JÉSSIKA POLIANA TEIXEIRA

PROTEOSTASIS AND ITS RELATIONSHIP WITH AUTISM: AN *IN SILICO* STUDY

LAVRAS – MG
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Thesis submitted to the Universidade Federal de Lavras, as part of the requirements of the Graduate Program in Agrochemistry, area of concentration in Chemistry/Biochemistry.

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Orientador

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PROTEOSTASIS AND ITS RELATIONSHIP WITH AUTISM: AN *IN SILICO* STUDY

PROTEOSTASE E SUA RELAÇÃO COM O AUTISMO: UM ESTUDO *IN SILICO*

Thesis submitted to the Universidade Federal de Lavras, as part of the requirements of the Graduate Program in Agrochemistry, area of concentration in Chemistry/Biochemistry.

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To God for never leaving me and for being my support in the moments when I thought of giving up. To my parents and my brother for always believing in me. To my fiancé, for all the love and understanding. And, all the people who believe in science.
I dedicate it.

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"Do everything with LOVE, for LOVE and to LOVE, that is the secret."
(Saint Therese of the Child Jesus)

RESUMO

As doenças neurológicas são um desafio evidente para o sistema de saúde mundial, havendo uma necessidade crescente de desvendar profundamente os mecanismos nelas envolvidos. O Transtorno do Espectro do Autismo (TEA), por exemplo, é um transtorno do neurodesenvolvimento que, por compreender um amplo espectro de origem e evolução, ainda é uma incógnita para os pesquisadores. Assim, o fato de ter uma etiologia variada e pouco elucidada, traz grandes dificuldades no diagnóstico, além da escassez de tratamentos que proporcionem melhorias significativas na qualidade de vida dos pacientes com TEA. Ao longo dos séculos, as desordens ligadas a quadros neurológicos sempre foram tratadas de forma diferente porque diferiam exatamente em seu mecanismo básico, origem e evolução. No entanto, pesquisas recentes vêm identificando vários pontos em comum entre eles, o que certamente levará ao avanço nos estudos relacionados, proporcionando o desenvolvimento de diagnósticos mais rápidos e tratamentos mais eficazes. A desregulação da proteostase, por exemplo, vem chamando a atenção, pois tem sido apontada como comum em doenças neurológicas como Autismo, Alzheimer, Parkinson e Esclerose Lateral Amiotrófica. No caso do TEA, a síntese e a manutenção de proteínas são citadas em vários trabalhos científicos, mas pouco se sabe sobre os mecanismos por trás de seu papel na doença. Este trabalho, portanto, traz um estudo que pretende ser aprofundado o suficiente para determinar a relação entre proteostase, síntese proteica, manutenção, morte e TEA. O objetivo principal é desvendar os mecanismos-chave no controle e manutenção de proteínas, a fim de encontrar alvos potenciais para tratar o TEA. Para tanto, o artigo faz uma revisão dos tópicos científicos mais recentes que relacionam o autismo à síntese de proteínas excitatórias e sugere três proteínas-alvo ligadas ao processo para estudos posteriores. A proteína eIF4E em específico, também chamada de fator de tradução eucariótica, foi um dos alvos mapeados durante o estudo de revisão, material base de todo o trabalho. As descobertas científicas sugerem que inibir a formação do complexo de iniciação eIF4E-eIF4G é uma atividade promissora para tratar alguns comportamentos relacionados ao autismo. Para tal, utilizou-se como alvo a proteína eIF4E, tendo-lhe sido aplicados alguns testes e estudos in silico, de forma a validar algumas hipóteses científicas, e encontrar potenciais inibidores da síntese proteica e da sua atividade excitatória. A prática começa com uma pesquisa de triagem virtual, baseada no ligante já testado e validado como inibidor, encontrado em vários trabalhos como inibidor da proteína em questão, o 4EIG-1. Após a seleção dos ligantes com sítio farmacofórico semelhante, por meio do processo de triagem virtual, foram realizados testes de absortividade, distribuição, metabolismo, excreção, toxicidade, docking e dinâmica molecular. Ao final do processo, são sugeridas moléculas que podem iniciar a linha de pesquisa de inibidores proteicos do fator de tradução eucariótico. Além de serem direcionados testes experimentais com os melhores inibidores, potencializando a possibilidade destes achados na criação de um medicamento para tratamento do TEA.

Palavras-chave: Triagem Virtual. Proteostasis. Química Computacional. Neurodesenvolvimento. mTOR.

ABSTRACT

Neurological diseases are a clear challenge to the world health system, and there is a growing need to thoroughly unravel the mechanisms involved in them. Autism Spectrum Disorder (ASD), for example, is a neurodevelopmental disorder, which, because it comprises a large spectrum of origin and evolution, is still a great challenge for researchers. Thus, the fact that it has a varied and poorly elucidated etiology, brings great difficulties in diagnosis, in besides the scarcity of treatments that provide significant improvements in the quality of life of patients with ASD. Throughout the centuries, the disorders linked to neurological conditions have always been treated differently because they differed exactly in their basic mechanism, origin, and evolution. However, recent research has been identifying several commonalities among them, which will certainly lead to progress in related studies, providing the development of faster diagnoses and more effective treatments. Dysregulation of proteostasis, for example, has been drawing attention, as it has been identified as a common feature in neurological diseases such as Autism, Alzheimer's, Parkinson's, and Amyotrophic Lateral Sclerosis. In the case of ASD, protein synthesis and maintenance are cited in several scientific papers, but little is known about the mechanisms behind its role in the disease. This paper, therefore, brings a study that aims to be thorough enough to determine the relationship of proteostasis, protein life, maintenance and death, and ASD. The main goal is to unravel the key mechanisms in protein control and maintenance in order to find potential targets to treat ASD. To this end, the paper provides a review of the most recent scientific topics that correlate autism to excitatory protein synthesis and suggests three target proteins linked to the process for further study. The eIF4E protein in specific, also called eukaryotic translation factor, was one of the targets mapped during the review study, the base material of all work. The scientific findings suggest that inhibiting the formation of the eIF4E- eIF4G initiation complex is a promising activity to treat some autism-related behaviors. To this end, the eIF4E protein was used as the target, and some in silico tests and studies were applied to it, in order to validate some scientific hypotheses, and find potential inhibitors of protein synthesis and its excitatory activity. The practice starts with a virtual screening search, based on the ligand already tested and validated as an inhibitor, found in several papers as an inhibitor of the protein in question, the 4EIG-1. After the selection of ligands with similar pharmacophore site, through the virtual screening process, tests of absorptivity, distribution, metabolism, excretion, toxicity, docking, and molecular dynamics were performed. At the end of the process, molecules are suggested that can start the research line of eukaryotic translation factor protein inhibitors. Besides being directed experimental tests with the best inhibitors, potentiating the possibility of these findings in the creation of a drug to treat ASD.

Keywords: Virtual Screening. Proteostasis. Computational Chemistry. Neurodevelopment. mTOR.

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SUMMARY

FISRT PART

1. INTRODUCTION

Neurological disorders are one of the leading causes of disability and death worldwide and account for 16.8% of deaths. The recognition of these pathologies as a global public health challenge is increasingly evident. The great challenge is to better understand the evolving mechanisms and to delineate the genetic and external factors involved in neurological disorders (APPELBAUM et al., 2022; FEIGIN et al., 2020; GUNATA; PARLAKPINAR; ACET, 2020).

Neurological diseases are said to have complex and varied etiologies, differing in their basic mechanisms of both origin and development, and taking these last facts into consideration, they have always been treated in different ways. The complexity and delicacy of the human brain has always represented a challenge in the studies and research related to its development, function, and disorders (PASKO et al, 2022; WANG, 2018).

Although the above notes take into account the distinction of neurological disorders, it is necessary to remember the fact that these diseases affect a common metabolic pathway. And, even with their peculiarities, studies and research prove that they may have more similarities than one might think. The discovery of these commonalities represents both a challenge and a breakthrough in research related to the diagnosis and treatment of neurodevelopmental disorders (JĘŚKO et al., 2020; LIU; TAN; TAN, 2023).

Proteostasis, or protein homeostasis, is responsible for keeping protein synthesis, folding, clustering, and degradation at the right place and in the right amount (LOTTES; COX, 2020; TEDESCO et al., 2023). Brain functions are highly dependent on proteostasis, because, unlike the other cells that make up living organisms, neurons have high durability and are formed by complex and varied structures, which requires strict control and maintenance. The control, maintenance and degradation of the machinery of protein synthesis is essential, any minimal dysregulation in any of these mechanisms results in neurological dysfunctions that can cause disorders such as Autism, Alzheimer's, Parkinson's (HUANG et al., 2023, JAYARAJ; HIPP; ULRICH HARTL, 2020; JOSHI et al., 2020).

In this sense, studies and research that unveil all the chemical and biological processes related to neurological disorders are necessary. By unveiling the mechanisms involved, highlighting the relationship between them, it will be possible to come up with proposals capable not only of treating, but also of diagnosing these pathologies.

The work is divided into two objectives. The first is a review study that aims to discuss the proteins that are related to autism and involved in the mTOR pathway, as well as potential targets for its treatment and possible diagnosis (AMORIM; LACH; GKOGKAS, 2018; TARAFDAR; PULA, 2018). The second objective is to find a potential inhibitor for one of the proteins found as target in the review study. The intent is to perform a thorough *in silico* study, covering virtual screening, molecular docking, toxicology tests, absorption, distribution, metabolism, excretion, and the molecular simulation test to propose a molecule that has the potential to treat autism.

2. OBJECTIVES AND GOALS

The main objective of this work is to investigate potential ligands to treat Autistic Spectrum Disorder (ASD), as well as to evaluate the role of protein synthesis in the development of neurological diseases.

Second, a thorough study of protein synthesis and the mechanisms essential for its control and maintenance was performed to find potential targets to treat ASD. eIF4E was the protein evaluated as a therapeutic target, aiming to inhibit the eIF4E- eIF4G complex, and new inhibitors were investigated.

Finally, ligands were previously selected by virtual screening and a theoretical study using computational methods and tools such as docking was performed in order to evaluate the interaction of the inhibitor (ligand) with the protein (eIF4E).

3. THEORETICAL REFERENCE

3.1 Neurological Disorders

Neurological disorders affecting neurodevelopment, or those characterized as neurodegenerative, have always been treated as distinct disorders because they differ in their basic mechanism, origin, and development. And indeed, each of these disorders affects a common metabolic pathway, but in a unique and distinctive way. Recent research has identified many similarities in this set of disorders, and has found that the shared

commonalities may lead to progress in research on the subject, and consequently to the development of more effective treatments (JĘŚKO et al., 2020).

Protein synthesis and its control mechanisms are among the essential processes for the proper functioning of the human body. Protein homeostasis, also called proteostasis, is responsible for keeping the life cycle of proteins balanced by ensuring that they fold, assemble, and break down at the right place and in the right amount. The three main functionalities that govern proteins are: the machinery of protein synthesis, the maintenance mechanisms, and the degradative pathways (LOTTES; COX, 2020).

Brain functions are closely related to proteostasis, as are other essential processes and functions for the body, any alteration in one of the three basic mechanisms of proteostasis can generate serious damage to health. Neurons specifically, distinguish themselves from other cell types by having a long-life span, as well as complex structures and diverse modeling, characteristics that make the protein maintenance process essential for their proper functioning. Dysfunction in proteostasis is extremely harmful to neurons, especially dendrites that require tightly controlled homeostasis for their development and maintenance. Dysregulation of proteostasis results in neurological dysfunctions that can lead to neurological disorders such as autism, Alzheimer's, amyotrophic lateral sclerosis, and Parkinson's (HUANG et al., 2023; JAYARAJ; HIPP; ULRICH HARTL, 2020; JOSHI et al., 2020; LOTTES; COX, 2020).

The most common neurodegenerative diseases are Alzheimer's disease (AD) and Parkinson's disease (PD). AD is characterized by progressive loss of neurons and neurotoxicity of proteins that tend to aggregate, such as tau protein and β-amyloid (Aβ). The degeneration seen in PD is also associated with protein (α-syn) dysfunction and deposition. The cerebral cortex and cerebellum of those with ASD demonstrate abnormalities in signaling pathways linked to PD. In addition, studies suggest that amyloid precursor protein (APP), presenilin-1 and tau may be involved in TEA. Therefore, important molecular pathways may be commonly involved in neurodegenerative diseases and neurodevelopmental disorders as in the case of ASD (ABRAHAM et al., 2019; JĘŚKO et al., 2020).

Studies show that metformin, an antihyperglycemic agent, has potential anti-inflammatory and immunosuppressive efficacy. The drug decreases beta-amyloid plaque deposition and chronic inflammation, in addition to increasing AMPK production and suppressing P65 NF-κB, mTOR, and S6K (add protein name) activation and consequently BACE-1 production, contributing to the improvement of neurological deficits (OU et al., 2018). This fact proves that by treating neurological diseases as a set of dysfunctions with

shared features, it is possible to more easily arrive at treatments that are effective in more than one neurological disease and that aid in prevention.

3.1.1 Autistic Spectrum Disorder (ASD)

ASD is a complex disorder related to neurodevelopmental and neurobehavioral issues. In ASD, changes in social, emotional, and psychological skills, restricted patterns of behavior and/or interests, and altered sensory processing are observed (MAROTTA et al., 2020; WANG et al., 2023).

Autism has a diverse group of etiologies, and because it covers a distinct group of disorders it receives the name Autistic Spectrum Disorder. Autism can be classified into two types, the syndromic type when it has known causative agents, as in the case of monogenic disorders, and the idiopathic type when its genetic causes are unknown. Among the known monogenic disorders that are related to autism are polyhydramnios, megalencephalic, symptomatic epilepsy syndrome (PMSE), phosphatase and tensing homolog (PTEN), fragile X syndrome (FXS), and neurofibromatosis (FAUS-GARRIGA; NOVOA; OZAITA, 2017; GANESAN et al., 2019).

External factors such as exposure to toxins, pesticides, infections, and contact with medications in the womb have also been pointed out as potential causes of autism. In addition, maternal depression, alteration of the mother's immune system during pregnancy, altered oxytocin levels, and protein regulation are also pointed out as contributors to ASD (LAMPIASI et al., 2023; MAROTTA et al., 2020; SINGH et al., 2023).

The molecular dysfunctions verified in autistic individuals interfere in the synthesis of synaptic proteins, which alters their development and plasticity, leading to autistic behaviors. In this sense, the deepening of protein synthesis and its regulation mechanisms become promising, and may contribute to the development of therapies for autism. The mTOR protein, for example, is considered the matriarch of the protein regulation process and, together with other proteins that make up its pathway, has been highlighted as a potential way to diagnose and treat ASD (KELLEHER; BEAR, 2008, PURUSHOTHAM et al 2022; WANG et al, 2033).

The eukaryotic translation initiation factor eIF4E is essential to sequence protein synthesis and is therefore among the key components of the mTOR pathway. Control of the eIF4E protein is related to many processes that include cell progression, survival, and

motility, as well as tumorogenesis, inflammation, immunity, and infection. Besides of course the relationship of eIF4E to neuronal cells, which reveal its uncontrolled function in neurodevelopmental disorders such as autism (AMORIM; LACH; GKOGKAS, 2018; PURUSHOTHAM et al 2022; SUN et al., 2023).

The research on ASD also investigates other proteins related in one way or another to proteostasis. Among them are mGLUR's and GRK5, the intention is to develop inhibitors/agonists of these proteins in order to improve the living conditions of individuals with ASD, and to enable early diagnosis of this disorder.

3.2 Computational Medicinal Chemistry

The technological development concomitant with the rise in increasingly powerful computational resources, make it possible to study the behavior of molecular systems. The ability to accurately simulate interactions between molecules is a powerful tool to unravel the properties of different types of substances, allowing faster and lower cost development of compounds for applications in various areas such as medicine, pharmacy, agriculture, technology, among others (CRAIG et al., 2020).

The model for predicting molecular properties depends on the methods that are used, based on this aspect they can be called quantum or classical. The quantum methods are those based on the Schrödinger equation and that allow to obtain information related to the electronic structure of the molecules (KREMS, 2019). However, it has the limitation of describing large molecular systems, as in the case of proteins, because they require high computational demands. Thus, this type of method is indicated for the description of smaller molecular systems (DURUMERIC et al., 2023; NAIRS; MINERS, 2014).

Among the computational methods developed based on the approximations of classical physics (Newton's equations) is Molecular Mechanics (MM). MM is used to treat large systems with a high number of atoms in their structure as in the case of proteins (Vennelakanti et al, 2022). This type of methodology does not treat electrons explicitly, which makes the process less time consuming because it requires less computational demand. Force fields are used to describe structures and conformations of molecules, and also predict the intra and intermolecular interactions between the particles that compose the systems, thus describing their potential energy. The choice of a specific force field depends on the type and properties of the system to be investigated (DURUMERIC et al., 2023; SANT’ANNA, 2009).

The force field allows the description of the total potential energy of the system V(r) (Eq. 1) described as the sum of the various energy terms, those for bonded atoms such as bond lengths and angles, dihedral angles, and the terms for unbonded atoms described by van der Waals and Coloumb interactions. The force field can be described using the equation

below quotes equation in the text:

$$V(r) = \sum V_l + \sum V_\theta + \sum V_\emptyset + \sum V_{vdW} + \sum V_{elet} \quad \text{Eq.1}$$

BOUND TERMS **UNBOUND TERMS**

The term V_l represents the stretching energy of the bond relative to its equilibrium, or ideal, value, V_θ is the strain energy of the bond angle with respect to its equilibrium value, $V_\emptyset$ is the torsion around the bond angle, V_{vdW} represents the energy of the interactions of van der Waals e V_{elet} represents the energy of interaction or electrostatic repulsion between two charges (NAMBA; DA SILVA; DA SILVA, 2008).

3.2.1 Virtual Screening

Virtual Screening is an efficient method in the search for new drugs, and when applied correctly, it is much faster and less expensive than experimental approaches. This approach occurs at an early stage of discovery, in which promising compounds are found in large databases of chemical substances (CARPENTER et al., 2018; COURNIA et al., 2020).

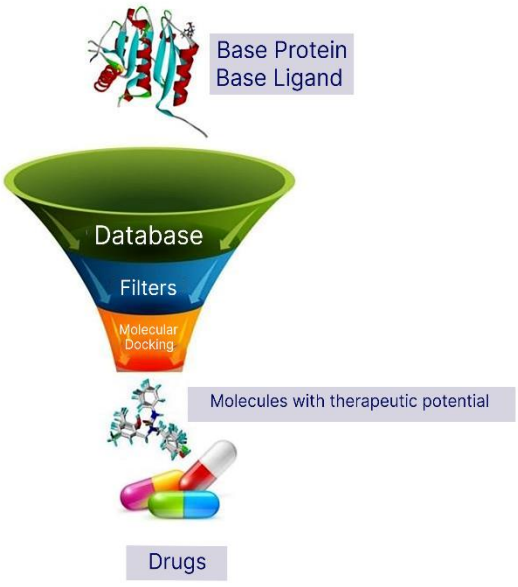
This search method is increasingly being used by large pharmaceutical companies. The aim is to find compounds for the target of interest, these searches are done in libraries that have accumulated millions of molecules over time, from internal research, company acquisitions, and publicly available chemical sources. The methods used in this virtual search vary, some of them relying on the similarity of known active molecules, such as the ligand. Others, however, use information based on the target protein, based on the structure. When both ligand and structure information is available, it is possible to combine methods to generate better results (COURNIA et al., 2020).

The ability to find active compounds using this methodology is highly dependent on the complexity of the target in question, proteins in most cases. When the target is well characterized and has many known active compounds, it is possible to find several additional

active molecules in a few seconds using the ligand-based method. However, when the protein in question is known, but there are no known active targets, the chance of finding assertive compounds is low. It is worth remembering that before taking any steps to analyze the interaction of protein and ligand, it is necessary to filter out the toxic compounds; it makes no sense to analyze a substance with toxic potential in view of developing a drug. For this filtering activity there are specific programs that use Lipinski's Rule of Five (CARPENTER et al., 2018; COURNIA et al., 2020; KANDEEL; AL-NAZAWI, 2020).

Virtual screening associated with other computational methods is being widely used to understand the molecular aspects of proteins, ligands and the interaction between them (COURNIA et al 2020). It also provides studies of the structural and morphological aspects mainly aiming at the development of new drugs. The use of this approach is frequent in the attempt of discovery for the treatment of fatal diseases (CARPTNER et al., 2018; COURNIA et al 2020).

Figure 1. Virtual Screening.



3.2.2 Molecular docking

Docking is a computational tool belonging to MM, important for the discovery of new drug candidates, because it is able to measure the degree of interaction between a ligand and the target molecule.

The docking method has the ability to generate 3D poses, which makes it possible to predict the conformation of ligands within the binding site of the macromolecule. In other words, through this method it is possible to investigate crucial molecular events, including binding mode and intermolecular interactions that are able to stabilize the ligand-receptor complex. Docking also provides quantitative energy predictions, ranking the coupled compounds according to the degree of affinity of the ligand-receptor interaction (FERREIRA et al., 2015; LI et al., 2021).

The affinity of the ligand for the receptor is measured by predicting the preferred orientation and minimum binding energy. The various types of non-covalent interactions are present in this approach among them are hydrogen bonds, ionic bonds, hydrophobic bonds, and van der Waals bonds. The steps for performing the docking consist of preparing the 3D structure of the proteins, preparing the ligands, estimating the energy of protein-ligand complex formation, and analyzing the results (RNAGARAJU; RAO.,2013).

At the time of the simulation, the ligand is at a certain distance from its molecular target, over time it manages to fit into the active site of the target in question. Rotational and translational movements, as well as internal changes in the structure of the ligand including torsional angle rotations, induce a total energy cost of the system, so after each movement the total energy of the system is calculated (KITCHEN et al., 2004).

Molecular docking can be classified into three different types. The first, called rigid docking, only the ligand has conformational freedom during the procedure. In the semi-rigid docking, besides the ligand, some residues of the receptor are kept free. In the flexible docking, all the species of the complex are kept free (FAN; FU; ZHANG, 2019).

In the process of molecular recognition between protein-ligand, both entropic and entropic contributions are considered (BROOIJMAS et al., 2003). These effects can be estimated by means of the Gibbs free energy of binding (ΔG^0_{lig}), which is related to the inhibition constant according to equations 1 and 2:

$$\Delta G^0_{lig} = \Delta H + T\Delta S \quad \text{Eq.1}$$

$$\Delta G^0_{lig} = RT\ln K_i \quad \text{Eq.2}$$

In the equations above, the ΔH represent the enthalpy change of the system, T the temperature, ΔS the entropy variation and R the universal constant for gases. For determining the ΔG^0_{lig} and understanding its behavior it is possible to predict with great certainty how the system will behave, which aids in explaining the processes important for molecular ligand-

receptor recognition (MAGALHÃES et al., 2007).

The value of ΔG^0_{lig} can be estimated by means of computational methods that use molecular dynamics simulations associated with the use of the classical molecular force field, the free energy perturbation method, and the thermodynamic integration method. The methods cited are classified as exact, but require a high computational demand, which limits their use (KOLLMAN, 1993; VAN GUNSTEREN *et al*., 1994; GENHEDEN *et al*., 2010; ZHAO *et al*., 2010).

The main objective of the software used for docking is the development of an evaluation model/scoring function capable of predicting as accurately as possible the ΔG^0_{lig}. Most docking software makes use of scoring functions, which are potential energy functions, most often based on force fields (HUANG e ZOU, 2010b). The values of the scoring function used for example by the MVD® (Molegro Virtual Docker) program is defined by the equation 3, below:

$$E_{score} = E_{inter} + E_{intra} \quad \text{Eq.3}$$

Where E_{inter} is the interaction energy between protein and ligand and E_{intra} is the internal of the ligand (SHAH et al., 2022). E_{inter} is calculated by the following equation:

$$E_{inter} = \sum_{ligand} \sum_{j=protein} [\, EPLP\,(rij) + 332.0 \frac{qiqj}{4r2ij}] \quad \text{Eq.4}$$

The term E_{PLP} refers to the "piecewise linear" potential which is based on two parameters, one for the approximation of the steric (van der Waals) term between atoms and another potential for the hydrogen bond. The MVD program is able to describe the electrostatic interactions between charged atoms (second term). The second term is the adaptation of Coulomb's Law to provide the energy value in Kcal/mol.

E_{intra} is calculated according to the equation (SHAH et al., 2022):

$$E_{intra} = \sum_{i=ligand} \sum_{j=protein} EPLP\,(rij) + \sum_{flexiblebond} A\,[1 - \cos(m\theta - \theta)] + Eclash \quad \text{Eq.5}$$

The first two sums refer to all pairs of atoms of the ligand that are not connected by two bonds. The second term refers to the torsion energy, where θ is the angle of torsion of the

bond. The last term, Eclash, assigns a penalty of 1000 if the distance between two heavy atoms (more than two bonds apart) is less than 2.0 Å, punishing non-existent conformations of the ligand (SHAH et al., 2022).

3.2.3 Molecular Dynamics

Molecular dynamics (MD) is a computational approach grounded in classical mechanics, is able to provide information about interaction and movement of atoms and molecules in time. MD is the most widely employed technique to understand and predict properties, structure, and function of biological macromolecules in time (BRAUN et al 2019).

The molecules that make up the systems in a MD simulation are treated as a collection of particles held together by elastic or harmonic forces, which makes it impossible to observe for example the interaction between orbitals, the breaking of a bond, the transfer of charges, and any other effect that involves electrons (BRAUN et al 2019).

A MD software has algorithms that represent the numerical solution of the equations of motion, providing a trajectory (coordinates and moments as a function of time) of the system under study. From the obtained trajectory, it is possible to extract properties such as energy, average number of hydrogen bonds formed during the simulation, RMSD, RMSF, average distance between atoms, spin radius, among others (VAN DER SPOEL *et al*., 2005). The technique can be employed both in electron, atom, or molecule systems, and in macromolecular systems.

In biomolecular systems such as proteins, simulations are performed inside a "water box". This is due to the need to minimize boundary effects. The replicas of the simulation box are arranged around the main cell so as to produce a system that tends to the thermodynamic limit. The number of molecules (N) and the volume (V) tend to infinity, but the number density (N/V) is kept constant. In the replicas, the particles move in the same way as in the central cell, so the motion of any particle is not limited to the walls of the box (MARK *et al*, 2000; MARTÍNEZ, 2007).

The Hamiltonian operator (H) is used to describe a classical molecular system, it is represented by the sum of the kinetic (K) and potential (V) energies as a function of the generalized coordinate series ql and all generalized moments pl of all N atoms of the system Eq. 6 (TUCKERMAN E MARTYNA, 2000).

$$H(\{\vec{q}_l, \vec{p}_l\}) = K(\{\vec{p}_l\}) + V(\{\vec{q}_l\}) \quad \text{Eq. 6}$$

Where: $\vec{q}_l = \vec{q}_1, \vec{q}_2, \vec{q}_3 \ldots\ldots\ldots, \vec{q}_N$ e $\vec{p}_l = \vec{p}_1, \vec{p}_2, \vec{p}_3 \ldots\ldots\ldots, \vec{p}_N$.

The potential energy term $V(\{\vec{q}_l\})$ describes the short and long range intra- and intermolecular interactions. To describe the kinetic energy of the system, Eq.7 is used, where mi represents the mass of the atom *i*.

$$K(\{\vec{p}_l\}) = \sum^{N}_{i=1} \vec{p}_l / 2m_i \quad \text{Eq.7}$$

The dynamics of particles can be described by equations of motion constructed from *H*, whose derivatives lead to Newton's equations of motion.

$$\dot{q}_i = \partial H/\partial p_i \quad \text{e} \quad \dot{p}_i = -\partial H/\partial q_i \quad \text{Eq. 8}$$

$$\dot{r}_i = p_i/ m_i = v_i \quad \text{Eq. 9}$$

$$\dot{p}_i = m_i \ddot{r} = -\partial V(\{\vec{r}_l\}) /\partial r_j = F_i \quad \text{Eq. 10}$$

The term $\ddot{r}$ represents acceleration of the atom *i* and *F* the force on the atom *i* (NAMBA *et al*., 2008). The DM technique consists of solving the equations numerically Eq. 9 e Eq. 10 and integrating them step by step in time, efficiently and accurately. It allows, therefore, to study the temporal evolution of the configurations of the system's constituents and, from the positions generated, to determine the macroscopic properties of the simulated system according to classical molecular mechanics.

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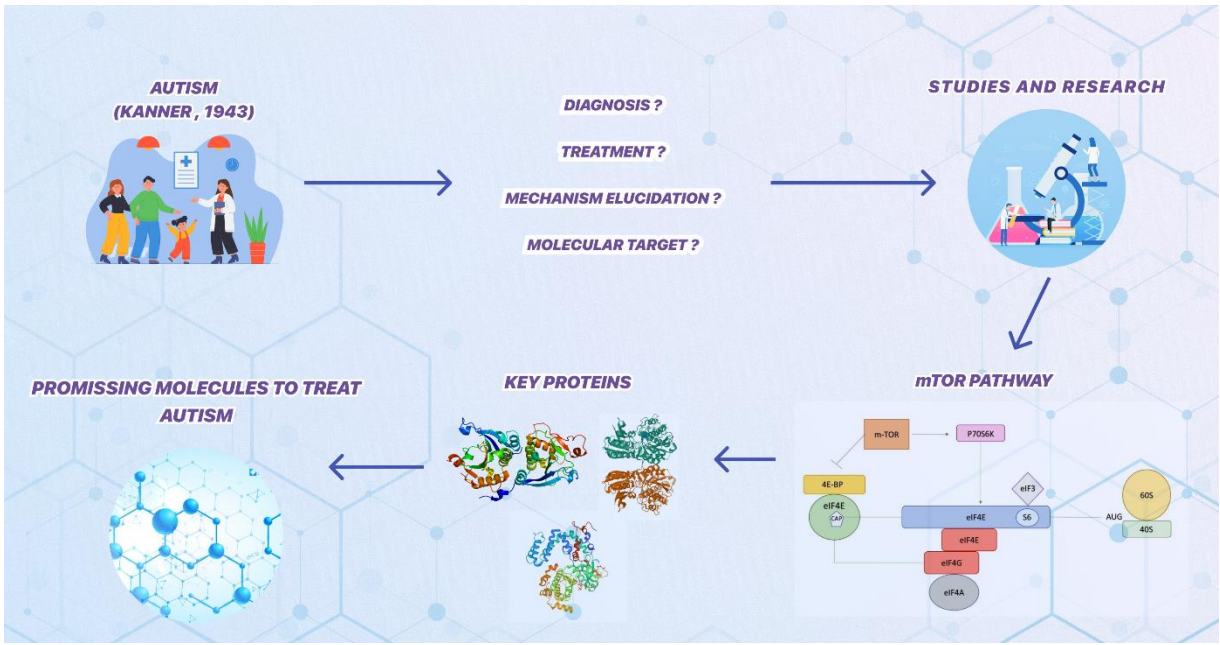
SECOND PART – PAPERS

PAPER 1 - Regulation of Protein Synthesis: an Approach to Treat Autistic Spectrum Disorder (ASD)

CURRENTE MEDICINAL CHEMISTRY
https://doi.org/10.2174/0929867328666210419125634

GRAPHICAL ABSTRACT



Keyword: neurodevelopment; translation; m TOR; e IF4E- e IF4G; mGluR and GRK5.

Abstract: Autistic Spectrum Disorder (ASD) is a disorder with different etiologies and poor elucidation, characterized by changes in social and cognitive skills. ASD has been characterized that affect large numbers of people in the world. In spite of its great importance, surprisingly just a modest progress has been achieved toward comprehending this pathology and designing new therapies. The molecular dysfunctions observed in people with autism are evidenced by the interference in the synthesis of synaptic proteins, which impairs their development and plasticity, leading to characteristics of individuals with ASD. The present work investigates in detail the mTOR pathway and the proteins related to its regulation and neurological functioning. The path of protein synthesis and translation is promising for the treatment of various disorders and its elucidation may, for example, result in drugs that facilitate the diagnosis and open the range of treatments, improving the quality of life of ASD

1. INTRODUCTION

Autistic spectrum disorder (ASD) is a neurodevelopmental disorder, characterized by changes in social, emotional and psychological skills. The delay in verbal communication, the prevention of social contact, restricted games and stereotyped behaviors are classic examples of symptoms observed in an individual with ASD [1,2].

The ASD was first diagnosed in 1943 by the child psychiatrist Leo kanner; at the time, he described some behavioral traits presented by a group of 11 children (ages from 2 to 8 years old), who were settled under his care at Johns Hopkins Hospital, in United States. Among the behaviors reported by Kanner are extreme loneliness, aversion to everything that comes from outside and away from your inner world, the explosion of behavior generated by any physical contact, movement or noise and the need to repeat behaviors. In addition, it was found that these children choose objects called encouraging, that reassure them and help them to face the world outside theirs [3,4,5]. Due to these behavioral patterns, the psychiatrist named the disorder as autistic affective contact disorder, borrowing the term autism, first used in 1908 by Eugen Bleuler, to describe the behavior of his schizophrenic patients. Kanner also observed macrocephaly in children with ASD, which is possibly due to an early brain development [6].

Kanner and coworkers developed the basis for current autism research and their findings have contributed to a major advance in child psychiatry and psychopathology. Their observations and clinical pictures are still used as references in the diagnosis of autism [3,7].

In fact, ASD has distinct etiologies, is little elucidated, and precisely because it encompasses a heterogeneous group of disorders, it receives the nomenclature Autism Spectrum Disorders. According to the Diagnostic and Statistical Manual of Mental Disorders IV (DMS IV), autism can be classified into syndromic types, when they have causative agents known as monogenetic disorders, and non-syndromic also called idiopathic, which do not have known genetic causes [8,9].

It is believed that much of the etiology of autism is influenced by the genome, once it is among the most inherited neuropsychiatric disorders. Among the related monogenic disorders, it is possible to mention the polyhydramnios, megalencephaly, and symptomatic epilepsy syndrome (PMSE), homostasis of phosphatase and tensin (PTEN), fragile X syndrome (FXS) and neurofibromatosis [6,8]. In addition, there is also much evidence that indicates environmental factors as significant agents in the causes of autism spectrum disorder, including: exposure to toxins, pesticides, infections and medications in the mother's

womb [6,8,10]. The activation of the mother's immune system at the time of pregnancy and modifications in the defense cells of the child are factors that can lead to changes in the neurodevelopment of the fetus, causing ASD [11].

Abnormal immune functions are directly related to the inflammation process, antibody and cytokine dysregulation, and imbalance in the amount of anti-brain autoantibodies, which are antibodies that attack proteins or cells in the body itself [10]. In a study carried out in 2012, Lintas and collaborators found that the dysregulation of the immune system would result in the production of reactive oxygen species, both before and after birth. During the gestation period, this change would result in increased neuronal production and migration, whereas reactive oxygen species associated with mitochondrial dysfunction, in the post-birth period, would lead to an abnormality in synaptic [11].

Maternal depression associated with the use of antidepressant, during pregnancy, especially those that inhibit serotonin reception, which act by blocking the serotonin transporter, can promote the accumulation of serotonin in the extracellular space. These inhibitors go through the placenta and are found in the amniotic fluid [11].

The neurotransmitter serotonin (5-hydroxytryptamine, 5HT) is one of the members of the monoamine family and is involved in several processes related to cell division and differentiation, neuronal migration, proliferation and cortical plasticity and synaptogenesis. In addition, it participates in several brain functions that include learning ability, memory and has an important role in modulating mood and sleep. Studies show that neurotypical children had high levels of 5HT when they are in the age group between 2 and 5 years, as the years pass these levels suffer potential decline. Children with ASD, on the other hand, do not show a decline in the rate of 5HT over time, the levels are lower than in neurotypical children aged 2 to 5 years and suffer a significant increase over time. The curious fact is that these increased levels of serotonin did not appear in any other intellectual deficiency or neuropsychiatric disorders. The selective inhibitors of 5HT reuptake (SSRIs), have a considerable efficacy in the treatment of repetitive symptoms in individuals with ASD, in particular, fluoxetine was able to significantly decrease the general symptoms presented by individuals with autism [12,13].

Although several studies have correlated the increase in serotonin with ASD, the elevation mechanism still remains uncertain [13].

Oxytocin also seems to play a role in the development of ASD, as it has effects on emotional and social behavior. Low levels of oxytocin were seen in children with autism, which could be related to their impaired abilities [1].

Autism is associated with several other neurological diseases whose origin is due to the mutation of a single gene and these are somehow related to negative regulation of protein synthesis [9].

There is evidence that the molecular dysfunctions seen in people with autism interfere with the synthesis of synaptic proteins, impairing their development, plasticity, and leading to behaviors characteristic of individuals with ASD, including cognitive impairment and Savant, in which, for some unclear reason, the person develops artistic and memory skills [6].

When discussing protein biogenesis, the matrix TOR, which, through its various pathways and proteins responsible for its regulation, controls fundamental cellular processes, should be taken into account. The dysfunction of this matrix contributes to pathological conditions, such as ASD and other neurological disorders. Accordingly, a detailed investigation of the TOR pathway and the proteins involved in this process is a promising approach in order to better understand these types of disorders and to seek more efficient treatments, which may result in improving the quality of life of patients.

2. mTOR AND ITS RELATIONSHIP WITH AUTISM

Proteostasis (protein homeostasis) is responsible for maintaining the normal and balanced conditions of the protein cycle, ensuring that they fold, group and degrade in place and in the correct amount. These regulatory mechanisms, also called the proteostasis network, are closely related to essential functions in the body, which includes brain functions. For, unlike other types of cells, neurons have a long life, in addition to exhibiting diverse morphology with complex architecture that can span a long distance, unique properties required in proteostasis [14,15,16].

The m-TOR (Mammalian Target of Rapamycin) is a serine / threonim protein kinase that, in response to the integration of signals that detect the availability of oxygen, amino acids, growth factors, energy levels and stress, is able to transcribe genes and translate proteins, which makes it responsible for cell growth and metabolism. In mammals, m-TOR is the central representative of two signaling complexes with different characteristics, m-TORC1 and m-TORC2 [17]. In addition, this protein works as a central element in the nutrient-sensitive signaling pathway. Jacinto et al. (2008) and Yang et al. (2018) reported that the mTOR signaling pathway represents a conserved mechanism by which yeast cells can actively respond to nutrients, suggesting that the nutrients actively detected around them are very important for yeast [18,19].

The m-TORC1 and m-TORC2 complexes are composed of common proteins such as mTOR, DEPTOR, SEC138 (mLST8), Tel2 and Tti1. What differentiates them is the specific addition of RAPTOR and PRAS40 in mTORC1 and RICTOR and mSIN1 in m-TORC2. Regarding the composition, specific inhibitors were created for the m-TORC1 and for the m-TORC1/m-TORC2 complex, such as rapamycin, but no specific inhibitors were found for m-TORC2, which has relevant contributions to brain functions [20,21,22]. Despite being insensitive to rapamycin, studies show that long-term exposure to this compound can disable the m-TORC2 pathway as a side effect [23].

The m-TORC1 complex promotes the synthesis of proteins and lipids, leading to anabolic cell metabolism, the suppression of catabolic cell metabolism (autophagy) and the increase in energy metabolism, regulating the useful life of proteins and organelles. The m-TORC1 activates global protein synthesis by phosphorylating S6 kinase 1 (S6K1) and eukaryotic initiation factor 4E binding protein 1 (4E-BP1), and by activating protein degradation through the ubiquitin-proteasome complex. In addition, it is able to inhibit autophagy by phosphorylating ULK1 / 2 complexes. Hyperactivation of m-TORC1 causes inhibition in the autophagy process and abnormal synapses (figure 1). The ubiquitin-proteasome systems and lysosome autophagy are essential for maintaining the proteostasis of neurons [20,24].

In a more detailed definition, autophagy is the mechanism that allows lysosomes of proteins and long-lived organelles to be degraded through the formation of autophagosomes and autolysomes, which collaborates with proteostasis. The dysfunction of this mechanism is associated with ASD, once it is relevant for an adequate synaptic pruning during the elimination of synapses, which occurs mainly between early childhood and puberty [25].

The ubiquitin-proteosome complex is responsible for directing short-lived, regulatory, poorly folded and damaged proteins for degradation. Through local synaptic activity, the complex directs synaptic proteins to degradation - a stage that occurs within dendritic spines - indicating their importance in structural and functional changes linked to synaptic plasticity. Inhibition of this system can lead to the accumulation of BDNF (brain derived neurotrophic factor), enhancing synaptic plasticity in the long run. In the same way, its deregulation is associated with aging and neurodegenerative diseases. Mutations in the genes responsible for their encoding are associated with ASD [13,26,27].

The m-TORC2 is a complex found in some compartments of the cell and intracellular membrane, including mitochondria and endosomal vesicles. Although it is less elucidated

than m-TORC1, it is known that m-TORC2 is capable of phosphorylating Akt, protein kinase C (PKC) and kinase regulated by serum, and glucorticoid (SGK); thus, influencing cell growth, proliferation, survival, migration and metabolism. Therefore, m-TORC2 plays a very important role in controlling portostasis [16]. In addition, it is a vital regulator of insulin, polymerization of actin and the formation and organization of the actin skeleton. Studies have shown that an increase in m-TORC2 activity restores long-term memory and plasticity performance, and its deregulation is associated with numerous diseases linked to aging, type 2 diabetes mellitus and cancer.

The activation of m-TORC2 can occur via Ptdlns (3,4,5) P3 (Phosphatidylinositol 3,4,5-triphosphate), plasma membrane tension and growth factors, including the insulin hormone PI3K signaling pathway. The m-TORC2 signaling can be inhibited by the m-TORC1 complex (Figure 2) [23, 28,29].

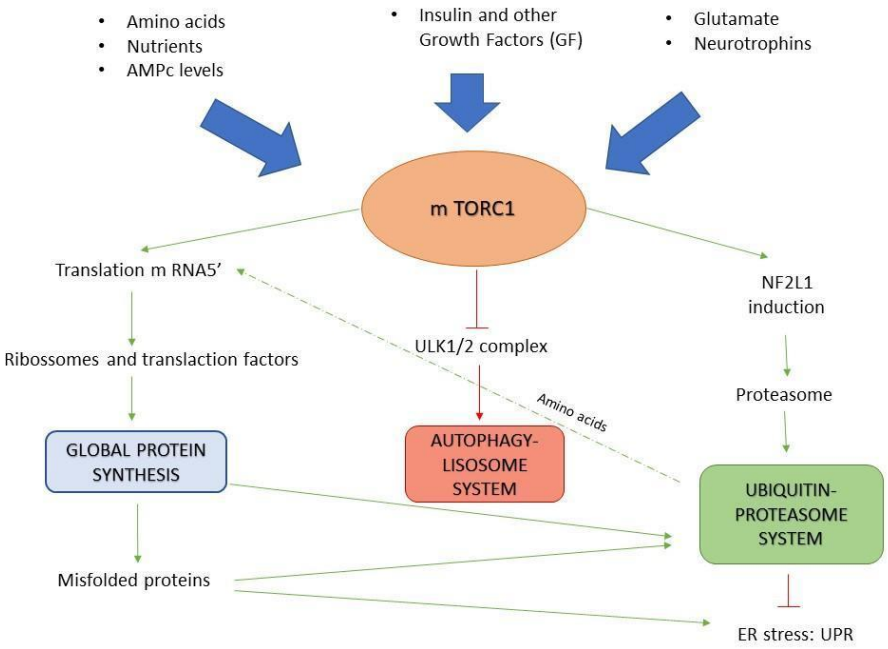


Figure 1. Activation of the m-TORC1 complex and its implications for proteostasis.

m-TORC1 is activated by the presence of nutrients, amino acids, AMPc: adenosine cyclic monophosphate, insulin, GF:growth factors, glutamate and neurotrophins. UPR: Unfolded protein response, ER: endoplasmic reticulum, ULK1/2: Serine/threonine-protein kinase ULK2, NF2L1: Nuclear factor erythroid 2-related factor 1. Adapted [9].

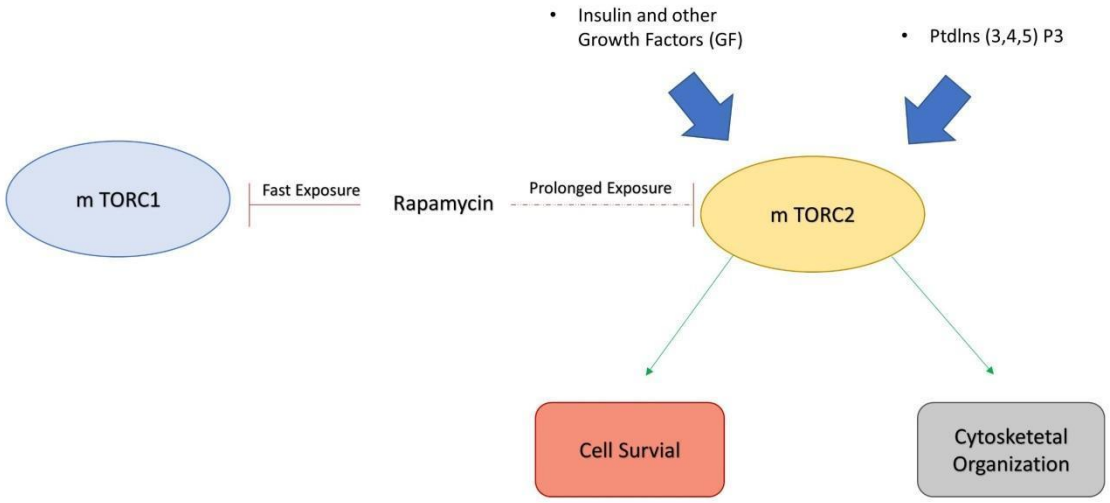


Figure 2. Activation of the m-TORC2 complex and its implications for proteostasis. m-TORC2 is activated by the presence of Growth Factors with insulin (PI3K) and by Ptdlns (3,4,5) P3 (Phosphatidylinositol-3,4,5-triphosphate). Adapted [29].

Changes in the genes involved in proteostasis and dysfunction in the autophagy process are related to some forms of autism, which indicates a direct relationship with the intracellular signaling of m-TOR. Because it is primarily responsible for controlling protein synthesis and degradation, m-TOR becomes essential for maintaining neurological cells and synaptic plasticity, enabling the nervous system to adapt to the stimuli received. The mRNAs located inside the dendrites provide the local translation of proteins, being essential in the identification and capture of synapses. The mRNA cannot enter by itself inside the dendrites; in this case, a complex of granules of messenger ribonucleoprotein (mRNP) is formed, composed by RNA, the key regulatory RBP's of the transcriptional process (translation / repression), along with splicing and some more accessory proteins. As examples of RBP's proteins, we have FMRP1 (mental retardation protein), ZBP1 (CEP-binding protein), CPEB's (cytoplasmic polyadenylation element-binding proteins), which are proteins that repress the process of translation when bound to mRNA, unlike Sam68 that promotes translation [13,30].

The proteostasis network is divided into three main functionalities that govern proteins, they are: protein synthesis machinery, maintenance mechanisms and degradative pathways. Dysfunction in any of these three pathways leads to serious problems in neurons, especially in dendrites that require strictly controlled homeostasis for their development and

maintenance. The dysregulation of proteostasis results in the dysregulation of neurological functions that can cause some neurological disorders such as autism [14,15,16].

The Fragile X syndrome (FX) pauses the transcription of the FMR1 gene; the role of the protein derived from that gene, the fragile mental retardation protein X (FMRP), is to bind to specific mRNAs and suppress protein translation. Thus, when the production of FMRP is silenced, we have an abrupt increase in protein synthesis. Another example of autosomal neurological disease is the tuberous sclerosis complex (TSC) caused by mutations in hemartin (TSC1) or in tuberin (TSC2). TSC1 along with TSC2 form a heterodidimeric complex that inhibits m-TORC1, which is one of the main regulators of cell growth and protein synthesis. The Phosphatidylinositol-3,4,5-triphosphate 3-phosphatase (PTEN) is also a negative regulator of protein synthesis and its loss of function also results in increased activity of m-TORC1; mutations in PTEN are associated with autism [6,24,31].

CPEB's from 1 to 4 are found mainly in dendrites, where they regulate synaptic plasticity; CPEB 4 is even associated with the transcription of risk genes for ASD. CPEB1 blocks the translation of mRNAs when they bind to the CPE (cytoplasmic polyadenylation element) present in the UTR3 (Untranslated Region), which is a non-coding region that represents a signal for the end of protein synthesis. CPB1 can also bind to neuroguidine, preventing the formation of the complexes e IF4E-e IF4G, which initiate protein translation. The beginning of the translation is the most limiting step in the process; thus, a favorable environment is required for the translation of the m RNA provided by the initial signaling pathways [13,30].

The alteration of mTOR signaling pathways can lead to megalocephaly, changes in the size of neurons, axonal dysregulation, proliferation of brain cells and variation of the dendritic spine in different regions of the brain, among others. In addition to the disruption of synaptogenesis regulation (corticogenesis and functions associated with neurons), studies indicate that the Akt/mTOR pathway for regulating translation in dendritic spines is a potent molecular substrate involved in autism [8].

The processes controlled by mTOR have been related to several specific functions in neurons. The mTOR plays a critical role in functions ranging from differentiation of precursors to synaptic plasticity, therefore, it is important in various neurological disorders [32].

For example, PIK3 / AKT / mTOR dysfunction is recognized as a root cause of neurodevelopmental and neuropsychiatric disorders with different origins and development as in the case of autism, epilepsy, brain injury and malformation. Studies show that mTOR

mutations detected in a severe type of microcephaly, hemimegalencephaly (HME) [30]. These brains demonstrate a lower level of raptor essential for mTORC1 activity, resulting in a decrease in the number and size of cells, thus displaying a small brain [32].

Loss of PTEN leads to an increase in the AKT / mTOR signaling pathway, a risk factor for macrocephaly, autism spectrum disorder and glioma. PTEN haploinsufficiency (+/-) leads to overgrowth of the brain and altered sizing of neurons with increased beta-catenin signaling, which indicates that PTEN and beta-catenin act together to control the number of cells and the process of brain growth [33].

Studies show that metformin decreases the deposition of beta-amyloid plaques and chronic inflammation, in addition to increasing the production of AMPK and suppressing the activation of P65 NF-κB, mTOR and S6K and consequently the production of Bace1 contributing to the improvement of neurological deficits [33].

The brain of a person with ASD is proven to have an altered size, it is most often larger than that of neurotypic individuals. This excessive growth is notably observed in early childhood [35]. The exacerbated activity of mTOR leads to an increase in external radial glial cells, a type of neuronal precursor cell NPCs, this is due to the mutation of the GABASE RAB39b gene that is associated with macrocephaly, autism spectrum disorder (ASD) and intellectual disability. Thus, the overproduction of PI3K-AKT-mTOR alters cortical neurogenesis, leading to macrocephaly and autistic behaviors [35].

Rosina et al. (2019) diagnosed that the increase in the mTOR and MAPK pathways leads to a significant increase in the amount of proteins such as rpS6, p-eIF4E, ERK1-2 and p-MNK1 in patients with ASD. This found suggests that the level of these proteins is correlated with the severity of autism. These aforementioned proteins are positive regulators of protein synthesis indicating an overactivation of protein synthesis in autism. There is solid evidence to prove that exacerbated protein synthesis is related to several neurodevelopmental disorders [36].

A study by Lieberman et al addresses mTOR as a regulatory pathway for the autophagy process and indicates the striatum as the controller of autophagic activity [37].

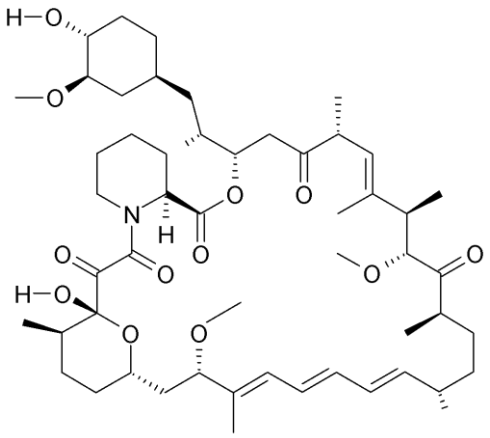
Therefore, the unbalanced activity of mTOR signaling is related to changes in proteostasis, autophagy and process deficits involving synaptic plasticity, that are characteristic of ASD. Autophagic dysfunction can also lead to the development of neurological diseases as in the case of autism spectrum disorder (ASD).

In view of the above, mTOR becomes an interesting target for autism mTORC1 is a specialized metabolism regulator, it acts by means of cellular signaling and is able to support

the growth, proliferation and storage of energy in macromolecules by promoting biomass production. This protein is the molecular key between catabolic and anabolic processes. After its activation, mTORFC1 is able to regulate several cellular processes that promote the synthesis of macromelecules and the growth of cells, in addition its inhibition is closely related to autophagy and cell recycling. Although there are advances in the study of this protein, there are several mechanisms that need to be better elucidated, its role in the treatment of cancer for example has been progressing. However, in the case of neurodevelopmenta diseases, which include autism, there is a great interest in this protein but very little is known about its performance in this type of disorder [38,39].

Rapamycin (**1**) and its derivatives Everolimus (**2**) make up the first generation of mTOR inhibitors, also called allosteric inhibitors. These types of inhibitors basically have the same mode of action, they bind to FKP12 forming a complex, which in turn bind to the FRB mTOR domain and alters the conformation of mTOR, thereby inhibiting the action of mTORC1. Compouds **1** to **22** represent mTOR inhibitors with potential treatment for tumors, including compounds **15** to **22,** which are natural inhibitors with great potential for treatment in various forms of cancer [40,41].

In addition, studies reveal that since there is this direct relationship between mTOR and autism, inhibitors capable of stopping m TOR may reveal beneficial effects in the treatment of ASD such as rapamycin and its derivatives such as Everolimus [42,43,44].



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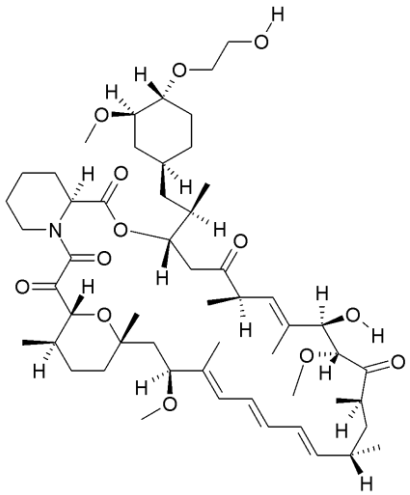

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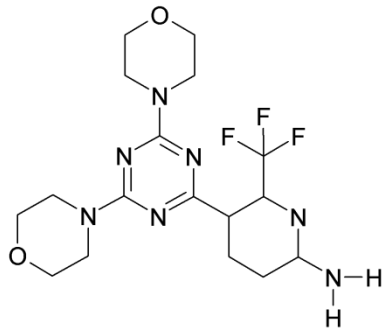

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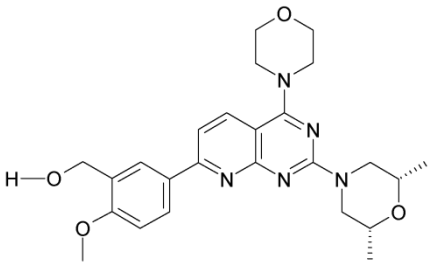

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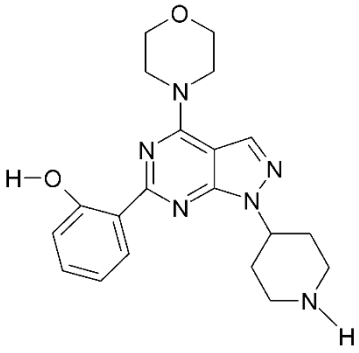

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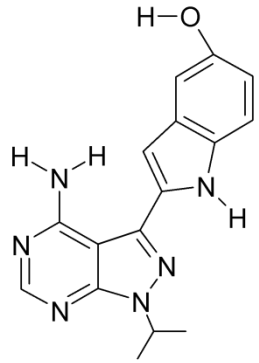

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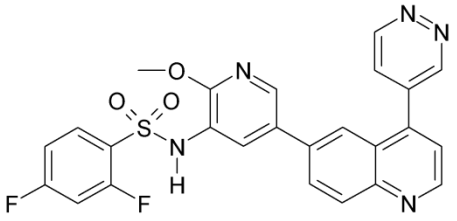

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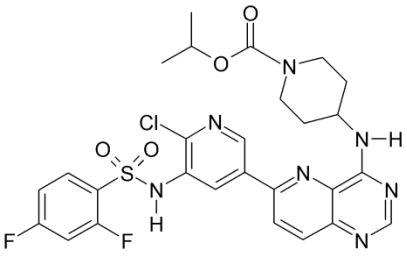

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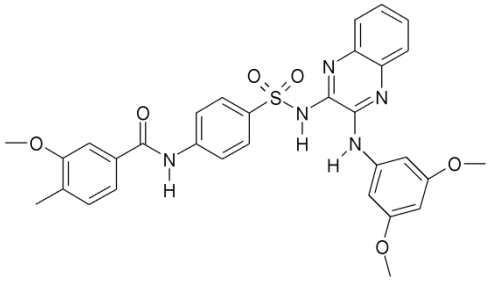

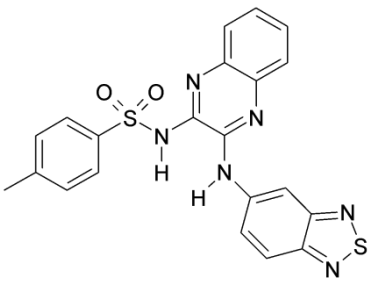

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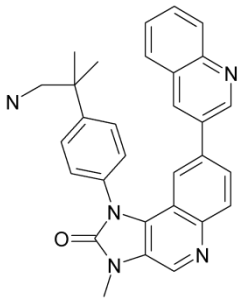

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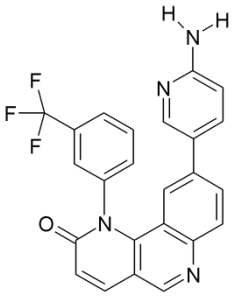

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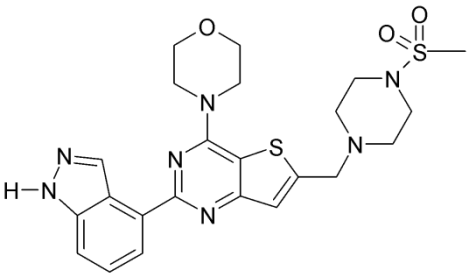

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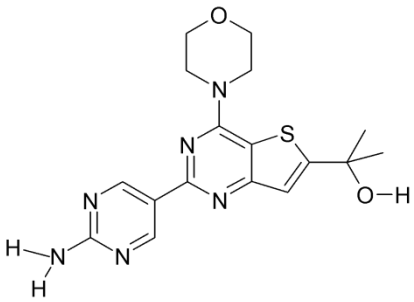

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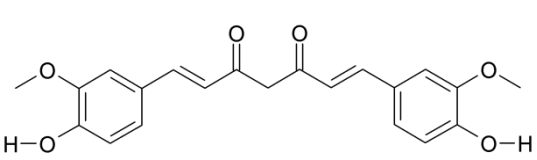

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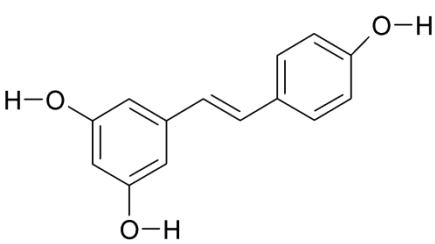

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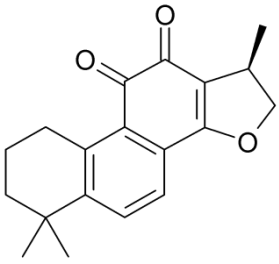

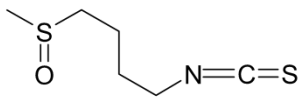

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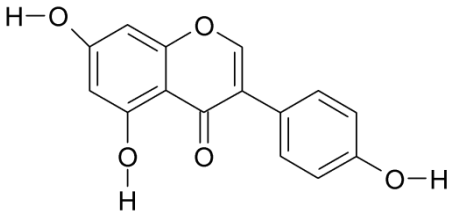

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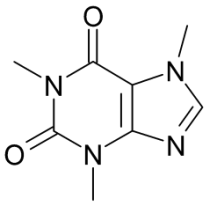

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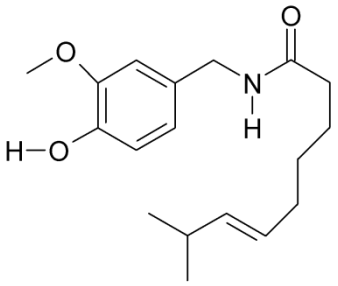

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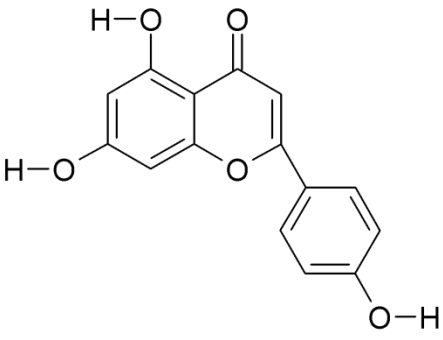

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22

2.1 e-IF4E

The initiation factor of eukaryotic translation is one of the essential components for sequencing protein synthesis. Translation is a complex mechanism and plays a very important role in monitoring gene expression; e-IF4E and 4E-BP1 are the main components of the mTOR signaling pathway [8,24].

The e-IF4E connects directly to the cap of the mRNA5 and binds to the scaffold of the protein e-IF4G and to the helicase of e-IF4A to form the heterotrimeric complex e-IF4F, allowing the recruitment of ribosomes and the start of translation. Each initiation factor is regulated by a different compound to finally control protein synthesis. The 4E-BPs are inhibitors of the formation of the complex e-I4EF/e-IF4G; in their non-phosphorylated forms, they interfere in this formation by binding to e-IF4E, thus, preventing it of binding to e-IF4G, blocking the formation of e-IF4E/e-IF4G (Figure 3) [45,46].

The ribosomal S70 p70 kinase 1 (p70S6K), as well as the 4E-BPs, plays an important role in mediating the mTOR in protein synthesis; when activated, it leads to the hyperphosphorylation of the ribosomal protein S6, a 40S ribosomal subunit, resulting in the selective translation of a unique family of mRNAs, which contains the oligopyrimidine tract at the 5 'transcriptional site (5′TOP), and the consequent protein synthesis (Figure 3) [8,47].

S6Ks are enzymes encoded by distinct, but quite homologous, genes that are linked to the size of cells. As a matter of fact, one of its isoforms has been shown to play a crucial role in cancer [48,49].

The eIF4G interacts with eIF4E for a reason of canonical binding to the 4E-BP moiety, and this same motif also appears in proteins 4E-BPs, which allows it to compete with e-IF4G for binding to eIF4E. The 4E-BMs acquire an α-helical conformation on the hydrophobic surface on the back of the eIF4E, located on the opposite side of the cover connection pouch. The existence of the sequences of a binding region associated with the presence of a non-canonical motif, located at the C-terminus of the canonical motif, increases the interaction between the 4E-BPs and eIF4E by repressing the translation. Proteins eIF4G also have canonical motifs that bind to the N-terminal of eIF4E, increasing their interaction, but leaving their lateral surface free to be attacked by 4E-BPs, which can bind there through an allosteric mechanism [50,51,52].

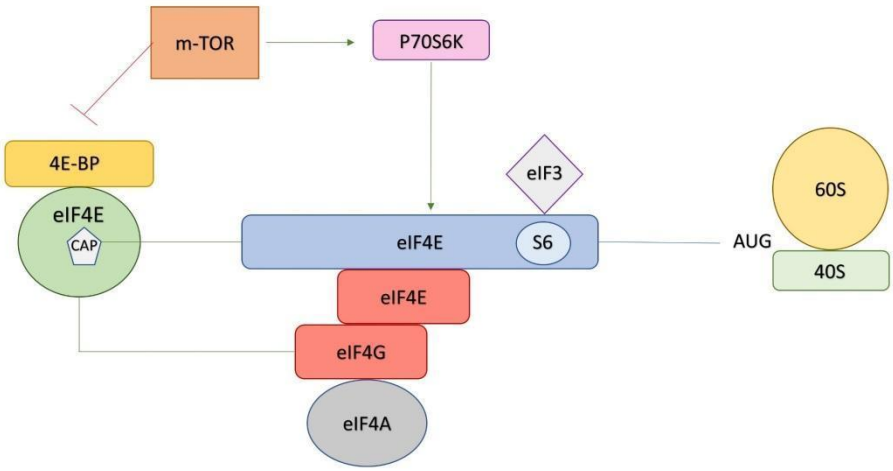


Figure 3. The mTOR mechanism is regulated by 4E-BP or P70S6K; 4E-BP when binding to eIF4E inhibits the initiation of translation, while the P6S6K S6 kinase binds to the 40S ribosomal subunit and leads to protein synthesis. The translation initiation factors, including eIF3, eIF4A, eIF4B, eIF4G and cap eIF4E binding protein regulate the start of translation.

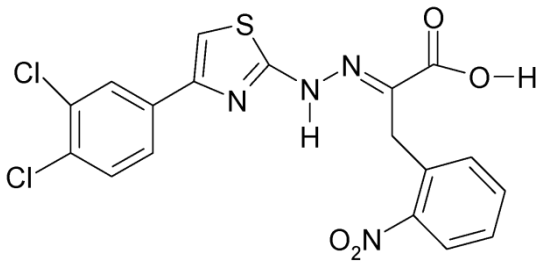
The activation of mTORC1 promotes the phosphorylation of 4E-BPs and their dissociation from eIF4E, allowing the formation of the complex with eIF4G, and proceeding with translation. In addition to mTORC1, other kinases can phosphorylate 4E-BPs by dissociating them from the eIF4E; however, the mechanisms of these alternative phosphorylation pathways remain unknown, revealing little about their action with the functioning of the organism [49,53].

When potentiating eIF4A activity, the initiation factor 4B (eIF4B) activates the complex eIF4F, whose activity is controlled by the mTOR or MAPK (mitogen-activated protein kinase) signaling pathway. eIF4F is phosphorylated by the activation of MNK (MAPK interacting with protein kinase), which also promotes the action of eIF4E. The signaling pathway of mTORC1 and the components of the complex eIF4E are present within the dendrites, hence their relevance in protein synthesis at the neuronal level.

The control of the protein eIF4E is related to many processes that include cell progression, survival and motility, as well as tumorogenesis, inflammation, immunity and infection. In addition, it is clear the relationship between eIF4E and the functionalities of neuronal cells, which reveal its uncontrolled function in neurodevelopmental disorders, as in the case of autism [54].

The eIF4E- eIFG inhibitors have also been studied as a form of treatment for autism, some of them previously tested in treatments for tumors [51]. A Class of 4EGI-1 inhibitors, structure **23**, shows improvements in the behavior of autistic mice [55,56].

The other structures, **24** to **27**, are proven to be eIF4E-eIFG inhibitors, but all attention is focused on the treatment of tumors, investing in their effectiveness for treating ASD may represent a good alternative [57].



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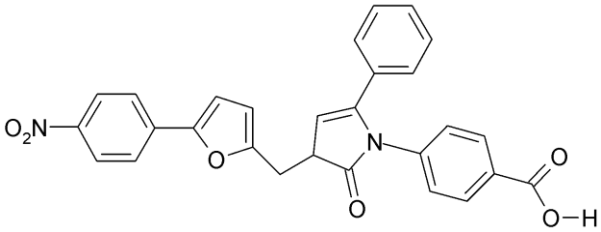

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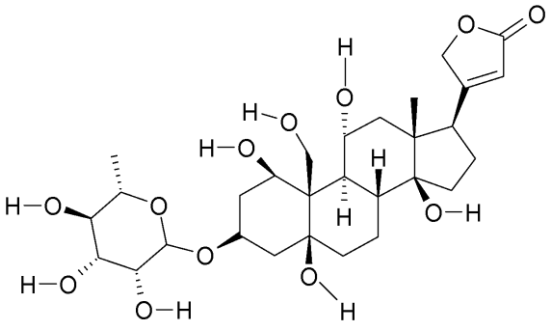

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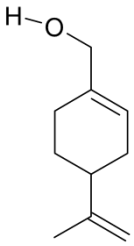

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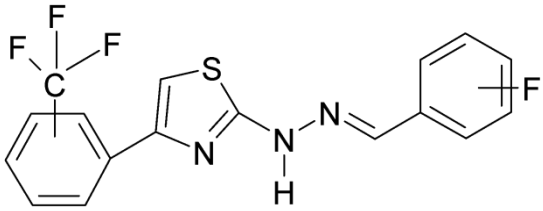

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2.2 mGluRs

The metabotropic glutamate receptors are responsible for numerous brain functions; in addition to regulating glutamatergic signaling, they are crucial for synaptogenesis, formation of neural circuits, during brain development, and have a very important role in synaptic plasticity. Their dysregulated function has been associated with neurological disorders such as ASD [58].

The SHANK and HOMER proteins support the mGluRs in a complex that comprises the metabotropic and ionotropic glutamate receptors. The ionotropic receptors (iGluRs) are the intermediaries of quick responses - they act as ion channels that open when they bind to glutamate, allowing the passage of cations. Examples of this class present in the nervous system are α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and cyanate receptors (KARs). The metabotripics slowly modulate neurotransmission, they belong to a family that is coupled to a G protein of class C, acting in the form of a GPCR dimer. The metabotripics are divided into three classes (group I, II, and III) according to the homology of their sequencing, ligand selectivity, coupling protein G and the second messenger pathways that start after its activation. Group I is formed by mGluR1 and mGluR5 located mainly in postsynaptic sites; group II comprises mGluR2 and mGluR3 in pre and post-synaptic; lastly, group III consists of mGluR4, mGluR6, mGluR7 and mGluR8 in presynaptic neurons, where they regulate the release of neurotransmitters. The mGluRs have a large N-terminal domain called Venus Fytrop (VFD) and an orthosteric binding site, which is a site recognized by the receptor's endogenous agonist. Each VFD has two lobes and the glutamate binds exactly in the gap between them [58,59,60].

The mGluRs, with the aid of the PIKE and HOMER scaffolding proteins, mediate the activation of PI3K, which subsequently activates ATK and inhibits the TSC1 / TSC2 complex. TSC2 inhibits RHEB and activates mTORC1, as a result of S6 kinase phosphorus FMRP. The FMRP, in turn, interacts with eIF4E, which, through specific CYFIP1 binding proteins to eIF4E, regulates the onset of translation of mRNAs [61,62].

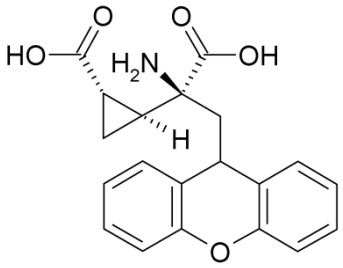
Changes in the expression of HOMER1 and mGluR5 genes have been identified as risk factors for ASD. The different studies and tests associate the HOMER proteins with synaptic plasticity that mediate mGluR5. The latter protein is directly associated with autism, since the signaling disorders mediated by them are characteristic of individuals with ASD [63,64].

The L-glutamate is the main excitatory neurotransmitter of the CNS (central nervous system), it is released mainly in the presynaptic glutamatergic terminals through the union of the membrane in the active zone and the synaptic vesicles. In the synaptic cleft, the neurotransmitter is activated by different receptors contained in the dendritic spines. The PSD, postsynaptic side, gets its name from its dense appearance in electrons due to the high concentration of proteins. Scaffolding proteins like PSD-95 are very important for the regulation and good positioning of receptors [60,63,65].

Neural functions, such as synaptic transmission, neuronal migration, excitability, plasticity, long-term potentiation (LTP) and long-term depression (LTD), depend on glutamatergic synapses [62].

There are several studies that point to the agonists and antagonists of some types of mGluRs as a target point in treatments for disorders of the Central Nervous System, including autism spectrum disorder [59].

Several studies have proposed structures with potential inhibition for mGluR. These inhibitors are indicated for the treatment of cognitive disorders, anxiety, schizophrenia, pain, depression, Parkinson's disease and may represent an alternative for the treatment of autistic spectrum disorders. Comopounds **28** to **47** represent structures capable of inhibiting mGluRs [66,67].



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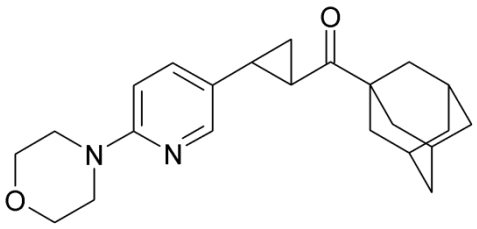

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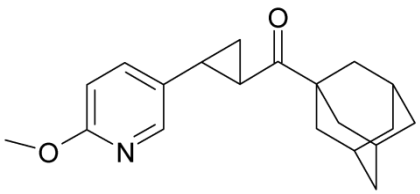

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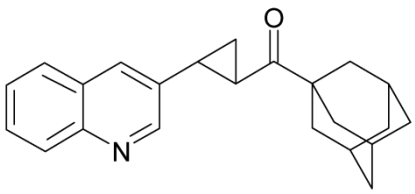

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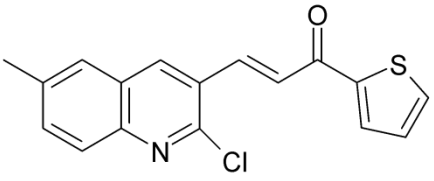

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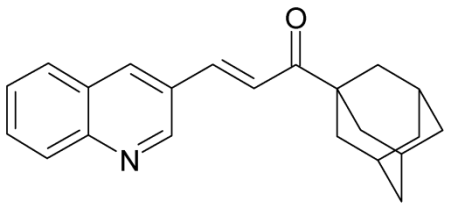

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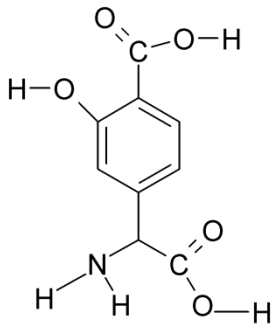

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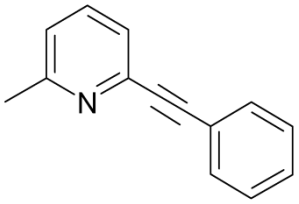

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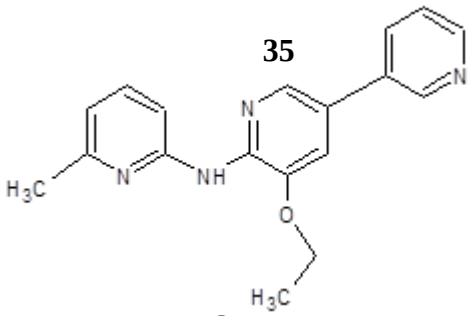

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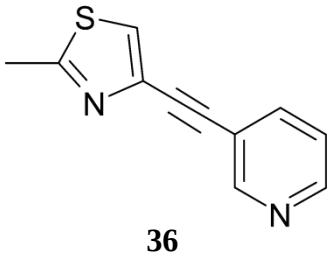

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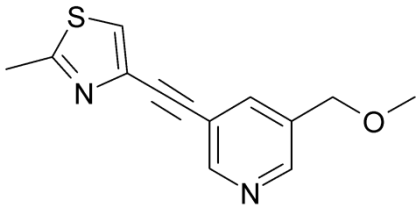

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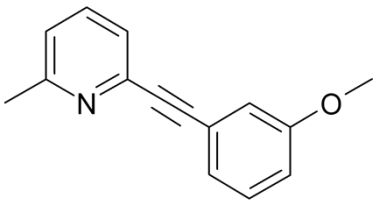

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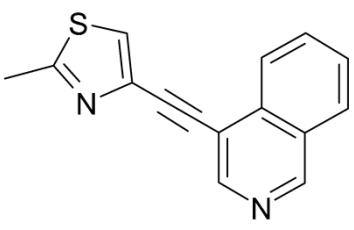

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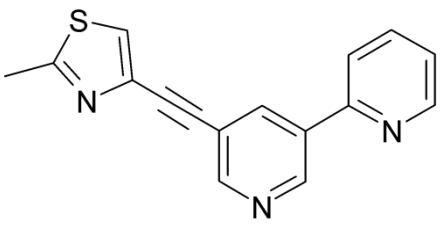

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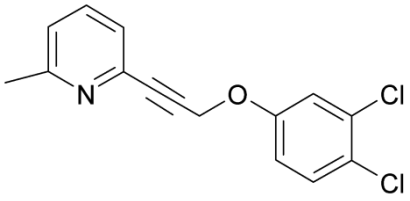

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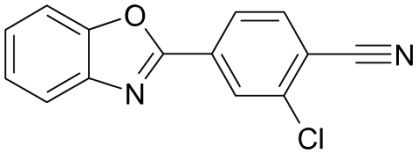

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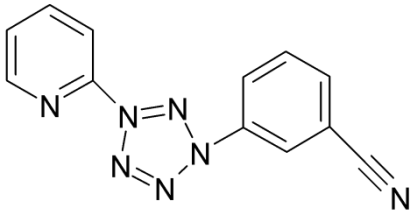

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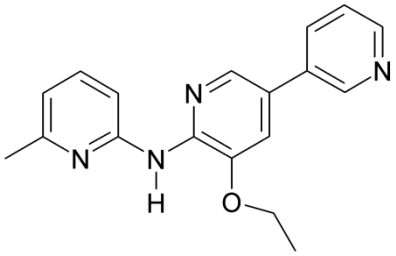

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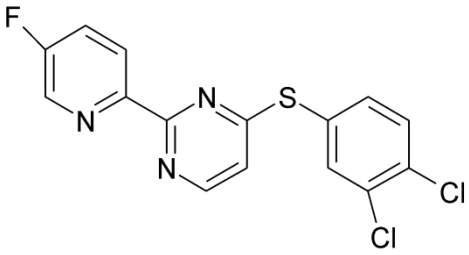

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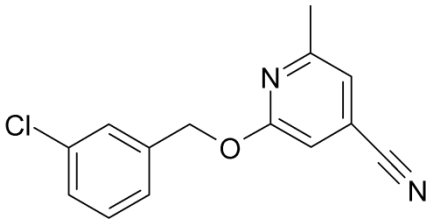

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2.3 GRK5

The kinase receptors coupled to protein G (GPCR) kinase (GRKs) belong to the serine/threonine kinase family and, through the phosphorylation of receptors occupied by agonists, as substrates, promote the decrease of GPCR sensitivity. GPRCs have been associated with the regulation of neurological and behavioral processes, such as learning and memory [66].

The GRK5 is widely expressed in different regions of the cortex; studies indicate its essential role in the regulation of mTORC1. The loss of GRK5 increases the activity of mTORC1, which makes it a negative regulator. It is part of the GRK's family and performs functions in cell cycles, such as apoptosis, regulation of the cytoskeleton and different substrates [69,70].

The Circuit dysfunctions in the medial prefrontal cortex (mPFC) along with social and cognitive disorders are associated with various psychiatric illnesses, such as ASD. In experiments with mice, it was found that the genetic interruption of GRK5 expression resulted in impaired social behaviors, unregulated signaling of the mTORC1 and synaptic transmission in the mPFC, indicating the essential role of GRK5 in the maintenance and modulation of the mPFC and social behaviors. GRK5 deficiency causes hyperactivation of G protein receptors [69].

The GRK5 is closely involved in the development of dendrites and is crucial for neuronal morphogenesis and functional neuronal circuits. Neuronal morphology occurs during the development of neurons, resulting in drastic changes in neuronal cell morphology that are crucial for the establishment of neuronal circuits and plasticity. The change in neuronal morphology is related to several disorders that affect cognition such as autism, mental retardation, fragile X syndrome and Down. GRK5 acts as a support for actin and stablishes the connection between the actin filament and the membrane rich in phosphatidylinositol-4,5 bisphosphate (PI (4,5) P2), during neuronal morphogenesis. Therefore, it promotes the formation of philopodia in neurons, neurite growth, dendrite branching and spine development [68].

The GCPR signaling in the brain is still undetermined, but it is known to be regulated by phosphorylation ordered by GRK5. It is a fact that GPCR receptors are involved in the synaptic plasticity, learning and memory; thus, performing essential tasks for the proper functioning of the brain. The kinases and phosphatases that modify GPCR are important for the recycling of these molecules and they contribute for the proper functioning of synaptic plasticity. The importance of such GPCR receptors is validated when associated with several genetic mutations found in GPCR genes that are related to ASD [70,71].

The various ASD-related genes converge on different canonical pathways. However, considering the complete pathway, systems like the second messenger cAMP and regulation of mGluR signaling, by GRK at synapses, are highly interconnected, despite being distinct routes, and still converge to synaptic functions, morphology and plasticity [71].

As for GRK5, there are several speculations that agonists of this protein may be effective for the treatment of ASD, but no potential chemical structure is found in the literature. Niu et al proved that GRK5 deficiency in mice caused a loss in their social behavior and revealing that this protein is promising for the treatment of diseases that involve deficiency in social behavior such as ASD [69].

3. Conclusion

The regulation of m-TOR, eIF4E, mGluR and GRK5 signaling are highly interconnected and converge to protein synthesis, synaptic, morphological and brain plasticity functions in a macro environment. This regulation interdependence makes ASD not only a multigenic disease, but also multiple pathway that connect. Hence, it is necessary to better understand these systems and the way they relate, so that it is possible to develop drugs that improve the quality of life of autistic individuals and other neurological disorders.

Autism is directly associated with cell growth, development and motility. The growth of axons, for example, controls neuronal connectivity and motility regulation, which are relevant points in neurological diseases; thus, the regulation and proper functioning of these processes guarantee an individual's neurological health.

To develop new drugs for the treatment of ASD, both *in vitro* and *in vivo* investigations become necessary in order to have a more complete and reliable analysis. Along with *in vitro* investigations, the computational chemistry and *in silico* methods are important allies to predict diverse pharmacological parameters, such as interaction modes and reactivity. These theoretical data may assist in the development of more efficient molecules for further testing of *in vitro* studies. This feature encouraged us to focus on some novel compounds to target the mTOR pathway and the proteins related to its regulation and neurological functioning. In an attempt to find new promising pharmacologically active molecules, we report here the a review article considering a detailed investigation of the TOR pathway and the proteins involved in this process as a promising approach in order to better understand these types of disorders and to explore more efficient treatments.

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PAPER 2 -Development, Application, and *in silico* studies of new eIF4E-eIF4G complex inhibitors to treat Autistic Spectrum Disorder

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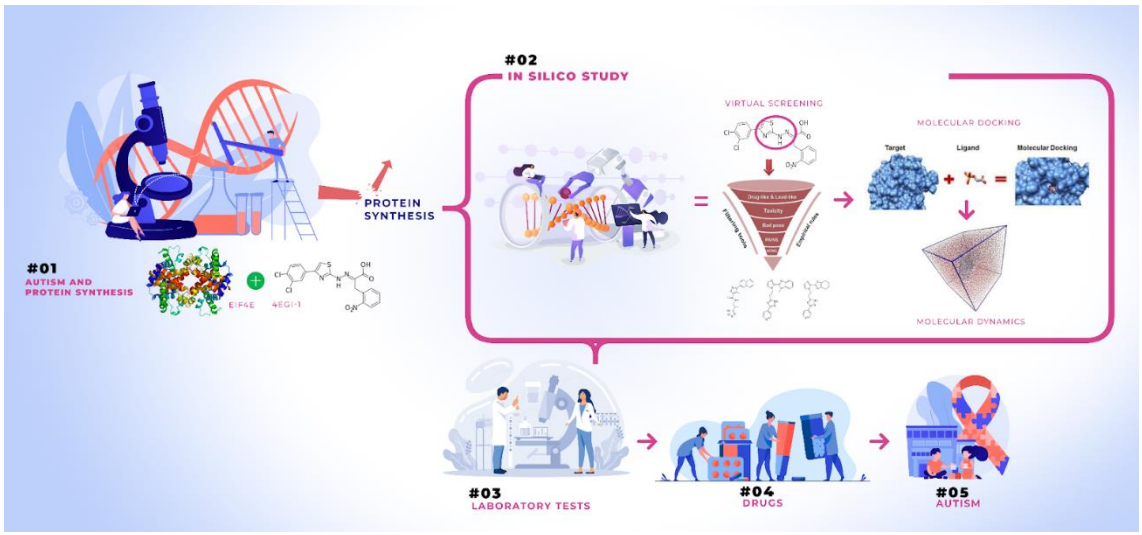
GRAPHICAL ABSTRACT



Keyword: neurodevelopmental disorders, proteostasis, inhibitors, autism and computational medicinal chemistry

Abstract: Autism Spectrum Disorders (ASD), involves a large group of etiologies and development, which complicates its diagnosis, treatment and possible cure. The study of possible pathways related to the disease is necessary, to facilitate the search for drugs that improve the living conditions of these patients, as well as early diagnosis. Dysfunction of protein synthesis, development, and motility, also called proteostasis has been frequently associated with neurological diseases. In this work, we seek to test compounds with the potential to inhibit the eukaryotic translation initiation factor, the eIF4E protein. There is strong evidence that the synthesis of excitatory proteins is associated with TEA. And, that finding potential inhibitors for this protein is a step forward on the path to treating ASD.

1. Introduction

The Autism Spectrum Disorders (ASD) encompasses a distinct group of disorders related to neurodevelopment, which complicates their diagnosis, treatment and possible cure. The difficulty in social interaction and communication, restricted and stereotyped behaviors, make up the behavioral pattern of individuals with ASD. The estimates indicate that 2% of the world's population has autism, with men being three times more affected than women and 30-45% of these people also having intellectual disabilities (Balasco; Bozzi, 2020; Bunt et al., 2020; Pangarazzi; Fry MD, 2020).

ASD represents a challenge to public health systems, both because of the increasing prevalence of this disorder since its first report by Kanner, and because of the difficulties in early diagnosis (Bertelli et al 2022). Another factor is the difficulty of finding information related to the etiology of the disorder, since autism comprises a large spectrum of origin and development. Most of the time, ADS is confused with other disorders that present similar phenotypes, which also delays the diagnosis (Fusar-Poli et al., 2020). The delay in diagnosis also results in a delay in working with the limitations arising from the disorder, which leads to a decrease in the quality of life of these individuals who already have characteristics that lead to completely impaired social inclusion.

The protein synthesis regulation is essential for the correct performance of all brain functions, which include the control of gene expression (Chalkiadaki et al., 2020). Impaired synaptic protein synthesis, for example, interferes with the development, plasticity, and death of neurons, leading to cognitive impairments and behaviors typical of autistic individuals. (Teixeira & Ramalho, 2021; Marotta et al, 2020; Kasherman et al 2020). In this sense, the eukaryotic translation initiation factor eIF4E is one of the essential components to trigger protein synthesis. The eIF4E attaches directly to the mRNA5' cap, binding with the eIF4G scaffold protein and the eIF4A helicase to form the eIF4F heterotrimeric complex, allowing ribosome recruitment and initiation of translation. Initiation factors are regulated by different compounds to ultimately control protein synthesis. The 4E-BPs are inhibitors of the eIF4E complex formation (Maracci et al, 2022). In their non-phosphorylated forms, they interfere in this formation by binding to eIF4E, preventing its binding with the eIF4G, and consequently blocking the formation of the eIF4E-eIF4G complex (Oblinger et al., 2018; Teixeira & Ramalho., 2021; Wiebe et al., 2019).

The regulation of eIF4E is related to numerous cellular processes, which include cell survival and motility as well as tumorigenesis, inflammation, and immunity (Negal et. al, 2023; Tian et al, 2023). Recent studies also show the importance of eIF4E-dependent translation in neuronal cell function, demonstrating its exacerbated activity in disorders related to the nervous system, such as neurodevelopment and neuropsychiatric conditions. The ASD is believed to originate from defects in synaptic functions, dysregulation of mRNA translation that leads to aberrant synthesis of local proteins, dysregulation of synaptic development and plasticity, and abnormalities in eukaryotic initiation factor eIF4E, since these characteristics have been identified in individuals with ASD (Amorim; Lachi; Gkogkas, 2018; Waltes et al., 2014).

In this context, the elucidation of the mechanisms involved in the protein synthesis regulation may represent great progress in the identification and treatment of diseases associated with neurodevelopment and neurodegeneration (Prashad & Gopal, 2020; Ileva, et al 2022). Understanding in depth the involvement of protein translation in the brain, especially taking into account the role of the eIF4E translation factor, is a challenge that can lead to promising approaches in the manufacture of drugs capable of treating neuropsychiatric diseases and mechanisms for identifying these pathologies. Thus, the objective of this study is precisely to test new inhibitors of the formation of the eIF4E-eIF4G complex through *in silico* assays evaluating the possibility and potential of the best inhibitors to become a drug that helps in the treatment of autism.

2.0 METHODOLOGY

2.1 Virtual Screening

The pharmacophoric model of the 4EGI-1 ligand was built three-dimensionally in the GaussView® program and optimized in Gaussian 09W®, this model was used as a reference for the virtual screening of eIF4E-eIF4G complex inhibitors. The data sources used for the searches were the PHARTMIT server (http://pharmit.csb.pitt.edu/) and the ZINC PHARMER database (http://zincpharmer.csb.pitt.edu/) (Viegas et al., 2019; Gimeno et al., 2019). To select molecules with promising pharmacokinetic profiles, Lipinski's rule of five associated with Veber's rule were applied (Lipinski et al., 2012; Kowalska et al., 2018).

The selected inhibitors went through a filtering step on the FAF-Drugs server 4 (https://mobyle.rpbs.univparisdiderot.fr/cgibin/portal.py#forms::FAFDrugs4),located inside

the MOBYLE PORTAL(https://mobyle.rpbs.univ-paris-diderot.fr/cgi-bin/portal.py#welcome), for the purpose of eliminating potentially toxic molecules, totaling covalent inhibitors and Pan Assay Interfering Compounds (PAINS).

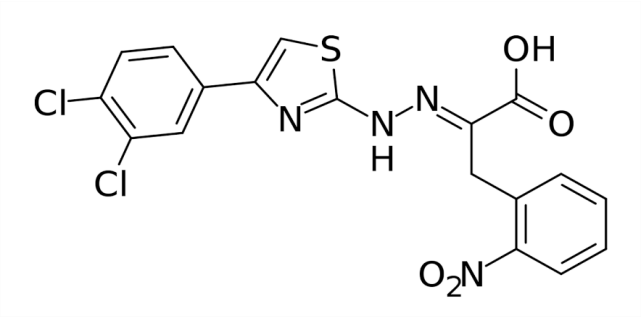


Figure 1. 2D structure of the 4EGI-1 ligand.

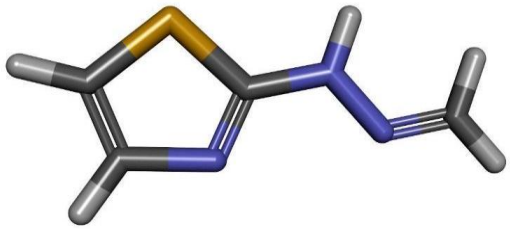


Figure 2. Main pharmacophoric portion of the 4EGI-1 ligand.

2.2 ROC Curve

For the construction of the ROC curve, at least five ligands were selected, in this case, inhibitors with known activity on the protein of interest. These compounds can be searched on the ChEMBL (https://www.ebi.ac.uk/chembl/), the exact values of biological activity were represented by the exact value of ki (Zeng et al 2020). The crystallographic structures were downloaded from this server, and then a table was created containing the smile formats of each structure and their respective ki and IC50. The protonation state of each compound was adjusted to physiological pH 7.4. To generate true-positives and false-positives (decoys), the DUD.E server was used (http://dude.docking.org/generate), and the decoys were found based on the smile formats generated in ChEMBL (Shoshan-Galeczki & Niv 2020; Irwin & Shoichet, 2016).

After generating the decoys, they were subjected to molecular docking calculation along with the active compounds, followed by the generation of the ROC curve. The ability of

the obtained pharmacophoric model was evaluated according to the area under the curve (AUC) to distinguish active from inactive compounds in terms of two parameters, sensitivity and specificity (Hadizadeh et al, 2022; Sing et al, 2005).

2.3 Molecular Docking

To continue the *in silico* analysis, and in order to investigate the interactions between the eIF4E protein and the ligands under study, molecular docking was performed with the aid of the Molegro Virtual Docker (MVD) (Bitencourt-Ferreira et al, 2020). For the analysis, the crystallographic structure of the eIF4E-eIF4G complex with the 4EGI-1 ligand (PDB: 4TPW) was extracted from the Protein Data Bank (https://www.rcsb.org/) (Westbrook & Burley, 2019). The protein preparation was carried out in the Discovery Studio, where the water molecules were removed, the charges were calculated and hydrogen was added.

For the ligands resulting from the virtual sorting, the molecules were previously optimized in order to leave the structure in its minimum energy conformation, for which the Gaussian 09W software was used with the calculation level DFT B3LYP/6-31G*. After optimization, the electrostatic charges calculation of the structures was performed in Spartan14, which served to better prepare the molecules that subsequently underwent molecular docking.

In this work, a total of 168 docking calculations were performed, the first calculation served to validate and standardize the methodology, the so-called redocking. The 4EGI-1 linker crystallized in the eIF4E structure was redocking within 11 Å of spherical restriction, within which the amino acid residues were considered flexible. The calculation generated a total of 30 different conformations which were later analyzed based on the best interaction energy and best superimposition parameters. Once parameterized, the same process was repeated for the 167 ligands found in the virtual screening, for each one of them a total of 30 poses were generated, which were again analyzed based on the same criteria. The 13 best binders were selected.

2.4 Toxicity test and Absorption, Distribution, Metabolism and Excretion Test (ADMET)

2.4.1 Toxicity Test

The site available on the internet allows the safe measurement of the toxicity of compounds based on computational methods. In this case, the ProTox-II 323 server (http://tox.chari te.de/ProTox-II) was used to determine the toxic effects of the 13 ligands with the best interaction energy and best overlap selected in molecular docking (Banerjee & Ulker, 2022). The server in question is able to measure the average lethal dose (LD50), immunotoxicity, and cytotoxicity, which are the toxicological endpoints, and organ toxicity such as hepatotoxicity, for example (Arulanandam et al, 2022; Luo et al, 2021).

2.4.2 Absorption, Distribution, Metabolism and Excretion Test (ADME)

Through the Swiss-ADME server (www.swissadme.ch/index.php), it was possible to analyze the solubility and pharmacokinetics of the 3 main suggested inhibitors suggested as drugs for the treatment of ASD (Fowler et al, 2021). The ADME analysis is an important test performed on molecules with potential to become a medicine (Luo et al, 2021). Many of the compounds with the potential to become a drug do not make it to the next tests due to the lack of their pharmacokinetic properties (Backchi et al 2022).

2.5 Molecular Dynamics

The coordinates and topologies files for the inhibitors verified in the toxicity and AMDE tests were generated in the Automated Topology Builder (ATB) server (https://atb.uq.edu.au/index.py). For the simulations, the force field GROMOS 96 54a7, GROMACS program (Version 5.1.2, Royal Institute of Technology and Uppsala University, Uppsala, Sweden) was used (Pall et al 2020; Sales et al 2022; Salvi et al, 2016). Employing the mentioned forcefield, the protein/inhibitor complexes (eIF4E-4EGI1, eIF4E-2 and eIF4E-3) were constructed in a cubic simulation box, with SPC water model as solvent. For energy minimization, the steepest descent algorithm was used, minimizing when the maximum force was <10.0 kJ/mol. After the minimization step, the complexes were submitted to molecular dynamics analysis for a time interval of 10 ns, obtaining 1000 conformations for each complex. The motion equations were integrated using the Leapfrog scheme. The results were analyzed using the VMD® program (version 1.9.2, University of Illinois at Urbana-Champaign, Champaign, IL, USA) and Discovery Studio® 3.5 (Accelrys, San Diego, CA, USA). Total energy, interaction, RMSD and hydrogen bond graphs were generated for

analysis of the results using the Origin® program (Version 3.5.0, Accelrys Software Inc., San Diego, CA, USA).

3. RESULTS and DISCUSSION

3.1 Virtual Screening

The screening model used was based only on the three-dimensional structure of the ligand, using its main pharmacophoric site as a search. The molport, mcule, drugbank and zinc databases used yielded 201, 1215, 0 and 201 structures, respectively (Gimeno et al., 2019; Veigas et al., 2019). The 1617 ligands resulting from the database search were reduced to 178 after passing through the filter of potentially toxic molecules on the FAF-Drugs server.

At the end of the process, a total of 178 structures were obtained, which were checked and analyzed one by one in order to eliminate those that were repeated. The final 160 molecules were directed to the molecular docking process.

3.2 ROC Curve

The ROC curve (Receiver Operating Characteristic) is a tool used to evaluate the reliability of prediction models. In the case of virtual screening, the curve is able to measure the probability of the ligands mapped by the mol port, mcule, drug bank and zinc pharmer databases to be eIF4E protein inhibitors. In general, the ROC curve is a graphical representation of the risks and limitations of a methodology (Janssens & Martens, 2020).

Figure 3 is a graphic display of the ROC curve, while the x axis represents the specificity, y is the sensitivity, two characteristic variables of the test. The sensitivity is the percentage of truly active compounds selected during virtual screening, while specificity is the percentage of truly inactive compounds identified by the test (Pshennikova et al, 2019).

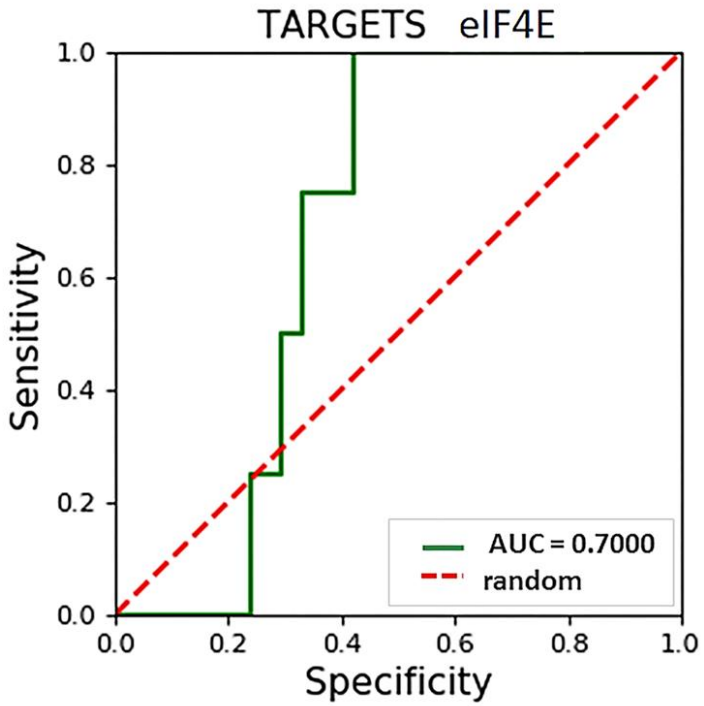


Figure 3. ROC Curve.

The AUC value, according to the literature, can vary between 0 and 1 (Maia et al, 2020). The closer to 1, the more effective the virtual screening workflow is in discriminating between active and inactive compounds. In general, when the AUC value is less than 0.5, it is considered a bad value, whereas values equal to and/or above 0.7 mean that the method is satisfactorily separating active from inactive ligands (Maia et al, 2020). In this case, the value obtained after the virtual screening methodology was 0.7, contemplating the values considered with a good degree of accuracy in the methodology.

3.3 Molecular Docking

To validates the methodology, as well as parameterize the calculations, the RMSD (Root-Mean-Square-Deviation) of the redocking was calculated. After superimposing the solid structures of two molecules, their atomic coordinates can be identified. This is the most common and effective method to assess structural and positional differences in *in silico* studies (Sargsyan, K et al, 2020). According to literature data, the redocking protocol is validated for RMSD values below 2 Å (Zubair et al 2020).

In redocking, the protein with the crystallized ligand extracted from the PDB (PDB:4TPW) is subjected to the molecular docking test, in this case using the MVD program. After extracting the pose with the best interaction energy and best pose, among the 30 generated, it was superimposed on the initial structure of the crystallized ligand. The RMSD was then calculated based on the difference in distance between them. (Vankayala et al, 2021).

Figure 5 shows the result of this superimposition whose RMSD value corresponds to 0.26 Å, validating the proposed protocol, as the value is well below that requested in the literature, 2 Å (Ferrari & Patrizio, 2021; Bhardwaj, P et al, 2019). In addition to establishing the ideal parameters for the other dockings, such as the constraining radius, coordinates and amount of flexible amino acids.

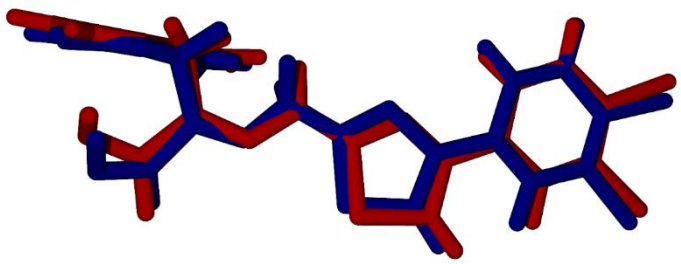
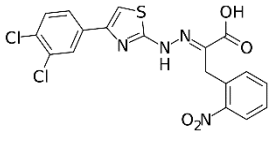
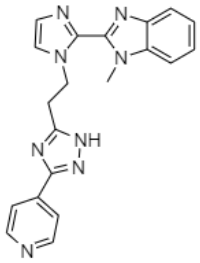
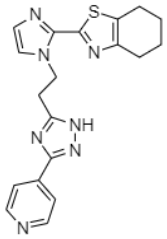
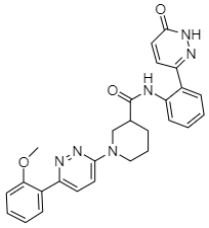
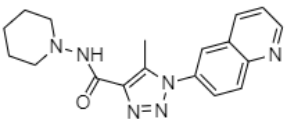
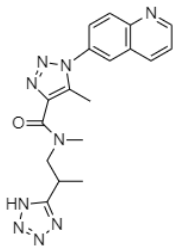
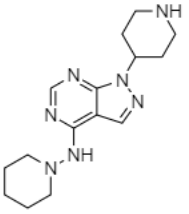
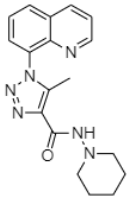
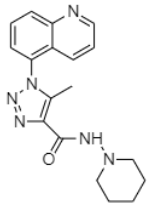
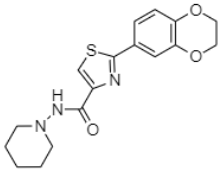
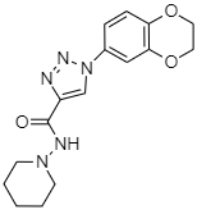
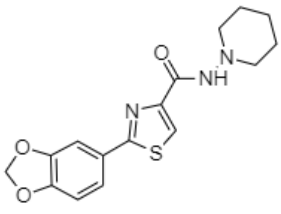


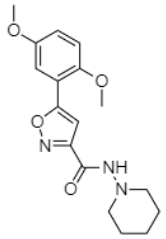
Figure 4. RMSD of crystallized ligand 4EGI-1 (red) and its best redocking position (blue).

After the validation and parameterization steps, 30 poses were generated for each docked ligand. In Table 1, it is possible to verify the structural formula, the relative interaction energy in $kJmol^{-1}$ and the molecular formula of each inhibitor candidate. The 4EGI-1 reference ligand in the screening, as well as the other ligands in Table 1, are the 12 best results of the 167 ligands evaluated. The selection of the best candidates was based on the lowest interaction energy and their generated poses. After all, the affinity of the ligand with the receptor is measured by predicting the preferred orientation and minimum binding energy (Shalzan, et al 2019). The number of ligands selected in the docking step, was directed to a sampling that addressed as much structural variation as possible and at the same time was a plausible number of structures to be subjected to ADMET testing.

Table 1. Molecular Docking Results of 4EGI-1 and 12 selected ligands from virtual screening.

Molecules	2D Structure	Relative Interaction Energy ($kJmol^{-1}$)	Formula
4EGI-1		31.908	$C_{18}H_{12}Cl_2N_4O_4S$
1		30.053	$C_{20}H_{18}N_8$
2		19.063	$C_{19}H_{23}N_7S$
3		59.901	$C_{27}H_{26}N_6O_3$
4		26.651	$C_{18}H_{22}N_6O$

5		35.298	$C_{18}H_{24}N_9O$
6		27.182	$C_{15}H_{24}N_7$
7		19.841	$C_{18}H_{22}N_6O$
8		17.841	$C_{18}H_{22}N_6O$
9		16.921	$C_{17}H_{20}N_3O_3S$
10		8.116	$C_{16}H_{18}N_3O_3S$
11		0.723	$C_{17}H_{23}N_5O_3$

12		0	$C_{18}H_{12}N_4O_4S$

The docking process is able to predict various types of intermolecular interactions such as hydrogen bonds, van der Waals and hydrophobic interactions and electrostatic forces (Salha et al, 2020). Figure 5 is the representation of interactions results of the molecular docking in A, B and C.

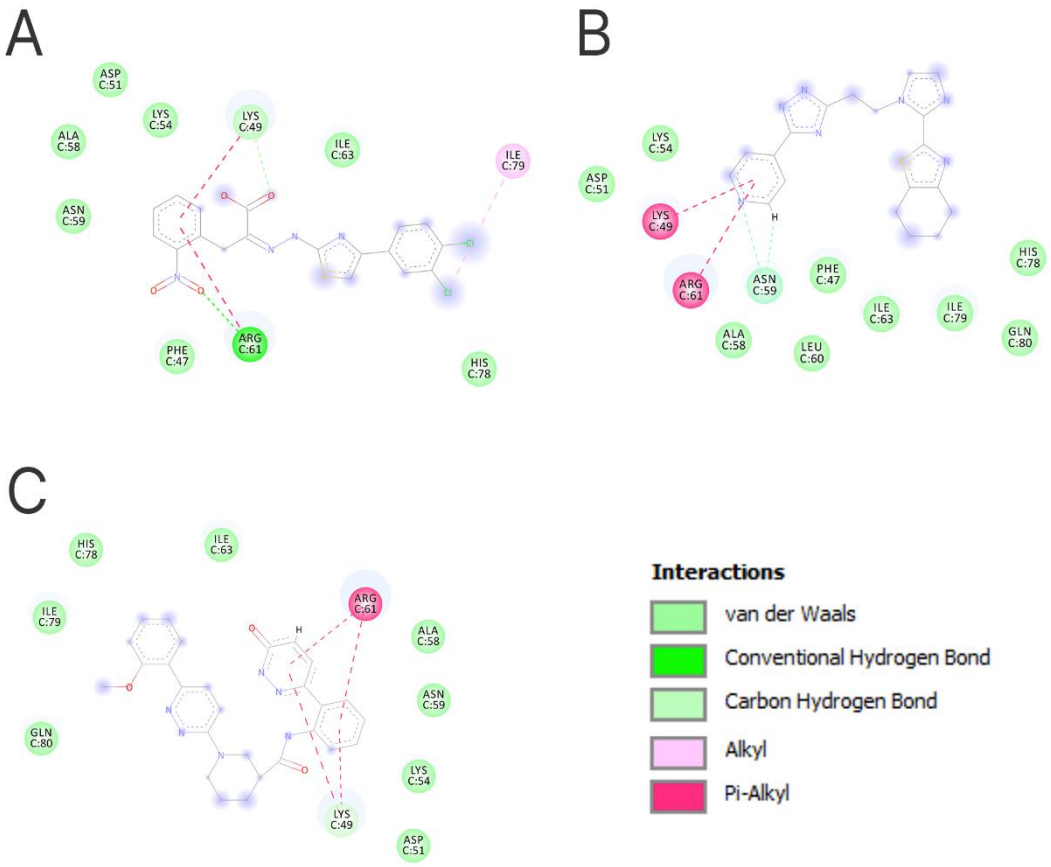


Figure 5. Interactions between eIF4E protein and inhibitors. **A.** Representation of intermolecular interactions captured in the molecular docking of the eIF4E-4EGI-1 complex. **B.** Intermolecular interactions between eI4FE-2. **C.** Intermolecular interactions between eI4FE-3.

In the 2D pharmacophore map, Figure 5A, it is possible to observe in A, **π**-alkyl interactions between one of the rings of the 4EGI-1 ligand and residues Lys 49 and Arg 61. Arg 61 also makes a hydrogen bond of length 2.28 Å with one of the terminal oxygens in the NO_2 group. Hydrogen bonds of lengths less than 2.5 Å characterize bonds with high stability, which have a large contribution to the stabilization of the molecule (Sales et al., 2017). Other alkyl and van der Waals type interactions can also be observed. A similar study was performed by Fischer et. al, 2021, in which the residues appearing on the pharmacophore map coincide with the residues mapped here, showing the likelihood of interactions occurring between them and the 4EGI-1 ligand (Fischer et al, 2021).

In images B and C, the energies of the interactions identified in the molecular docking suggest that the sum of interactions such as van der Waals, electrostatic and hydrophobic interactions are responsible for keeping the inhibitor stable at the protein binding site. In B,

two **π**-**π**-type interactions between ligand **2** and residues Lys 49 and Arg 61, and a carbon-hydrogen bond with Asn 59 are highlighted. For C, **π**-**π**-type interactions were also identified at the same residues Lys 49 and Arg 61 and a carbon-hydrogen interaction with residue Lys 49. No hydrogen bond formation could be identified in images B and C, however the energies of the interactions identified in molecular docking suggest that the sum of interactions such as van der Waals, electrostatic and hydrophobic interactions are responsible for keeping the inhibitor stable at the protein binding site.

4EGI-1 is a study-proven inhibitor of eukaryotic eIF4E-eIF4G translation complex formation. This inhibitor was the first small molecule that was able to stop the proliferation of various types of cancer by inhibiting the formation of the mentioned complex (Maracci, C et al, 2022). The crystal structure of eIF4E/4EGI-1 (PDB:4TPW) shows that the ligand binds in a hydrophobic portion of the protein between the β2 strand of Leu60-Thr68 and the α1 helix of Glu69-Asn77, and away from the eIF4G binding site, thus promoting allosteric inhibition (Fan, A & Sharp, P.P, 2021; Fischer, D.P et al, 2021).

Once allocating to the site, the inhibitor promotes a conformational change in the α1 helix of the protein, preventing it from binding to eIF4G, forming the eukaryotic translation complex, and consequently initiating protein synthesis (Fischer, D.P et al, 2021).

After selecting the best ligands, it is important to analyze the ADMET of these compounds, as these are possible drug candidates, knowing the potential for absorption, distribution, metabolism, excretion and toxicity is a basic requirement for the study to go ahead (Kesharwani et al, 2020). Subsequently, it is possible to more accurately determine the types of protein-ligand interactions that remain over time, this will make it possible to select a potential drug candidate based on *in silico* studies.

Toxicity Test

For a chemical compound to be directed to clinical tests in the future, it is of paramount importance that it be submitted to a toxicity test. The mapped parameters are some of the basic requirements for a drug that has the potential to treat a disease to actually become a medicine (Banerjee et al., 2018; Luo et al 2021). In Table 2, it is possible to evaluate the

toxicity results for the 4EGI-1, ligands **2** and **3** compounds in the ProToxII server, which were the less toxic compounds among all of the tested compounds. The toxicity results of all of the ligands presented in Table 1 are shown in the supplementary material.

Table 2. Toxicity resulting from data generated on the ProTox-II server.

Endpoint	**Target**	**4EGI-1**	**2**	**3**
Organ Toxicity	Hepatotoxicity	Active	Inactive	Inactive
Toxicity end Points	Carcinogenicit Immunotoxicit Mutagenicit Cytotoxicity LD50 (mg/kg) Toxicity Class	Active Inactive Inactive Inactive 1000 4	Inactive Inactive Inactive Inactive 200 3	Inactive Inactive Inactive Inactive 800 4
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR) Androgen Receptor (AR)	Inactive	Inactive	Inactive
Tox21-Stress response pathways	Heat shock factor response element (HSE)	Inactive	Inactive	Inactive

In Table 2, it is possible to see that the 4EGI-1 ligand was hepatotoxic and carcinogenic, while its lethal dose is 1000 mg/kg when ingested orally, classifying it as toxicity level 4. Ligand **2** did not show any type of toxicity, but its L50 is 200mg/kg, which makes it grade 3 on the toxicity scale. On the other hand, ligand **3** did not show any type of

toxicity and its lethal dose is 800 mg/kg, which guarantees it a grade 4 on the scale of toxic compounds. Compounds that are shown to be toxic to any type of tissue in addition to a low L50 value are usually declared harmful compounds if ingested. However, to state with greater degrees of clarity which compound is closer to the required characteristics of a drug, it is necessary to carry out the ADME (Baberjee et al 2018). Out of the 13, only 2 of these compounds did not present a potential toxicological threat, including 4EGI-1 shown to be carcinogenic and hepatotoxic.

The complete result can be evaluated in the supplementary material of this article.

After the toxicity test, the pharmacophore map resulting from the docking, Figure 5, was constructed. For which only the base ligand and the drug candidates that did not show any organ toxicity and toxicity endpoints were selected. The purpose of the evaluation of the pharmacophore maps is to diagnose the types of interactions that occur between the eIF4E protein, the base ligand 4EGI-1, and ligands **2** and **3**.

3.5 Absorption, Distribution, Metabolism and Excretion Test (ADME)

Swiss-ADME is a platform that provides parameters such as lipophilicity (WLOGP, TPSA), water solubility (ESOL Log S), drug similarity rules and medicinal chemistry (Bakchi et al, 2022). The ADME prediction study provided physical-chemical properties of the potential oral drug candidates based on the combination of Lipinski, Veber, Ghose, Egan and Muegge rules.

Table 3. ADME Results.

Molecule	MW (g/mol)	Rotable Bonds	H-bonds aceptors	H-bonds donors	ESOL LogS	TPSA (Å²)	WLOGP	GI absorption	log kp (cm/s)
2	**377.47**	**5**	**5**	**1**	**-3.94**	**113.41**	**3.31**	**High**	**-6.90**
3	**482.53**	**7**	**6**	**2**	**-4.50**	**113.10**	**3.19**	**High**	**-7.40**
4EGI-1	**451.28**	**7**	**6**	**2**	**-6.44**	**148.64**	**5.45**	**Low**	**-4.74**

The ADME shows that in relation to molecular mass all three ligands are within the required range to be a drug, according to the Muegge Rule, the MW values should be between 200 and 600 g/mol. When we analyze the rotational bonds, none of the three ligands does not comply with the rules proposed by the scholars, which says that to have pharmacological potential the rotational bonds should not be greater than 15. The number of hydrogen acceptors should not be more than 10 and the number of hydrogen donors should not be more than five. For solubility, the higher the log value of S, the more soluble the compound is. The TSA value should be less than or equal to 140 A, the WlogP value should be less than or equal to 5.88, the absorptive GI should be high as it is about the solubility in the gastrointestinal. To be a good drug the drug should be water soluble and liposoluble, in this case the most hydrophobic ligand is 4EGI-1 and the most hydrophilic ligand is ligand 2, showing higher water solubility of the three ligands presented. The kp is the permeability coefficient, the log of kp is the skin permeation, the more negative the value of log kp, the less skin permeating the molecule is, i.e., the more negative the lower the chance of the compound crossing the skin (Bragana et al, 2022; Nelluta et al, 2023; Protti et al, 2021). The ADME results point out that the ligand that most afflicts the proposed rules for a molecule to be a drug, is the ligand 4EGI-1. This compound violates 2 of the rules proposed by Lipinski, Veber, Ghose, Egan, and Muegge rules, which indicates that it is not a good candidate for oral drug, so for the molecular dynamics step only ligands **2** and **3** will be studied.

3.6 Molecular Dynamics (MD)

The MD simulations are able to predict the behavior of the atoms that make up a system over time, based on models that govern the laws of physics, the famous Newton's equations. The trajectory resulting from this simulation describes the atomic configuration of the system at each point during the proposed simulation interval (Hollingsworth & Dor, 2018).

The evaluation parameters RMSD, RMSF, number of hydrogen bonds and interatomic distances between the protein and ligand are some of the data that can be extracted in a molecular simulation (Sinha & Wang, 2020). From the RMSD, it is possible to identify at what point a given system has reached equilibrium. In Figure 6, graph A demonstrates that at instant 4000 ps the eIF4E protein starts to decrease its oscillations in time, that is, begins to reach the thermodynamic equilibrium. The same occurs with the RMSD of the ligands, in

graph B it is observed that at 4000 ps both the ligands **2** and **3** begin to stabilize, and at 6000 ps the oscillations decrease even more, which indicates that they reach their equilibrium.

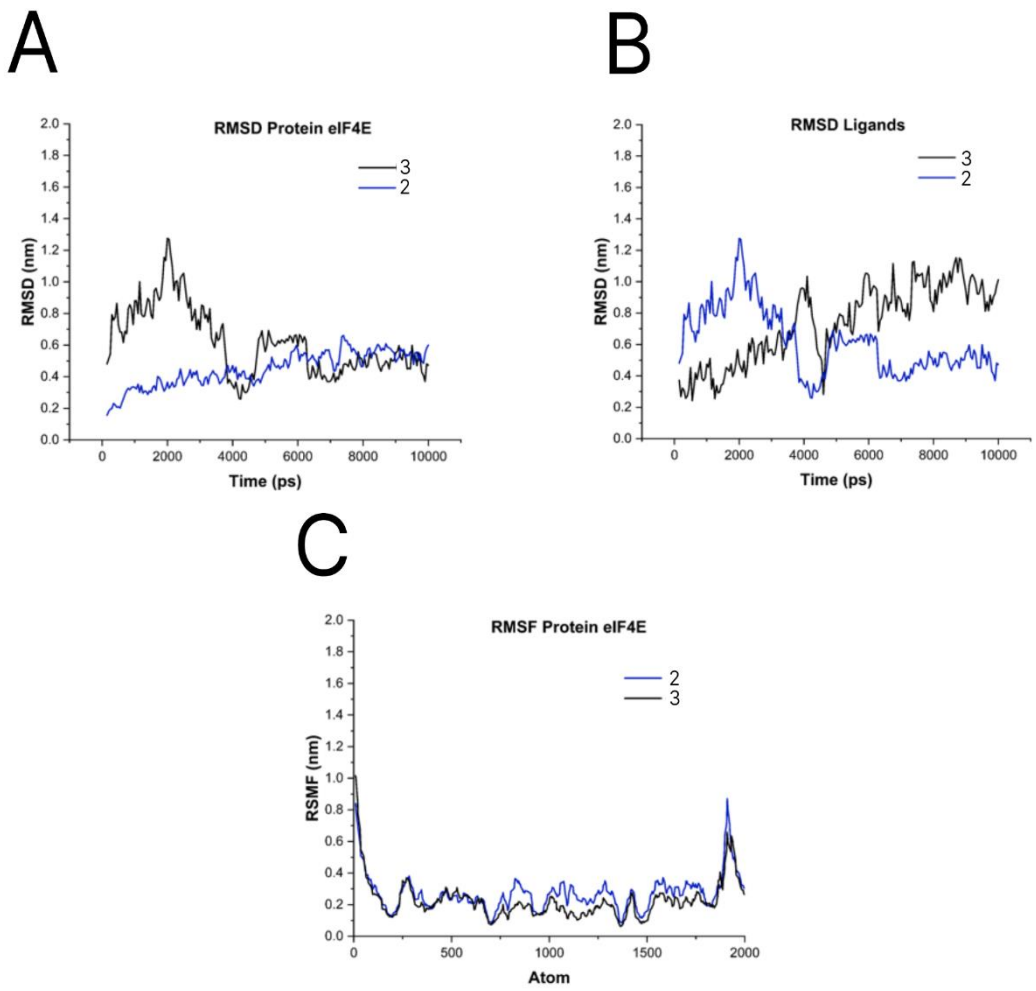


Figure 6. (A) Root Mean Square Deviation (RMSD) of the eIF4E protein (B) and RMSD for the ligands **2** and **3** in complex with the translation initiation factor eIF4E. (C) Root Mean Square Fluctuation (RMSF) of protein eIF4E.

Starting at 4.000 ps it is possible to observe in Figure 6 that the protein starts to decrease its oscillations, verified by an RMSD of 0.5 nm until the end of the simulation which indicates that the system probably entered into equilibrium. Figure 6 graph C provides information on the root mean square fluctuation, the RMSF. The RMSF plot shows the fluctuation of each residue of the eIF4E protein over the simulation time interval. Studying the protein regions with the highest flexibility can facilitate understanding of their interaction with the ligands. Additionally, the flexibility of the terminal residues and surface loop regions is generally greater than in the core of the protein (Bhardwa et al, 2022; Sales et al 2022).

In Figure 6 graph C in the 2000 ps band it can be observed that a large fluctuation occurs, which leads one to believe that and a terminal region and/or that a possible interaction occurs in this band. The crystallized structure of the eIF4E protein (PDB: 4TPW) used as a basis in this work shows the docking region of the 4EGI-1 ligand closer to the surface, so the area of noticeable fluctuation in this region may also be the region of interaction with the ligand.

The variation of the total energy obtained for both the eIF4E-2 and eIF4E-3 systems is shown in Figure 7 (graph A and B). Throughout the simulation the values remained balanced in both systems, showing that stabilization occurred in both A and B. Figure 7 also shows the interaction relationship between the ligands and the protein (graph C and D). For eIF4E-2, the total interaction energy was -218.32 ± 4.89 $kJmol^{-1}$, where -109.83 ± 3.04 $KJmol^{-1}$ corresponds to the Coulombic interactions and -108.50 ± 1.85 $KJmol^{-1}$ to Lennard-Jones interactions, which shows an equivalent contribution of LJ and electrostatic energies. In the eIF4E-3 system, the average value of the total interaction was -180.91 ± 2.50 $KJmol^{-1}$. This value corresponds to the sum of the short-range coulombic (electrostatic) interactions, and the short-range Lennard-Jones interactions (Roe & Brooks, 2022). These energy values were-148.58 ± 0.95 $kJmol^{-1}$ and -32.33 ±1.54 $KJmol^{-1}$ respectively. In this complex, it is possible to observe a greater contribution of electrostatic interactions in the total interaction energy between amino acids of the eIF4E protein and the atoms of the ligand 3.

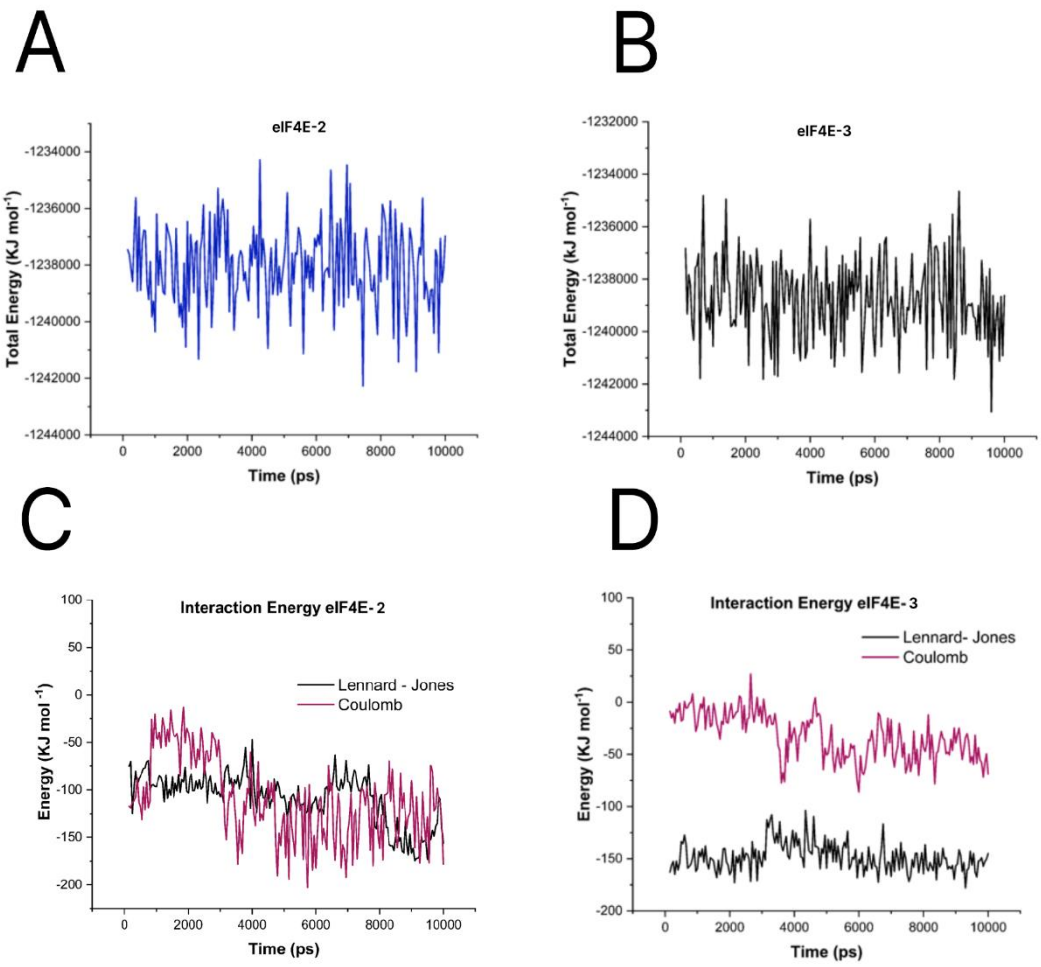


Figure 7. Energy graphs extracted from MD simulations. (A) Variation of the total energy for the eIF4E-2 system. (B) Variation of the total energy for the eIF4E-3 system. (C) Interaction energy plot for the eIF4E-3 complex. (D) Interaction energy plot for the eIF4E- 2 complex.

Hydrogen bonds in molecular dynamics are also identified after simulating the trajectory of the molecules, based on a set of geometric criteria. The most common criterion used is the distance of the donor atom from the H acceptor, in this sense the distance between them must be equal or less than 0.35 nm, another criterion evaluated is the angle of the donor hydrogen from the receptor, which must be less than 30 degrees. (Zhang et al, 2022; Wohlert et al 2022). For this work, the hydrogen bonds performed between the two ligands and the protein which were up to 0.35 nm in length were mapped during the simulation. Figure 8 illustrates the behavior of the two complexes during the 10000 ps. In graph A, the process starts with 4 hydrogen bonds that reaches up to 10 around 8200 ps and drops to 6 in the final moments of the simulation. In B, the simulation starts evidencing 3 hydrogen interactions that remain during the first 2000 ps, by the end of the simulation process the formation of 4

hydrogen bonds is evident. In both graphs it is possible to notice a trend of behavior, so as to keep the interactions at the numbers of 6 and 4 for the hydrogen interactions, in the complexes eIF4E-2 and eIF4E- 3, respectively.

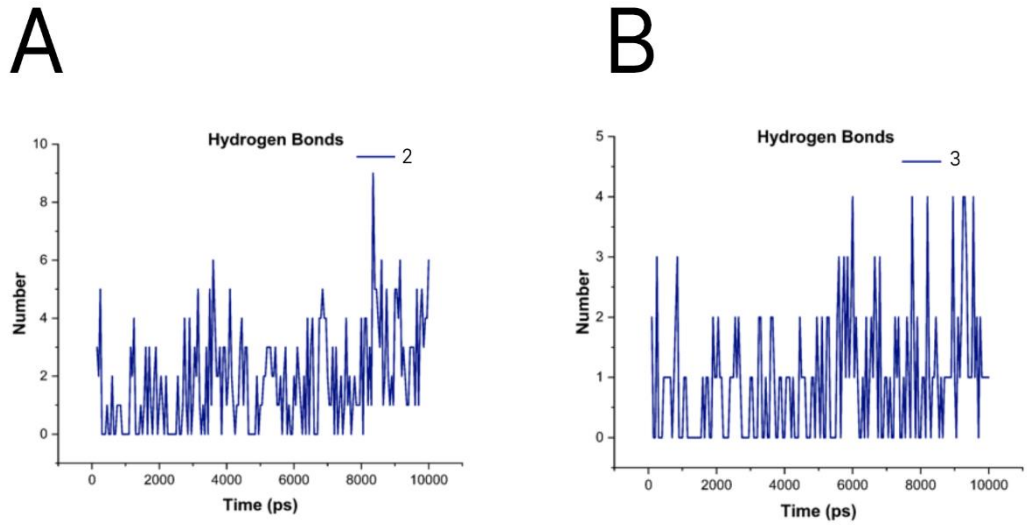


Figure 8. (A) Hydrogen bonds formed between eIF4E and 2 during the molecular dynamics simulation. (B) Hydrogen bonds formed between eIF4E and 3 during the molecular dynamics simulation.

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tudies and research in the literature point to some residues as important, which are shown in Figure 9, for molecular interactions considering the reference compound. In the residue plots illustrated below, those that remain most of the simulation with distance less than or equal to 0.25 nm indicate the presence of interactions (Wang et al, 2022). The ligand 2 is represented in blue in the graphs in Figure 9. Amino acids Phe 47, Lys 49, Asn 59, Arg 61, Ile 63 and His 78 remain closest to the ligand 2 during the simulation, with distance equal or less than 0.25 nm during the simulation. In Figure 5B and 10A, it is possible to confirm the **σ-π** and **π-π** interactions at residues Phe 47 and Lys 49 and Arg 61 respectively. In the figure 9, the black lines in the graphs represent the distance of the residues of the eIFE4 protein with the ligand 3. After analysis it is possible to verify that the amino acids Phe47, Arg 61, His 78, Tyr 79 remain at a distance less than or equal to 0.25 nm from the ligand during the entire simulation. This behavior indicates high probability of interaction between protein and ligand 3, which can contribute to the stability of the ligand in the enzyme binding site. The **π-π** type interactions are observed at residues Lys 49 and Arg 61 in Figure 5 in B and C respectively, obtained from docking simulations.

In the Figure 10 B, which show the **π-π** interactions for the frame of the ligand obtained from MD, it is possible to observe that the interaction of residue Arg 61 is also present.

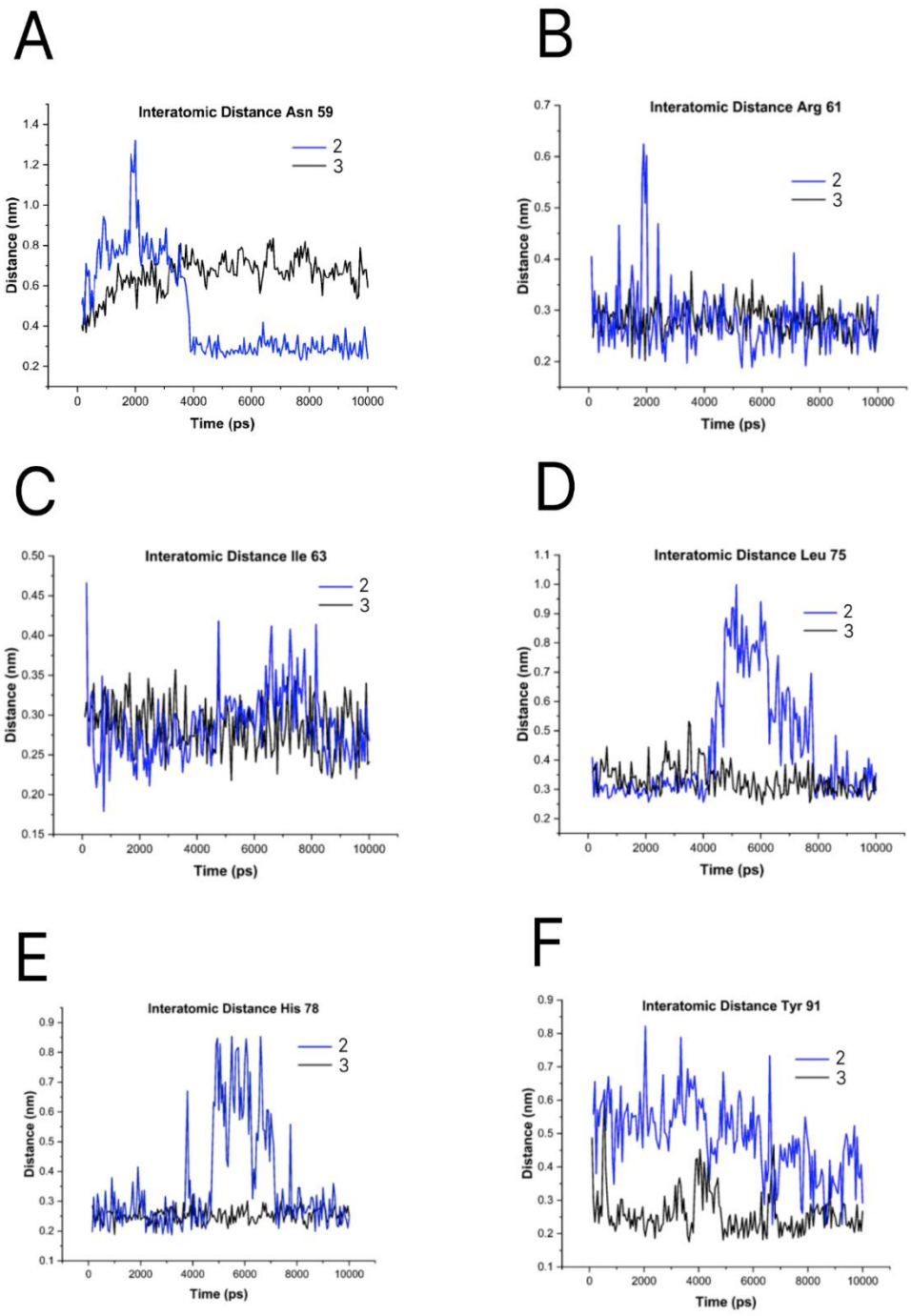

Figure 9. Interatomic distance of the main amino acid residues in the vicinity of ligands 2 and 3.

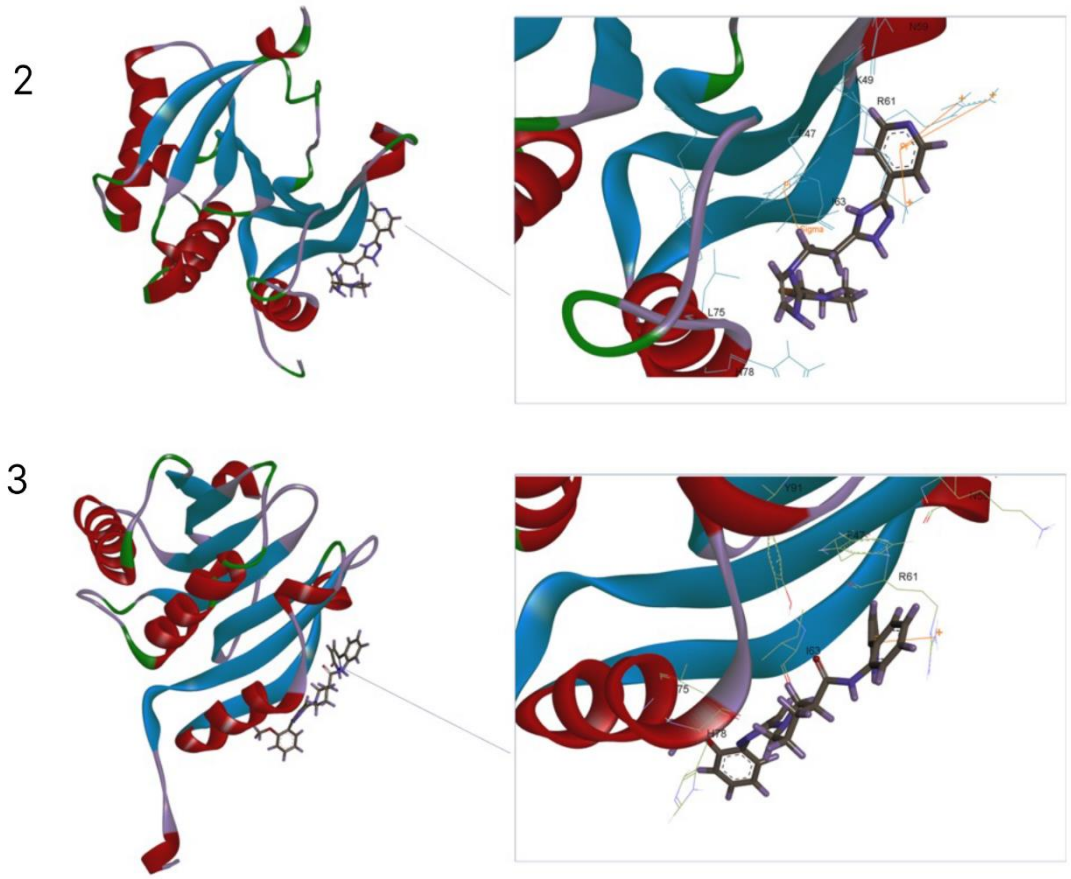

Figure 10. Interactions mapped in molecular dynamics for the complexes A. eIF4E-2 and B. eIF4E-3**.**

The MD simulations allow a better visualization of the protein-ligand interaction behavior over a pre-established time step. By associating the results obtained in this simulation with the docking results with the drug candidate ligands, it is possible to see that the ligand 2 is likely to show a higher stability in the IF4E protein binding site. So, it is imagined that being in close proximity to the amino acids considered to be the protagonists of the eIF4E-4EGI-1 interaction, there are strong indications that interactions occur between ligand 2 and the eIF4E, enabling the protein stabilization. The molecular dynamics calculations also showed that this same ligand makes a considerable number of hydrogen bonds, which remain during almost the entire simulation time. From the results, we strongly believe that these high number of interactions are the major contributors to the stabilization of the ligand in the protein site.

CONCLUSION

The process of building and validating a drug is a lengthy one, since it requires *in silico* studies, experimental and clinical tests. The purpose of the works that make use of computational chemistry methods is to facilitate the process, in order to contribute to findings that help, for example, in the treatment of diseases that are still a mystery to science, such as autism. It is important to mention that further experimental studies must be carried to validate the theoretical results presented here. Other important aspect as the route of synthesis and obtainment, the economic feasibility and possible forms of administration of these candidates should be also evaluated. With this in mind, and certain that the results elucidated here are only the beginning of a hard work that requires further tests and studies, the ligand 2 is proposed as a potential molecule to inhibit the excitatory synthesis of labeled proteins in autistic individuals. Keeping in mind that by controlling the production of neurons and synapses, the autistic symptoms can also be alleviated and/or controlled. The ligand 2 could also be the base ligand for a subsequent study of a new visual screening, since it was not toxic and complied with the screening principles proposed by Lipinski, Veber, Ghose, Egan and Muegge in ADMET.

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CONFLIT OF INTEREST

The authors declare no conflict of interest.

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SUPPLEMENTAR MATERIAL

Toxicity Analysis

Endpoint	Target	4EGI-1
Organ Toxicity	Hepatotoxicity	Active
Toxicity end Points	Carcinogenicity	Active
	Immunotoxicity	Inactive
	Mutagenicity	Active
	Cytotoxicity	Inactive
	LD50 (mg/kg)	1000
	Toxicity Class	4
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	Inactive
	Androgen Receptor (AR)	
Tox21-Stress response pathways	Heat shock factor response element (HSE)	Inactive

Endpoint	Target	Ligand 1
Organ Toxicity	Hepatotoxicity	Inactive
Toxicity end Points	Carcinogenicity	Active
	Immunotoxicity	Inactive
	Mutagenicity	Inactive
	Cytotoxicity	Inactive
	LD50 (mg/kg)	1200
	Toxicity Class	4
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	Inactive
	Androgen Receptor (AR)	
Tox21-Stress response pathways	Heat shock factor response element (HSE)	Inactive

Endpoint	Target	Ligand 2
Organ Toxicity	Hepatotoxicity	Inactive
Toxicity end Points	Carcinogenicity	Inactive
	Immunotoxicity	Inactive
	Mutagenicity	Inactive
	Cytotoxicity	Inactive
	LD50 (mg/kg)	200
	Toxicity Class	3
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	Inactive
	Androgen Receptor (AR)	
Tox21-Stress response pathways	Heat shock factor response element (HSE)	Inactive

Endpoint	Target	Ligand 3
Organ Toxicity	Hepatotoxicity	Inactive
Toxicity end Points	Carcinogenicity	Inactive
	Immunotoxicity	Inactive
	Mutagenicity	Inactive
	Cytotoxicity	Inactive
	LD50 (mg/kg)	800
	Toxicity Class	4
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	Inactive
	Androgen Receptor (AR)	
Tox21-Stress response pathways	Heat shock factor response element (HSE)	Inactive

Endpoint	Target	Ligand 4
Organ Toxicity	Hepatotoxicity	Active
Toxicity end Points	Carcinogenicity	Active
	Immunotoxicity	Inactive
	Mutagenicity	Active
	Cytotoxicity	Inactive
	LD50 (mg/kg)	500
	Toxicity Class	4
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	Inactive
	Androgen Receptor (AR)	
Tox21-Stress response pathways	Heat shock factor response element (HSE)	Inactive

Endpoint	Target	Ligand 5
Organ Toxicity	Hepatotoxicity	Active
Toxicity end Points	Carcinogenicity	Active
	Immunotoxicity	Inactive
	Mutagenicity	Active
	Cytotoxicity	Inactive
	LD50 (mg/kg)	500
	Toxicity Class	4
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	Inactive
	Androgen Receptor (AR)	
Tox21-Stress response pathways	Heat shock factor response element (HSE)	Inactive

Endpoint	Target	Ligand 6
Organ Toxicity	Hepatotoxicity	Active
Toxicity end Points	Carcinogenicity	Active
	Immunotoxicity	Inactive
	Mutagenicity	Inactive
	Cytotoxicity	Inactive
	LD50 (mg/kg)	1300
	Toxicity Class	4
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	Inactive
	Androgen Receptor (AR)	
Tox21-Stress response pathways	Heat shock factor response element (HSE)	Inactive

Endpoint	Target	Ligand 7
Organ Toxicity	Hepatotoxicity	Active
Toxicity end Points	Carcinogenicity	Active
	Immunotoxicity	Inactive
	Mutagenicity	Active
	Cytotoxicity	Inactive
	LD50 (mg/kg)	500
	Toxicity Class	4
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	Inactive
	Androgen Receptor (AR)	
Tox21-Stress response pathways	Heat shock factor response element (HSE)	Inactive

Endpoint	Target	Ligand 8
Organ Toxicity	Hepatotoxicity	Active
Toxicity end Points	Carcinogenicity	Active
	Immunotoxicity	Inactive
	Mutagenicity	Active
	Cytotoxicity	Inactive
	LD50 (mg/kg)	500
	Toxicity Class	4
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	Inactive
	Androgen Receptor (AR)	
Tox21-Stress response pathways	Heat shock factor response element (HSE)	Inactive

Endpoint	Target	Ligand 9
Organ Toxicity	Hepatotoxicity	Active
Toxicity end Points	Carcinogenicity	Active
	Immunotoxicity	Inactive
	Mutagenicity	Active
	Cytotoxicity	Inactive
	LD50 (mg/kg)	500
	Toxicity Class	4
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	Inactive
	Androgen Receptor (AR)	
Tox21-Stress response pathways	Heat shock factor response element (HSE)	Inactive

Endpoint	Target	Ligand 10
Organ Toxicity	Hepatotoxicity	Active
Toxicity end Points	Carcinogenicity	Active
	Immunotoxicity	Inactive
	Mutagenicity	Inactive
	Cytotoxicity	Inactive
	LD50 (mg/kg)	1000
	Toxicity Class	4
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	Inactive
	Androgen Receptor (AR)	
Tox21-Stress response pathways	Heat shock factor response element (HSE)	Inactive

Endpoint	Target	Ligand 11
Organ Toxicity	Hepatotoxicity	Active
Toxicity end Points	Carcinogenicity	Active
	Immunotoxicity	Inactive
	Mutagenicity	Inactive
	Cytotoxicity	Inactive
	LD50 (mg/kg)	500
	Toxicity Class	4
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	Inactive
	Androgen Receptor (AR)	
Tox21-Stress response pathways	Heat shock factor response element (HSE)	Inactive

Endpoint	Target	Ligand 12
Organ Toxicity	Hepatotoxicity	Active
Toxicity end Points	Carcinogenicity	Inactive
	Immunotoxicity	Inactive
	Mutagenicity	Inactive
	Cytotoxicity	Inactive
	LD50 (mg/kg)	500
	Toxicity Class	4
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	Inactive
	Androgen Receptor (AR)	
Tox21-Stress response pathways	Heat shock factor response element (HSE)	Inactive

Endpoint	Target	Ligand 13
Organ Toxicity	Hepatotoxicity	Active
Toxicity end Points	Carcinogenicity	Active
	Immunotoxicity	Inactive
	Mutagenicity	Active
	Cytotoxicity	Inactive
	LD50 (mg/kg)	574
	Toxicity Class	4
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	Inactive
	Androgen Receptor (AR)	
Tox21-Stress response pathways	Heat shock factor response element (HSE)	Inactive

ATTACHMENT

Works developed during the doctorate:

- **Future Therapeutic Perspectives into the Alzheimer's Disease Targeting the Oxidative Stress Hypothesis**
  Jéssika P. Teixeira, Alexandre A. de Castro, Flávia V. Soares, Elaine F. F. da Cunha and Teodorico C. Ramalho

  (Molecules)
  https://doi.org/10.3390/molecules24234410

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Review

Future Therapeutic Perspectives into the Alzheimer's Disease Targeting the Oxidative Stress Hypothesis

Jéssika P. Teixeira [1], Alexandre A. de Castro [1], Flávia V. Soares [1], Elaine F. F. da Cunha [1] and Teodorico C. Ramalho [1,2,*]

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Abstract: Alzheimer's disease (AD) is a neurodegenerative disease that is usually accompanied by aging, increasingly being the most common cause of dementia in the elderly. This disorder is characterized by the accumulation of beta amyloid plaques (Aβ) resulting from impaired amyloid precursor protein (APP) metabolism, together with the formation of neurofibrillary tangles and tau protein hyperphosphorylation. The exacerbated production of reactive oxygen species (ROS) triggers the process called oxidative stress, which increases neuronal cell abnormalities, most often followed by apoptosis, leading to cognitive dysfunction and dementia. In this context, the development of new therapies for the AD treatment is necessary. Antioxidants, for instance, are promising species for prevention and treatment because they are capable of disrupting the radical chain reaction, reducing the production of ROS. These species have also proven to be adjunctive to conventional treatments making them more effective. In this sense, several recently published works have focused their attention on oxidative stress and antioxidant species. Therefore, this review seeks to show the most relevant findings of these studies.

Keywords: Alzheimer's disease; oxidative stress; antioxidants; free radicals; cellular respiration

- **Regulation of Protein Synthesis: An Approach to Treat Autism Spectrum Disorder (ASD)**

  Jéssika P. Teixeira and Teodorico C. Ramalho

  (Current Medicinal Chemistry)
  https://doi.org/10.2174/0929867328666210419125634

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REVIEW ARTICLE

Regulation of Protein Synthesis: An Approach to Treat Autism Spectrum Disorder (ASD)

Jéssika Poliana Teixeira[1] and Teodorico Castro Ramalho[1,2,*]

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Abstract: Autism Spectrum Disorder (ASD) is a disorder with different etiologies and poor elucidation, characterized by changes in social and cognitive skills. ASD impacts a large number of people in the world. Surprisingly, in spite of its great importance, just modest progress has been achieved towards comprehending this pathology and designing new therapies. The molecular dysfunctions observed in people with autism are evidenced by the interference in the synthesis of synaptic proteins, which impairs their development and plasticity, leading to characteristics of individuals with ASD. The present work investigates the mTOR pathway and the proteins related to its regulation and neurological functioning. The path of protein synthesis and translation is promising to treat various disorders and its elucidation may, for example, result in drugs that facilitate the diagnosis and broaden the range of treatments, improving the quality of life of ASD patients.

Keywords: Neurodevelopment, translation, mTOR, eIF4E-eIF4G, mGluR, GRK5.

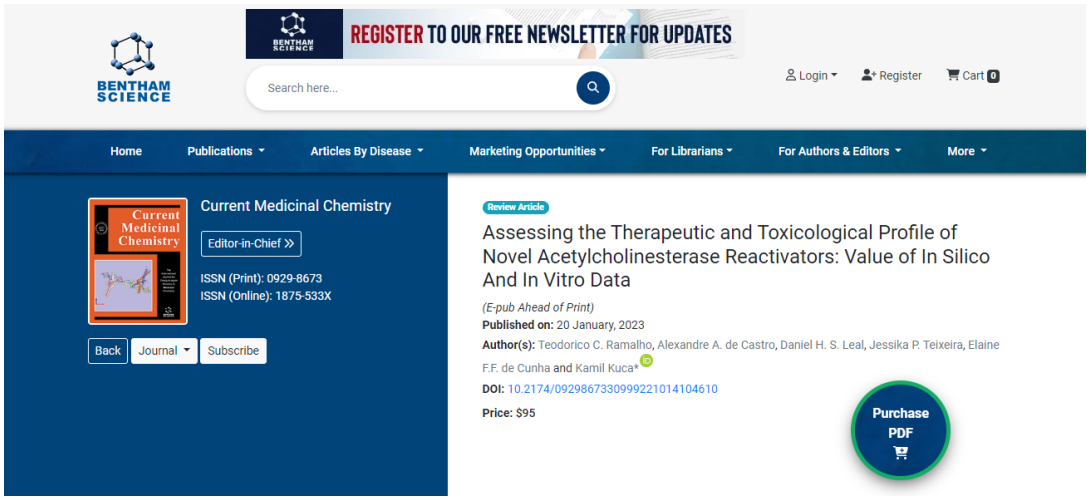
1. INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevel- reassure them and help them to face the world outside their own [3-5]. Due to these behavioral patterns, the

- **Assessing the therapeutic and toxicological profile of novel acetylcholinesterase reactivators: value of in silico and in vitro data.**

Teodorico C. Ramalho, Alexandre A. de Castro, Daniel. H. S. Leal, Jéssika P. Teixeira, Elaine F. F. da Cunha and Kamil Kuca

(Currente Medicinal Chemistry)
DOI: 10.2174/0929867330999221014104610



- Coumarin/beta-cyclodextrin inclusion complexes reveal acceleration and improve of wound healing

Patent

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Depositante 1 de 5

Nome ou Razão Social: UNIVERSIDADE FEDERAL DE SÃO JOÃO DEL REI

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CPF/CNPJ: 21186804000105

Inventor 12 de 20

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Nacionalidade: Brasileira

Qualificação Física: Doutorando

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Cidade: LAVRAS

Estado: MG

CEP:

País: BRASIL

Telefone:

- Development, Application and *in silico* studies of new eIF4E-eIF4G complex inhibitors to treat Autistic Spectrum Disorder

  Journal of Biomolecular Structure and Dynamics

  Jéssika P. Teixeira, Thaís A. Sales, Létícia, A and Teodorico C. Ramalho

- mGlur5 protein inhibitors, a new proposal to treat autism. In silico methods to propose new drugs.

  Jéssika P. Teixeira and Teodorico C. Ramalho

  Work in development