Original Article

Virtual Screening of Novel Non-Lactam Inhibitors Against CTX-M-15 β-Lactamase for Enhancement and Remodeling of Antibiotic Efficiency

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Abstract

Background: The CTX-M-15 are beta-lactamases that break down practically all the antibiotics that belong to the beta-lactam group resulting in antibiotic resistance in bacteria. Several beta-lactam inhibitors can be used in combination with different cephalosporin antibiotics to treat infections caused by microbes that produce the CTX-M-15 enzyme.

Objective: This study aims to use a multi-step virtual screening strategy to screen three new non—lactam inhibitors against CTX-M-15.

Methods: Study was designed and conducted in the Department of Biotechnology at the University of Central Punjab. A multi-step virtual screening strategy was used to screen three new non— lactam inhibitors against CTX-M-15. The CTX-M-15 binding sites were explored to determine the possible target sites of the inhibitors. Compounds from the E-LEA3D were subjected to virtual screening, and their performance was evaluated based on the binding energies and various other factors. Using Auto Dock Vina, the docking complexes were formed and visualized in Pymol where their RMSD values and binding energies were compared.

Results: The best results were found in the case of Isoproterenol as it inhibited the lactamase activity of CTX-M 15 by forming a stable docked complex. The molecular docking simulations using Autodock Vina revealed favorable binding interactions, indicating the ability of Isoproterenol to bind to the active site.

Conclusion: The lactamase activity of CTX-M 15 was inhibited by Isoproterenol as they form a stable docked complex. Designing inhibitors against the CTX-M-15 type β-lactamase represents a promising avenue for combating drug-resistant bacteria.

Keywords: Beta-Lactamase, Pymol, Virtual Screening, Drug Resistance, Antibiotics

Introduction

The introduction of antibiotics in this world was one the greatest achievements of all time. The Beta-lactam group was the main reason that first ever antibiotic penicillin G worked against pathogenic bacteria and the identification of the beta-lactam group gave rise to the production of all today the antibiotics we use such as cephalosporin, carbapenems, and monobactams. However, the excess use of these antibiotics has led to resistance in bacteria against the beta-lactam group by the production of the enzyme beta-lactamase. CTX-M 15 beta-lactamase is of one the enzyme contributing to multi-drug resistance against antibiotics.[1,2]

The mechanism of action of beta-lactamase is the hydrolysis of the beta-lactam ring causing antibiotics to cease

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to work. To overcome the issue, much research is being done to design inhibitors against beta beta-lactamase enzyme to produce a novel antibiotic that can be effective against resistant bacterial strains. As a result, techniques for identifying new anti-lactamase drugs with modes of operation are urgently required. Furthermore, virtual screening technologies like molecular docking and molecular dynamics simulations have made it easier to screen huge drug libraries for potential inhibitors. High-throughput screening has also played a crucial role in identifying novel inhibitors by screening diverse chemical libraries. Generally, β-lactamase inhibitors work in two ways; they might bind as the substrate forming unfavorable interactions like acyl-enzymes or may inactivate the enzyme permanently by undergoing secondary reactions with the enzyme at the active site. For instance, NDM-1 has a flexible hydrolysis mechanism, making the inhibition very difficult. Certain covalent inhibitors have been found to have high potency, yet their lack of specificity and high toxicity renders them inappropriate for use as adjuvants.[2,3]

A virtual screening approach is being used to screen novel β-lactam inhibitors against CTX-M-15. Bioinformatics and experimental approaches are also being utilized to establish an understanding of the binding of CTX-M-15 with inhibitors. RSCB Protein Data Bank can be used as a source for the crystal structure of CTX-M-15. Various computational tools were used for different processes, such as Discovery Studio 2.5 was used to remove the water molecules and add hydrogen atoms to the enzyme, the conformation of the receptor-ligand complex was predicted by Patch Dock and Autodock Vina in molecular docking, docking validation was carried out using iMods.[4]

A potential inhibitor for CTX-M-15 type beta-lactamases which was found due to the virtual screening approach is Isoproterenol. Further, molecular docking was performed, and following that post molecular docking analysis or molecular dynamic simulation to study the interaction between the protein-ligand complex. Binding affinity, RMSD value, hydrogen bonds, and salt bridges were analyzed and the best interaction between the inhibitor and enzyme was chosen based on these scores. The potential efficacy of the inhibitor Isoproterenol against CTX-M-15 carrying bacterial strains to inhibit b-lactamases was validated.[4]

The newly designed inhibitor against CTX-M-15 beta-lactamase through in- silico approaches show promising results overcoming antibiotic resistance. Furthermore, new strategies and mechanisms can be utilized to develop such inhibitors against beta-lactamase that exhibits higher potency, selectivity, and kinetic properties. The structural activity relationship can be enhanced in designing new drugs that displays efficiency against antibiotic drug-resistant bacterial strains.[5]

This research was designed to employ a multi-step virtual screening approach to identify novel non-lactam inhibitors against CTX-M-15, a β-lactamase known for conferring antibiotic resistance in bacteria.

Methods

First, the crystal structure of the natural CTX-M-15 extended-spectrum beta-lactamase enzyme (PDB: 4HBT) was obtained from the RCSB PDB Protein Data Bank (https://www.rcsb.org). By using the Discovery Studio Visualizer. 6 (Studio, 2009), All water molecules and ligands were removed, and polar hydrogen atoms were introduced. The binding sites were determined using residue 1 Å (Angstrom) surrounding the crystal structure.

To identify the possible ligand compounds virtual screening was performed using e-LEA3D (https://chemoinfo.ipmc.cnrs.fr) – drug design server.[7] The de novo drug design and virtual screening tool was used to design new ligands to optimize the interaction between the ligand and enzyme. All the screening compounds are molecules that fall within Lipinski's rule of five i.e., molecular mass < 500 Da, hydrogen bond donors ≤ 5, hydrogen bond acceptors ≤10, octanol–-water partition coefficient log P (Clog P) ≤ 5.

To predict the conformation of ligand-receptor complex, Autodock Vina[8,9] and PatchDock[10] (http://bioinfo3d.cs.tau.ac.il/PatchDock/) were used. Next, conformation having the best fitness scores were was extracted from each docking clusters. The exhaustiveness setting in each docking was set to 8 and the simulation box (number of points in x, y, and z dimensions) was set to 40× 40×40. Autodock Vina was used to prepare pdbqt files of protein and ligand. Polar hydrogen atoms were added to create a charge for efficient binding and their binding affinity was calculated using the scoring function of the tool Autodock Vina. Pymol (https://pymol.org/2/) was used to visualize the docking complexes.

Post-docking analysis or molecule dynamic simulations

were performed to check the stability of the ligand within the enzyme's active site. For the molecular dynamic simulation studies, iMods[11] was used. IMods (https://imods.iqfr.csic.es/) is a tool that performs Normal Mode Analysis (NMA). Along with iMods, to study the molecular interaction between protein-ligand complex Protein - Ligand Interaction Profiler (PLIP) (https://plip-tool.biotec. tu-dresden.de/plip-web/plip/index) was used.

Results

The best results were found in the case of Isoproterenol as it inhibited the lactamase activity of CTX-M 15 by forming a stable docked complex. The molecular docking simulations using Autodock Vina revealed favorable binding interactions, indicating the ability of Isoproterenol to bind to the active site.

The virtual screening of FDA-approved drugs was performed to find the appropriate inhibitor that would form the docking complex with the prior mentioned molecule to inhibit its activity. Compounds from the E-LEA3D were subjected to virtual screening, and their performance was evaluated based on the binding energies and various other factors. Using Auto Dock Vina, the docking complexes were formed and visualized in Pymol where their RMSD values and binding energies were compared. Among the available options, the best results were found in the case of Isoproterenol, which can be used as a potential inhibitor for CTX-M-15 type beta-lactamases (Fig I, II). The hydrolytic activity was seen no more hence a promising future against antibiotic resistance. As a result, the suggested putative inhibitor might be employed as lead molecules for future therapeutic candidates against bacteria that produce beta-lactamases.

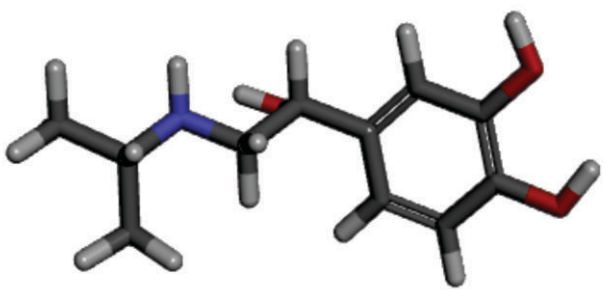
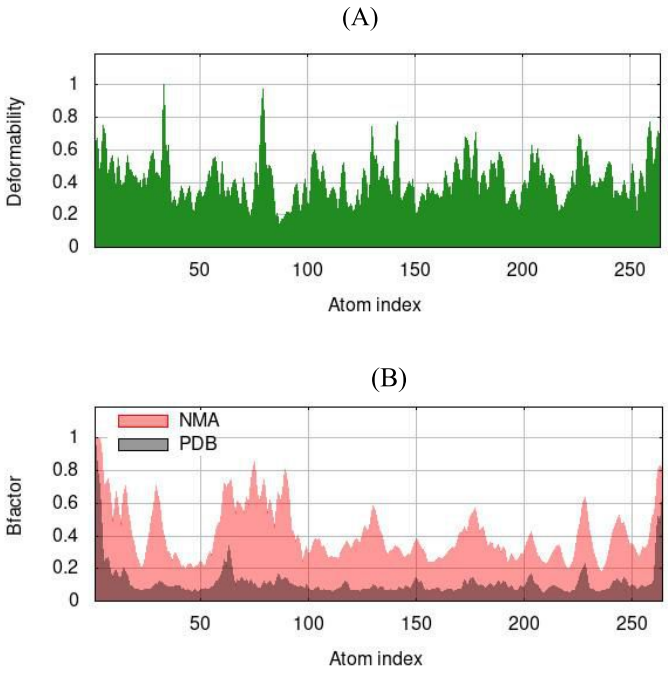


Figure I: *Structure of Isoproterenol used as an inhibitor for beta-lactamase CTX-M-15 found through virtual screening using Le3D*

Figure II: *Docked complex between CTX-M-15 and Isoproterenol visualized in Pymol*

Molecular Dynamic (MD) simulations involve computational techniques to check various parameters. For this purpose, iMods (Internal coordinates normal mode analysis server) was used as it is the best free online tool available that helps in Normal Mode Analysis (NMA). Patch Dock was used for online docking between the enzyme and inhibitor. The complex was selected based on score. The selected complex from Patch Dock was saved in .pdb format and then uploaded on iMods for simulation studies. Figure III shows the MD Simulation of the docked complex formed between CTX-M-15 and Isoproterenol. The detailed results are mentioned



below in figure III.

The molecular interactions in a protein-ligand complex

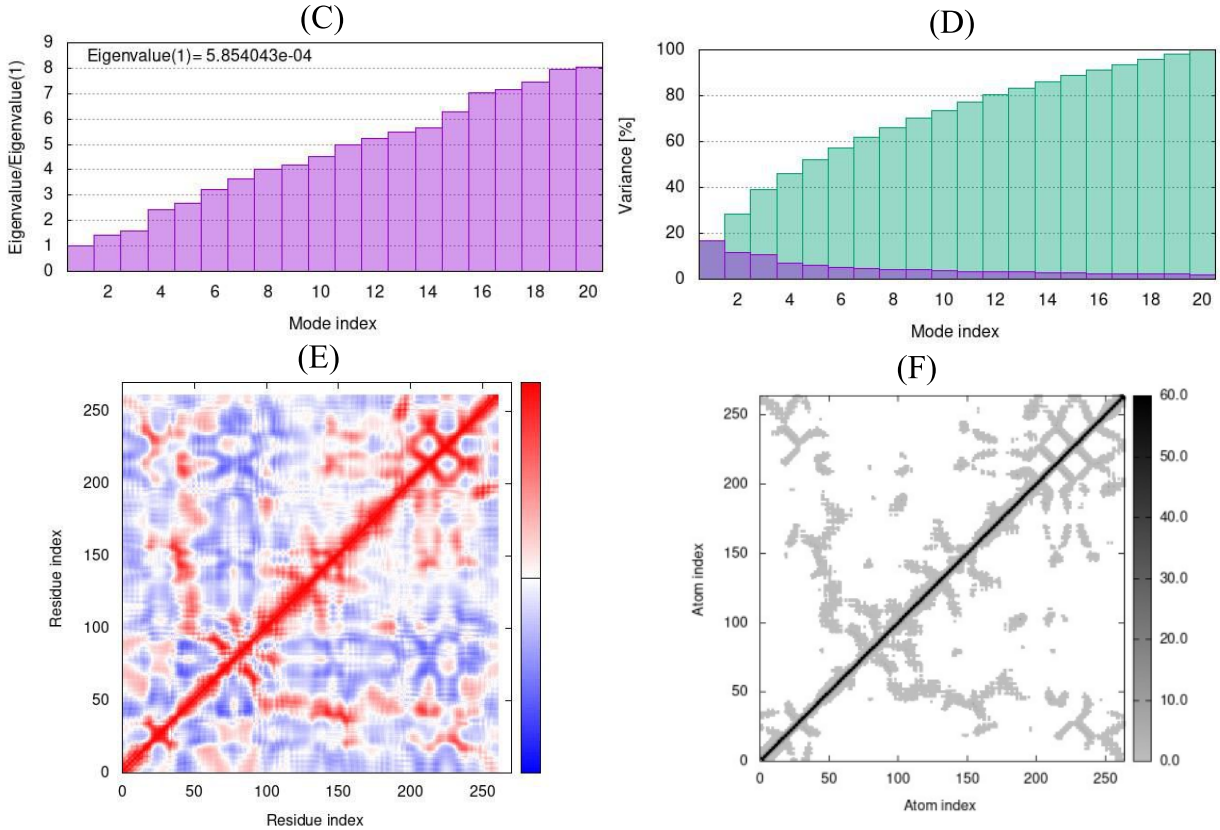
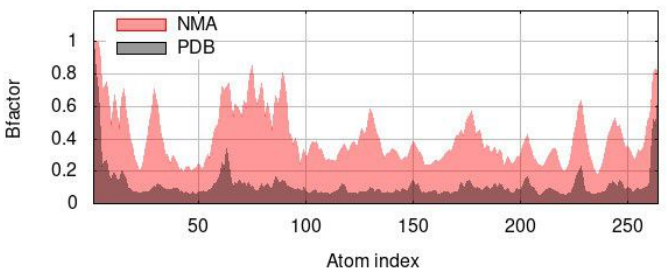


Figure III: *MD Simulations by IMODs*

A) B-factor/Mobility of the docked complex formed between CTX-M-15 and Isoproterenol B) A comparison between the already present PDB docked complex with no B-factor and the docked complex under study. C) Eigenvalue of the docked complex. D) %Variance of the docked complex between CTX-M-15 and Isoproterenol. E) Covariance matric of the docked complex - uncorrelated (white) correlated (red) or anti-correlated (blue) motions. F) Elastic network model of the docked complex - darker color means stiffer spring



can be studied through Interaction Profile Generation using tools like Protein Protein-Ligand Interaction Profiler (PLIP). PLIP generates various small molecule interactions and sulphate sulfate interactions. Only two of those interactions; small molecule interaction (EDO-A-308) and sulphate sulfate (SO4-A-301) are presented respectively. Figure IV represents the visual representation of the docked complex analyzed by PLIP. While figure V provides the Visual representation of SO4-A-301 (sulfate interactions) - hydrogen bonds (dark blue rods), water bridges (light blue rods), and salt bridges (yellow dotted line)

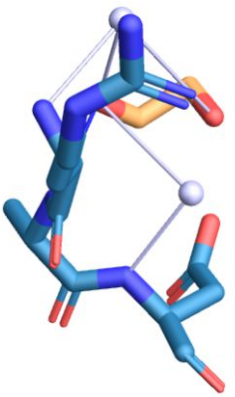


Figure IV: *Visual representation of EDO-A-308 (small molecule interactions) hydrogen bonds (dark blue rods) and water bridges (light blue rods)*

Table I: *Hydrogen Bonds*

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Sidechain	Donor Atom	Acceptor Atom
1	62A	ALA	1.93	2.89	167.09		283 [Nam]	2058 [O3]

Table II: *Water Bridges*

Index	Residue	AA	Dist. A-W	Dist. D-W	Donor Angle	Water Angle	Donor Atom	Acceptor Atom	Water Atom
1	61A	ARG	3.20	2.85	165.78	108.66	272 [Nam]	2058 [O3]	2303
2	61A	ARG	3.97	2.85	165.78	81.44	272 [Nam]	2056 [O3]	2303
3	63A	ASP	3.59	2.92	154.34	104.59	288 [Nam]	2058 [O3]	2186

Table III: *Hydrogen Bonds*

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Donor Atom	Acceptor Atom
1	70A	SER	2.73	3.29	117.48	351 [O3]	2026 [O3]
2	130A	SER	1.82	2.74	157.69	815 [O3]	2026 [O3]
3	237A	SER	3.52	4.04	114.62	1612 [Nam]	2026 [O3]

Table IV: *Water Bridges*

Index	Residue	AA	Dist. A-W	Dist. D-W	Donor Angle	Water Angle	Donor Atom	Acceptor Atom	Water Atom
1	70A	SER	3.17	3.12	144.22	108.58	2026 [O3]	351 [O3]	2246
2	70A	SER	4.06	2.99	139.64	77.56	346 [Nam]	2025 [O3]	2166
3	130A	SER	2.88	3.12	144.22	73.81	2026 [O3]	813 [O2]	2246
4	236A	GLY	3.45	2.80	147.00	102.56	2025 [O3]	1611 [O2]	2139

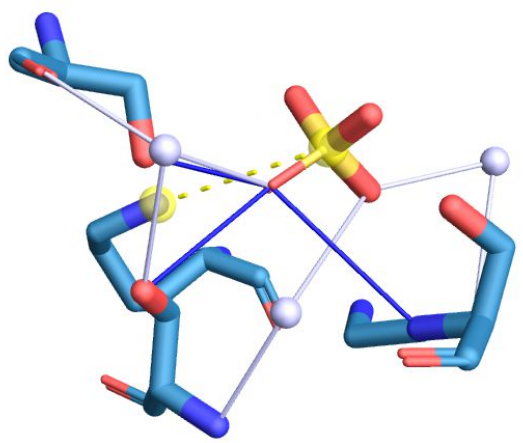


Figure V: *Visual representation of SO4-A-301 (sulfate interactions) - hydrogen bonds (dark blue rods), water bridges (light blue rods), and salt bridges (yellow dotted line)*

Table V: *Salt Bridges*

Index	Residue	AA	Distance	Ligand Group	Ligand Atoms
1	234A	LYS	4.75	Sulfate	2022, 2022

Binding Affinity of CTX-M-15 With Isoproterenol

The binding affinities were found by docking through Autodock vina. The structure with the highest negative value (smallest value) was chosen for further analysis in Imods.

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-6.5	0.000	0.000
2	-6.4	1.426	2.206
3	-6.3	2.610	3.995
4	-6.3	2.351	3.339
5	-6.1	1.850	2.186
6	-5.3	3.001	4.559
7	-5.1	25.455	27.072
8	-5.1	25.125	26.220
9	-5.1	25.314	26.367

Figure VI: *Binding affinities of the docking complexes formed between CTX-M-15 and Isoproterenol*

The first RMSD (i.b. and u.b.values) should be zero in case of a successful docking.

Discussion

The emergence and spread of antibiotic-resistant bacteria pose a significant threat to global public health. The pace of discovering new antibiotics is sluggish, necessitating innovative approaches for effective treatment of bacterial infections. One promising strategy involves designing drug combinations to mitigate the emergence of antibiotic resistance. By formulating combinations where resistance to one drug coincides with increased susceptibility to another, we can potentially thwart the development of resistance and enhance treatment efficacy. CTX-M 15 β-lactamase, because of its hydrolytic activity, plays a critical role in producing resistance to β-lactam antibiotics. Bacteria carrying the CTX-M-15 gene exhibit significant resistance to β-lactam antibiotics, such as penicillin and cephalosporins.[12] In this study, the potential inhibitory activity of Isoproterenol was explored to inactivate CTX-M 15 β-lactamase, as the previously available inhibitors and their residual binding are no longer effective.[4]

Isoproterenol, a non-selective beta-adrenergic agonist, is proposed as a CTX-M-15 β-lactamase inhibitor. It has several medicinal applications. It is often used to treat bradycardia and heart block by raising heart rate and cardiac output, as well as a bronchodilator. These characteristics, together with its current clinical application, show its potential as a dual-purpose therapeutic drug.

The use of Isoproterenol as an inhibitor against CTX-M 15 β-lactamase is a novel approach that has not been extensively investigated. Isoproterenol docking with CTX-M 15 β-lactamase shows that different residues bind with the inhibitor and have different affinities. Ser70 is known to form hydrogen bonds with the inhibitor whereas Ser273 and Ser130 form a stable docked complex by establishing hydrogen bonds.[13] Different bioinformatics sites and tools have been utilized to check the potential of docking between CTX-M 15 β-lactamase and the inhibitor. As for ligand and inhibitor's crystal structure retrieval RCSB PDB Protein Data Bank and e-LEA3D – drug design server have been used respectively.[7] Autodock Vina was used for docking and iMods for the analysis. The NMA graph is far higher than the PDB graph showing that the complex is stable with a binding affinity of -6.5.[14,15]

The CTX-M-15 enzyme, belonging to the CTX-M-1 subgroup, is widely prevalent among Gram-negative pathogens. In the current study, molecular docking simulations using Autodock Vina revealed favorable binding interactions, indicating the ability of Isoproterenol to bind to the active site. The lactamase activity of CTX-M 15 was inhibited by Isoproterenol as they form a stable docked complex. The hydrolytic activity was seen no more hence a promising future against antibiotic resistance.[16]

Based on the results obtained, further experimental studies should be conducted to validate the inhibitory activity of Isoproterenol against CTX-M 15 β-lactamase. Additionally, understanding the structural basis of the interaction between the inhibitor and CTX-M-15 β-lactamase can aid in the design of more effective compounds. Overall, the development of inhibitors against CTX-M-15 β-lactamase represents a promising strategy for combating drug-resistant bacteria and provides hope for the future in the fight against antibiotic resistance.[17] Future studies should focus upon inhibitor development by different mechanisms which that involve irreversible inhibition, competitive inhibition, and metallo-beta-lactamase inhibition. Strategies to enhance inhibitor potency, selectivity, and pharmacokinetic properties must be explored, including structure-activity relationship studies and prodrug approaches to increase the effectiveness of the drug.[18] In vitro assays, such as enzyme inhibition assays, can provide quantitative data on the inhibitory potency of Isoproterenol. Moreover, in vivo studies, such as animal models or clinical trials, are crucial to assess the effectiveness and safety of Isoproterenol as an inhibitor.[19,20]

Conclusion

Based on these findings, the study concluded that Isoproterenol offers a high possibility of binding to and strongly inhibiting the lactamase activity of CTX-M-15, as supported by the molecular docking experiment aided by AutoDock Vina. The favorable binding interactions supported the ranked mapping of Isoproterenol with the active site of the enzyme. Thus, the data obtained are indicative of the fact that attempts to develop inhibitors against CTX-M-15 β-lactamase should be considered as one of the relevant approaches to addressing the issue of increasing bacterial resistance to antibiotics. More development in any such inhibitor could contribute greatly to the continued fight against drug-resistant infections.

Conflict of Interest: None

Funding Disclosure: None

Ethical Consideration: The study was approved by the ethical review board. Informed written consent was obtained from the participants, and the confidentiality of their data was clearly explained.

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Authors Contribution

All the authors contributed equally in accordance with ICMJE guidelines and are accountable for the integrity of the study.

A: Conception of idea & design, acquisition, analy-sis & interpretation of data, final approval of the manu-script

MS: Acquisition of data, drafting the article, analysis and reporting, final approval of the manuscript

AR: Analysis & interpretation of data, drafting the article, critical review

AH: Conception of idea & design, interpretation of data, results writeup, final approval of the manuscript

RA: Acquisition of data, drafting the article, analysis and reporting, final approval of the manuscript

SMI: Analysis & interpretation of data, drafting the article, critical review

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