (1) represent cephalixin binding through ASN 132, SER 70, ALA 237, SER 130, THR 235, LYS 234 amino acids, figure (2) the compound 2a binding with ASN 132, ASP 104, VAL 216 amino acids, figure (3) the compound 2b binding with ARG 241, GLY 242, THR 235, ALA 237, SER 70 amino acids.

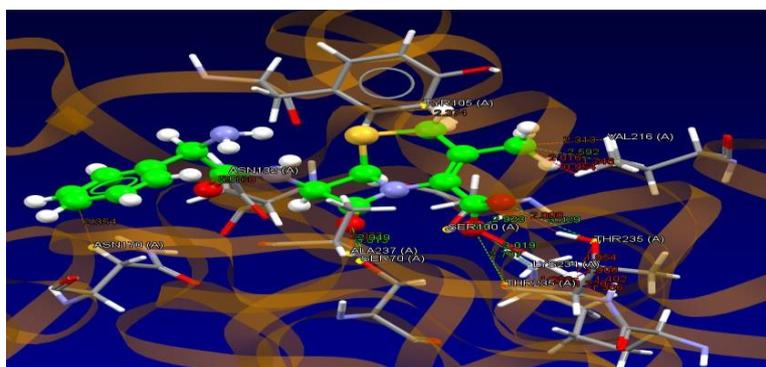


Figure 1: H-bond and short contact interaction profile for cephalixin binding to the beta lactamase enzyme in the conventional medication cephalixin (PDB code: 4R3B). H-bond interaction between cephalixin and amino acid residues [cephalexin is shaped like a ball and stick, whereas amino acids are capped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

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