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# *IN SILICO* DOCKING OF AMINOPYRIMIDINES TARGETING RECEPTOR FOR ADVANCED GLYCATION END PRODUCTS (RAGE)

**Vimalavathini R.*, Thamizharasi S., Vishvaja S., Srinithi S. and Yuvaraj G.**

College of Pharmacy, Mother Theresa Post Graduate and Research Institute of Health Sciences,Gorimedu, Puducherry, India - 605006.

***Corresponding Author: Dr. Vimalavthini R.**
Assistant Professor, Department of Pharmacology, College of Pharmacy, Mother Theresa Post Graduate and Research Institute of Health Sciences, Gorimedu, Puducherry, India – 605006.

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## ABSTRACT

RAGE is a multi-ligand trans membrane receptor belonging to immunoglobulin superfamily involved in various physiological and pathological pathways of various diseases like cardio vascular diseases, cancer, neurodegenerative and auto immune diseases. Nearly 10 drugs of aminopyrimidine group (flucytosine, pyrimethamine, sulfadiazine, cytarabine, cidofovir, zalcitabine, lamivudine, capectitabine, sulfadimethoxine and sulfadimidine) were imported from PubChem database. The amino acids serine, histidine were predicted as active sites for RAGE receptor (PDB ID: 3CJJ) and the amino acids asparagine, lysine, glutamic acid, tryptophan, arginine, and tyrosine were predicted as active sites for RAGE receptor (PDB ID: 3O3U) using PDBSum database. The molecular docking were performed with RAGE receptor (PDB ID: 3CJJ and PDB ID: 3O3U) both with and without the selection of active sites, as rigid and flexible docking respectively. The docking studies were done using Autodock Tools 4.2 (Version 1.5.6rc3) software. The drugs sulfadimethoxine, sulfadimidine, capectitabine and pyrimethamine showed good docking profiles for PDB ID: 3CJJ whereas sulfadimidine, pyrimethamine, sulfadiazine and sulfadimethoxine showed good docking profiles for PDB ID: 3O3U. Finally the result from the study demonstrates the above mentioned drugs can be presumed as inhibitors of RAGE.

**KEYWORDS:** RAGE, aminopyrimidine, PubChem, PDBSum, molecular docking, AutoDock.

## INTRODUCTION

Excessive glycation leads to formation of advanced glycation end products (AGE) which interferes with the normal structure and functions of biomolecules thus upsetting their molecular structure, modifying enzymatic activity, interfering with receptor functioning, cell signaling and gene expression. AGEs mediate their pathological effects by activating signaling cascades through the receptor for advanced glycation end products (RAGE).[1] RAGE is a multi-ligand trans membrane receptor belonging to immunoglobulin family, expressed on smooth muscle cells, macrophages, endothelial cells and astrocytes. Though it has physiological role in pathological conditions[2] it unleashes aberrations of series of signal transduction cascades and is implicated in pathology of diabetes, arthritis, Alzheimer's disease, arteriosclerosis, acute respiratory failure, sepsis and cancer.[3]

Docking is the process used to predict the binding orientation of a ligand to their protein targets so that the free energy of the overall system is minimized.[4] Aminopyrimidines are those pyrimidine ring system which possess amino group ($-NH_2$) present in the 2, 4, 5 and 6$^{th}$ positions of the ring. Aminopyrimidines are used in the treatment of cardiovascular, central nervous system, inflammatory, metabolic and oncology diseases. The pyrimidine ring is the basic structural moiety of many natural compounds, drugs herbicides and dyes.[5] Mechanistic and flexible docking studies reported that 2-aminopyrimidines were inhibitors of RAGE and also act as potential therapeutics for Alzheimer's disease.[6] Based on the above evidences, it is inferred that aminopyrimidines may exhibit RAGE inhibitor activity. Hence at the point of drug repurposing, nearly 10 aminopyrimidine (flucytosine, pyrimethamine, sulfadiazine, cytarabine, cidofovir, zalcitabine, lamivudine, capectitabine, sulfadimethoxine and sulfadimidine containing drugs were docked with RAGE protein using AutoDock Tools 4.2 (Version 1.5.6rc3).[7]

## MATERIALS AND METHODS

### Design of small molecules (Ligand): ligands

The ligands were mined from the NCBI PubChem databases.

Flucytosine (PubChem ID: 3366),
Pyrimethamine (PubChem ID: 4993),
Sulfadiazine (PubChem ID: 5215),
Cytarabine (PubChem ID: 6253),
Cidofovir (PubChem ID: 60613),
Zalcitabine (PubChem ID: 24066),
Lamivudine (PubChem ID: 60825),

Capectitabine (PubChem ID: 60953)
Sulfadimethoxine (PubChem ID: 5323)
Sulfadimidine (PubChem ID: 5327)

The structures of ligands were imported from PubChem in sdf format. The imported .sdf files were converted to pdb format using OpenBabel-2.3.1 and saved in pdb format. The active sites of the protein were determined using the PDBSum database. The actives sites were listed as follows,
PDB ID: 3CJJ: Ser156(A); His158(A).

PDB ID: 3O3U: Asp14(N); Lys15(N); Glu44(N); Trp62(N); Asp65(N); Arg66(N); Glu111(N); Glu153(N); Tyr155(N).
The ligands were docked with the RAGE using AutoDock Tools 4.2 (Version 1.5.6rc3). The efficacy of the RAGE inhibition is screened for the given ligands in the Table 1.

## METHODOLOGY

### Preparation of Receptor for docking

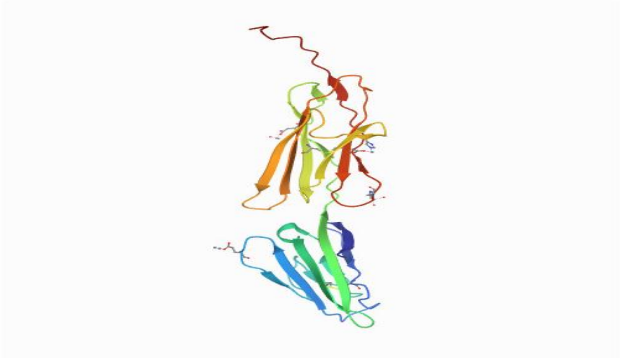

**Fig. 1: Crystal structure of human rage ligand-binding domain (PDB ID: 3CJJ)**

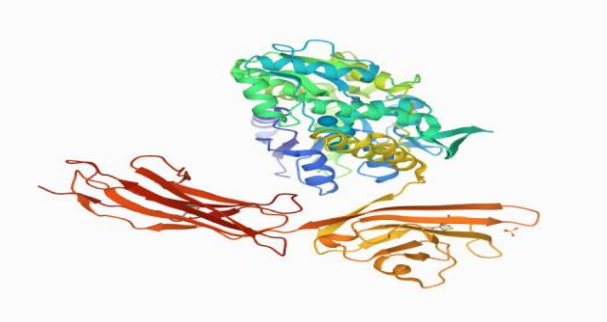

**Fig. 2: Cystal structure of human Receptor for Advanced Glycation Endproducts (RAGE) (PDB ID: 3O3U)**

The RAGE receptor was imported from the RCSB Protein Data Bank (https://www.rcsb.org/) (PDB ID: 3CJJ), (PDB ID: 3O3U). The heteroatoms of the protein were removed and was saved in the pdb format as appears in the figures 1 and 2.

### Molecular Docking studies by AutoDock Tools 4.2 (Version 1.5.6rc3)

Receptor and ligand interactions can be predicted using docking software, AutoDock. This tool prepare the receptors by adding all hydrogen atoms (polar only) to the macromolecule to correct the calculation of partial atomic charges. Gasteiger charges are calculated for each of the atom in the RAGE receptor molecule in AutoDock 4.2. 3-D affinity grids of size 60*60*60 Å with 0.375 Å spacing were centered on the geometric center of the target protein. The dimensional affinity grid was fixed as specified above, to run the Autogrid file. When the docking is done using the active sites, the additional step of selecting the active sites was done and the grid parameters are saved. The selected important docking parameters for the Lamarckian Genetic Algorithm (LGA) as follows: population size of 150 individuals, 2.5 million energy evaluations, maximum of 27000 generations, number of top individuals to automatically survive to next generation of 1, mutation rate of 0.02, crossing over of 0.8, 25 docking runs and randominitial positions and conformations. The ligands were docked with RAGE receptor using AutoDock 4.2 (Version 1.5.6rc3) software with the above mentioned procedure. The drug score, binding frequencies, active residues interaction were simulated. The procedure was adopted with certain modifications.[8]

## RESULTS AND DISCUSSION

### Flexible docking with 3CJJ

Sulfadimethoxine and Sulfadimidine exhibited higher binding energy of -5.04 kcal/mol and -4.77 kcal/mol respectively (Table 1). Sulfadimethoxine formed hydrogen bonds with the residues Val199, Glu94, Ala152, Gly153, and Sulfadimidine with the residues Gly153, Thr177 as shown in the Table 2. Sulfadimethoxine, Sulfadimidine, Capectitabine, Pyrimethamine, Flucytosine and Sulfadiazine showed higher inhibition constants of 201.23, 321.38, 519.08, 705.14, 875.81 and 902.97 respectively in µM units (Table 1).

**Table 1. The binding energy, inhibition constant and number of hydrogen bonds for various aminopyrimidines docked with RAGE receptor (3CJJ and 3O3U).**

| Sr. No | Pub Chem ID | Name | 3CJJ docked without active sites | | | 3CJJ docked with active sites | | | 3O3U docked without active sites | | | 3O3U docked with active sites | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | B.E | Inhib. constant | No. H-bond | B.E | Inhib. constant | No. H-bond | B.E | Inhib. constant | No. H-bond | B.E | Inhib. constant | No. H-bond |
| 1. | 3366 | Flucytosine | -4.17 | 875.81 µM | 4 | -4.2 | 837.92 µM | 4 | -3.87 | 1.44 mM | 3 | -3.87 | 1.46 mM | 3 |
| 2. | 4993 | Pyrimethamine | -4.3 | 705.14 µM | 1 | -4.63 | 405.83 µM | 1 | -8.09 | 1.17 µM | 3 | -8.1 | 1.16 µM | 3 |
| 3. | 5215 | Sulfadiazine | -4.15 | 902.97 µM | 2 | -4.0 | 1.18 mM | 1 | -7.51 | 3.11 µM | 3 | -7.52 | 3.07 µM | 3 |
| 4. | 6253 | Cytarabine | -2.83 | 8.36 mM | 2 | -3.43 | 3.06 mM | 3 | -5.15 | 168.25 µM | 5 | -5.47 | 97.04 µM | 3 |
| 5. | 60613 | Cidofovir | -2.6 | 12.49 mM | 4 | -2.82 | 8.62 mM | 4 | -4.84 | 283.91 µM | 2 | -5.88 | 48.68 µM | 7 |
| 6. | 24066 | Zalcitabine | -3.72 | 1.89 mM | 3 | -3.5 | 2.7 mM | 3 | -6.56 | 15.5 µM | 5 | -6.59 | 14.66 µM | 6 |
| 7. | 60825 | Lamivudine | -3.63 | 2.19 mM | 3 | -4.01 | 1.15 mM | 2 | -5.67 | 70.28 µM | 4 | -5.64 | 73.8 µM | 3 |
| 8. | 60953 | Capectitabine | -4.48 | 519.08 µM | 4 | -4.62 | 408.25 µM | 3 | -6.81 | 10.13 µM | 4 | -7.08 | 6.43 µM | 3 |
| 9. | 5323 | Sulfadimethoxine | -5.04 | 201.23 µM | 4 | -5.18 | 159.9 µM | 3 | -7.33 | 4.24 µM | 3 | -7.46 | 3.39 µM | 3 |
| 10. | 5327 | Sulfadimidine | -4.77 | 321.38 µM | 2 | -4.93 | 241.44 µM | - | -8.48 | 609.89 nM | 3 | -8.53 | 562.16 nM | 3 |

*B.E- Binding energy (kcal/mol); Inhib. constant- Inhibition constant; No.H-bond- Number of hydrogen bonds.*

**Table: 2 Hydrogen bonds formed between the aminopyrimidines and the amino acids of the RAGE receptor (3CJJ and 3O3U).**

| Sl.No | Pub Chem ID | Name | Hydrogen Bonds | | | |
|---|---|---|---|---|---|---|
| | | | 3CJJ(docked without active sites) | 3CJJ(docked with active sites) | 3O3U(docked without active sites) | 3O3U(docked with active sites) |
| 1. | 3366 | Flucytosine | 3cjj:A:Ile120:O<br>3cjj:A:Ile120:HN<br>3cjj:A:Ile120:O<br>3cjj:A:Arg29:HH12 | 3cjj:A:Ile120:O<br>3cjj:A:Arg29:HH12<br>3cjj:A:Ile120:HN<br>3cjj:A:Ile120:O | 3o3u:N:Glu44:OE1<br>3o3u:N:Glu44:OE2<br>3o3u:N:Leu43:HN | 3o3u:N:Glu44:OE2<br>3o3u:N:Glu44:OE1<br>3o3u:N:Leu43:HN |
| 2. | 4993 | Pyrimethamine | 3cjj:A:Val117:O | 3cjj:A:Leu155:O | 3o3u:N:Glu44:OE2<br>3o3u:N:Glu153:OE2<br>3o3u:N:Glu45:OE2 | 3o3u:N:Glu45:OE2<br>3o3u:N:Glu153:OE2<br>3o3u:N:Glu44:OE2 |
| 3. | 5215 | Sulfadiazine | 3cjj:A:Glu94:OE1<br>3cjj:A:Val117:O | 3cjj:A:Tyr150:OH | 3o3u:N:Asp65:OD2<br>3o3u:N:Trp340:O<br>3o3u:N:Arg66:HE | 3o3u:N:Trp340:O<br>3o3u:N:Asp65:OD2<br>3o3u:N:Arg66:HE |
| 4. | 6253 | Cytarabine | 3cjj:A:Glu94:OE1<br>3cjj:A:Gly213:O | 3cjj:A:Ser211:OG<br>3cjj:A:Gly213:O<br>3cjj:A:His217:O | 3o3u:N:Asp66:OD1<br>3o3u:N:Arg66:HH21<br>3o3u:N:Trp62:HE1<br>3o3u:N:Glu44:OE2<br>3o3u:N:Glu153:OE1 | 3o3u:N:Glu44:OE2<br>3o3u:N:Trp62:HE1<br>3o3u:N:Arg66:HH21 |
| 5. | 60613 | Cidofovir | 3cjj:A:Gly153:HN<br>3cjj:A:Ala152:HN<br>3cjj:A:Val117:O<br>3cjj:A:Glu94:OE2 | 3cjj:A:Pro212:O<br>3cjj:A:Gly213:O<br>3cjj:A:Gly213:O<br>3cjj:A:Thr154:OG1 | 3o3u:N:Glu153:OE1<br>3o3u:N:Trp62:HE1 | 3o3u:N:Glu45:OE2<br>3o3u:N:Tyr341:OH<br>3o3u:N:Asp65:OD2<br>3o3u:N:Glu44:OE2<br>3o3u:N:Glu153:OE1<br>3o3u:N:Tyr155:HN<br>3o3u:N:Asp65:OD1<br>3o3u:N:Trp62:HE1 |
| 6. | 24066 | Zalcitabine | 3cjj:A:Glu94:O<br>3cjj:A:Arg179:HH22<br>3cjj:A:Ile91:O | 3cjj:A:Ser211:OG<br>3cjj:A:His217:HN<br>3cjj:A:Gly213:O | 3o3u:N:Arg66:HH21<br>3o3u:N:Glu44:OE2<br>3o3u:N:Tyr155:HN<br>3o3u:N:Arg66:HE<br>3o3u:N:Glu153:OE1 | 3o3u:N:Glu153:OE1<br>3o3u:N:Arg66:HH21<br>3o3u:N:Glu44:OE2<br>3o3u:N:Tyr341:OH<br>3o3u:N:Tyr155:HN<br>3o3u:N:Arg66:HE |

| | | | | | | |
|---|---|---|---|---|---|---|
| 7. | 60825 | Lamivudine | 3cjj:A:Val117:O<br>3cjj:A:Gly153:HN<br>3cjj:A:Glu94:OE2 | 3cjj:A:Glu94:OE1<br>3cjj:A:Arg179:HE | 3o3u:N:Glu44:OE2<br>3o3u:N:Arg66:HE<br>3o3u:N:Trp62:HE1<br>3o3u:N:Asp65:OD1 | 3o3u:N:Glu44:OE2<br>3o3u:N:Trp62:HE1<br>3o3u:N:Arg66:HE |
| 8. | 60953 | Capectitabine | 3cjj:A:Tyr150:OH<br>3cjj:A:Ala152:HN<br>3cjj:A:Tyr150:OH<br>3cjj:A:Gly153:HN | 3cjj:A:Gly153:O<br>3cjj:A:Gly153:O<br>3cjj:A:Gly153:HN | 3o3u:N:Glu153:OE1<br>3o3u:N:Trp62:HE1<br>3o3u:N:Asp65:OD1<br>3o3u:N:Asp65:OD1 | 3o3u:N:Glu111:OE1<br>3o3u:N:Lyz15:HZ1<br>3o3u:N:Tyr155:HN |
| 9. | 5323 | Sulfadimethoxine | 3cjj:A:Val117:O<br>3cjj:A:Glu94:OE2<br>3cjj:A:Ala152:HN<br>3cjj:A:Gly153:HN | 3cjj:A:Gly153:HN<br>3cjj:A:Thr177:HG1<br>3cjj:A:Ala152:HN | 3o3u:N:Asp65:OD1<br>3o3u:N:Trp62:HE1<br>3o3u:N:Arg66:HE | 3o3u:N:Asp65:OD1<br>3o3u:N:Trp62:HE1<br>3o3u:N:Arg66:HE |
| 10. | 5327 | Sulfadimidine | 3cjj:A:Gly153:O<br>3cjj:A:Thr177:HG1 | -no hydrogen bonds formed- | 3o3u:N:Arg66:HE<br>3o3u:N:Glu153:OE1<br>3o3u:N:Asp65:OD2 | 3o3u:N:Glu153:OE1<br>3o3u:N:Asp65:OD2<br>3o3u:N:Arg66:HE |

**Rigid docking with 3CJJ**

Sulfadimethoxine and Sulfadimidine showed higher binding energy of -5.18 kcal/mol and -4.93 kcal/mol respectively (Table 1). Sulfadimethoxine formed hydrogen bonds with the residues Gly153, Thr177, Ala152 and Sulfadimidine showed no bonding as shown in the Table 2. Sulfadimethoxine, Sulfadimidine, Pyrimethamine, Capectitabine and Flucytosine, showed inhibition constants of 159.9, 241.44, 405.83, 408.25 and 837.92 respectively in µM units (Table 1).

**Flexible docking with 3O3U**

Sulfadimidine and Pyrimethamine shows higher binding energy of -8.48 and -8.09 respectively (Table 1). Sulfadimidine formed hydrogen bonds with the residues Arg66, Glu153, Asp65 while Pyrimethamine with the residues Glu44, Glu45, Glu153 as shown in the Table 2. Sulfadimidine, Pyrimethamine, Sulfadiazine and Sulfadimethoxine showed inhibition constants of 609.89 nM, 1.17 µM, 3.11 µM and 4.24 µM respectively (Table 1).

**Rigid docking with 3O3U**

Sulfadimidine and Pyrimethamine shows higher binding energy of -8.53 kcal/mol and -8.1 kcal/mol respectively (Table 1). Sulfadimidine formed hydrogen bonds with the residues Arg66, Glu153, Asp65 while Pyrimethamine with the residues Glu44, Glu45, Glu153 as shown in the Table 2. Sulfadimidine, Pyrimethamine, Sulfadiazine and Sulfadimethoxine shows inhibition constant of 562.16 nM, 1.16 µM, 3.07 µM, and 3.39 µM respectively (Table 1).

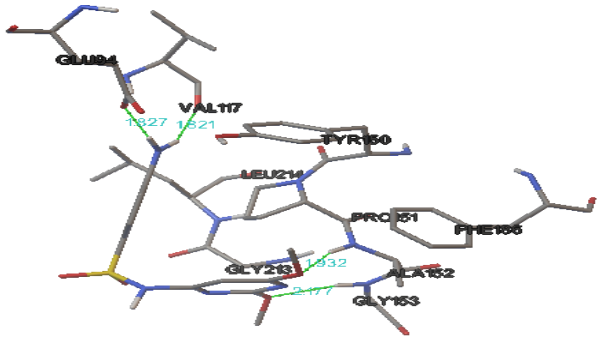


**Fig 3: Sulfadimethoxine docked with 3CJJ active sites.**

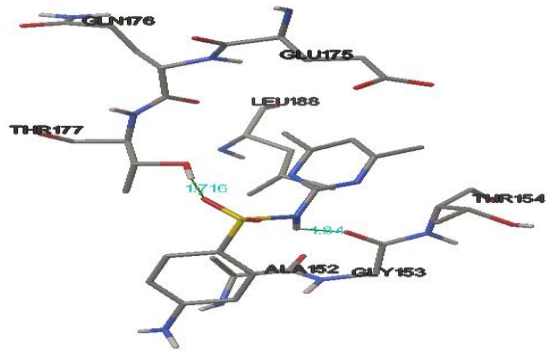


**Fig 4: Sulfadimidine docked with 3CJJ active sites.**

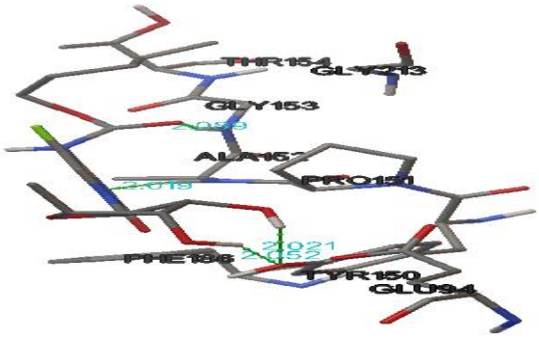


**Fig 5: Capectitabine docked with 3CJJ without active sites.**

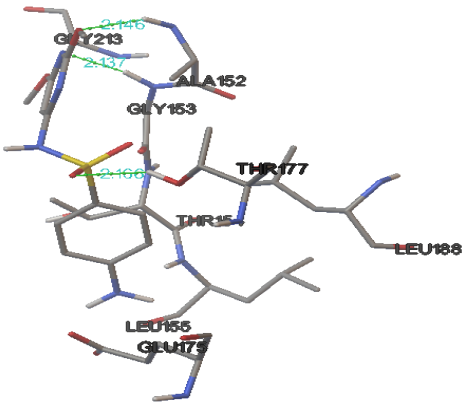


**Fig 6: Sulfadimethoxine docked with 3CJJ with active sites.**

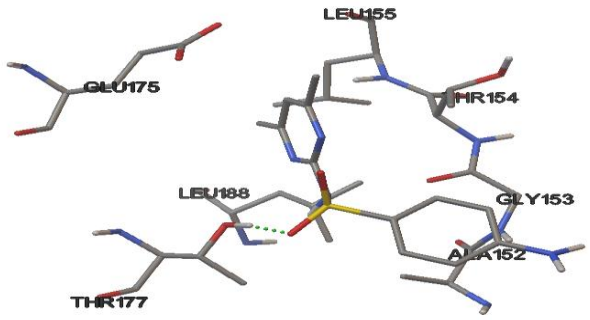


**Fig 7: Sulfadimidine docked with 3CJJ docked with active sites.**

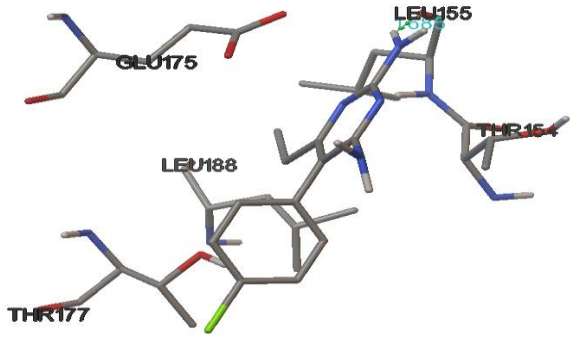


**Fig 8: Pyrimethamine docked with 3CJJ with active sites.**

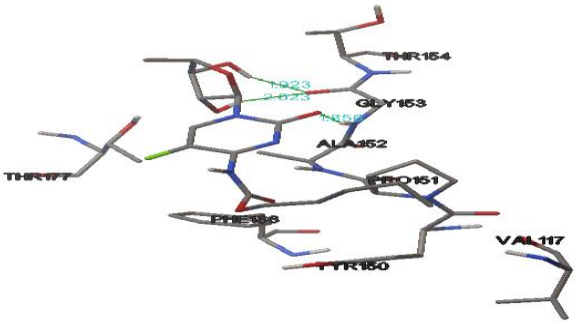


**Fig 9: Capectitabine docked with 3CJJ with active sites.**

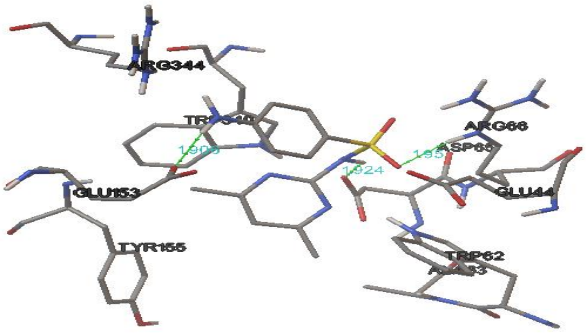


**Fig 10: Sulfadimidine docked with 3O3U active sites.**

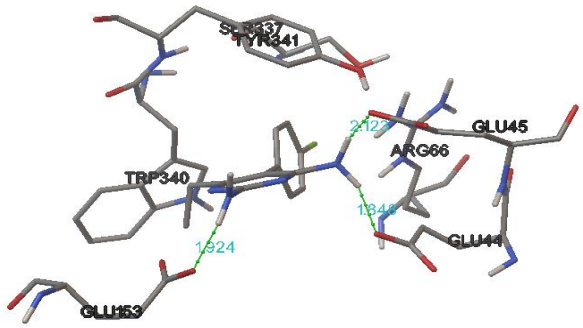


**Fig 11: Pyrimethamine docked with 3O3U docked active sites.**

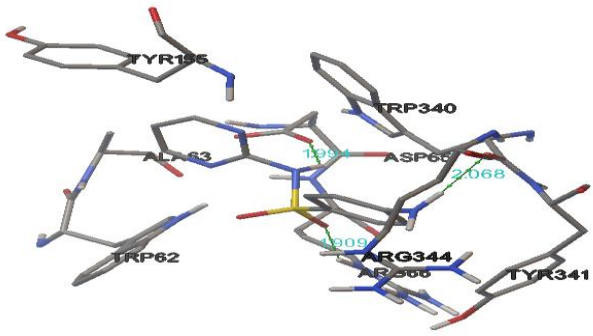


**Fig 12: Sulfadiazine docked with 3O3U active sites.**

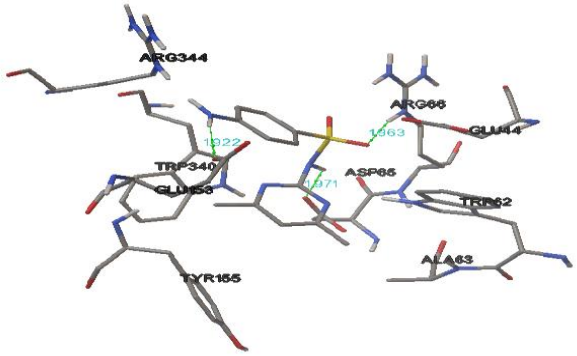


**Fig 13: Sulfadimidine docked with 3O3U with active sites.**

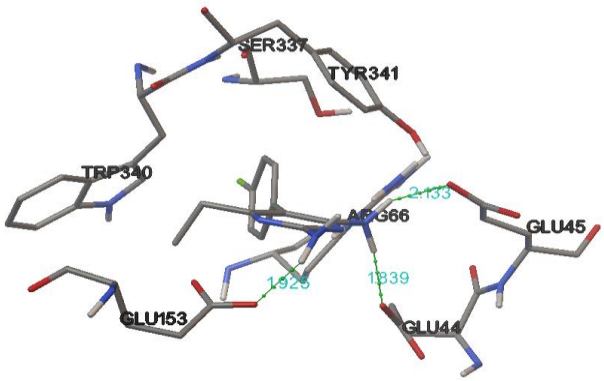


**Fig 14: Pyrimethamine docked with 3O3U with active sites.**

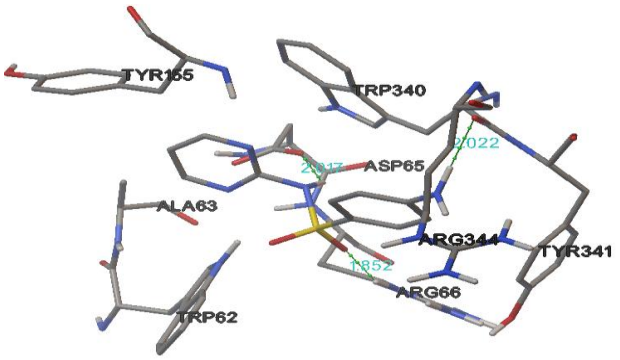


**Fig 15: Sulfadiazine docked with 3O3U with active sites.**

## CONCLUSION

The *Insilico* docking studies of 10 drugs were performed by docking with the RAGE receptors (3CJJ and 3O3U) and the results were analysed. The inference of the study reveals that Sulfadimidine, Pyrimethamine, Sulfadimethoxine, Sulfadiazine, Flucytosine, Capectitabine shows RAGE inhibitory activity. Therefore this study recommends that the above mentioned drugs can be profiled for further in vitro and in vivo studies for the therapeutics of diseases which involves the RAGE.

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