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Structure-Based Virtual Screening of Small Molecules Targeting Key Proteins of *Neisseria gonorrhoeae*

Saumya Sachan and Ruchi Yadav*

Amity University Uttar Pradesh, Lucknow Campus, Uttar Pradesh, India

Abstract

Objectives: To identify potential small molecule inhibitors against key resistance-associated proteins of *Neisseria gonorrhoeae* using a molecular docking-based virtual screening approach, addressing the urgent need for novel therapeutics in light of rising drug resistance.

Materials and Methods A computational study employing virtual screening of phytochemicals and standard antibiotics against selected *N. gonorrhoeae* proteins using SwissDock and CB-Dock, followed by interaction analysis through Discovery Studio Visualizer.

Interventions. Docking of 30 structurally diverse phytochemicals and four standard antibiotics against four essential proteins of *N. gonorrhoeae*: Penicillin-binding Protein 2 (PBP2; PDB ID: 3equ), Hypothetical Protein with bound ppGpp (PDB ID: 5vog), Adhesin Complex Protein (PDB ID: 6gq4)

Main Outcome Measures: Evaluation of binding affinities, docking scores, and molecular interaction patterns to determine the most promising small molecule inhibitors.

Results: Several phytochemicals exhibited strong binding affinities and favorable interactions with the target proteins, in some cases outperforming standard antibiotics. Detailed interaction profiles revealed key residues involved in ligand binding and supported the potential of selected compounds as inhibitors.

Conclusion: The in silico findings highlight promising phytochemical candidates for further experimental validation. These results contribute to the development of novel therapeutic strategies to combat extensively drug-resistant *N. gonorrhoeae* strains and support the role of natural compounds in antimicrobial drug discovery.

Keywords: *Neisseria gonorrhoeae*, Docking, phytochemical , Discovery Studio, Drug discovery

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*Corresponding author

1. Introduction

Neisseria species are Gram-negative, facultatively intracellular diplococci that typically colonize the mucosal membranes of mammals and reptiles. These organisms, measuring approximately 0.6–1 µm in diameter, are most often observed in adjacent pairs with flattened sides—a characteristic feature of the genus. An exception is *Neisseria elongata*, which forms small rod-shaped cells (diplobacilli) and arranges in short chains. Members of the *Neisseriaceae* family are aerobic, chemoorganotrophic bacteria with a genomic GC content between 46.5% and 53.5%, and they utilize organic compounds as their primary energy source. (1) Notably, *Neisseria* species are naturally competent for transformation, allowing horizontal gene transfer and genomic plasticity, which underlies their adaptability and evolution. (2)

Among these, *Neisseria gonorrhoeae*—the etiological agent of gonorrhoea—is a highly adapted, obligate human pathogen known for its strict nutritional and environmental requirements, thriving optimally at 35–37°C. The bacterium targets mucosal epithelial cells in the urogenital, rectal, and pharyngeal tracts, causing clinical manifestations such as urethritis, cervicitis, pelvic inflammatory disease (PID), and infertility if untreated. In neonates, vertical transmission may lead to ophthalmia neonatorum, a preventable cause of blindness. (3,4)

Despite being the second most prevalent bacterial sexually transmitted infection (STI) globally after *Chlamydia trachomatis*, gonorrhoea frequently remains asymptomatic, especially in women, complicating early

detection and increasing transmission risk. (5) Gonorrhoea has a well-established link with increased HIV acquisition and transmission, particularly in high-risk populations such as men who have sex with men (MSM) and individuals with multiple sexual partners. (6,7)

A growing global concern is the rapid and widespread emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *N. gonorrhoeae* strains. Over the decades, the bacterium has successively developed resistance to nearly all classes of first-line antibiotics, including penicillins, tetracyclines, fluoroquinolones, macrolides, and extended-spectrum cephalosporins (ESCs). (8,9) Mechanisms of resistance include point mutations in key genes such as *penA*, *gyrA*, *parC*, and 23S rRNA; overexpression of efflux pumps like MtrCDE; and the acquisition of plasmid-encoded resistance determinants such as *TetM*. (10-14) The continued rise of XDR strains—some of which show high-level resistance to both ceftriaxone and azithromycin—has led to treatment failures and prompted global health authorities to reinforce surveillance and containment strategies. (15,16)

Several international initiatives such as the WHO's Global Gonococcal Antimicrobial Surveillance Programme (GASP), CDC's Gonococcal Isolate Surveillance Project (GISP), and Euro-GASP by the ECDC have revealed rising resistance trends across continents including Asia, Europe, North America, and Oceania. (17,18) In India, where diagnostic and reporting challenges persist, prevalence among high-risk groups is estimated at 2–3%, though likely underestimated. (19)

The challenge of antibiotic resistance in *N. gonorrhoeae* is compounded by its antigenic variability, immune evasion strategies, and the absence of an effective vaccine or reliable animal model for research. (20) As conventional therapeutic avenues dwindle, the need for novel and targeted treatments becomes imperative. In this context, structure-based virtual screening (SBVS) has emerged as a powerful computational strategy to identify new small-molecule inhibitors. SBVS enables the rapid screening of large compound libraries against the three-dimensional structures of target proteins, providing insights into ligand-receptor interactions, binding affinities, and potential inhibitory activity. (21)

This study focuses on the application of molecular docking to identify phytochemical and synthetic small molecules capable of binding to resistance-associated and virulence-related proteins of *N. gonorrhoeae*, including Penicillin-Binding Protein 2 (PBP2), hypothetical proteins involved in stringent response, and adhesins. By leveraging in silico methods such as SwissDock and CB-Dock, the goal is to prioritize promising drug candidates for further preclinical validation, addressing the urgent need for alternative therapies in the fight against gonococcal antimicrobial resistance.

2. Materials and Methods

In this study, four key proteins of *Neisseria gonorrhoeae* were selected for molecular docking analysis based on their essential roles in bacterial survival, pathogenicity, and antimicrobial resistance. These proteins included Penicillin-Binding Protein 2 (PBP2; PDB ID: 3EQU), Adhesin Complex Protein (Ng-ACP; PDB ID: 6GQ4), a Hypothetical Protein complexed with ppGpp (PDB ID: 5VOG), and Type IV Pilin or Fimbrial Protein (PDB ID: 2HI2). PBP2 is a critical enzyme involved in the synthesis of peptidoglycan, a vital component of the bacterial cell wall, and serves as a primary target for β-lactam antibiotics. Structural alterations in this protein are associated with resistance to cephalosporins. The Ng-ACP protein facilitates adherence to host epithelial surfaces, promoting colonization and contributing to infection, thus playing a pivotal role in bacterial virulence. The 5VOG structure represents a hypothetical protein co-crystallized with the alarmone ppGpp, suggesting its involvement in the stringent response and stress adaptation, which are crucial for bacterial survival under adverse host conditions. The fourth target, Type IV Pilin, is a major subunit of the pili apparatus responsible for bacterial motility, host cell interaction, and immune evasion—mechanisms that enhance persistence and pathogenicity. The three-dimensional structures of these proteins were obtained from the RCSB Protein Data Bank (https://www.rcsb.org/). Prior to docking, each protein structure was prepared by removing all heteroatoms, water molecules, and co-crystallized ligands, followed by the addition of polar hydrogen atoms. Energy minimization was performed where required to optimize protein geometry. These structurally and functionally diverse targets were selected to ensure comprehensive virtual screening, with the aim of identifying promising small-molecule inhibitors that could disrupt multiple stages of the *N. gonorrhoeae* infection cycle.

Table 1. Proteins targets of Neisseria gonorrhoea used for docking

S.No	PDB ID	Protein name	Functions
1	3EQU	Penicillin-Binding Protein 2 (PBP2)	Involved in peptidoglycan synthesis; target for βlactam antibiotics.
2	6GQ4	Adhesin Complex Protein (Ng-ACP)	Facilitates bacterial adhesion to host cells, promoting colonization and infection.
3	5VOG	Hypothetical Protein (N. gonorrhoeae) with bound ppGpp	Possibly involved in bacterial stress response and survival mechanisms.
4	2HI2	Fimbrial Protein (Type IV Pilin)	Essential for bacterial motility, host cell interaction, and immune evasion.

The molecular docking study followed a systematic workflow comprising several key steps as shown in figure 1. Total 30 phytochemicals were retrieved from the PubChem database for docking with target proteins.

Molecular docking was carried out using SwissDock and CB-Dock tools. Docking results were evaluated in terms of binding affinities, molecular interactions, and docking scores. Based on these evaluations, the top five protein-ligand complexes were shortlisted as top-ranking molecules. To gain deeper insight into the interaction patterns, the docking results were visualized using Discovery Studio, which enabled detailed interaction analysis.

Finally, the selected top candidates underwent further analysis and interpretation for their potential in drug development, incorporating ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiling and lead optimization steps to assess their pharmacokinetic properties and suitability as therapeutic agents.

Figure 1. Methodology followed for docking proteins of Neisseria gonorrhoea with 30 phytochemicals

Table 2. Names of Phytochemicals used for docking with the proteins

Ligand Name	PubChe m ID	M.W (g/mol)	Phytochemical Class	Plant Source	Scientific Name
Combretastatin A4	5351341	316.35	Stilbenoid	Bushwillows	Combretum caffrum
7-Epi-Isogarcinol	25242772	612.63	Xanthone	Mangosteen	Garcinia mangostana
30-Hydroxylup-20(29)-en-3-one	15038289	440.71	Triterpenoid	Brazilian Cerrado Plants	Byrsonima crassifolia
Isoorientin	114776	448.38	Flavonoid	Bamboo leaves, Passionflower	Phyllostachys nigra, Passiflora edulis
Chebulinic acid	72284	954.70	Tannin	Terminalia chebula (Haritaki)	Terminalia chebula
Chebulagic acid	250397	956.70	Tannin	Terminalia chebula (Haritaki)	Terminalia chebula
Punicalagin	16129719	1084.71	Tannin	Pomegranate	Punica granatum
Gallic acid	370	170.12	Phenolic Acid	Grapes, Tea leaves, Oak bark	Vitis vinifera, Camellia sinensis
Ellagic acid	5281855	302.19	Phenolic Acid	Pomegranate, Strawberries	Punica granatum, Fragaria × ananassa
Hernanol	6917899	286.34	Cinnamic Acid Derivative	Wild Plants (TBA)	-
Crassiflorone	11674902	278.30	Cinnamic Acid Derivative	African Medicinal Plants	Milletia griffoniana
Lupeol	259846	426.72	Triterpenoid	Mango, Aloe, Olives	Mangifera indica, Aloe vera, Olea europaea
Arctigenin methyl ether	384877	374.41	Lignan	Burdock	Arctium lappa
Pinocembrin chalcone	6474295	256.27	Flavonoid	Honey, Propolis	Apis mellifera (Bee-derived)
Plumbagin	10205	188.20	Quinone	Black Walnut, Leadwort	Juglans nigra, Plumbago zeylanica
4-Methoxybenzyl isothiocyanate	123197	179.22	Isothiocyanate	Moringa	Moringa oleifera
Podorhizol	9931715	272.34	Phenolic	Wild Ginger	Zingiber zerumbet
Vomifoliol (+)-	5280462	194.23	Sesquiterpenoi d	Pine Needles, Tomato	Pinus species, Solanum lycopersicum
Esculetin	5281416	178.14	Coumarin	Chicory, Horse Chestnut	Cichorium intybus, Aesculus hippocastanum
3,4-Dicaffeoylquinic acid	6474309	516.43	Phenolic Acid	Ar/choke, Echinacea	Cynara cardunculus, Echinacea purpurea
Cyclolanceaefolic acid methyl ester	637426	514.84	Triterpenoid	Tradi/onal Chinese Medicinal Plants	Cyclocarya paliurus
Penduletin	5320462	300.27	Flavonoid	Propolis, Various Herbs	Apis mellifera, Salvia species
Berberine	2353	336.36	Alkaloid	Goldenseal, Barberry	Hydrastis canadensis, Berberis vulgaris
Aloin	12305761	418.40	Anthraquinone	Aloe Vera	Aloe vera
Phthalic acid	1017	166.13	Phenolic Acid	Various Plants (Common Metabolite)	-
Rhein	10168	284.22	Quinone	Rhubarb	Rheum palmatum
Pinocembrin	68071	256.27	Flavonoid	Propolis, Ginger	Apis mellifera, Zingiber officinale
Salidroside	159278	300.30	Phenylpropano id Glycoside	Rhodiola	Rhodiola rosea
Rosavin	9823887	554.60	Lignan	Rhodiola	Rhodiola rosea

Phytochemicals used for docking study has been listed in table 2 these phytochemicals show promising potential in combatingNeisseria gonorrhoeae, especially with rising antibiotic resistance.

The compiled table provides a comprehensive overview of 30 phytochemicals, highlighting their molecular properties, phytochemical classes, plant sources, and scientific names. The selected ligands belong to multiple phytochemical classes, including flavonoids, tannins, alkaloids, stilbenoids, terpenoids, phenolic acids, lignans, and quinones.

3. Result and Discussion

Molecular docking of 30 selected phytochemicals with four target proteins of *Neisseria gonorrhoeae* revealed several promising candidates with significant binding affinities. Among these, **Chebulinic acid (PubChem ID: 72284)** demonstrated the strongest binding across all targets, with the highest docking score of **–10.4 kcal/mol** against Penicillin-Binding Protein 2 (3EQU) and **–10.0 kcal/mol** against the ppGpp-bound hypothetical protein (5VOG). Similarly, **Punicalagin (PubChem ID: 16129719)** exhibited a notably high docking score of **–11.3 kcal/mol** against PBP2 (3EQU), making it another potential candidate for further investigation as shown in table 3.

Table 3. Docking Scores of Selected Phytochemicals Against Target Proteins

S.No	Ligand Name	PubChem ID	5VOG	2HI2	6GQ4	3EQU
1	7-Epi-Isogarcinol	25242772	-8.9	-6.2	-7.1	-8.3
2	Combretastatin A4	5351341	-5	-5	-5.5	-6.5
3	30-Hydroxylup-20(29)-en-3-one	15038289	-6.4	-6.4	-6.4	-7.9
4	Isoorientin	114776	-4.8	-4.8	-6.5	-8.1
5	Chebulinic acid	72284	-10	-7.8	-7.9	-10.4
6	Chebulagic acid	250397	-9	-7.1	8	-9.8
7	Punicalagin	16129719	-8.5	-7.1	-6.8	-11.3
8	Gallic acid	370	-6	-5.8	-5.5	-6.7
9	Ellagic acid	5281855	-7.7	-6.9	-6.6	-8.6

Chebulagic acid (PubChem ID: 250397) also showed excellent binding affinity, particularly with 3EQU (**–9.8 kcal/mol**) and 5VOG (**–9.0 kcal/mol**). Other compounds such as **7-Epi-Isogarcinol**, **30-Hydroxylup-20(29)-en-3-one**, and **Ellagic acid** displayed moderate to good affinities, ranging between **–6.4 to –8.9 kcal/mol** across various targets.

Interestingly, reference compound **Combretastatin A4 (PubChem ID: 5351341)** exhibited lower docking scores (**–5.0 to –6.5 kcal/mol**), suggesting that several of the tested phytochemicals may offer better binding potential than known drugs.

3.1 Protein-Ligand Interaction Analysis

Structural analysis using Discovery Studio Visualizer revealed key interactions—hydrogen bonds, hydrophobic contacts, and π–π stacking—in top *S. aureus* protein-ligand complexes, providing insights into binding orientation and inhibitory potential. This visualization aids in validating docking results and identifying promising phytochemicals for further development.

Chebulinic acid exhibited a strong binding affinity toward 5VOG with a docking score of **-9.0 kcal/mol**. As shown in the interaction map, the ligand formed key hydrogen bonds with residues **ARG 89, GLY 144, and ASN 146**, and showed electrostatic interactions with **ASP 141**. Hydrophobic contacts were also noted with **VAL 85 and TYR 87**, suggesting a stabilized binding conformation. The network of polar and non-polar contacts supports the potential of Chebulinic acid to inhibit the function of this hypothetical stress response protein.

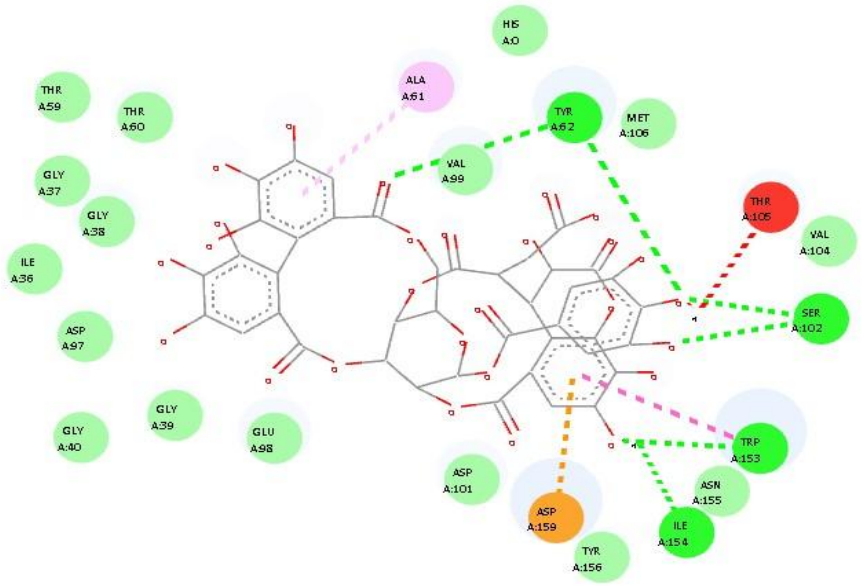
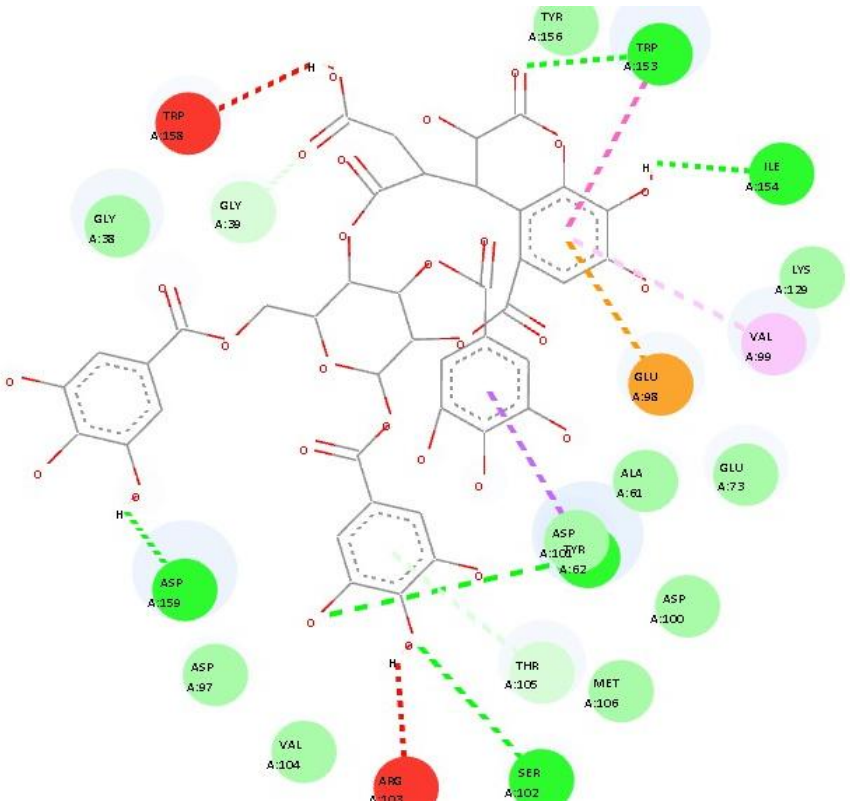
Chebulagic acid demonstrated the **strongest docking score of -10.0 kcal/mol** among the tested ligands. The interaction map revealed multiple hydrogen bonds involving **ARG 89, GLY 144, and ASN 146**, similar to Chebulinic acid, and additional contacts with **ASP 141 and ASN 83**. This dense interaction network reflects a stable ligand binding in the active site, further supported by π-π stacking interactions with **TYR 87**, indicating the compound's high affinity and potential inhibitory role.

Punicalagin interacted favorably with 5VOG, displaying a docking score of **-8.5 kcal/mol**. The ligand formed strong hydrogen bonds with **ASN 83, ARG 89, and GLY 144**, with additional van der Waals and electrostatic contacts involving **ASP 141 and ASN 146**. Hydrophobic interactions with **VAL 85** contributed to the stabilization of the complex. These findings highlight Punicalagin as a promising molecule for targeting bacterial stress response pathways mediated by 5VOG.

4. Conclusion

This study identified several small molecules with strong binding affinities against *Neisseria gonorrhoeae* target proteins, surpassing standard drugs in some cases, highlighting their potential as novel therapeutic candidates. Given the alarming rise in antibiotic resistance, especially in *N. gonorrhoeae*, developing new, target-specific inhibitors is essential. Among the ligands analyzed, **Chebulagic acid** showed the strongest interaction with 5VOG, followed closely by **Chebulinic acid** and **Punicalagin**, as evidenced by their binding affinities and comprehensive interaction profiles. All three ligands formed multiple hydrogen bonds and

hydrophobic contacts with residues such as **ARG 89, GLY 144, ASP 141, and ASN 146**, which are critical for maintaining the integrity of the binding pocket. These natural phytochemicals demonstrate promising inhibitory potential against the hypothetical protein 5VOG of *N. gonorrhoeae*, suggesting their relevance in future antimicrobial drug design.

Protein-Ligand Complex	**Interaction Analysis**
5VOG- 250397 (Chebulinic acid) Complex	
5VOG-72284 (Chebulagic acid) Complex	

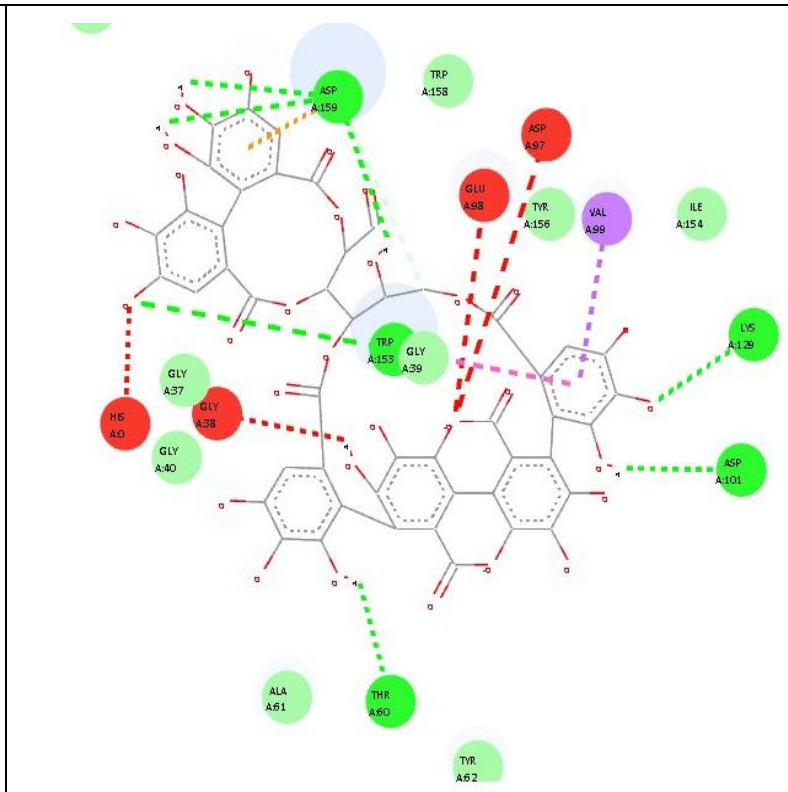
5VOG- 161297192 (Punicalagin) Complex	

Figure 2. Protein-ligand interaction map of the target proteins. The map highlights various interacting bonds such as hydrogen bonds, hydrophobic interactions, electrostatic interactions, and π-π stacking

A multidisciplinary approach combining computational modeling, structural analysis, and experimental validation is vital to accelerate drug discovery. Exploring synergistic effects with existing antibiotics and investing in advanced screening and design techniques will be key to overcoming resistance and ensuring effective long-term treatment strategies.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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