ORIGINAL ARTICLE

Molecular Docking Studies of Schiff Bases with Azetidinone Against Dihydrofolate Reductase Enzyme as Potential Anti-cancer Agents

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Abstract

Background: In recent times, cancer has emerged as a major health concern. It was established that every antagonist of the dihydrofolate reductase exhibits anti-cancer activity. For anti-cancer action, several Schiff-based derivatives with azetidinone rings were designed and docked against the dihydrofolate reductase protein (PDB id:6CXK) in the current work. The ligands were compared to those of standard antagonists of dihydrofolate reductase, that is, trimethoprim and pyrimethamine. **Materials and Methods:** The ligands were drawn in.mol format using ChemSketch software and converted to.pdb format using Avogadro software. The iGEMDOCK software was utilized to conduct molecular docking investigations, and Discovery Studio Visualizer was ultimately used to visualize the results. **Results and Discussion:** Most compounds have demonstrated a better affinity for binding to the dihydrofolate reductase. Most of the ligands have demonstrated nearly the same binding affinities as that of the standard dihydrofolate reductase, such as trimethoprim (−102.1 kcal/mol) and pyrimethamine (−91.8 kcal/mol). The top 2 compounds 3A8B (−100.6 kcal/mol) and 3A9B (−94.6 kcal/mol) were chosen for visualization. **Conclusion**: Schiff base derivatives with azetidinone ring have the potential to be a promising class of drugs for the treatment of anti-cancer action since they have a higher binding affinity to the dihydrofolate reductase than standard antagonists.

Key words: Anti-cancer, dihydrofolate reductase, discovery studio visualizer, iGEMDOCK software, molecular docking, Schiff base

INTRODUCTION

Schiff base

Schiff bases are a significant class of medications, for the therapy of numerous diseases. They have been gaining importance since Hugo Schiff originally characterized Schiff's base 160 years ago. A ketone or an aldehyde that contains a carbonyl group and has a nitrogen-based moiety is called a Schiff base. It is created by condensing a primary amine with the carbonyl group and substituting the carbonyl group with an imine group known as azomethine.[1-3] Particularly adaptable compounds with C = N (imine) groups are aniline-Schiff bases, which have been shown to exhibit a wide range of biological functions,[4-7] antibacterial, antifungal,[8,9] anti-cancer,[10] and anti-inflammatory.

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Azetidinone

Since the discovery of penicillin by Sir Alexander Fleming in 1928 and the subsequent discovery of cephalosporin, both of which were employed as effective antibiotics, the chemistry of ß-lactams has assumed a significant role in organic chemistry. The emergence of bacterial resistance to commonly used antibiotics of this kind continues to encourage research in this field. Functionalized ß-lactams or novel active principles in the ß-lactam series are required. ß-lactam has antiviral,[11,12] antifungal,[13,14] antibacterial,[15,16] and anti-cancer activities.[17-20] Penicillins, cephalosporins, carbapenems, nocardicin, and monobactams are among the broad spectrum ß-lactam antibiotics[21,22] that share the 2-azetidinone (ß-lactam) ring as a structural characteristic.

MATERIALS AND METHODS

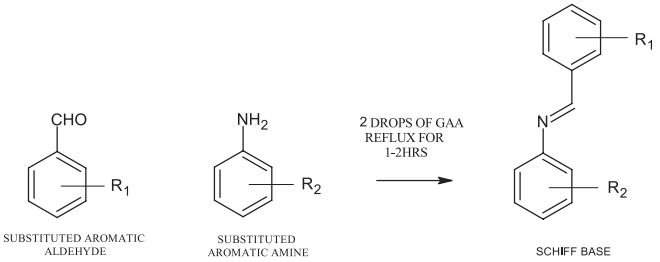
Step 1

Schiff bases are the condensation products of aldehydes and amine compounds in the presence of glacial acetic acid

Table 1: The interactions and binding energies of the top 10 ligands with the enzyme dihydrofolate reductase

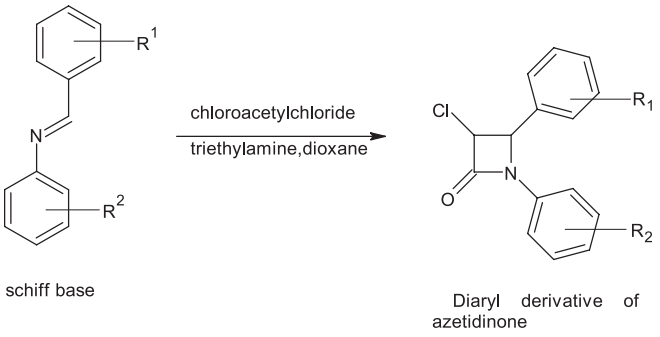
Compound Code	Binding Energy Kcal/mol	Interacting active site amino acid residues
3A8B	−100.6	ARG: 57 [2.61], ASN: 18 [3.10], TYR: 100 [2.59], ALA: 7 [2.94], LEU: 28, LEU: 54, MET: 16, PHE: 31, and ALA: 6
3A9B	−94.6	ASN: 18 [2.80], ARG: 57 [2.77], LEU: 28, LEU: 54, ILE: 94, MET: 16, ASP: 27, and PHE: 31,
3A5B	−93.6	ASN: 18 [3.88], ARG: 57 [6.23], TYR: 100 [7.33], ILE: 94 [4.87], LEU: 54, LEU: 28, MET; 16, PHE: 31, LYS: 32, PRO: 55, MET: 20, GLU: 17, SER: 49, ILE; 50, ILE: 5, ALA; 6, and THR: 46
2A5B	−90.6	ASN: 18 [4.12], ARG: 57 [6.28], ILE; 94 [4.39], LEU: 54, LEU: 28, PHE: 31, MET: 16, TYR: 100, THR: 46, ILE: 50, MET; 20, and LYS; 32
2A10B	−90.2	ASP: 27 [4.76], THR: 113 [4.31], PHE: 31, ALA: 6, ALA; 7, ILE; 5, LEU: 28, TRP; 30, ILE: 50, THR; 46, MET: 20, GLU; 17, and LEU: 54
4A8B	89.8	ARG: 57 [6.34], LEU: 28, PHE: 31, ILE: 50, LEU: 54, ARG: 52, ASN: 18, ILE: 94, THR: 46, MET: 16, LYS: 32, ad PRO: 55,
7A8B	89.6	ASN: 18 [4.79], ILE: 50 [4.98], ARG: 57 [6.48], PHE: 31, LEU: 54, ARG: 52, ILE: 94, THR: 46, MET: 16, LEU: 28, LYS: 28, and PRO: 55
3A4B	−89.5	ASN: 18 [3.79], ARG: 57 [6.05], TYR: 100 [7.63], PHE: 31, MET: 16, LEU: 28, LEU: 54, LYS: 32, PRO: 55, MET: 20, GLU: 17, SER: 49, THR: 46, ILE: 50, ALA: 6, ALA: 7, and ILE: 94
4A10B	−88.9	ASN: 18 [4.21], ARG: 57 [6.19], MET: 16, LYS: 32, PRO: 55, LEU: 54, LEU: 28, ILE: 50, GLU: 17, MET: 20, ALA: 6, ILE: 5, TYR: 100, ALA: 7, ad ILE: 94
3A3B	−88.7	ASN: 18 [4.21], ARG: 57 [6.19], MET: 16, PHE: 31, ASP: 27, LYS: 32, PRO: 55, LEU: 54, LEU: 28, ILE: 94, ALA: 7, TYR: 100, MET: 20, ALA: 6, ILE: 5, GLU; 17, and ILE: 50
Trimethoprim	−102.1	ASP: 27 [2.82], THR: 113 [3.26], PHE: 31, ALA: 7, ILE: 5, ILE: 50, MET: 16, and LEU: 28
Pyrimethamine	−91.8	TYR: 100 [2.60], ILE: 5 [2.60], PHE: 31, ILE: 50, ILE: 94, and ALA: 7
Co-crystalized ligand (Dihydrofolate)	−101.8	ASP: 27 [3.90], ILE: 5 [3.81], MET: 16 [3.59], ARG: 57, LYS: 32, PHE: 31, ALA; 6, ALA: 7, TRP: 22, MET: 20, GLU: 17, ASN: 18, THR: 46, LEU: 54, PRO: 55, LEU: 28, GLU: 95, TRP: 30, THR: 113, ILE: 94, and TYR: 100

and ethanol refluxed for 4 h after cooling the product and recrystallized by ethanol.



Step 2

Schiff base in the presence of chloroacetyl chloride, triethylamine, and dioxane gives azetidinone-derived Schiff base.

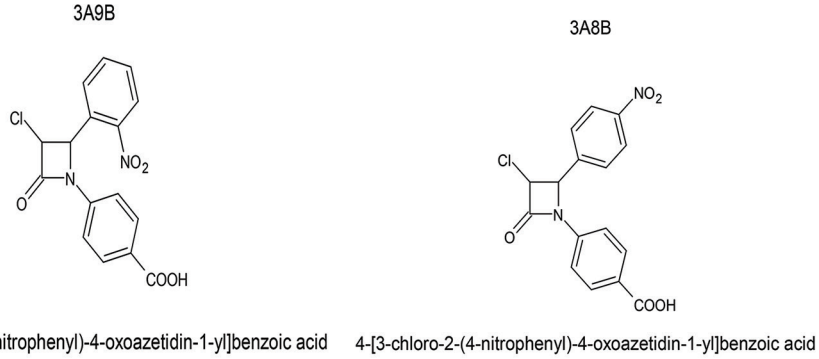


The Schiff base synthesis techniques were derived from the literature.[23-25] There have also been reports of alternative Schiff base synthesis techniques using azetidinone.[26-29] The method indicated above was used to select several substituted aromatic aldehydes and aromatic amines. Schiff bases have been designed by adding an azetidinone moiety, and the final products were designed by the approach. Using Swiss ADME software,[30-32] the ADME properties of designed ligands were predicted after they were screened using TopKat software[31-33] for *in silico* toxicity. Designed compounds with good ADME properties and anticipated non-carcinogenic and non-toxic compounds were chosen for molecular docking.

Molecular docking

The target was chosen based on the SWISS target prediction software.[31,34,35] Most of the compounds have shown dihydrofolate reductase as a potential target. Hence, dihydrofolate reductase is used for molecular docking.

ChemSketch software was used to sketch the ligand's 2D structures, which were then saved in.mol format. Using the Avogadro tool,[31,35,36] the ligand structures in .mol format were converted into the .pdb format. Docking studies were conducted for the safe, non-carcinogenic developed compounds with good ADME features to evaluate binding poses and interactions. Hence, the present study aims to evaluate Schiff base derivatives for anti-cancer activity.



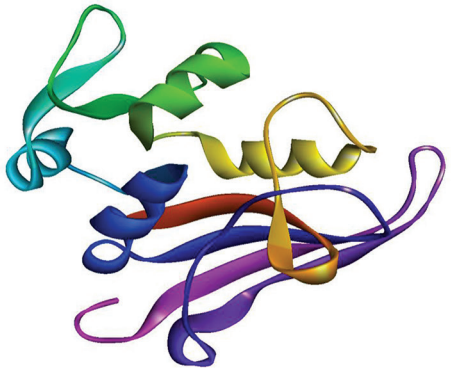
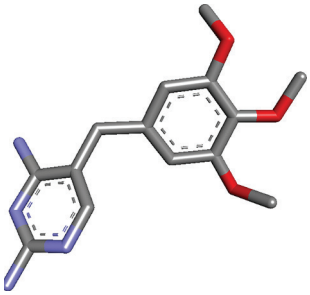
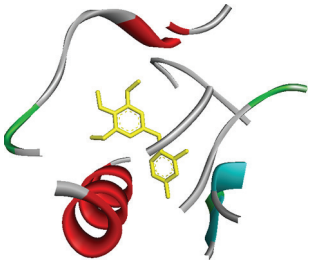


Figure 1: Cleaned dihydrofolate reductase enzyme – PDB ID: 6CXK

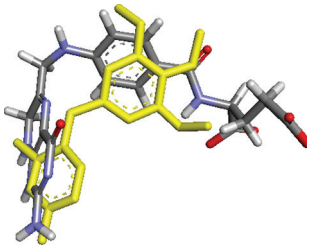
Table 2: Docking and visualization data of standard antagonist trimethoprim against dihydrofolate reductase enzyme



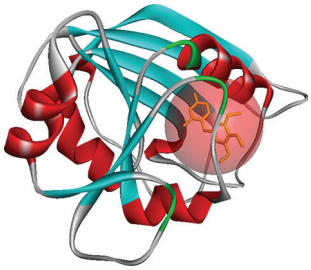
Trimethoprim ligand



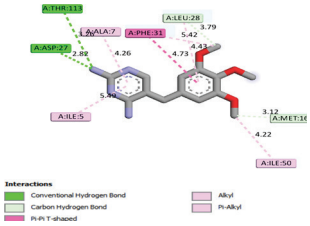
Trimethoprim ligand+dihydrofollate reductase enzyme complex



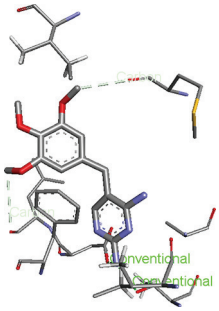
Trimethoprim ligand+co-crystal ligand overlap



Trimethoprim ligand+whole dihydrofolate reductase enzyme

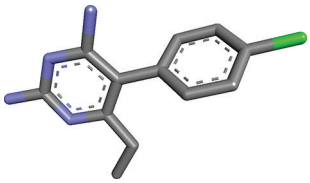


Trimethoprim ligand+2D interaction with dihydrofolate reductase protein

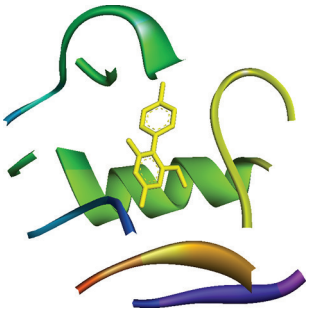


Trimethoprim 3D interactions with dihydrofolate reductase enzyme

Table 3: Docking and visualization data of standard antagonist pyrimethamine against dihydrofolate reductase enzyme



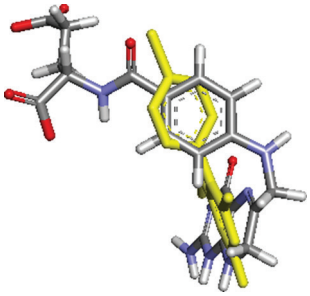
Pyrimethamine ligand



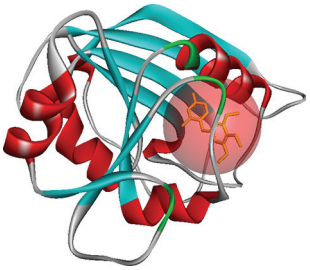
Pyrimethamine ligand+dihydrofollate reductase enzyme complex

(Contd...)

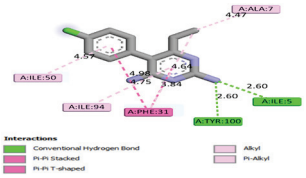
Table 3: *(Continued)*



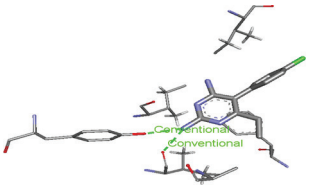
Pyrimethamine ligand+co-crystal ligand overlap



Pyrimethamine ligand+whole dihydrofolate reductase enzyme

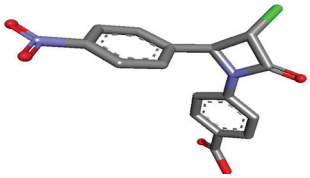


Pyrimethamine ligand+2D interaction with dihydrofolate reductase enzyme

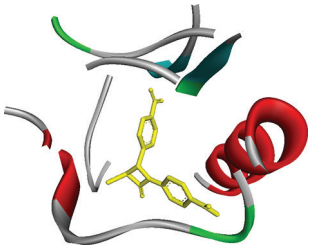


Pyrimethamine 3D interactions with dihydrofolate reductase enzyme

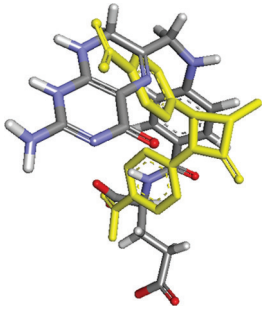
Table 4: Docking and visualization data of 3A8B ligand against dihydrofolate reductase enzyme



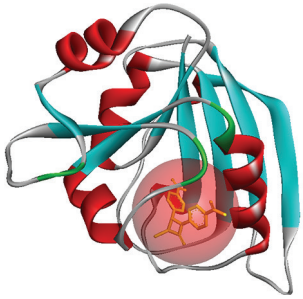
3A8B Ligand



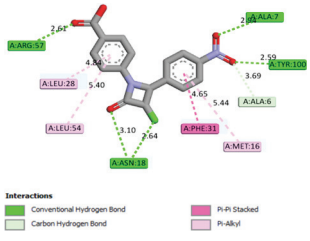
3A8B Ligand+dihydrofollate reductase enzyme complex



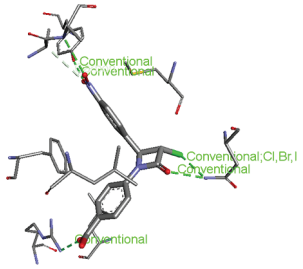
3A8B Ligand+co-crystal ligand overlap



3A8B Ligand+whole dihydrofolate reductase enzyme

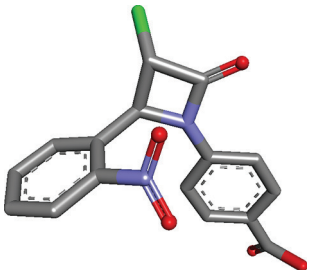


3A8B ligand 2D interaction with dihydrofolate reductase protein

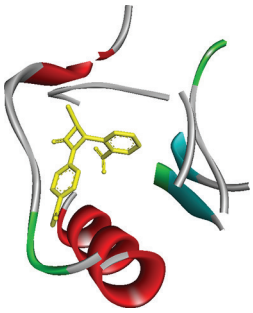


3A8B ligand 3D interactions with dihydrofolate reductase enzyme

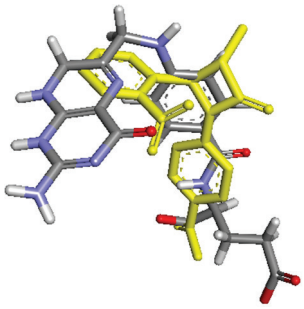
Table 5: Docking and visualization data of 3A9B ligand against dihydrofolate reductase enzyme



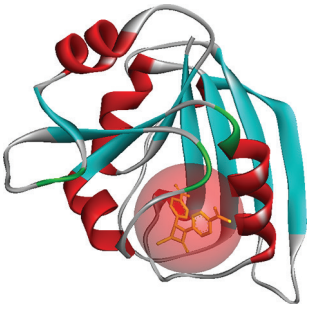
3A9B ligand



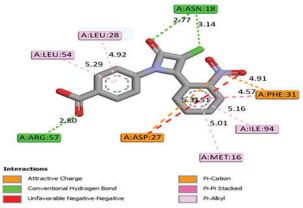
3A9B ligand+dihydrofollate reductase enzyme complex



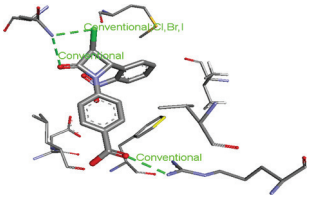
3A9B ligand+co-crystal ligand overlap



3A9B ligand+whole dihydrofolate reductase enzyme

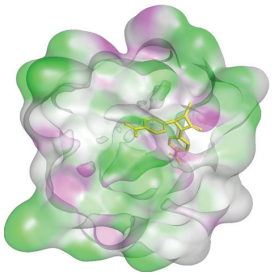


3A9B ligand+2D interaction with dihydrofolate reductase protein

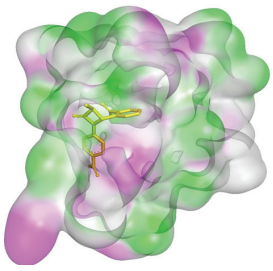


3A9B ligand 3D interactions with dihydrofolate reductase enzyme

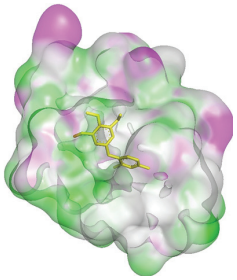
Table 6: Pocket analysis and binding modes of 3A8B, 3A9B, trimethoprim, and pyrimethamine



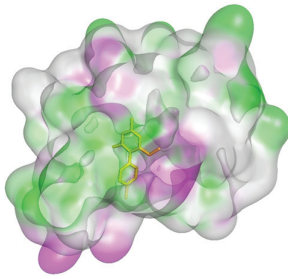
3A8B



3A9B



Trimethoprim



Pyrimethamine

iGEMDOCK was the program utilized for docking.[31,35,37] This software calculates the orientation and structure of ligands concerning the protein's active site. To assess the molecular interactions of the chosen safe chemicals with the dihydrofolate reductase (Figure 1, PDB ID:6CXK) using a co-crystallized ligand inhibitor dihydrofolate that was retrieved from the protein data bank, *in silico* docking simulation studies were carried out.

The Discovery Studio Visualizer (Biovia) was used for visualization. An accurate docking method was chosen, and a standard docking protocol was adhered to. The optimal docking solutions were examined based on the scoring function. The scoring function uses a combination of hydrogen bonding, van der Waals, and electrostatic energies. To determine the interactions between the ligands and the target protein, post-docking interaction profile analysis of the best poses was carried out. Using Insilco toxicity prediction, safe and non-carcinogenic compounds were found and molecular docking was performed along with standard dihydrofolate reductase inhibitors such as trimethoprim[38-44] and pyrimethamine.[45-51] To assess binding affinities and

molecular interactions, docking simulations were performed. For the post-docking interaction investigation, the top 2 compounds were selected based on their superior binding energies and molecular interaction profiles.

RESULTS AND DISCUSSION

The top 2 ligands' structures that have superior binding energies have been chosen for visualization.

CONCLUSION

Table 1 In conclusion, the binding energies of all of the top 10 compounds were nearer to the binding energies of the standard antagonists of the Dihydrofolate reductase enzyme. The binding energies of the top two compounds 3A8B (-100.6 k.cal/mol) and 3A9B (-94.6 k.cal/mol) were nearer to those of the standard Dihydrofolate reductase enzyme Inhibitors, such as Trimethoprim (-102.1 k.cal/mol) and pyrimethamine(91.8 k.cal/mol) and hence selected for visualization.

In the visualization process, the top 2 ligands were compared with the co-crystallized ligand [Dihydro folic acid] for structural similarity. The ligand binding site in the whole protein is also visualized. In 3d interaction, the number of conventional hydrogen bonds was visualized. 2d interaction gives us a clear-cut idea of the interacting amino acid residues and their distance from that of the ligand at the active pocket site.

Table 2 Trimethoprim has Two Hydrogen Bond Interactions Namely ASN:18 [2.80], ARG:57 [2.77] Table 3 Pyrimethamine also has two hydrogen bond interactions namely TYR:100 [2.60], ILE:5 [2.60]. Table 4 Compound 3A8B has four conventional hydrogen bond connections through the amino acid residue ARG:57 [2.61], ASN:18 [3.10], TYR:100 [2.59], and ALA:7 [2.94].Table 5 Compound 3A9B has two conventional hydrogen bond interactions with the receptor through the amino acid residues ASN:18 [2.80], and ARG:57 [2.77]. There are two conventional hydrogen bond interactions displayed by the standard Inhibitors. Trimethoprim and 3A8B has four amino acid residues in common ALA:7, LEU:28, MET:16, PHE:31. Trimethoprim and 3A9B has four amino acid similar ASP:27, PHE:31, MET:16, LEU:28. Pyrimethamine and 3A8B have three amino acid residues in common TYR:100, ALA:7, and PHE:31. Pyrimethamine and 3A9B have two amino acid residues common PHE:31 and ILE:94.

BINDING POCKET ANALYSIS

Table 6 the standard antagonists Trimethoprim, Pyrimethamine and the top ligands 3A8B,3A9B were docked in the centre of the binding pocket. This might have been the reason for their better binding energy. The top compounds 3A8B and 3A9B contains one electron-withdrawing group NO2 and one electron-withdrawing group COOH, which might have contributed to their better binding energies. since the compounds 3A8B and 3A9B had near-binding energies as that of the standard Dihydrofolate reductase enzyme inhibitors Trimethoprim and pyrimethamine, they can be further synthesized and used for further studies.

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