Towards effective advance treatment Strategies: Screening and synergism analysis of drug combinations for Alzheimer's disease

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Abstract
Alzheimer's disease (AD) remains a significant challenge in modern medicine due to its progressive cognitive decline and the absence of effective treatments. This neurodegenerative disorder is characterized by the accumulation of amyloid β protein (Aβ) and the formation of neurofibrillary tangles. Molecular docking, a computational technique, has emerged as a powerful tool in drug discovery by predicting ligand-receptor interactions. Virtual high throughput screening (vHTS) facilitates the rapid evaluation of potential drug candidates against AD-associated targets. Given the complexity of aging mechanisms implicated in AD, a multi-target strategy has garnered attention as a promising approach. Combination therapy utilizing existing medications offers a cost-effective alternative to traditional drug development.

In this study, the synergistic effects of specific drug combinations including N-acetyl cysteine + Curcumin, Mitoquinone + Butylated hydroxy anisole and Carnosine + Kynurenic Acid Cocktail of Drugs, were evaluated against AD using Compusyn software. Understanding the synergism, antagonism and additive effects of these combinations provides insights into potential therapeutic avenues for AD. This integrated approach combining computational modelling, drug screening and combination therapy represents a promising strategy in the quest for effective AD treatments.

Keywords: Amyloid beta protein, Alzheimer's disease, synergism, combination index.

Introduction

Alzheimer's disease (AD) is the most prevalent neurodegenerative illness that causes a steady deterioration in cognitive function and is characterized by the development of neurofibrillary tangles and senile plaque in the brain. The amyloid β protein (Aβ) is the primary component of extracellular amyloid plaques in AD. The amyloid precursor protein (APP) is converted into Aβ through the successive cleavage of β- and γ-secretase[23]. Through molecular docking, one can verify a prospective drug's specificity against homologous proteins. Virtual high throughput screening based on docking is faster than traditional screening and is less costly than typical high throughput screening. A method called "molecular docking" is used to match ligands to their binding sites, or receptors, in the most optimal way possible.

There are two dependable approaches for drug screening: *in vitro* and *in vivo*. The aim of this study is in vitro research. Following drugs screening, certain drug combinations may have a beneficial effect on Alzheimer's disease. Determining the synergism, antagonism and additive effect of a combination drugs is essential. The Chou-Talalay method is employed for determining synergism.

Material and Methods

Screening of drugs by molecular docking: The crystal structure of $A\beta_{1-42}$ was extracted from the Protein Data Bank (PDB ID: 1IYT). The 3D structure of multiple drugs was taken from Pub chem.

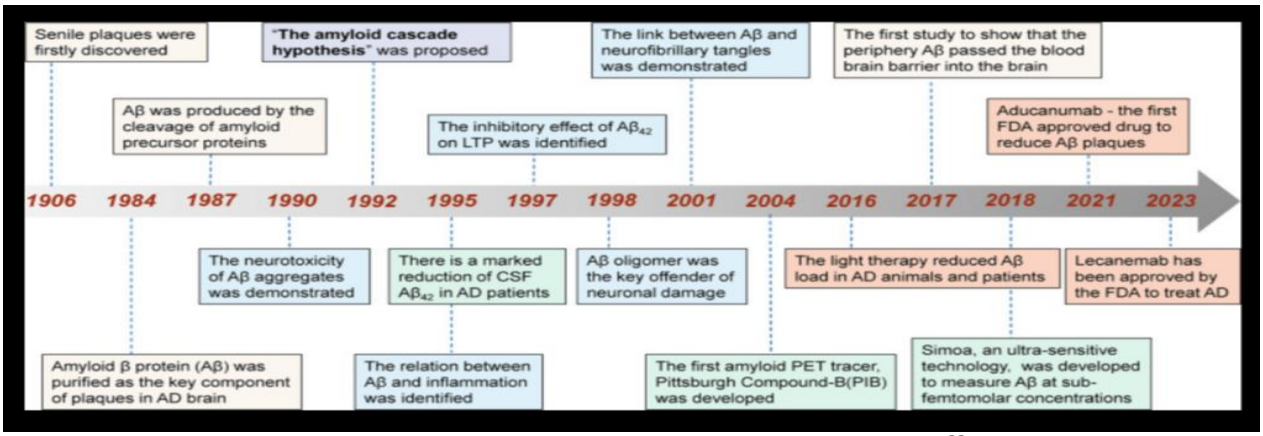


Figure 1: Amyloid cascade hypothesis and application[22]

Drugs and their mode of action[10,13,18]

Compounds	Mode of action
Mito Q	It is an oral antioxidant that can specifically target malfunctions in the mitochondria.
N- acetylcysteine	It is an antioxidant that is applied to cancer therapy.
Butylated Hydroxy-anisole	It's a synthetic substance that's used to cure genetic disorders and preserve food.
Metformin	It is a metabolic regulator that enhances resilience to stress.
Lithium Chloride	This class of medication is called a mood stabilizer. It is a salt that is prescribed as mental health medicine, mostly for bipolar illness. It belongs to the anti-aggregation and metabolic modulator class.
Curcumin	It is an anti-inflammatory, anti-aggregation and antioxidant drug. It stimulates the stress response gene and is resistant to stress.
Carnosine	It is used to stop the aging process and diabetes-related complications such renal problems and nerve damage
Kynurenic acid	It has anticonvulsant and anti-excitatory properties. Neuroactive activity is seen in this acid.

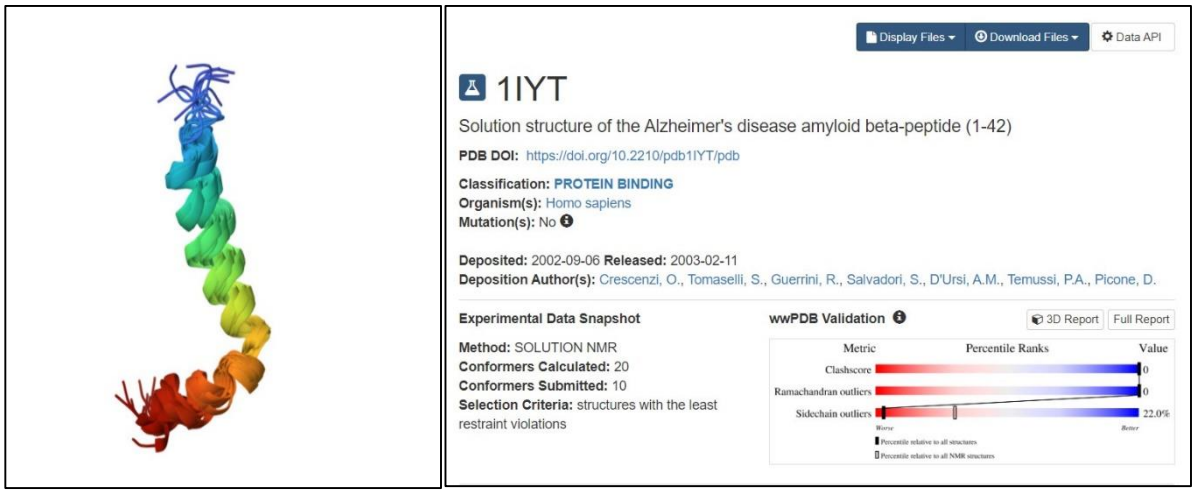


Figure 2: 1IYT Solution structure of the Alzheimer's disease amyloid beta-peptide (1-42)[6]

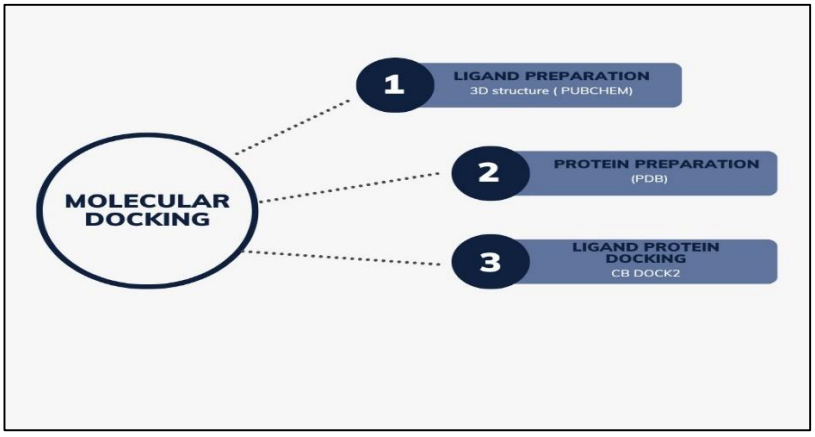


Figure 3: Steps for Docking

CB-Dock: The enhanced protein-ligand blind docking tool, CB-Dock, automatically determines the binding sites, computes their center and size, adjusts the docking box size based on the query ligands and then uses Auto Dock Vina to carry out molecular docking.

Determination of synergism: The median-effect equation, which is derived from the mass-action law principle, serves as the common link between a single entity and several entities and first- and higher-order dynamics form the basis of the Chou-Talalay approach for drugs combination. The Michaelis-Menten, Hill, Henderson-Hasselbalch and Scatchard equations from biochemistry and biophysics are all included in one universal equation. A quantitative definition of additive effect (CI = 1), synergism (CI < 1) and antagonism (CI > 1) in pharmacological combinations can be found in the resulting combination index (CI) theorem of Chou-Talalay[4]. Additionally, as indicated by the CI plot and

isobologram, respectively, this theory offers techniques for automatic computer simulation of synergism and/or antagonism at every impact and dosage level. The synergism for N-acetyl cysteine+ Curcumin, Mitoquinone + Butylated hydroxy anisole and Carnosine + Kynurenic Acid Cocktail of drugs against Alzheimer's disease was determined in this case using the Compusyn software.

Method of using software

Loading Compusyn: There were two options available in the open main window: New Experiment and Recall Experiment.

Single drug editing:

- Enter the drug's name, abbreviation and units after opening the drug editor window by clicking on New Drug or Edit Drug in the main window.
- There must be two or more data points for each drug.
- To enter data point, type dose concentration (Positive number) and enter an effect (a number between 0 and 1) representing fraction of population affected by the treatment at specified dose.

Drug Combo Editing:

- In order to construct a new drug combo, one must decide which drug to include in it. Click on every drug in the combo to bring up the drug selection dialog, then click on the constant ratio.
- One needs to provide the ratio at which the drugs are mixed or sequentially added for combos with constant ratios.

Report Generation:

- Choose the combination index table, combination index plot, log (Fa – CI) and isobologram to create a report for the combination drug.

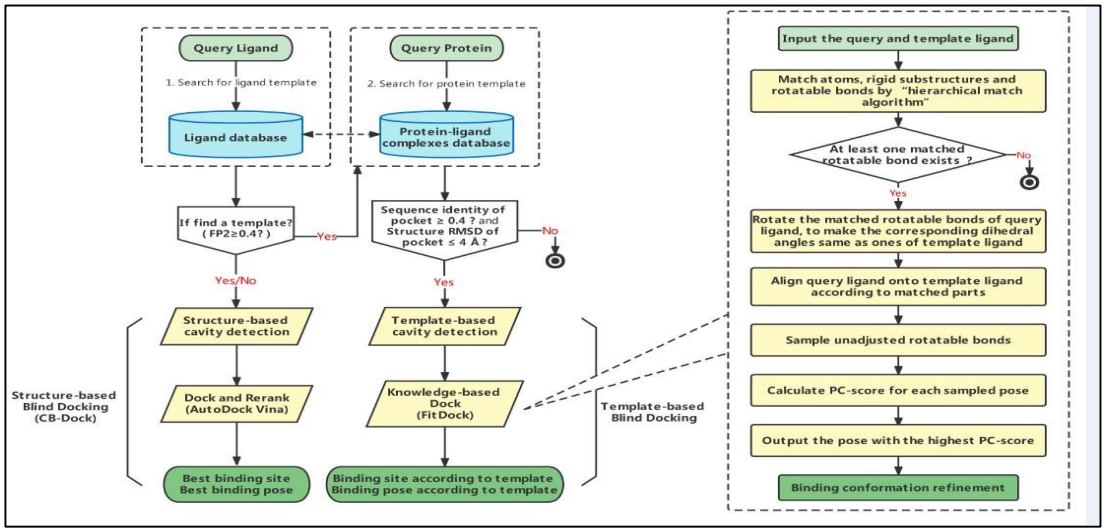


Figure 4: CB Dock: a web server working process[9]

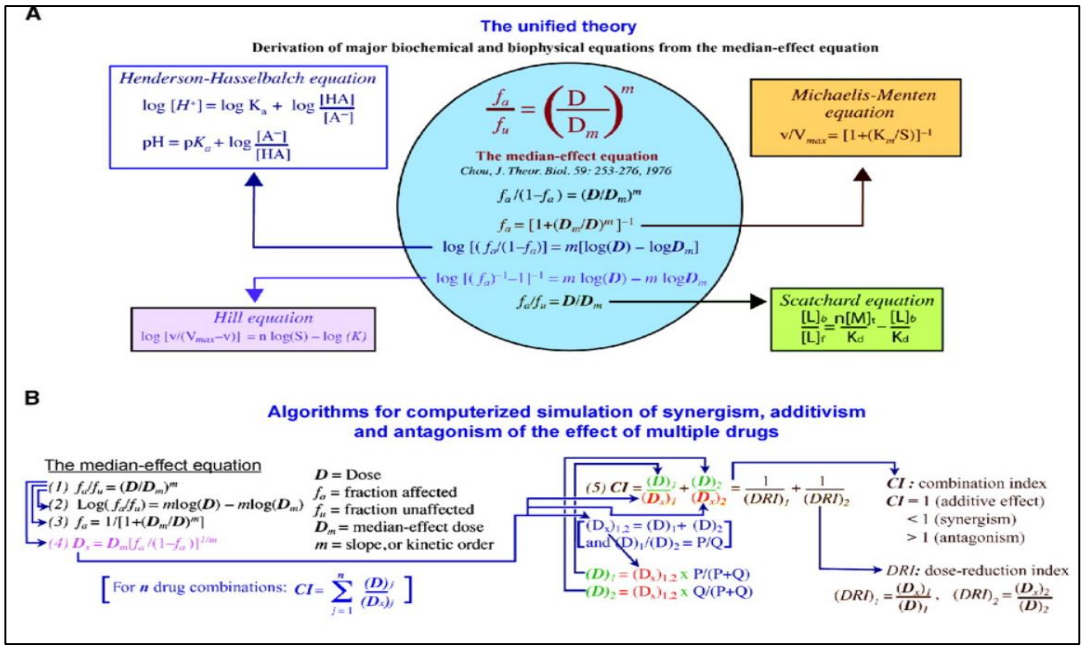


Figure 5: Compusyn software for combination index using Chou-Talalay approach[4]

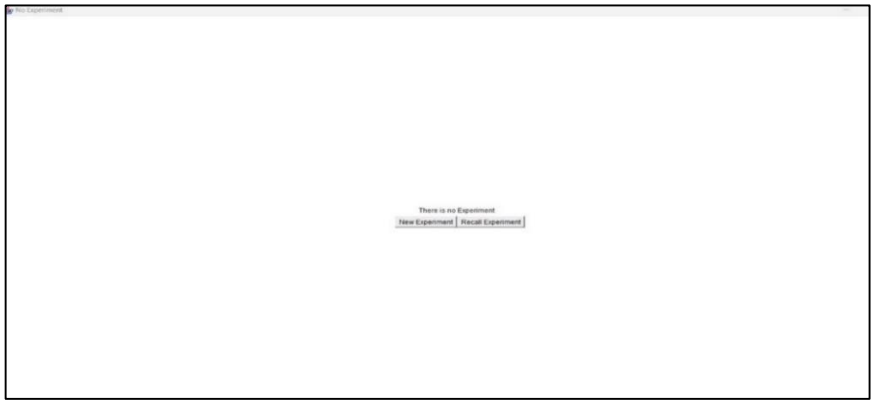


Figure 6: Compusyn software Main window

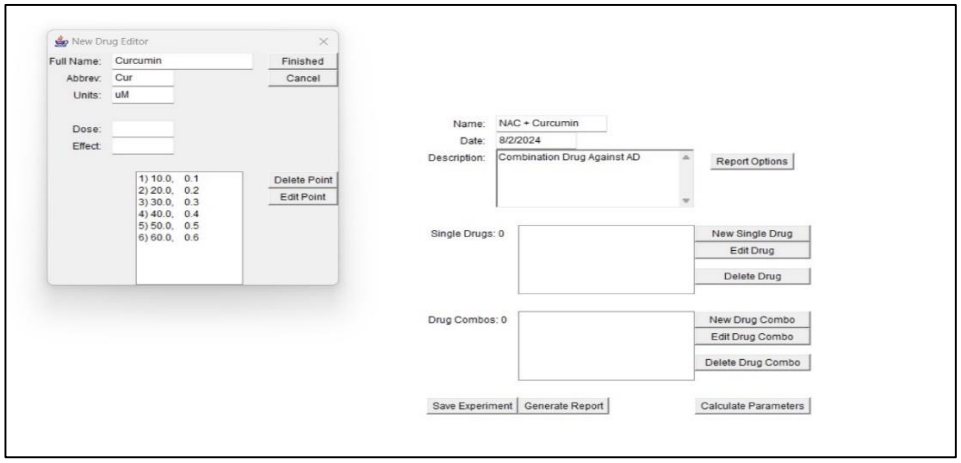


Figure 7: Single drug editing

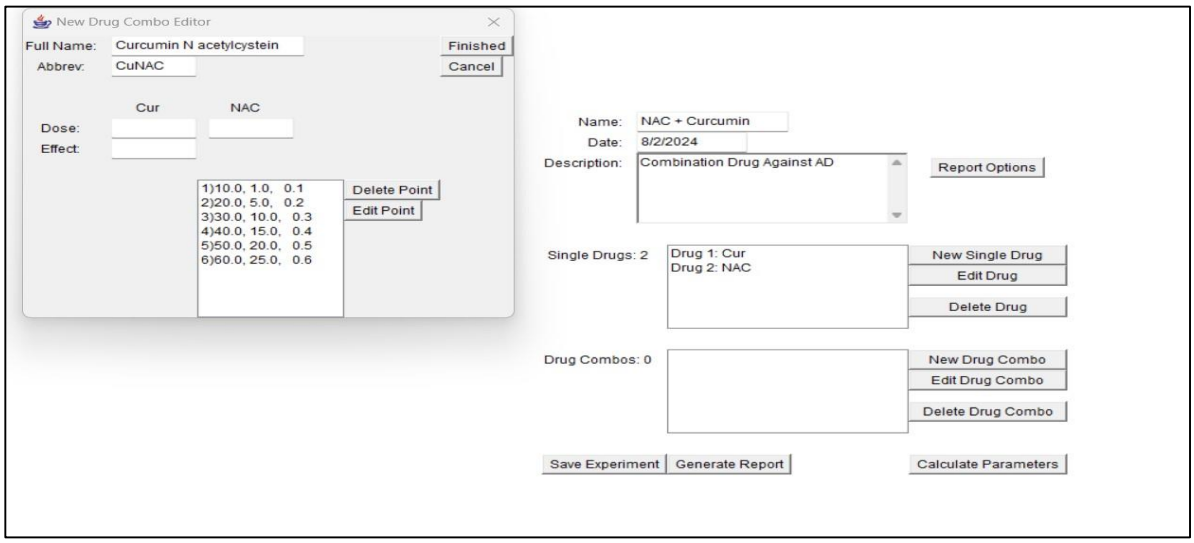


Figure 8: Combo drug editing

Results

Molecular docking was employed as an *in vitro* approach for high throughput drug screening. During virtual screening, cavity detection guided protein-ligand blind docking with CB Dock, a web server, used to create the structure of drugs bound to $A\beta_{1-42}$. Six drugs can be chosen for cocktail drug formation based on Vina score. The above results demonstrate that lithium chloride and metformin are excluded from the drug combination development process.

Synergism analysis of drugs

Combination index for N acetyl cysteine + Curcumin (2:1): One of the main causes of Alzheimer's disease (AD) is oxidative stress coupled with glutathione depletion. In animal models of AD, the glutathione precursor N-acetylcysteine (NAC) has neuroprotective effects[1]. It has been discovered that a neuroprotective drug such as N-acetyl cysteine (NAC) preserves the tissue environment around the neurons, potentially promoting the growth of neuronal tissue

following injury. By raising glutathione levels, NAC prevents inflammation-related neuronal damage that can result in AD and other dementia-causing illnesses by lowering reactive oxygen species[8]. The main ingredient in Curcuma longa, curcumin, has demonstrated promising results in the major prevention or treatment of AD. In the past ten years, researchers have concentrated on improving curcumin's pharmacokinetic characteristics and optimizing its medicinal qualities[3].

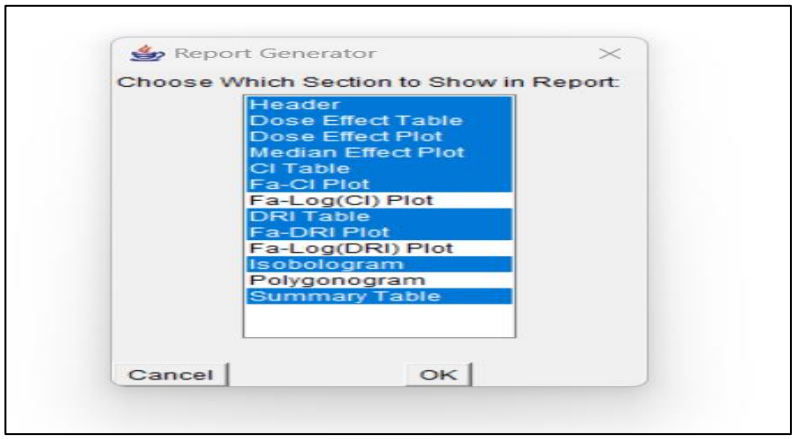
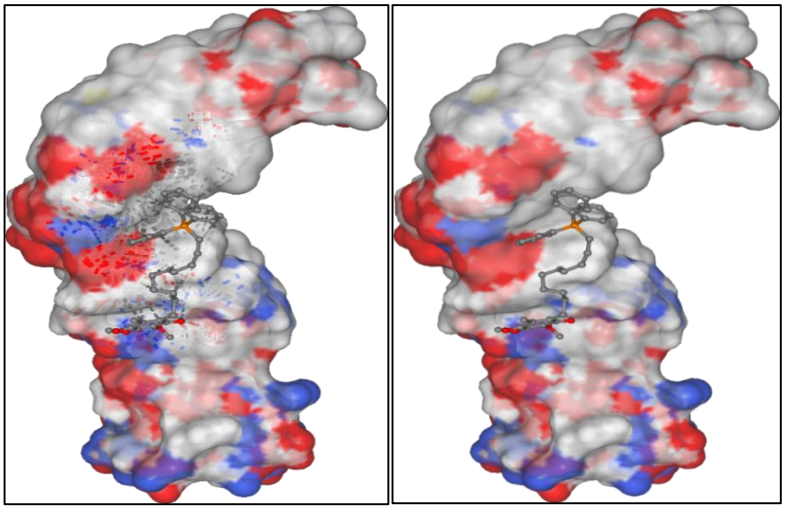


Figure 9: Report generator

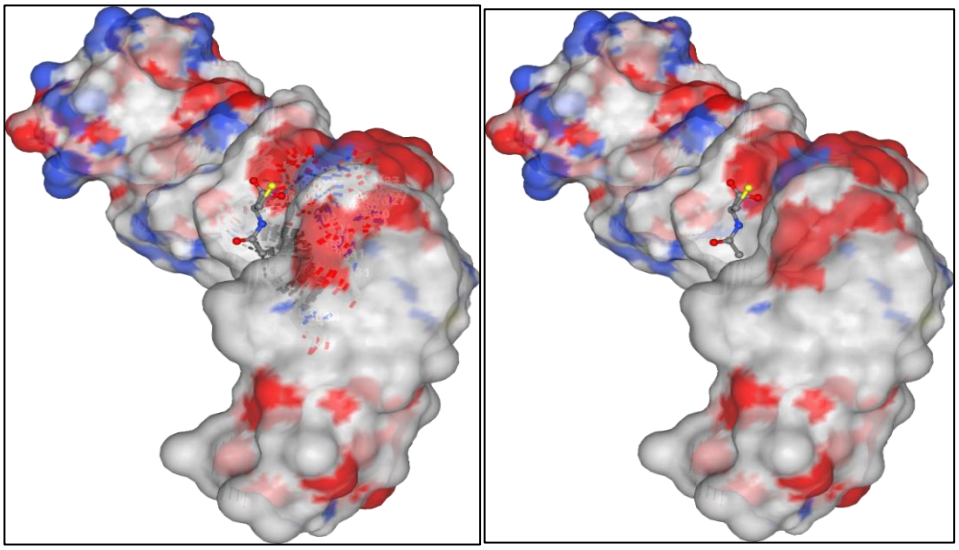


Curpocket ID	Vina Score	Cavity volume (A^{03})	Center (x,y,z)	Docking Size (x,y,z)
C4	**-32.0**	**13**	**0,-3,-8**	**30,30,30**
C5	-30.7	8	2,1,-8	30,30,30
C3	-24.3	15	-2,-3,13	30,30,30
C1	-19.9	20	-6,7,17	30,30,30
C2	-19.7	17	-8,-5,17	30,30,30

Pocket: C4 & Score: -32.0

Chain A: VAL12 LYS16 PHE19 PHE20 ASP23 VAL24 ALA30 ILE31 LEU34 VAL12 GLN15 LYS16 PHE19 PHE20 ASP23 VAL24 ASN27 ALA30 ILE31 LEU34 VAL12 LYS16 PHE19 PHE20 ASP23 VAL24 ASN27 ALA30 ILE31 ILE32 LEU34 GLN15 LYS16 PHE19 PHE20 ASP23 VAL24 ALA30 ILE31 VAL12 LYS16 PHE19 PHE20 ASP23 VAL24 ALA30 ILE31 LEU34 PHE19 ASP23 ASN27 ALA30 ILE31 ILE32 LEU34 GLN15 LYS16 PHE19 PHE20 ASP23 ASN27 ILE31 LEU34 GLN15 PHE19 PHE20 ASP23 VAL24 ASN27 ALA30 ILE31 LEU34 GLN15 PHE19 PHE20 ASP23 VAL24 ALA30 ILE31 GLY33 LEU34 GLN15 PHE19 ASP23 ASN27 ALA30 ILE31 ILE32 LEU34

Figure 10: Mito – Q with 1IYT protein

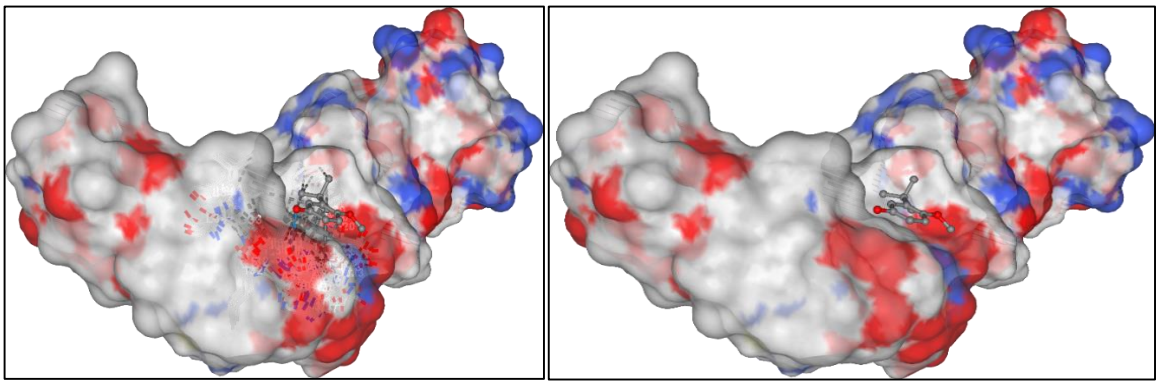


Curpocket ID	Vina Score	Cavity volume (A^{03})	Center (x,y,z)	Docking Size (x,y,z)
C4	**-15.7**	**13**	**0,-3,-8**	**17,17,17**
C5	-15.3	8	2,1,-8	17,17,17
C1	-11.6	20	-6,7,17	17,17,17
C2	-10.1	17	-8,-5,17	17,17,17
C3	-5.5	15	-2,-3,13	17,17,17

Pocket: C4 & Score: -15.7

Chain A: PHE20 ASP23 VAL24 ILE31 LEU34 PHE20 ASP23 VAL24 ASN27 ILE31 LEU34 PHE20 ASP23 VAL24 ASN27 ALA30 ILE31 PHE20 ASP23 VAL24 ASN27 ALA30 ILE31 PHE20 ASP23 VAL24 ILE31 LEU34 ASP23 VAL24 ASN27 ALA30 ILE31 PHE19 ASP23 ASN27 ILE31 LEU34 PHE20 ASP23 VAL24 ASN27 ALA30 ILE31 PHE20 ASP23 VAL24 ASN27 ALA30 ILE31 LEU34 ASP23 ASN27 ALA30 ILE31

Figure 11: NAC with 1IYT Protein

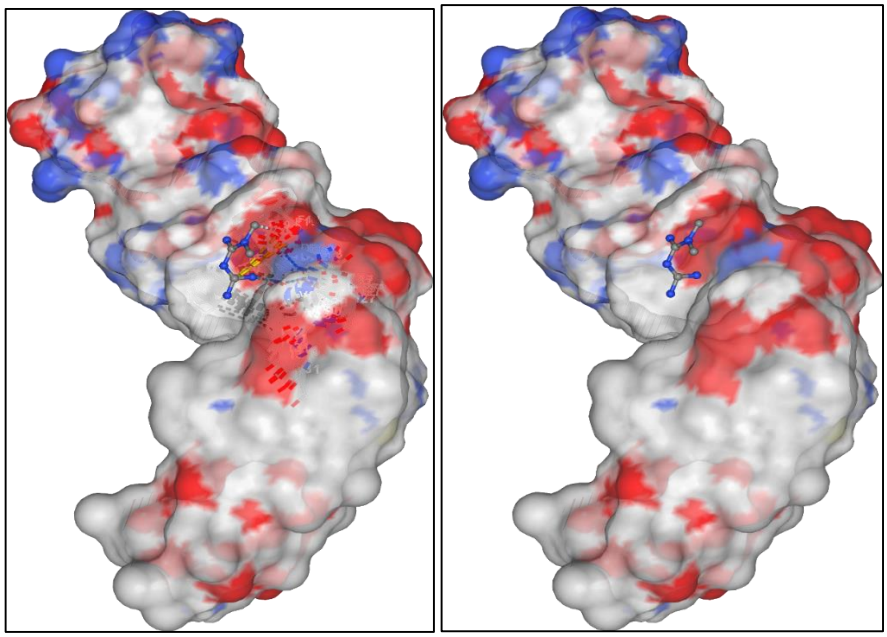


Curpocket ID	Vina Score	Cavity volume (A^{03})	Center (x,y,z)	Docking Size (x,y,z)
C4	**-23.1**	**13**	**0,-3,-8**	**17,17,17**
C5	-20.6	8	2,1,-8	17,17,17
C1	-16.2	20	-6,7,17	17,17,17
C3	-12.4	15	-2,-3,13	17,17,17
C2	-11.9	17	-8,-5,7	17,17,17

Pocket: C4 & Score: -23.1

Chain A: PHE20 ASP23 VAL24 ALA30 ILE31 PHE20 ASP23 VAL24 ASN27 ALA30 ILE31 PHE20 ASP23 VAL24 ASN27 ALA30 ILE31 ILE32 LEU34 PHE20 ASP23 VAL24 ALA30 ILE31 PHE20 ASP23 VAL24 ALA30 ILE31 ILE32 LEU34 ASP23 ASN27 ALA30 ILE31 ILE32 LEU34 ASP23 ASN27 ALA30 LEU34 PHE20 ASP23 VAL24 ASN27 ALA30 ILE31 LEU34 PHE20 VAL24 ALA30 ILE31 GLY33 LEU34 ASN27 ALA30 ILE31 ILE32 LEU34

Figure 12: BHA with 1IYT Protein

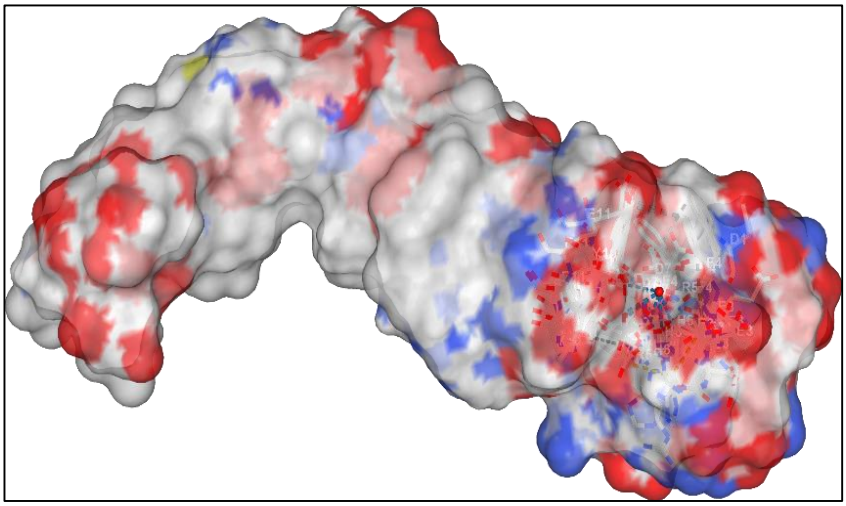


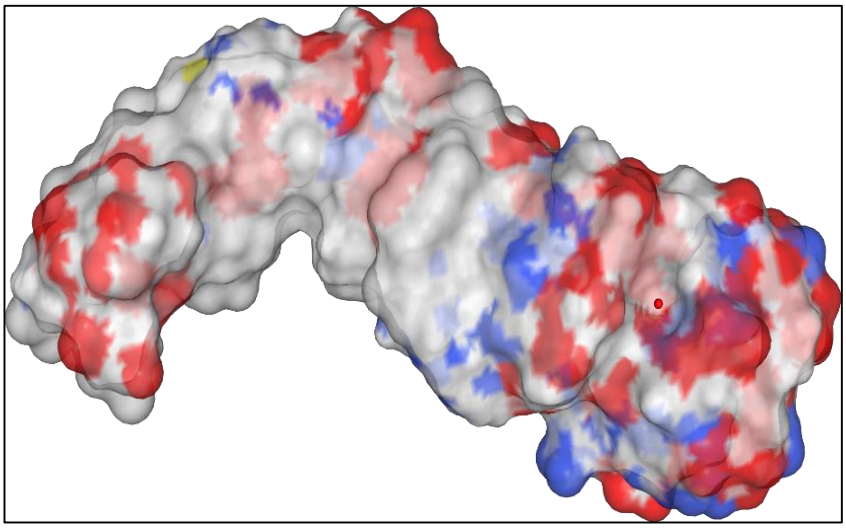
Curpocket ID	Vina Score	Cavity volume (A^{03})	Center (x,y,z)	Docking Size (x,y,z)
C4	**-15.3**	**13**	**0,-3,-8**	**16,16,16**
C5	-13.7	8	2,1,-8	16,16,16
C3	-11.3	15	-2,-3,13	16,16,16
C1	-11.2	20	-6,7,17	16,16,16
C2	18.6	17	-8,-5,17	16,16,16

Pocket: C4 & Score: -15.3

Chain A: PHE20 ASP23 VAL24 ILE31 PHE19 PHE20 ASP23 VAL24 ASN27 ILE31 PHE20 ASP23 VAL24 ASN27 ILE31 PHE20 ASP23 VAL24 ILE31 PHE20 ASP23 VAL24 ILE31 ASP23 VAL24 ASN27 ALA30 ILE31 PHE19 ASP23 ASN27 ILE31 PHE20 ASP23 VAL24 ASN27 ILE31 PHE20 ASP23 VAL24 ASN27 ILE31 ASP23 VAL24 ASN27 ALA30 ILE31

Figure 13: Metformin with 1IYT



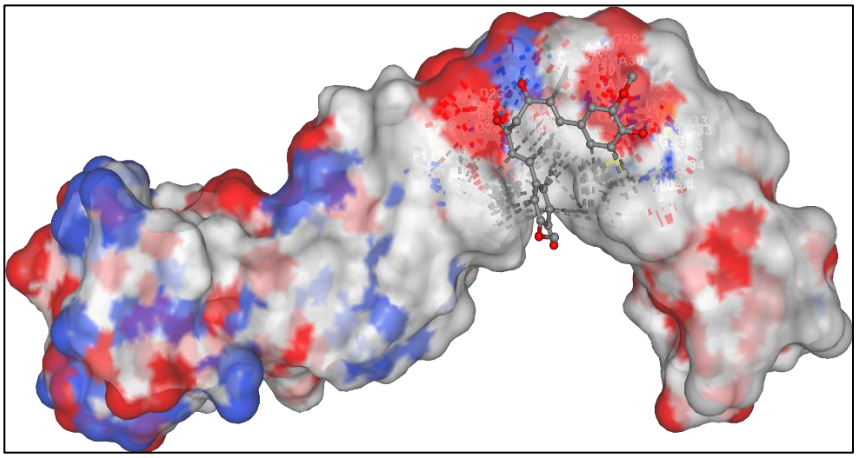


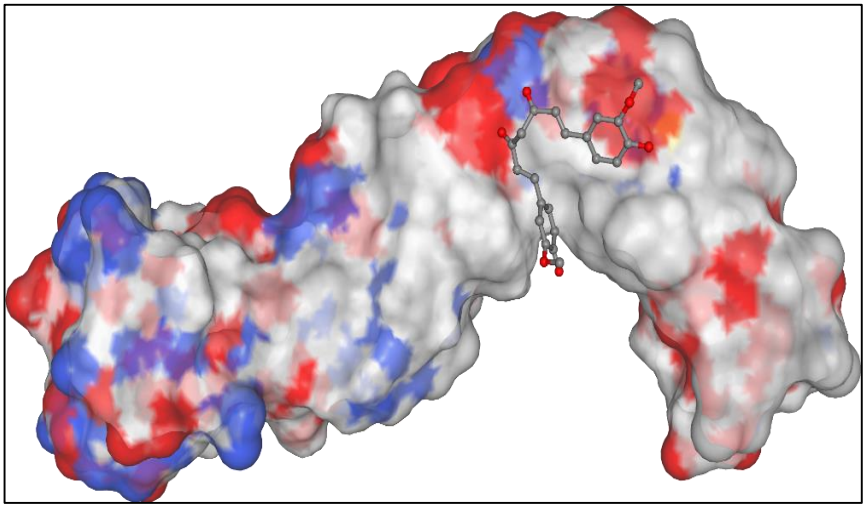
Curpocket ID	Vina Score	Cavity volume (A^{03})	Center (x,y,z)	Docking Size (x,y,z)
C1	**-3.9**	**20**	**-6,7,17**	**11,11,11**
C4	-3.5	13	0,-3,-8	11,11,11
C2	-2.8	17	-8,-5,17	11,11,11
C3	-2.7	15	-2,-3,13	11,11,11
C5	23.2	8	2,1,-8	11,11,11

Pocket: C1 & Score: -3.9

Chain A: GLU3 HIS6 ASP7 TYR10 GLU3 PHE4 ARG5 ASP7 HIS6 ASP7 SER8 TYR10 GLU3 PHE4 HIS6 ASP7 GLU11 ASP1 GLU3 PHE4 ASP7 GLU11 ARG5 HIS6 ASP7 TYR10 HIS6 ASP7 TYR10 GLU3 PHE4 ASP7 TYR10 GLU3 ASP7 TYR10 HIS6 ASP7 TYR10

Figure 14: Licl with 1IYT Protein



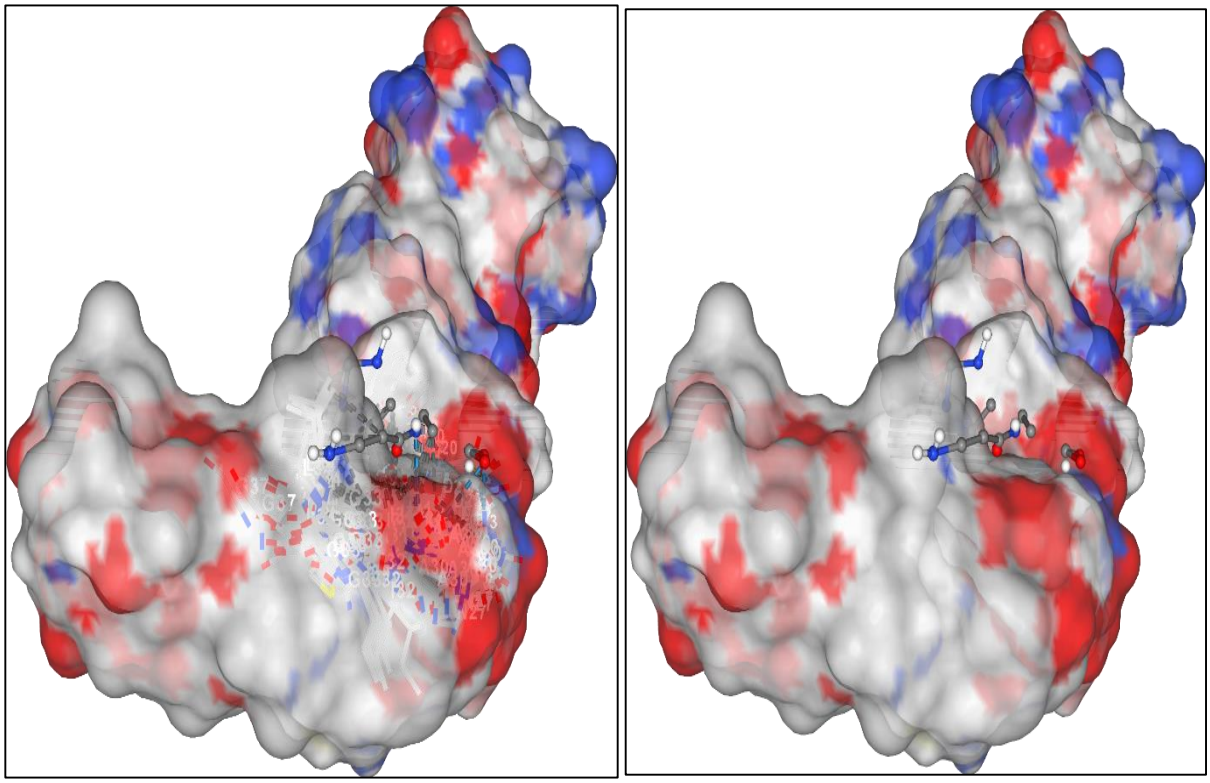


Curpocket ID	Vina Score	Cavity volume (A^{03})	Center (x,y,z)	Docking Size (x,y,z)
C4	**-30.2**	**13**	**0,-3,-8**	**26,26,26**
C5	-29.9	8	2,1,-8	26,26,26
C3	-26.2	15	-2,-3,13	26,26,26
C1	-21.9	20	-6,7,17	26,26,26
C2	-18.0	17	-8,-5,17	26,26,26

Pocket: C4 & Score: -30.2

Chain A: PHE20 ASP23 VAL24 ASN27 ALA30 ILE31 GLY33 LEU34 PHE20 ASP23 VAL24 ASN27 ALA30 ILE31 GLY33 LEU34 PHE20 ASP23 VAL24 ASN27 ALA30 ILE31 ILE32 GLY33 LEU34 PHE20 ASP23 VAL24 ALA30 ILE31 ILE32 LEU34 PHE20 ASP23 VAL24 ASN27 ALA30 ILE31 ILE32 LEU34 ASP23 ASN27 GLY29 ALA30 ILE31 ILE32 LEU34 ASP23 ASN27 ALA30 ILE31 LEU34 PHE20 ASP23 VAL24 ASN27 ALA30 ILE31 GLY33 LEU34 PHE20 ASP23 VAL24 ASN27 ALA30 ILE31 GLY33 LEU34 ASP23 ASN27 ALA30 ILE31 ILE32 LEU34

Figure 15: Curcumin with 1IYT Protein

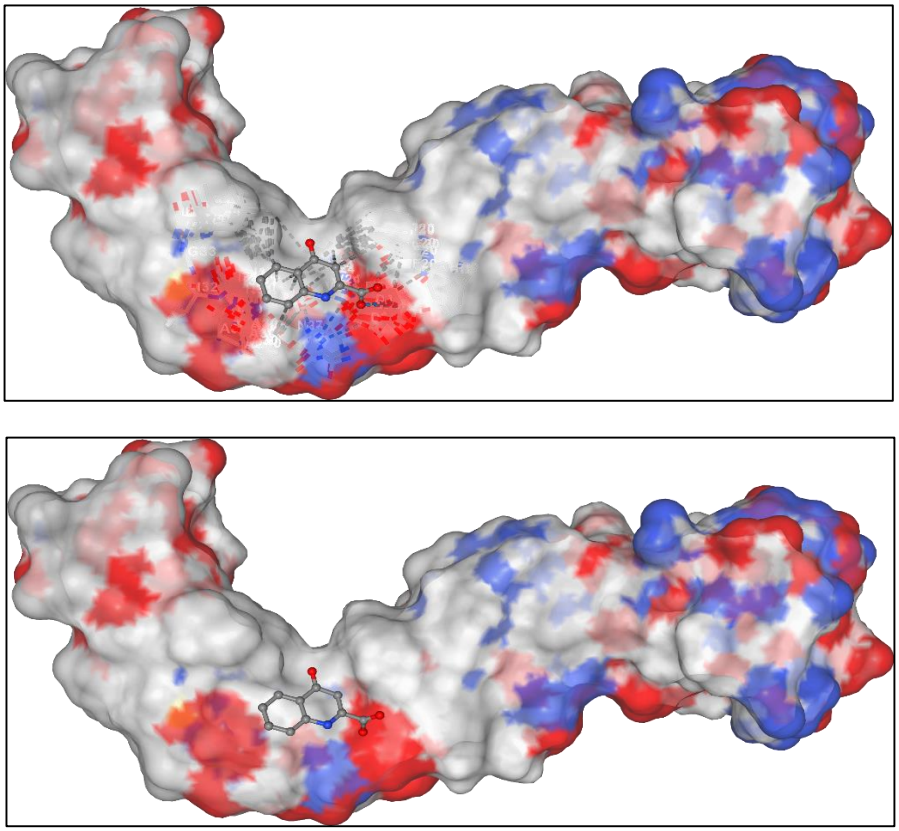


Curpocket ID	Vina Score	Cavity volume (A^{03})	Center (x,y,z)	Docking Size (x,y,z)
C4	**-28.6**	**13**	**0,-3,-8**	**23,23,23**
C5	-28.2	8	2,1,-8	23,23,23
C3	-21.5	15	-2,-3,13	23,23,23
C1	-20.7	20	-6,7,17	23,23,23
C2	-19.0	17	-8,-5,17	23,23,23

Pocket: C4 & Score: -28.6

Chain A: PHE20 ASP23 VAL24 ASN27 ALA30 ILE31 GLY33 LEU34 PHE20 ASP23 VAL24 ASN27 ALA30 ILE31 GLY33 LEU34 PHE20 ASP23 VAL24 ASN27 ALA30 ILE31 ILE32 GLY33 LEU34 PHE20 VAL24 ASN27 ALA30 ILE31 ILE32 LEU34 PHE20 ASP23 VAL24 ASN27 ALA30 ILE31 ILE32 GLY33 LEU34 ASP23 ASN27 ALA30 ILE31 ILE32 GLY33 LEU34 PHE20 ASN27 ALA30 ILE31 GLY33 LEU34 GLY37 PHE20 ASP23 VAL24 ASN27 ALA30 ILE31 GLY33 LEU34 PHE20 VAL24 ASN27 ALA30 ILE31 GLY33 LEU34 ASN27 ALA30 ILE31 ILE32 GLY33 LEU34

Figure 16: Carnosine with 1IYT Protein



Curpocket ID	Vina Score	Cavity volume (A^{03})	Center (x,y,z)	Docking Size (x,y,z)
C4	**-24.2**	**13**	**0,-3,-8**	**18,18,18**
C5	-22.4	8	2,1,-8	18,18,18
C1	-17.1	20	-6,7,17	18,18,18
C3	-14.8	15	-2,-3,13	18,18,18
C2	-14.2	17	-8,-5,17	18,18,18

Pocket: C4 & Score: -24.2

Chain A: PHE20 ASP23 VAL24 ALA30 ILE31 LEU34 PHE20 ASP23 ASN27 ALA30 ILE31 LEU34 PHE20 ASP23 VAL24 ASN27 ALA30 ILE31 ILE32 PHE20 ASP23 VAL24 ALA30 ILE31 PHE20 ASP23 VAL24 ALA30 ILE31 LEU34 ASP23 ASN27 ALA30 ILE31 PHE19 ASP23 ASN27 ALA30 LEU34 PHE20 ASP23 VAL24 ASN27 ALA30 ILE31 ASP23 ASN27 ALA30 ILE31 LEU34 ASP23 ASN27 ALA30 ILE31

Figure 17: KYNA with 1IYT Protein

Summary of Vina score

Drug name	Vina Score
Mito Q	-32.0
NAC	-15.7
BHA	-23.1
Metformin	-15.3
Lithium chloride	-3.9
Curcumin	-30.2
Carnosine	-28.6
Kynurenic acid	-24.2

Through its anti-inflammatory, anti-apoptotic and anti-oxidative effects, curcumin has been shown to mitigate spatial memory[15]. When NAC and curcumin are combined at a concentration of 3 uM and a dosage effect value (Fa) of 0.1, the combination index value is 0.96, indicating that the two medications work in concert. Synergism is indicated by a log (Fa-CI) plot below a log (CI) value and by a classic isobologram on the lower left of the hypotenuse.

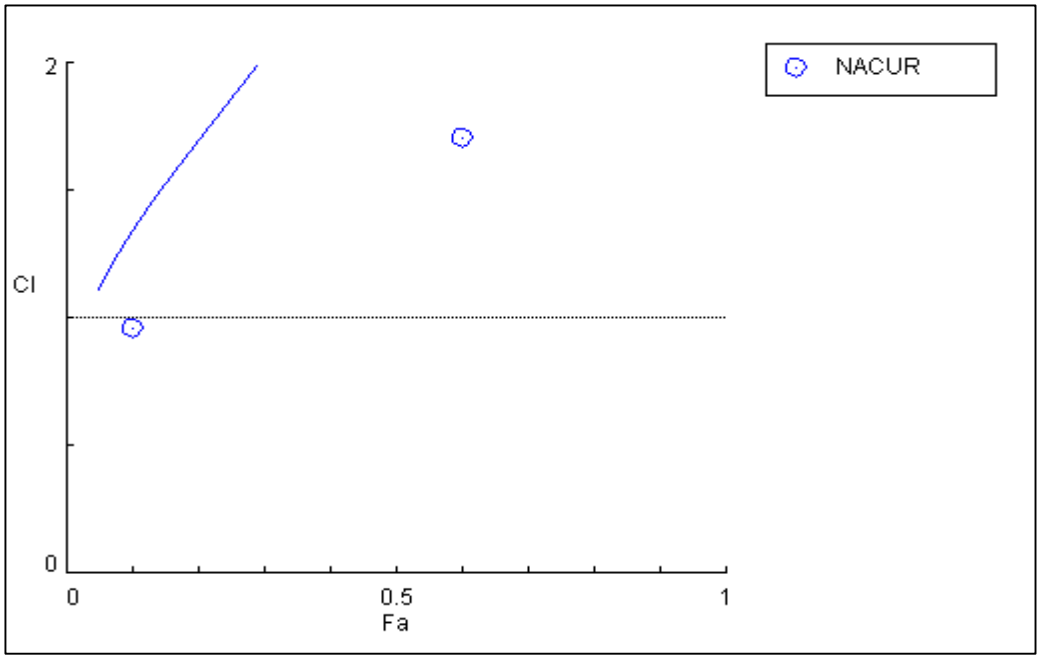


Figure 18: Combination index plot (NAC+ Curcumin)

Combination index value table (NAC+ Curcumin)

Total Dose (uM)	Fa (Dose effect)	CI value
3	0.1	**0.96**
30	0.2	3.77
45	0.3	3.11
60	0.4	2.58
75	0.5	2.12
90	0.6	1.70

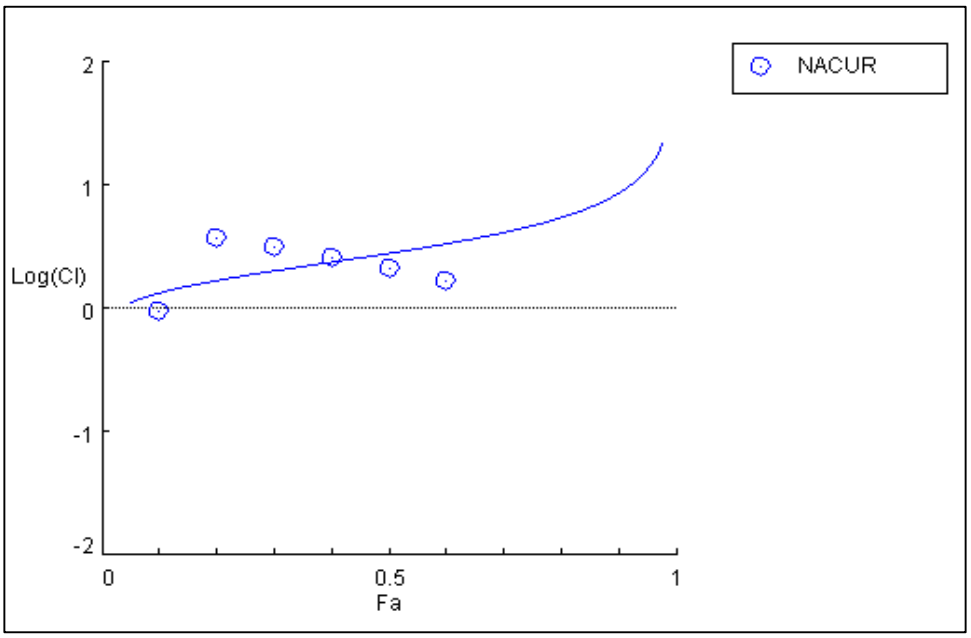


Figure 19: Log (Fa – CI) Plot (NAC+ Curcumin)

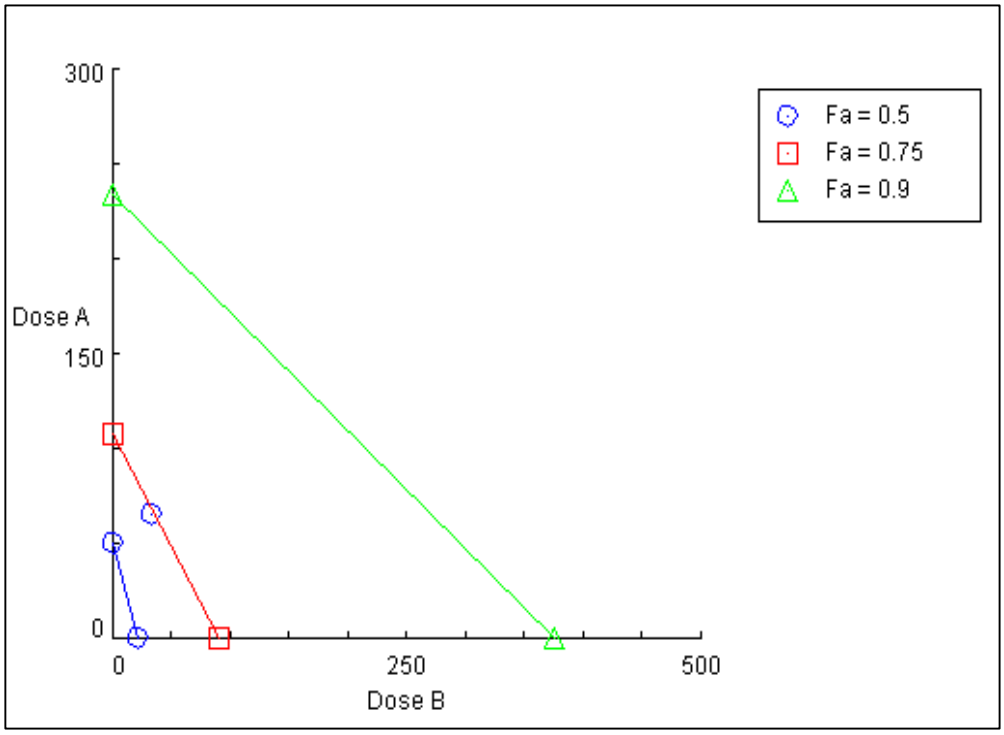


Figure 20: Isobologram (NAC+ Curcumin)

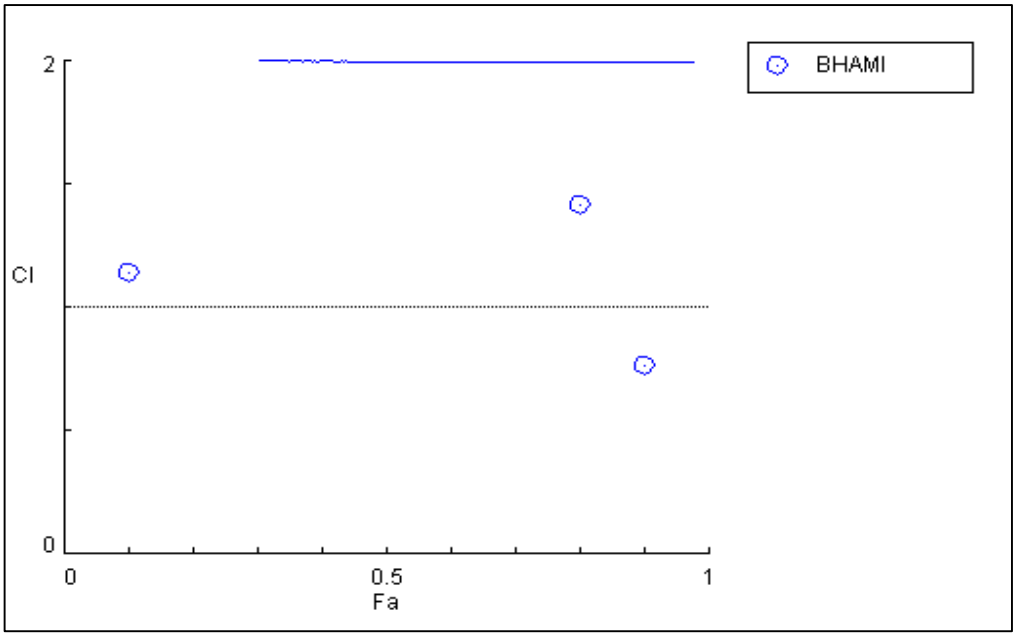


Figure 21: Combination index plot (BHA+ MitoQ)

Combination index value table (BHA+ MitoQ)

Total Dose (uM)	Fa	CI value
1.1	0.1	1.13
5.5	0.2	2.67
11.0	0.3	3.24
16.5	0.4	3.22
22.0	0.5	2.94
27.5	0.6	2.52
33.0	0.7	2.0
38.5	0.8	1.41
44.0	0.9	0.76

Combination Index for Butylated hydroxy anisole + Mito Q (5:0.5): Either external sources or the body's regular, necessary metabolic activities produce free radicals. Extremely reactive radicals are common. They are important for the breakdown of food and biochemical pathways. They have an impact on food's color, flavor, odor and nutritional content as well. There are two common synthetic antioxidants used in food: butylated hydroxytoluene (BHT) and butylated hydroxy anisole (BHA). Compounds known as antioxidants shield living systems from the damaging effects of ROS and free radicals[20]. Even at extremely low quantities, these biologically active substances have an impact. They mitigate the effects of free radicals and ROS from radiation, industrial pollutants, bad eating practices and break the auto-oxidative chain reaction that ROS started. Tert-butylhydroquinone, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are the most widely utilized antioxidants[14].

Early synaptic disruptions and neuropathology that underlie memory impairments in Alzheimer's disease (AD) are significantly influenced by mitochondrial dysfunction and abnormal release of reactive oxygen species (ROS). The antioxidant medication mitoquinone mesylate, which targets mitochondria, reversed the synaptic deficits caused by hAβ1-42[11].

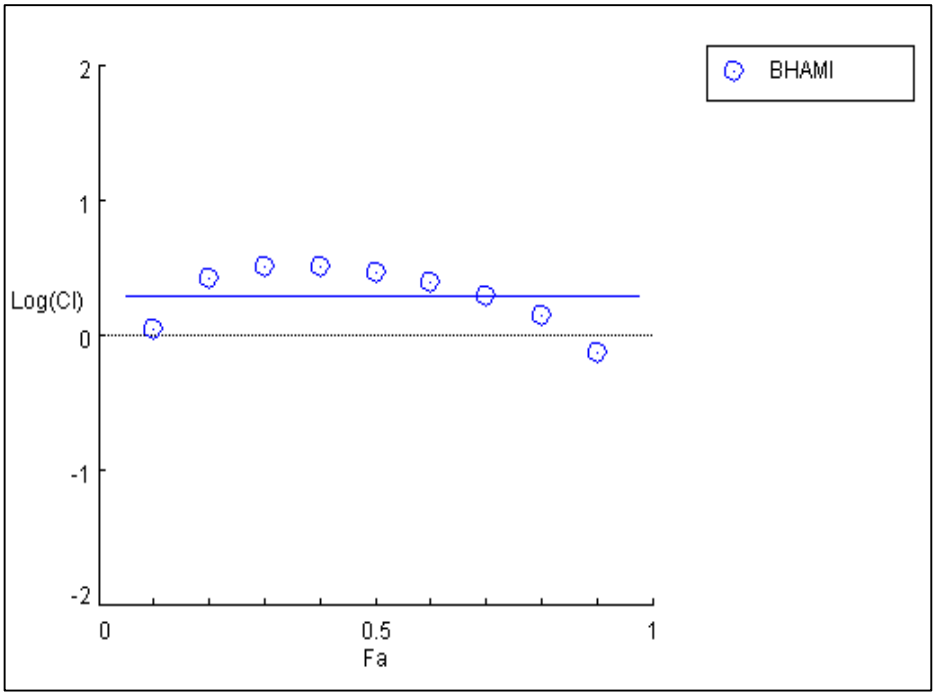


Figure 22: Log (Fa – CI) Plot (BHA+ MitoQ)

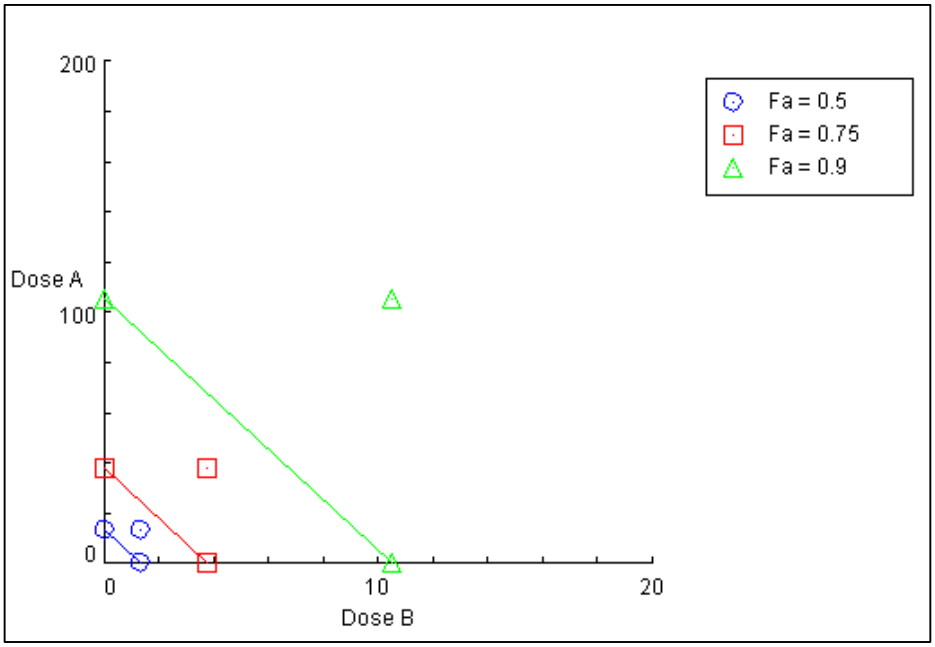


Figure 23: Isobologram (BHA+ MitoQ)

In a mouse model of AD, MitoQ reduces oxidative stress in the brains of the animals, preventing or at least delaying the disease's progression. To ascertain whether MitoQ treatment has a similar effect on human AD and to potentially address the role of mitochondrial ROS production in the genesis of the illness, if any, clinical trials with MitoQ will be required[21]. When BHA and Mito Q are combined at a concentration of 44 uM and a dosage effect value (Fa) of 0.9, the combination index value is 0.76, indicating that the two medications work in concert. Synergism is indicated by a log (Fa-CI) plot below a log (CI) value and by a Classic isobologram on the lower left of the hypotenuse.

Combination index for Carnosine + Kynurenic acid (1:1): More than a century ago, carnosine, a naturally occurring, endogenous molecule, was identified as a plentiful, nitrogen-containing, non-protein component of meat. However, because of its potentially positive impacts on human health, it has been the subject of intense research in recent years. Along with its analogues homocarnosine, anserine and ophidine/balenine, carnosine (β-alanyl-L-histidine) is a histidine-containing dipeptide (HCD) that is extensively found in mammalian tissues[16]. Carnosine is a naturally occurring dipeptide composed of β-alanine and L-histidine that is extensively present in mammalian tissues. The brain, skeletal and cardiac muscles contain the highest concentration of this dipeptide. Moreover, certain invertebrate species possess carnosin[2].

Tryptophan (TRP) is metabolized by the kynurenine pathway (KP) to produce kynurenic acid (KYNA). In neurodegenerative diseases like Alzheimer's disease (AD), this pathway is active. Mostly produced by astrocytes, KYNA is thought to have neuroprotective properties[7]. A promising therapeutic strategy involves raising KYNA levels, either by inhibiting the KP enzymes or by using prodrugs or analogs with strong potency and high bioavailability. KYNA has the potential to be a significant therapeutic for neurodegenerative illnesses, either by itself or in combination with other chemicals that precisely influence specific populations of neurons[12].

When kynurenic acid and carnosine are combined at a concentration of 10 uM, 20uM, 30uM, 40 uM and a dosage effect value (Fa) of 0.5,0.6,0.7,0.8, the combination index value is 0.70, 0.98, 0.99, 0.81 indicating that the two medications work in concert. Synergism is indicated by a log (Fa-CI) plot below a log (CI) value and by a classic isobologram on the lower left of the hypotenuse.

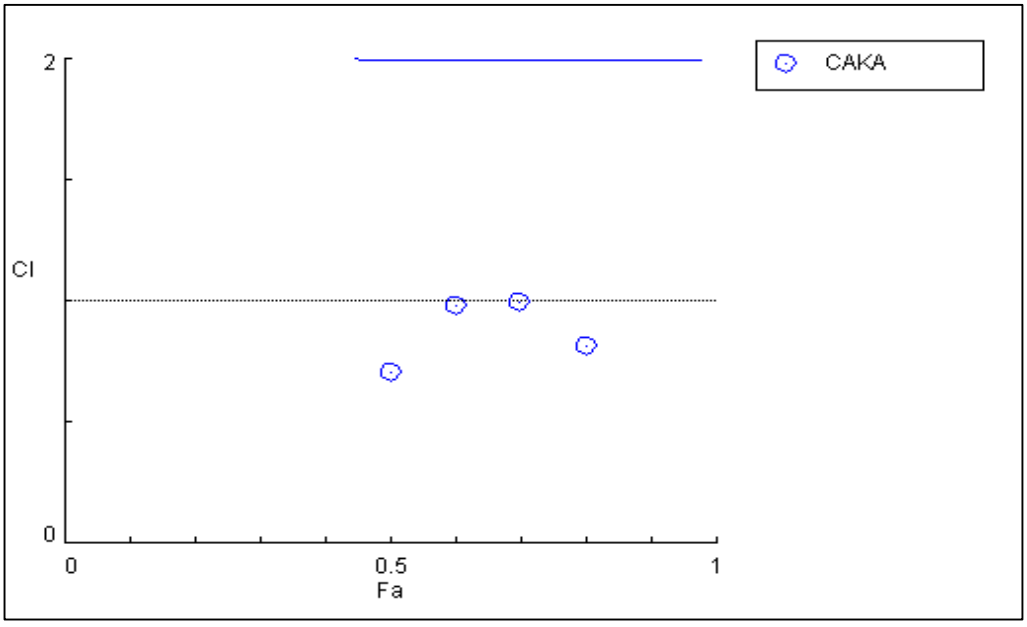


Figure 24: Combination index plot (Carnosine + Kynurenic acid)

Combination index value table (Carnosine + Kynurenic acid)

Total Dose (uM)	Fa(Dose effect)	CI value
10	0.1	5.03
20	0.2	4.88
30	0.3	4.52
40	0.4	4.00
10	**0.5**	**0.70**
20	**0.6**	**0.98**
30	**0.7**	**0.99**
40	**0.8**	**0.81**

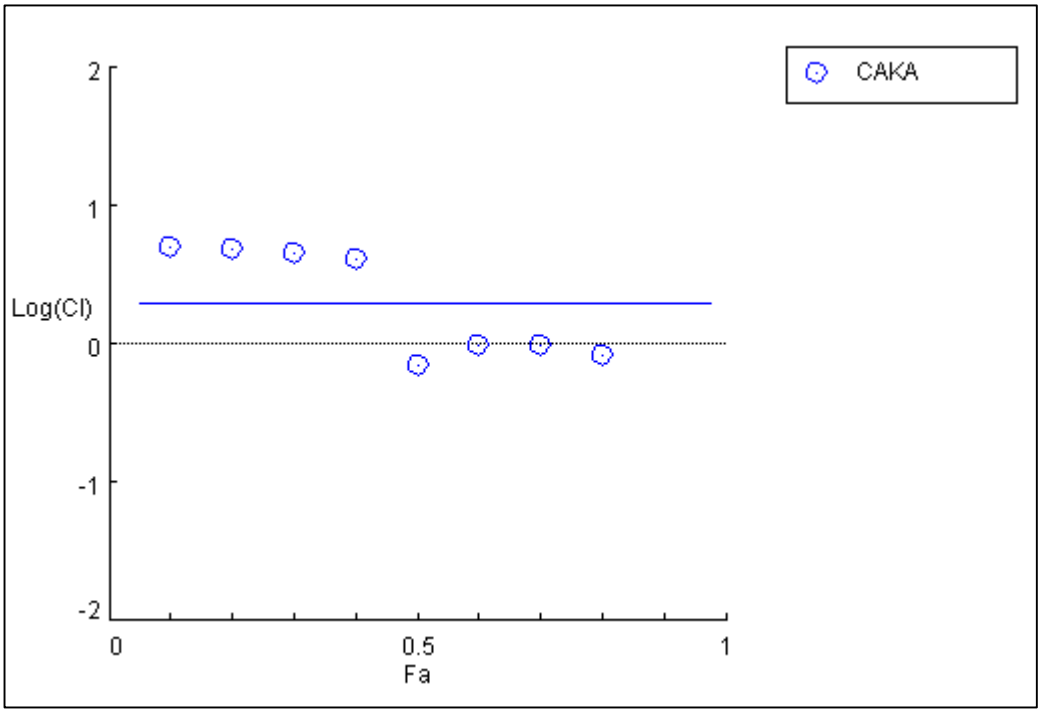


Figure 25: Log (Fa – CI) Plot (Carnosine + Kynurenic acid)

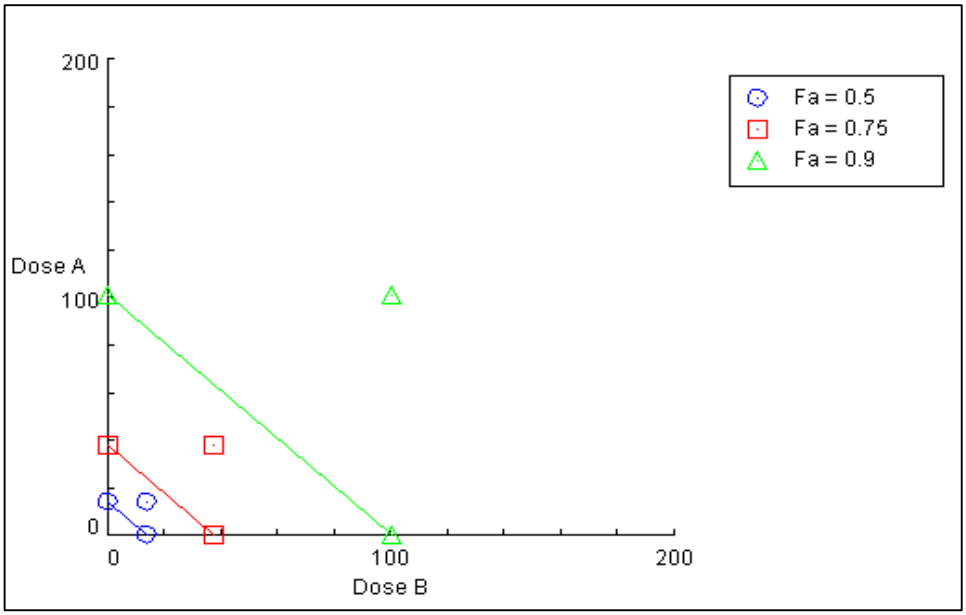


Figure 26: Isobologram (Carnosine + Kynurenic acid)

Conclusion

- *In vitro* molecular docking was used for high throughput screening of drugs. In virtual screening, the structure of drugs bound to $A\beta_{1-42}$ was generated using CB Dock. A web-based server for cavity detection guided protein-ligand blind docking. Above results shows that lithium chloride and metformin had been less effective as compared to all other drugs. It can further be employed for de novo design of combination drug against a amyloid beta disease-causing protein target.
- In order to address the many aging mechanisms and increase resilience to aging, a multi-target strategy may be helpful[9]. Combining existing medications has gradually grown to be a more desirable and economical alternative because creating new medicines is expensive, time-consuming and fraught with failure[17]. The combination of drugs carnosine + kynurenic acid, mitoquinone + butylated hydroxy anisole and N-acetyl cysteine + curcumin works synergistically to prevent Alzheimer's disease. In an *in vivo* experiment, three combined drugs might demonstrate synergism.
- Additionally, it has the potential to regenerate a drug molecule for a protein target, which might lead to the drug's potential to reuse in *in vivo* experiments on *Caenorhabditis elegans* Alzheimer's disease model**.**

Discussion

The findings presented regarding the efficacy of various drugs against amyloid beta (Aβ1-42) in Alzheimer's disease, as well as the exploration of combination therapies and drug repurposing, provoke several important points for discussion. The identification of less effective drugs, such as lithium chloride and metformin, underscores the challenges associated with finding effective treatments for Alzheimer's disease. Despite extensive research, many single-drug approaches have shown limited success in clinical trials, highlighting the need for alternative strategies.

The rationale behind combining existing medications lies in the complex nature of Alzheimer's disease. A multi-target approach acknowledges the diverse mechanisms underlying the disease's pathogenesis, aiming to address multiple pathological processes simultaneously.

By targeting different aspects of the disease, combination therapies may offer synergistic effects, potentially enhancing efficacy while minimizing adverse effects. This approach aligns with the growing recognition of Alzheimer's disease as a multifactorial disorder.

The shift towards combination therapies is driven not only by therapeutic considerations but also by economic and practical factors. Developing new drugs from scratch is a time-consuming and costly endeavor, often fraught with high rates of failure in clinical trials. In contrast, combining existing medications offers a more efficient and cost-effective approach to drug development. By leveraging drugs that have already undergone extensive safety testing and regulatory approval, combination therapies can accelerate the translation of research findings into clinical practice.

The mention of drug regeneration and repurposing highlights the potential of existing compounds to be repositioned for new therapeutic indications. This strategy capitalizes on the wealth of knowledge accumulated from previous drug development efforts, potentially unlocking new therapeutic applications for known compounds. However, drug repurposing also presents challenges including the need for rigorous preclinical and clinical validation to ensure safety and efficacy in the new indication. Additionally, intellectual property and regulatory considerations may impact the feasibility of repurposing efforts.

While *in silico* molecular docking studies provide valuable insights into potential drug-target interactions, experimental validation is essential to confirm the efficacy and safety of proposed treatments. *In vivo* experiments, particularly using animal models of Alzheimer's disease, play a crucial role in assessing the translational potential of candidate therapies. Furthermore, the use of models such as *Caenorhabditis elegans* Alzheimer's disease model provides a valuable platform for studying drug effects in a whole-organism context, facilitating the evaluation of complex physiological responses and potential side effects.

Acknowledgement

We are grateful to the Government of Gujarat for providing financial aid under the Scheme of Developing High Quality Research (SHODH) – MYSY. We are thankful to CVM University and Dr. Basudeb Bakshi, Principal of Natubhai V Patel College of Pure and Applied Sciences for providing support and infrastructure. We are thankful to CVM University for sanctioning grant under Student Startup and Innovation policy (SSIP).

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(Received 22nd May 2024, accepted 25th July 2024)