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Research Article

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Molecular Docking Mediated Virtual Drug Screening for $GABA_A$ Receptor: Promising Digoxin Derivatives

Moleküler Yanaştırma Yöntemiyle GABAA Reseptörü için Sanal İlaç Tarama: Umut Veren Digoksin Türevleri

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ABSTRACT

In the central nervous system (CNS) of mammalian species, γ-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter due to it regulates neuronal development through leading neural differentiation, proliferation, migration, etc. GABAA receptor is the major GABA receptor since it has the highest expression level among the other GABA receptors within CNS. Many pieces of evidence prove that the defects in the GABAergic pathway might give rise to serious diseases such as schizophrenia, epilepsy, anxiety, depression, insomnia, etc. In this study drug library with a totally of 8170 ligands consists of three distinct datasets which are FDA-approved Drugs, Drugs Approved by World but not FDA, and Non-human Metabolites have been screened for the allosteric site of the GABAA receptor with PyRx Virtual Screening Tool and ligand-receptor interactions have been analyzed with Biovia Discovery Studio software. Results reveal that Digoxin and its two distinct derivatives (DD1 and DD2), as well as Conivaptan, are promising in the treatment of GABAergic pathway-based disorders. The findings of this report should be verified with further molecular dynamics (MD) simulations and the ligands should be tested by both *in vitro* and *in vivo* studies.

Key Words

Virtual drug screening, molecular docking, GABA, GABAergic pathway, central nervous system, nervous disorders.

ÖZ

Memeli türlerinin merkezi sinir sisteminde (MSS) γ-aminobütirik asit (GABA), nöral farklılaşma, çoğalma, göç vb. yolakları düzenleyen nöronal gelişim için birincil inhibitör nörotransmiterdir. $GABA_A$ reseptörü MSS içindeki diğer GABA reseptörleri arasında en yüksek ekspresyon seviyesine sahip olduğu için majör GABA reseptörüdür. GABAerjik yolaktaki bozuklukların şizofreni, epilepsi, anksiyete, depresyon, uykusuzluk gibi ciddi hastalıklara yol açabileceğini gösteren pek çok kanıt bulunmaktadır. Bu çalışmada FDA Onaylı İlaçlar, Dünyaca Onaylı Ama FDA Onaylı Olmayan İlaçlar ve İnsan Dışı Metabolitler olarak üç farklı verisetinden oluşan toplam 8170 ligand içeren ilaç kütüphanesi $GABA_A$ reseptörünün allosterik bölgesi için PyRx Virtual Screening Tool ile taranmış ve ligand-reseptör etkileşimleri Biovia Discovery Studio yazılımı ile analiz edilmiştir. Sonuçlar, Digoksin ve iki farklı türevinin (DD1 ve DD2) ve ayrıca Conivaptan'ın GABAerjik yolak temelli bozuklukların tedavisinde umut verici olduğunu ortaya koymaktadır. Bu raporun bulguları daha ileri moleküler dinamik (MD) simülasyonları ile doğrulanmalı ve ligandlar hem in vitro hem de in vivo çalışmalarla test edilmelidir.

Anahtar Kelimeler

Sanal ilaç tarama, moleküler yanaştırma, GABA, GABAerjik yolak, merkezi sinir sistemi, sinir sistemi bozuklukları.

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INTRODUCTION

γ-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the mammalian central nervous system (CNS). Through the GABAergic pathway, GABA acts a significant role in neuronal development by regulating neural differentiation, migration, proliferation, etc [1], [2]. Fundamentally, the functions of GABA are exhibited by binding to three distinct receptors which are $GABA_A$, $GABA_B$, and $GABA_C$ receptors [3]. While $GABA_A$ and $GABA_C$ are chloride ion (Cl^-) transporter ionotropic and ligand-gated receptors, $GABA_B$ is a metabotropic receptor [4]. Differentiation in GABA expression, GABA metabolism, synaptic receptors' activity, and differentiation on Cl^- ion concentration across the cell membrane is the primary parameters defining the regulation of neuronal activity [5].

Among the GABA receptors, $GABA_A$ has the highest expression level along with the CNS and is primarily responsible for fast inhibition in the brain [6]. The chemical structure of $GABA_A$ receptors contains five distinct subunits which are $\alpha_{(1-6)}$, $\beta_{(1-3)}$, $\gamma_{(1-3)}$, δ, ϵ, Θ, π, $\rho_{(1-3)}$ [7]. In general, $GABA_A$ receptors that are widely expressed in the mammalian brain contain two α, two β, and one γ chains, and this conformation of $GABA_A$ receptors enables phasic inhibition which is fast and temporaneous through rapid desensitizing of postsynaptic receptors. In addition, tonic (long-term) activation within mammalian organisms is mediated by extrasynaptic $GABA_A$ receptors containing α5 subunit in the hippocampus and π subunit in the brain [8]–[10]. This tonic activation acts a prominent role in neuronal development since it regulates neural cell growth, formation of synapses, migration, and proliferation. Furthermore, $GABA_A$ receptors' localization over the synapses provides strong communication between neural cells [11]. As such, $GABA_A$ receptor expression profile, construction of its structure, and regular process of GABAergic pathway through these receptors are the main parameters for neuronal network creation and development within CNS [12].

Many discoveries demonstrate that the defects of the GABAergic pathway might cause serious diseases [13]. Among these diseases, schizophrenia takes the lead as one of the most significant neurodevelopmental disorders [14]. The primary symptoms of schizophrenia such as psychotic thoughts, hallucinations, delusions, and behavioral alterations are sourced from disrupted neuronal communications [15]. Various researches have reported that the inhibition of α5 subtype $GABA_A$ receptor by enhanced binding affinity of GABA gives rise to schizophrenic behavior patterns [16]. In addition, enhanced immune reactivity of α2 subtype $GABA_A$ receptor which is sourced from prenatal infections also might create schizophrenic pathogenesis [17]. Besides, the observation of attenuation on $GABA_A$ receptor expression and GABAergic pathway might cause other serious neuronal disorders such as epilepsy [18], anxiety [19], depression [20], insomnia [21], etc. For instance, it's been reported that extreme anxiety, avoidance behavior, and traumatic memories may be sourced by inhibition of $GABA_A$ receptors and be treated with to increase GABAergic transmission by various chemical compounds such as benzodiazepines [22]. Moreover, surprisingly it's recently been discovered that GABA metabolites secreted by B-cells inhibit $CD8^+$ T cell activity and suppress anti-tumor immune response through binding to $GABA_A$ receptors expressed on the surface of $CD8^+$ T cells [23]. Those findings indicate the great potential of $GABA_A$ receptors to treat several diseases from neuronal disorders to cancer and to date, various inhibitors have been developed such as Suramin [24], Broflanilide [25], Clozapine [26], etc.

In this study, virtual drug screening targeting the $GABA_A$ receptor's allosteric site has been carried out with a molecular docking-based approach. As such, 1609 FDA-Approved Drugs, 4254 Drugs Approved by World but not FDA, and 2307 Non-human Metabolites -a total of 8170 ligands- have been investigated during the virtual screening. Furthermore, the ligand that is included in the chemical structure of $GABA_A$ receptor (Benzamidine) has been re-docked and 7 distinct known inhibitors have been analyzed in order to validate the research. Findings demonstrate that two distinct Digoxin derivatives (ZINC000118915215 (DD1) and ZINC000118915217 (DD2)) from the Drugs Approved by World but not FDA dataset have the highest binding affinity to the allosteric site of $GABA_A$ receptor. In addition, the Digoxin drug (ZINC000242548690) from the FDA-Approved Drugs dataset has been revealed as one of the ligands having the highest binding affinity after Conivaptan (ZINC000012503187). Therefore, Digoxin and its derivatives are evaluated as promising compounds in the treatment of GABAergic pathway-based diseases.

MATERIALS and METHODS

Receptor Preparation

Crystal structure of $GABA_A$ receptor and grid box sizes selection were carried out by following the research published by Sahila and his colleagues in 2015 [27]. The $GABA_A$ crystal structure with PDB ID: 4COF was retrieved in .pdb format from Protein Data Bank (PDB). X-ray diffraction analysis of the selected $GABA_A$ receptor has a 2.97 Å resolution, 0.226 R-value (free), and 0.206 R-value (observed). The downloaded receptor structure was imported in UCSF Chimera software version 1.16. The ligands and heteroatoms of the $GABA_A$ receptor were removed from the structure and the receptor was prepared by following the Dock Prep module of UCSF Chimera [28]. During preparation solvent molecules (water) were removed, hydrogen atoms and partial charges were added, and the side chains were replaced by using Dunbrack 2010 rotamer library. Once the preparation had been completed, the prepared receptor was exported in .pdb format and imported into PyRx Virtual Screening Tool so that to be used in molecular docking studies.

Ligand Preparation

In order to perform virtual drug screening, a library containing a total of 8170 ligands was prepared including FDA-Approved Drugs dataset consisting of 1609 ligands, Drugs Approved by the World but not FDA dataset consisting of 4254 ligands, and Non-human Metabolites dataset consisting of 2307 ligands. The ligands constituting datasets were retrieved from the ZINC15 database. The datasets were imported in PyRx software separately, and the ligands were prepared by following the energy minimization module of the PyRx Virtual Screening Tool [29].

Molecular Docking

Virtual drug screening was conducted on the AutoDock Vina package loaded in PyRx Virtual Screening Tool by docking all ligand datasets to the allosteric site of the $GABA_A$ receptor [30]. During the molecular docking strategy, the file format of ligands was converted to .pdbqt, and grid box coordinates were defined as x= -20.224, y= -20.175, z= 126.3505 by following the Sahila et. al.'s publication and considering the benzamidine ligand location in the crystal structure of the protein [27]. Grid box dimensions were set to x= 40.143, y= 40.138, z= 40.053. Once the molecular docking had been completed, the data demonstrating binding affinity, rmsd/ub, and rmsd/lb of each dataset were exported in .csv format. The ligands with the highest binding affinity and mode with 0 value of each rmsd/ub, and rmsd/lb parameters had been selected, and receptor-ligand interactions' analysis was carried out in Biovia Discovery Studio Visualiser software.

Validation

In order to validate the molecular docking strategy, the structure of Benzamidine ligand found in the crystal structure of the receptor had been exported as .pdb format and re-docked to the receptor by following ligand preparation and molecular docking protocols. Besides, 7 known GABA antagonists which are Broflanilide (CHEBI: 131598), Clozapine (CHEBI: 3766), Flumazenil (CHEBI: 5103), Hydrochloride (CHEBI: 145121), Picrotoxinin (CHEBI: 8206), Suramin (CHEBI: 45906), Xenovulene A (CHEBI: 66336) were downloaded from ChEBI database and docked by conducting the same procedure.

ADME Study and Toxicity Profile

Absorption, Distribution, Metabolism, and Excretion (ADME) and toxicity properties of the three ligands exhibiting the highest binding affinity within the Drugs Approved by the World but not FDA and Non-human Metabolites datasets were analyzed with both OSIRIS Property Explorer tool [31] and swissADME server [32]. As such, physico-chemical properties (formula, molecular weight, molar refractivity, and topological polar surface area (TPSA)), lipophilicity parameters (iLOGP, XLOGP3, WLOGP, MLOGP, SILICOS-IT, and Consensus Log Po/w), solubility properties (Log S, SILICOS-IT solubility in both mg/ml and mol/l units, and solubility class), druglikeness properties (druglikeness and drug-score), pharmacokinetics parameters (GI absorption, BBB permeant, P-gp substrate, CYP1A2 inhibitor, CYP2C19 inhibitor, CYP2C9 inhibitor, CYP2D6 inhibitor, and CYP3A4 inhibitor), and toxicity profiles (mutagenicity, tumorigenicity, irritant effects, and reproductive effects) of related ligands were revealed. There was no requirement for ADME and toxicity analysis of the FDA-Approved Drugs dataset now that the ligands had been tested by FDA previously.

RESULTS and DISCUSSION

In order to reveal possible promising GABA antagonists, molecular docking mediated virtual drug screening was carried out. As such, a ligand library with a total of 8170 ligands consisting of three distinct datasets which are FDA-Approved Drugs (1609 ligands), Drugs Approved by World but not FDA (4254), and Non-human Metabolites (2307 ligands) were docked to the allosteric site of $GABA_A$ receptor. The binding affinity values and interacting receptor residues of the best 20 ligands of FDA-Approved Drugs, Drugs Approved by World but not FDA, and Non-human Metabolites are shown in Table 1, Table 2, and Table 3, respectively.

The molecular docking strategy was validated by re-docking the Benzamidine compound found in the crystal structure of the receptor. In this scope, the Benzamidine compound was exported as a separate file and re-docked to the allosteric site of the receptor. The binding affinity and the RMSD values of re-docked Benzamidine was determined as -6.7 kcal/mol, and 0.0 (rmsd/ub, and rmsd/lb), respectively. Furthermore, the Benzamidine-receptor interactions in both the downloaded crystal structure and the re-docked structure were performed in Biovia Discovery Studio (Figure 1). Results demonstrate that while Benzamidine in the crystal structure interacts with Tyr62, Phe200 residues through pi-pi stacked interactions and Tyr97, Glu155, Ser156, Tyr157, residues through conventional hydrogen bonds, re-docked Benzamidine interacts with Tyr62, Phe200 through pi-pi stacked interactions, Tyr97, Ser156, Tyr157 through conventional hydrogen bonds, Glu155, Tyr205 residues of the receptor through the salt bridge and pi cation interactions. Similarities of interacting residues of the receptor and the types of interactions demonstrate the sufficient accuracy of the molecular docking approach conducted in the study.

Furthermore, 7 known GABA antagonists were docked to the $GABA_A$ receptor's allosteric site and binding affinities were analyzed (Table 4). The chemical structures of the three best-scored ligands from each dataset were illustrated in Figure 2. Inhibitor docking findings demonstrate that the Suramin ligand has the highest binding affinity with -10.4 kcal/mol value. In addition, Broflanilide has -9.5 kcal/mol binding affinity, and Clozapine has -8.3 kcal/mol binding affinity. The receptor interactions of these three ligands and remaining inhibitors were analyzed in order to reveal common interacting residues of the $GABA_A$ receptor with GABA antagonists. As such it's revealed that Suramin might create conventional hydrogen bonds with Ala45, carbon-hydrogen bonds with Ser46, Asp48, Asn100, Lys102, Arg180, halogen (fluorine) interactions with Ile47, Asp48, Met55, alkyl and pi-alkyl interactions with Ala45, Leu99, Ala135, Met137, pi-cation interaction with Arg180 residues, Broflanilide might create carbon-hydrogen bonds and pi-anion interactions with Asp43, pi-pi stacked interaction with Tyr62, alkyl and pi-alkyl interactions with Tyr62, Leu99, Phe200, Ala201 residues, Clozapine might create carbon-hydrogen bond with Asp43, pi-sigma interactions with Tyr62, Phe200, conventional hydrogen bonds with Gln64, alkyl and pi-alkyl interactions with Leu99, Ala201, halogen (fluorine) interactions with Lys173 residues of the $GABA_A$ receptor (Figure 3). Considering the findings and interactions of remaining GABA antagonists, the common residues were revealed as; Asp43, Ala45, Ser46, Ile47, Tyr62, Leu99, Ala135, Met137, Tyr157, Arg180, Ile181, Phe200, Ala201. The number of common amino acids and their percentages in interaction have been analyzed by considering 20 best ligands of the three datasets as well as the inhibitors. The results are demonstrated in Table 5.

Among 1609 FDA-Approved Drugs, 7 drugs (ZINC000012503187, ZINC000242548690, ZINC000036701290, ZINC000203757351, ZINC000004099009, ZINC000095617679, ZINC000011679756) exhibited higher binding affinity then -10.4 kcal/mol which is the Suramin's binding affinity. In particular, the three best-scored ligands from the FDA-Approved Drugs dataset that are ZINC000012503187 (Conivaptan), ZINC000242548690 (Digoxin), ZINC000036701290 (Ponatinib) have been evaluated as promising with the binding affinities of -11.8 kcal/mol, -11.6 kcal/mol, and -10.9 kcal/mol, respectively. The receptor interactions of these ligands are demonstrated in Figure 4. Conivaptan is a Vasopressin receptors (V1a and V2) inhibitor [33] and is widely used in decreasing sodium levels in the blood and heart failure diseases [34]. Findings demonstrate that Conivaptan might create conventional hydrogen bonds with Asp43, carbon-hydrogen bonds with Ala45, Alkyl, and pi-alkyl interactions with Ala45, Leu99, Ala135, Ala201, pi-pi stacked, and pi-pi t-shaped interactions with Tyr157, Phe200, pi-sigma interaction with Asn100, and pi-cation interaction with Arg207. Digoxin is a molecule extracted from Digitalis lanata [35] and is used in the treatment of many cardiac diseases since it has activity on increasing

Table 1. Best 20 ligands, their binding affinities and interacting receptor residues of FDA-Approved Drugs dataset.

FDA-Approved Drugs		
Ligand Name	Binding Affinity (kcal/mol)	Receptor Residues Interacting with Ligand
ZINC000012503187	-11.8	ASP43, ALA45, LEU99, ASN100, ALA135, TYR157, PHE200, ALA201, ARG207
ZINC000242548690	-11.6	ALA45, SER46, MET49, VAL53, MET55, TYR62, LEU99, ALA135, PRO184
ZINC000036701290	-10.9	ASN41, ASP43, ALA45, TYR62, GLN64, LEU99, PHE200, ALA201, ARG207
ZINC000203757351	-10.8	ASP43, ALA45, LEU99, ASN100, LYS102, ALA135, ARG180, ARG196, PHE200, ALA201, ARG207
ZINC000004099009	-10.6	ALA45, SER46, ASP48, ASP101, LYS102, ALA135, GLU182
ZINC000095617679	-10.6	ASP43, ALA45, TYR62, LEU99, MET115, TYR157, ARG180, PHE200, THR202, ARG207
ZINC000011679756	-10.5	ASN41, ASP43, TYR62, TYR157, PHE200, ALA201, THR202, TYR205
ZINC000006745272	-10.4	ASP43, ALA45, SER46, ASP48, TYR62, GLN64, LEU99, ASN100, ASP101, ALA135, PHE200
ZINC000100378061	-10.3	ALA45, TYR62, LEU99, ARG180, PHE200
ZINC000000538658	-10.2	ALA45, TYR62, LEU99, ARG180
ZINC000004097344	-10.2	ASP43, ALA45, LEU99, ALA135, MET137, GLU155, ALA201, THR202, TYR205
ZINC000040430143	-10.2	ASN41, ASP43, TYR62, PHE200, ALA201
ZINC000052955754	-10.2	ALA45, TYR62, MET137, ARG180, GLU182, PHE200, ARG207,
ZINC000068202099	-10.2	ASN41, ASP43, TYR62, TYR157, ARG169, GLY170, PHE200, ALA201
ZINC000169621231	-10.2	ALA45, ASN100, ARG180
ZINC000001493878	-10.1	ALA45, SER46, ASP48, TYR62, LEU99, ASP101, ARG129, GLU155, TYR157, PHE200, TYR205,
ZINC000006716957	-10.1	ALA45, LEU99, ALA135, MET137, THR151, GLU153, GLU182, LEU183, PRO184
ZINC000026985532	-10.1	ASP43, SER46, TYR62, LEU99, ASN100, ALA135, MET137, ARG180, PHE200, ARG207
ZINC000064033452	-10.1	ASP43, TYR62, SER156, TYR157, PHE200, ALA201
ZINC000070466416	-10.1	ASP43, ALA45, SER46, TYR62, ALA135, MET137

Table 2. Best 20 ligands, their binding affinities, and interacting receptor residues of Drugs Approved by World but not FDA dataset.

Drugs Approved by World but not FDA		
Ligand Name	Binding Affinity (kcal/mol)	Receptor Residues Interacting with Ligand
ZINC000118915215	-11.9	ILE47, MET55, ALA135, MET137, ARG180, GLU182, LEU183, PRO184
ZINC000118915217	-11.9	MET49, TYR97, ALA135, MET137, ARG180, PRO184
ZINC000001542146	-11.3	ALA45, ILE47, TYR62, LEU99, ALA135, MET137, ILE181, GLU182, PHE200
ZINC000257362202	-11.2	ALA45, SER46, ASP48, MET49, GLU52, VAL53, MET55, LEU99, LYS102, ALA135, THR151, PRO184
ZINC000150339052	-10.9	ALA45, ASP48, MET55, LEU99, ALA135, CYS136, MET137, THR151, PRO184
ZINC000256630457	-10.9	ASP43, ALA45, TYR97, LEU99, ASN100, ALA135, MET137, GLU182, PHE200, ALA201
ZINC000118915214	-10.8	ILE47, VAL50, ASN54, ALA135, MET137, GLU153, LEU183, ARG196
ZINC000011616152	-10.7	ASP43, ALA45, TYR62, ARG180, PHE200, ALA201
ZINC000118915216	-10.7	ASP43, ASP48, VAL53, MET55, LEU99, ALA135, MET137, ARG180, LEU183, PRO184
ZINC000256109538	-10.7	SER46, ILE47, ASP48, MET49, GLU52, VAL53, MET55, ALA135, PRO184
ZINC000885764928	-10.7	TYR62, ALA135, ARG180, PHE200
ZINC000008143788	-10.6	TYR62, GLN64, PHE200
ZINC000095618690	-10.6	ASP48, TYR62, LEU99, ALA135, MET137, ARG180, GLU182
ZINC000000577115	-10.5	TYR62, GLU155, TYR157, PHE200, ALA201, TYR205
ZINC000043195321	-10.5	ASP43, ALA45, TYR62, LEU99, ARG180, PHE200, ALA201,
ZINC000100054749	-10.5	ALA45, SER46, ASP48, LEU99, ASN100, ASP101, LYS102, ALA135, CYS136, MET137, ASN149, THR151
ZINC000224657532	-10.5	ASP43, GLN64, GLU155, PHE200, ALA201, TYR205
ZINC000000537940	-10.4	ASN41, GLU155, TYR157, ARG169, ALA174, PHE200, ALA201, THR202, TYR205
ZINC000095618689	-10.4	TYR62, GLY127, TYR157, LYS173, GLU179, PHE200, ALA201, TYR205
ZINC000003872494	-10.3	TYR62, TYR97, GLU155, TYR157, PHE200, ALA201, TYR205

Table 3. Best 20 ligands, their binding affinities and interacting receptor residues of Non-human Metabolites dataset.

Non-human Metabolites		
Ligand Name	Binding Affinity (kcal/mol)	Receptor Residues Interacting with Ligand
ZINC000028642721	-10.1	ALA45, SER46, ILE47, THR60, ASN100, ASP101, ALA135, CYS136, MET137, ARG180, GLU182
ZINC000100029436	-10.0	ALA45, SER46, ILE47, ASP48, TYR62, LEU99, ILE181, PHE200, ALA201, ARG207
ZINC000004098622	-9.9	ASP43, ALA45, SER46, TYR62, TYR97, LEU99, ASN100, ASP101, ARG180, PHE200, TYR205
ZINC000085432705	-9.8	ALA45, SER46, ASP48, TYR62, LEU99, ALA135, MET137, PHE200, ALA201
ZINC000003870412	-9.7	ALA45, SER46, ASP48, ASN54, MET55, LEU99, ASN100, ASP101, ALA135, MET137
ZINC000004096846	-9.7	SER46, ASP48, ASN54, ASN100, ASP101, ALA135, MET137, ARG180
ZINC000169335484	-9.6	ASP43, ALA45, ASP48, ASN54, MET55, LEU99, LYS102, ALA135, CYS136, MET137, GLU153, ARG180, GLU182, ARG207
ZINC000003874317	-9.5	ASN41, TYR62, GLN64, GLU155, SER156, TYR157, THR176, PHE200, ALA201, TYR205
ZINC000003255767	-9.4	ASP43, ALA45, TYR62, GLN64, LEU99, TYR157
ZINC000032052445	-9.4	ALA45, SER46, ASN100, ASP101, ALA135, ARG180
ZINC000038231587	-9.4	TYR62, TYR157, PHE200, ALA201, TYR205
ZINC000072180374	-9.4	TYR62, LEU99, ASN100, MET137, ASN149, CYS150, THR151, PHE200
ZINC000096006026	-9.4	ASP41, SER46, ASP48, MET55, GLN64, ALA135, CYS136, MET137, GLU153, TYR157, ARG180, GLU182
ZINC000100256265	-9.4	ASP43, ALA45, TYR62, LEU99, ASN100, CYS136, ASN149, THR151, ARG180, PHE200
ZINC000256095149	-9.4	ILE47, ASP48, MET137, ARG196, SER209
ZINC000257693609	-9.4	ASP43, ALA45, SER46, ASP48, TYR62, LEU99, ASN100, ARG180, GLU182, PHE200
ZINC000000156701	-9.3	ASN41, ASP43, TYR97, TYR157, ALA201, TYR205
ZINC000008215411	-9.3	ASP43, ALA45, SER46, ASP48, VAL53, ASN54, MET55, THR60, LEU99, ASN100, ASP101, CYS136, ARG180, GLU182, PRO184
ZINC000085552319	-9.3	ASN41, ASP43, GLN64, TYR97, GLU155, VAL198, VAL199, PHE200, ARG207
ZINC000096015174	-9.3	ILE47, ASP48, ASN149, ARG180, ILE181, GLU182, SER211

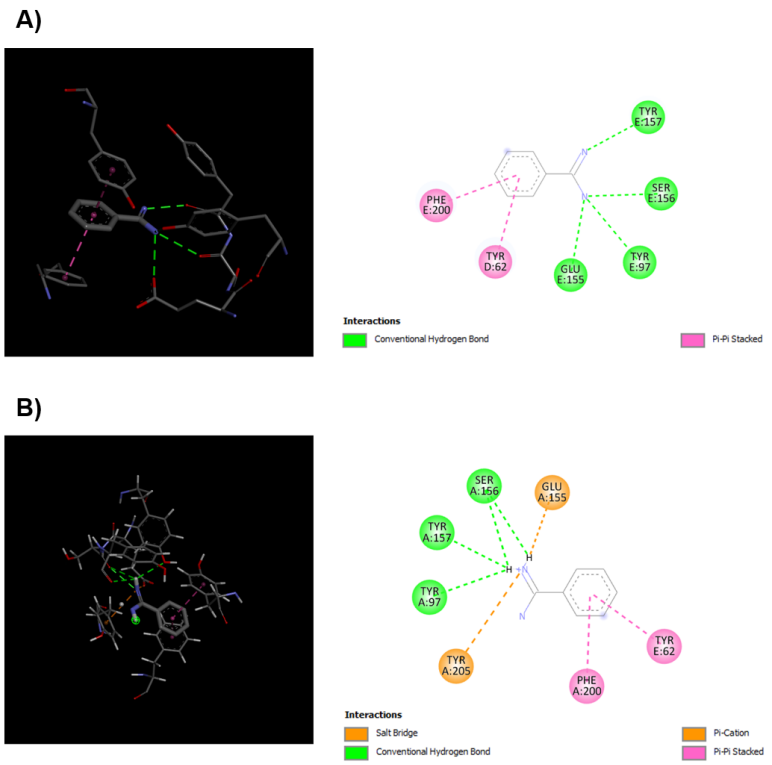

Figure 1. Interactions between the allosteric site of the GABAA receptor with Benzamidine; A) in crystal structure analysis B) re-docked result analysis.

Table 4. The ligands, their binding affinities, and interacting receptor residues of Inhibitors dataset including re-docked Benzamidine ligand.

Inhibitors Including Re-docked Benzamidine		
Ligand Name	Binding Affinity (kcal/mol)	Receptor Residues Interacting with Ligand
Suramin	-10.4	ALA45, SER46, ALA135, CYS136, MET137, ASN149, CYS150, THR151, GLU153, ARG180, VAL194, ARG196, PHE200, ARG207, SER209
Broflanilide	-9.5	ALA45, SER46, ILE47, ASP48, MET55, LEU99, ASN100, LYS102, ALA135, MET137, ARG180
Clozapine	-8.3	ASP43, TYR62, LEU99, PHE200, ALA201
Hydrochloride	-7.9	ASP43, ALA45, LEU99, MET115, TYR157, THR176, ALA201
Xenovulene	-7.3	ALA45, SER46, ILE47, ILE181
Flumazenil	-7.1	ASP43, TYR62, GLN64, LEU99, LYS173, PHE200, ALA201
Picrotoxinin	-6.8	SER46, ILE181
Benzamidine	-6.7	TYR62, TYR97, GLU155, SER156, TYR157, PHE200, TYR205

Table 5. The analysis of the frequency features of interacting common aminoacid residues of the allosteric site of $GABA_A$ receptor within three datasets and the inhibitors.

Common Aminoacid Residues' Frequencies		
Aminoacid Name	Number	Percentage
ASP43	28	% 8.48
ALA45	37	% 11.21
SER46	23	% 6.96
ILE47	10	% 3.03
TYR62	35	% 10.61
LEU99	34	% 10.30
ALA135	30	% 9.10
MET137	24	% 7.27
TYR157	17	% 5.17
ARG180	26	% 7.87
ILE181	5	% 1.52
PHE200	37	% 11.21
ALA201	24	% 7.27
Total	330	% 100

myocardial contractility, blood pleasure, stroke volume, and reducing heart rate [36]. Results put forward that Digoxin might create alkyl and pi-alkyl interactions with Ala45, Met49, Leu99, Ala135, Pro184, carbon-hydrogen bonds with Ser46, Tyr62, Pro184, pi-sulfur bonds with Met55, conventional hydrogen bonds with Val53, and Met55 residues of the receptor. Ponatinib is the third FDA-approved drug among the ones with the highest binding affinity. It was approved by the FDA in 2012 as a tyrosine-kinase inhibitor [37] and is used in the treatment of leukemia [38]. It's revealed that Ponatinib might create conventional hydrogen bonds with Asn41, Asp43, Arg207, carbon-hydrogen bonds with Ala45, Gln64, pi-pi stacked, and pi-alkyl interactions with Ala45, Tyr62, Leu99, Phe200, and Ala201 residues of the receptor.

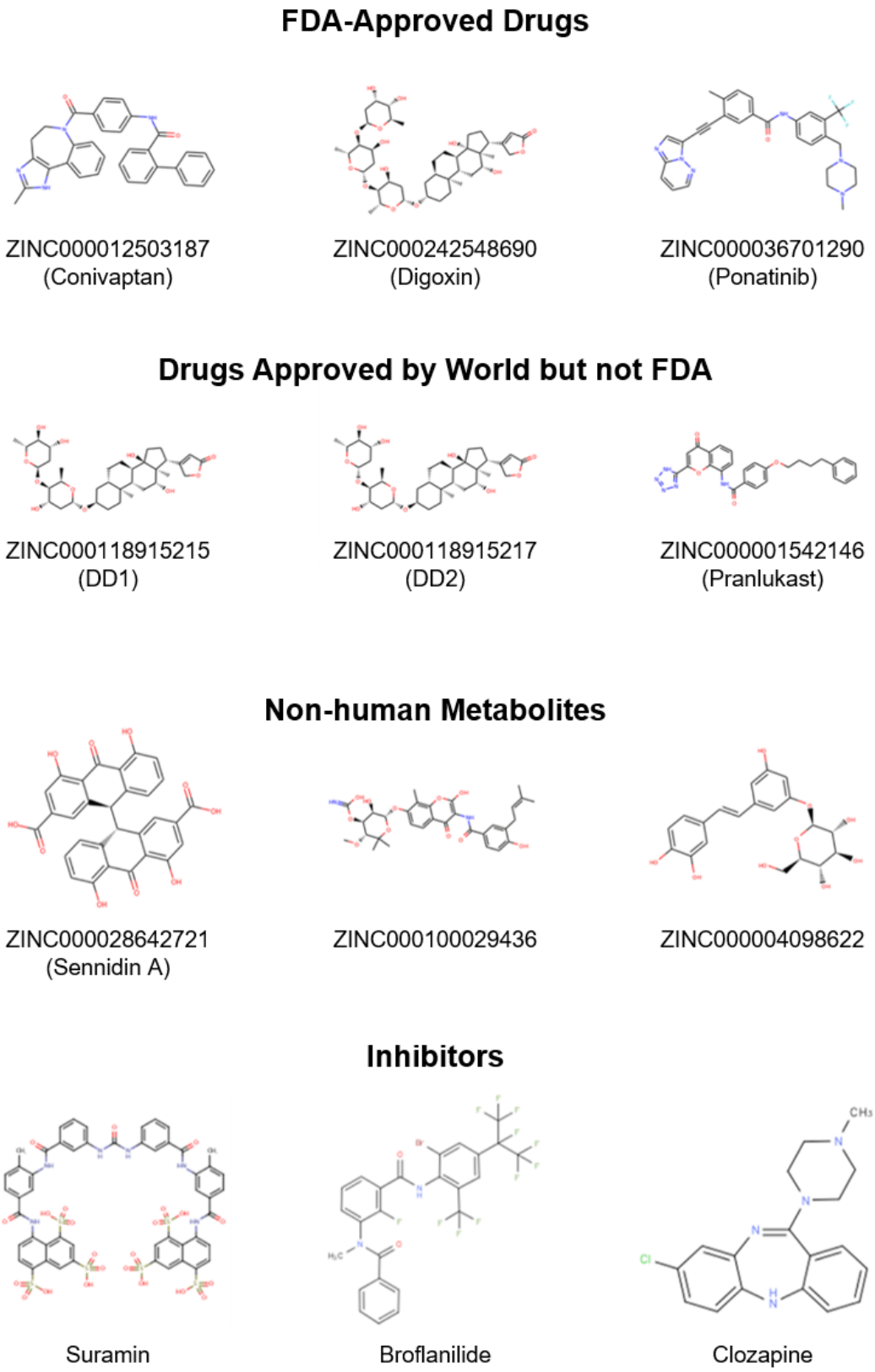

Figure 2. Three ligands with the highest binding affinity of each dataset from the molecular docking results.

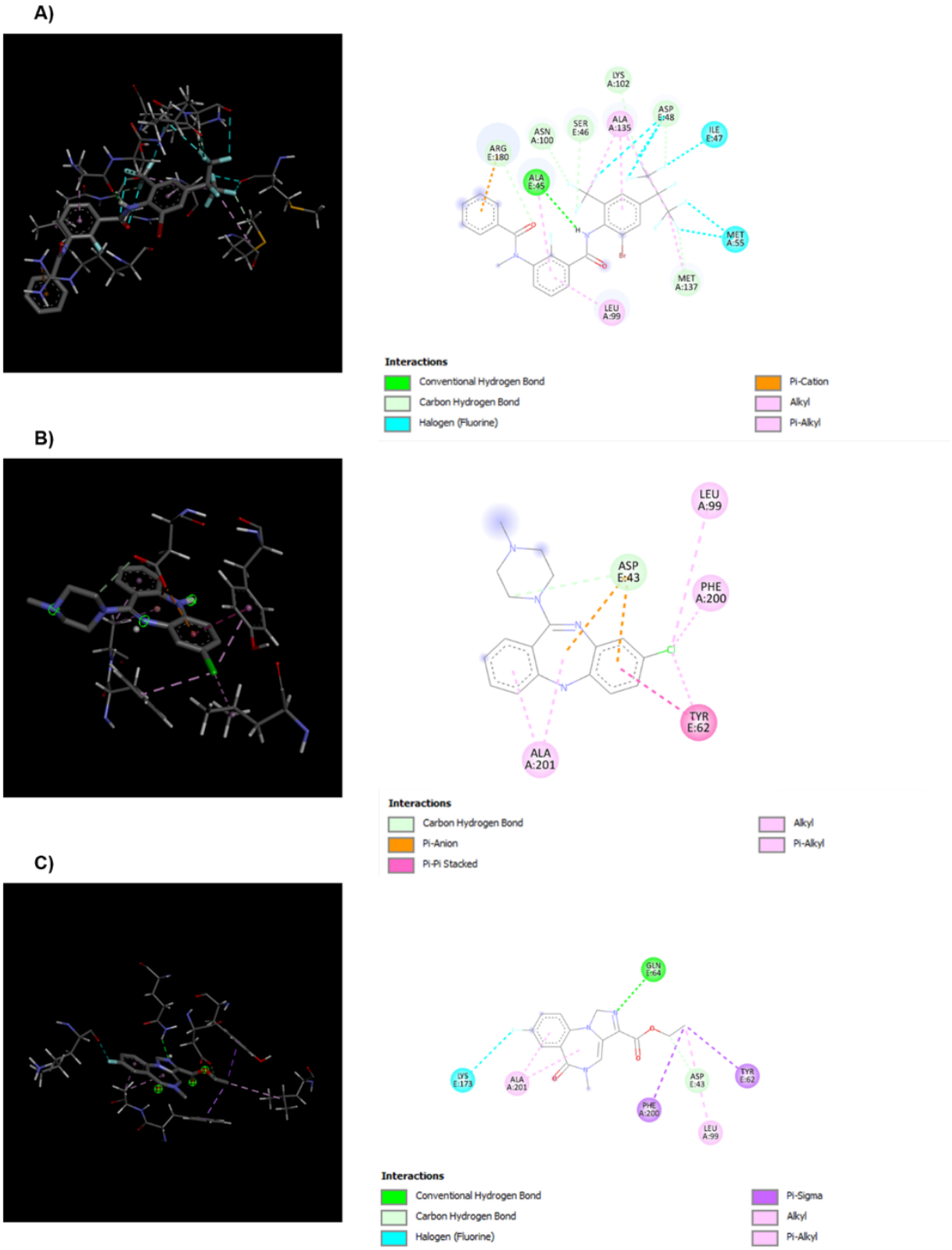

Figure 3. Interactions between the allosteric site of the $GABA_A$ receptor with the three best-scored ligands of Inhibitors; A) Suramin, B) Broflanilide, C) Clozapine.

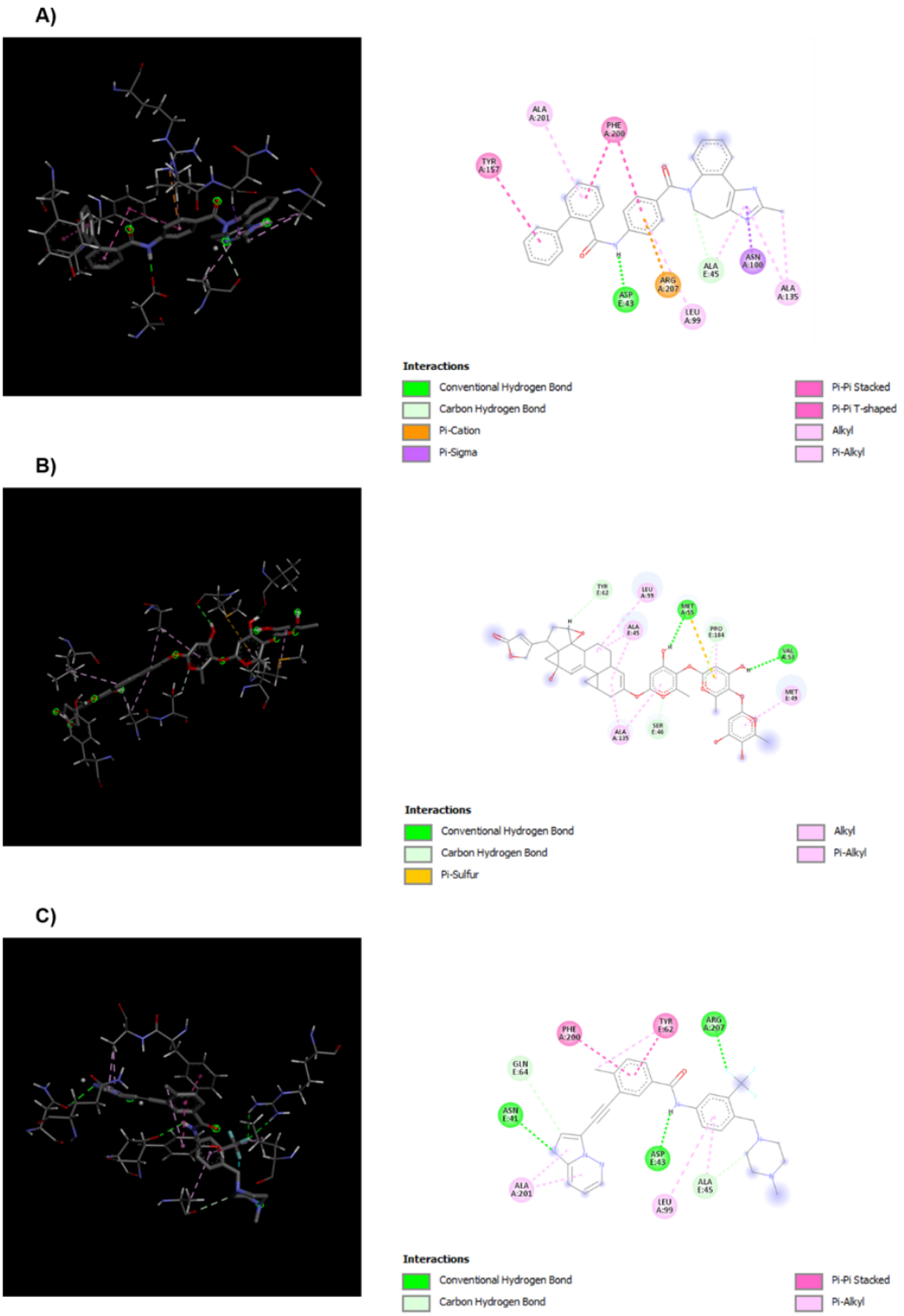

Figure 4. Interactions between the allosteric site of the GABAA receptor with three best-scored ligands of the FDA-Approved Drugs; A) ZINC000012503187 (Conivaptan) B) ZINC000242548690 (Digoxin), and C) ZINC000036701290 (Ponatinib).

In addition, 4254 ligands from the Drugs Approved by World but not FDA dataset had been docked to the allosteric site of the $GABA_A$ receptor, and 17 (ZINC000118915215, ZINC000118915217, ZINC000001542146, ZINC000257362202, ZINC000150339052, ZINC000256630457, ZINC000118915214, ZINC000011616152, ZINC000118915216, ZINC000256109538, ZINC000885764928, ZINC000008143788, ZINC000095618690, ZINC000000577115, ZINC000043195321, ZINC000100054749, ZINC000224657532) drugs were discovered with higher binding affinity than Suramin which is the best-scored ligand of the inhibitors. The three best-scored ligands which are ZINC000118915215 (DD1), ZINC000118915217 (DD2), and ZINC000001542146 (Pranlukast) were analyzed since they have the binding affinities of -11.9 kcal/mol, -11.9 kcal/mol, and -11.3 kcal/mol, respectively. The ligand-receptor interactions of the related ligands are demonstrated in Figure 5. While DD1 and DD2 are distinct derivatives of the Digoxin molecule, Pranlukast is a widely used drug as antiallergic and antiasthmatic since it's a cysteinyl leukotriene receptor-1 antagonist [39], [40]. Ligand-receptor interaction studies revealed that DD1 creates conventional hydrogen bonds with Ile47, Glu182, pi-sulfur bonds with Met55, alkyl and pi-alkyl interactions with Ala135, Met137, Leu183, Pro184, and carbon-hydrogen bond with Arg180, DD2 creates alkyl and pi-alkyl interactions with Met49, Ala135, Met137, Pro184, pi-sulfur bond with Met55, conventional hydrogen bond with Tyr97, carbon-hydrogen bond with Arg180, Pranlukast creates alkyl and pi-alkyl interactions with Ala45, Tyr62, Leu99, Ala135, Met137, Ile181, Phe200, conventional hydrogen bonds with Ile47, Glu182, pi-pi stacked interaction with Tyr62, pi-donor hydrogen bond with Met137, and carbon-hydrogen bond with Ile181 residues of the receptor.

Eventually, ligands of the Non-human metabolites dataset had been docked to the $GABA_A$ receptor and no ligand with a binding affinity higher than Suramin's was revealed among the 2307 ligands. However, 7 ligands (ZINC000028642721, ZINC000100029436, ZINC000004098622, ZINC000085432705, ZINC000003870412, ZINC000004096846, ZINC000169335484) had exhibited higher binding affinity than -9.5 kcal/mol which is the Broflanilide's (second-best inhibitor) binding affinity. Docking studies revealed that the three best-scored ligands of the Non-human Metabolite dataset were ZINC000028642721 (Sennidin A), ZINC000100029436, and ZINC000004098622 with the binding affinities of -10.1 kcal/mol, -10.0 kcal/mol, and -9.9 kcal/mol, respectively. The receptor interactions of these ligands are demonstrated in Figure 6. The data demonstrate that ZINC000028642721 (Sennidin A) might create pi-sigma interaction with Ala45, conventional hydrogen bonds with Ser46, Ile47, Asp101, Ala135, Met137, Glu182, carbon-hydrogen bonds with Thr60, Asn100, Cys136, pi-alkyl interactions with Ala135, Arg180, ZINC000100029436 might create alkyl and pi-alkyl interactions with Ala45, Leu99, Phe200, Ala201, carbon-hydrogen bonds with Ser46, Asp48, Ile181, conventional hydrogen bonds with Ile47 pi-pi stacked interactions with Tyr62, Phe200, and ZINC000004098622 might create conventional hydrogen bonds with Asp43, Ser46, Tyr97, Leu99, carbon-hydrogen bond with Ala45, pi-alkyl interactions with Ala45, Leu99, pi-pi stacked interactions with Tyr62, Phe200, unfavorable donor-donor and unfavorable acceptor-acceptor interactions with Asp101, Arg180, pi-donor hydrogen bonds with Asn100, Tyr205 amino acid residues of the receptor.

In the light of the findings, 30 drugs with the highest binding affinity and their datasets were listed in Table 5. Data put forward that the five best ligands are DD1, DD2 (from Drugs Approved by World but not FDA), Conivaptan, Digoxin (from FDA Approved Drugs), and Pranlukast (from Drugs Approved by World but not FDA) with the binding affinities of -11.9 kcal/mol, -11.9 kcal/mol, -11.8 kcal/mol, -11.6 kcal/mol, and -11.3 kcal/mol, respectively. Since the Digoxin drug and its two distinct derivatives exhibited quite binding affinity, these compounds were evaluated as rather promising in order to be used in the treatment of GABAergic disorders. In addition, there is no evidence in literature demonstrating the binding profile of Digoxin to $GABA_A$ receptors' allosteric site, Gautam et al. have reported that GABAergic agent production increases during dose-dependent Digoxin treatment [41]. As such, the findings might keep light on the molecular mechanism of the increases in GABAergic agent production, and provide significant evidence in order to test Digoxin and its derivatives (DD1, and DD2) in the treatment of GABAergic disorders. Furthermore, Vasopressin receptors inhibitor Conivaptan and cysteinyl leukotriene receptor-1 antagonist Pranlukast might be repurposed for treatment of GABAergic disorders since they have quite binding affinity to the allosteric site of the $GABA_A$ receptor.

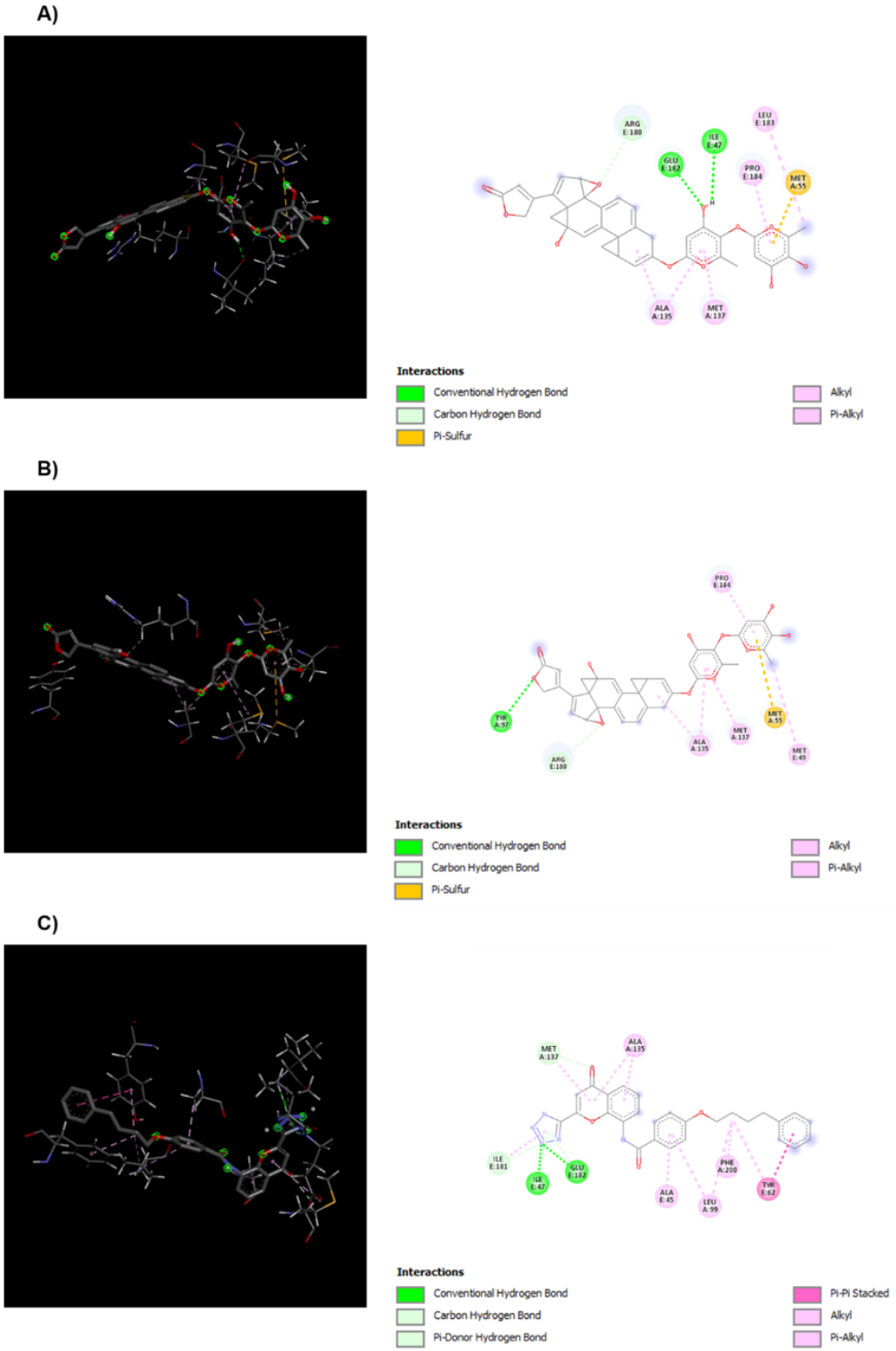

Figure 5. Interactions between the allosteric site of the GABAA receptor with three best-scored ligands of the Drugs Approved by World but not FDA; A) ZINC000118915215 (DD1), B) ZINC000118915217 (DD2), and C) ZINC000001542146 (Pranlukast).

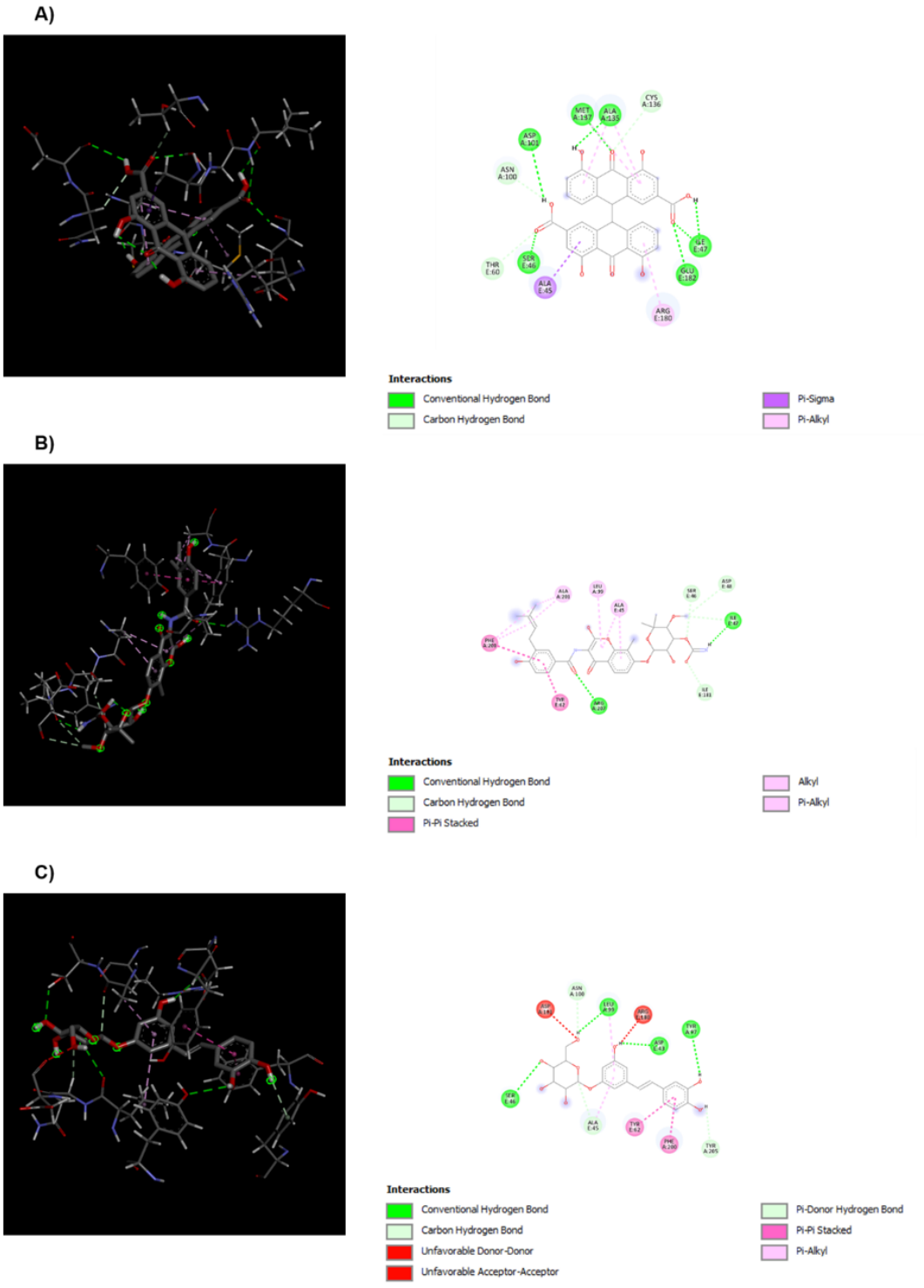

Figure 6. Interactions between the allosteric site of the GABAA receptor with three best-scored ligands of the Non-human Metabolites; A) ZINC000028642721 (Sennidin A), B) ZINC000100029436, and C) ZINC000004098622.

Table 6. The scores, datasets, interacting receptor residues of the best scored 30 ligands from the molecular docking results.

30 Ligands with Best Scores			
Ligand Name	Score (kcal/mol)	Dataset	Receptor Residues Interacting with Ligands
ZINC000118915215	-11.9	Drugs Approved by World but not FDA	ILE47, MET55, ALA135, MET137, ARG180, GLU182, LEU183, PRO184
ZINC000118915217	-11.9	Drugs Approved by World but not FDA	MET49, TYR97, ALA135, MET137, ARG180, PRO184
ZINC000012503187	-11.8	FDA Approved Drugs	ASP43, ALA45, LEU99, ASN100, ALA135, TYR157, PHE200, ALA201, ARG207
ZINC000242548690	-11.6	FDA Approved Drugs	ALA45, SER46, MET49, VAL53, MET55, TYR62, LEU99, ALA135, PRO184
ZINC000001542146	-11.3	Drugs Approved by World but not FDA	ALA45, ILE47, TYR62, LEU99, ALA135, MET137, ILE181, GLU182, PHE200
ZINC000257362202	-11.2	Drugs Approved by World but not FDA	ALA45, SER46, ASP48, MET49, GLU52, VAL53, MET55, LEU99, LYS102, ALA135, THR151, PRO184
ZINC000036701290	-10.9	FDA Approved Drugs	ASN41, ASP43, ALA45, TYR62, GLN64, LEU99, PHE200, ALA201, ARG207
ZINC000150339052	-10.9	Drugs Approved by World but not FDA	ALA45, ASP48, MET55, LEU99, ALA135, CYS136, MET137, THR151, PRO184
ZINC000256630457	-10.9	Drugs Approved by World but not FDA	ASP43, ALA45, TYR97, LEU99, ASN100, ALA135, MET137, GLU182, PHE200, ALA201
ZINC000118915214	-10.8	Drugs Approved by World but not FDA	ILE47, VAL50, ASN54, ALA135, MET137, GLU153, LEU183, ARG196
ZINC000203757351	-10.8	FDA Approved Drugs	ASP43, ALA45, LEU99, ASN100, LYS102, ALA135, ARG180, ARG196, PHE200, ALA201, ARG207
ZINC000011616152	-10.7	Drugs Approved by World but not FDA	ASP43, ALA45, TYR62, ARG180, PHE200, ALA201
ZINC000118915216	-10.7	Drugs Approved by World but not FDA	ASP43, ASP48, VAL53, MET55, LEU99, ALA135, MET137, ARG180, LEU183, PRO184
ZINC000256109538	-10.7	Drugs Approved by World but not FDA	SER46, ILE47, ASP48, MET49, GLU52, VAL53, MET55, ALA135, PRO184
ZINC000885764928	-10.7	Drugs Approved by World but not FDA	TYR62, ALA135, ARG180, PHE200
ZINC000008143788	-10.6	Drugs Approved by World but not FDA	TYR62, GLN64, PHE200
ZINC000004099009	-10.6	FDA Approved Drugs	ALA45, SER46, ASP48, ASP101, LYS102, ALA135, GLU182
ZINC000095617679	-10.6	FDA Approved Drugs	ASP43, ALA45, TYR62, LEU99, MET115, TYR157, ARG180, PHE200, THR202, ARG207
ZINC000095618690	-10.6	Drugs Approved by World but not FDA	ASP48, TYR62, LEU99, ALA135, MET137, ARG180, GLU182
ZINC000000577115	-10.5	Drugs Approved by World but not FDA	TYR62, GLU155, TYR157, PHE200, ALA201, TYR205

Table 6. Continue

30 Ligands with Best Scores			
Ligand Name	Score (kcal/mol)	Dataset	Receptor Residues Interacting with Ligands
ZINC000011679756	-10.5	FDA Approved Drugs	ASN41, ASP43, TYR62, TYR157, PHE200, ALA201, THR202, TYR205
ZINC000043195321	-10.5	Drugs Approved by World but not FDA	ASP43, ALA45, TYR62, LEU99, ARG180, PHE200, ALA201,
ZINC000100054749	-10.5	Drugs Approved by World but not FDA	ALA45, SER46, ASP48, LEU99, ASN100, ASP101, LYS102, ALA135, CYS136, MET137, ASN149, THR151
ZINC000224657532	-10.5	Drugs Approved by World but not FDA	ASP43, GLN64, GLU155, PHE200, ALA201, TYR205
ZINC000000537940	-10.4	Drugs Approved by World but not FDA	ASN41, GLU155, TYR157, ARG169, ALA174, PHE200, ALA201, THR202, TYR205
ZINC000006745272	-10.4	FDA Approved Drugs	ASP43, ALA45, SER46, ASP48, TYR62, GLN64, LEU99, ASN100, ASP101, ALA135, PHE200
ZINC000095618689	-10.4	Drugs Approved by World but not FDA	TYR62, GLY127, TYR157, LYS173, GLU179, PHE200, ALA201, TYR205
ZINC000003872494	-10.3	Drugs Approved by World but not FDA	TYR62, TYR97, GLU155, TYR157, PHE200, ALA201, TYR205
ZINC000011616153	-10.3	Drugs Approved by World but not FDA	ASP43, ALA45, SER46, ASP48, MET55, TYR62, ARG180, PHE200, ALA201
ZINC000022058728	-10.3	Drugs Approved by World but not FDA	ALA45, MET55, LEU99, ALA135, GLU182, PRO184, ARG207

Once the virtual drug screening had been completed, ADME analysis and toxicity profile of the three best-scored ligands from the Drugs Approved by World but not FDA and Non-human Metanolites datasets were carried out with swissADME server and OSIRIS Property Explorer tool. Since they had been tested by FDA previously, the ligands from FDA-Approved Drugs were not investigated in ADME and toxicity studies. The physico-chemical properties, lipophilicity, water-solubility, druglikeness properties, pharmacokinetics, and toxicity results of the ligands are listed in Table 6. Since DD1 and DD2 are structural isomers, the ADME and toxicity profiles of these derivatives have similar properties. Both derivatives were analyzed as water-soluble, they have no inhibitory activity on CYP isoforms, they have no permeation activity from the blood-brain barrier (BBB), and they have no possible side effects such as mutagenicity, tumorigenicity, irritant effects, and reproductive effects. While Pranlukast was found as insoluble in water as well as not permeant from BBB, its inhibitory effects on CYP2C19, CYP2C9, and CYP3A4 were observed. In addition, ADME and toxicity studies of Non-human Metabolites ligands revealed that ZINC000028642721 (Sennidin A) is poorly soluble, has a CYP2C9 inhibition activity, has no undesired effect, ZINC000100029436 is moderately soluble in water, has no CYP isoform inhibition activity, but has mutagenicity effect, and ZINC000004098622 is water-soluble, has no CYP isoform inhibition activity, but has a reproductive effect. In the light of the information on binding affinities, chemical interactions with the amino acid residues of the receptor, ADME analysis, and toxicity profiles of the ligands, FDA approved Digoxin drug and its derivatives (ZINC000242548690, ZINC000118915215, and ZINC000118915217) as well as Conivaptan which was approved by FDA and Pranlukast are evaluated as promising in order to be used in the treatment of GABAergic disorders.

Table 7. ADME and toxicity analysis of the best three ligands from Drugs Approved by World but not FDA and Non-human Metabolites datasets.

ADME and Toxicity Analysis							
Properties		Drugs Approved by World but not FDA			Non-human Metabolites		
Physico-chemical properties	Ligand Name	ZINC000118915215	ZINC000118915217	ZINC000001542146	ZINC000028642721	ZINC000100029436	ZINC000004098622
	Formula	C35H54O11	C35H54O11	C27H23N5O4	C30H18O10	C31H36N2O11	C20H22O9
	Molecular Weight (g/mol)	650.80	650.80	481.50	538.46	612.62	406.38
	Molar Refractivity	166.04	166.04	135.27	138.55	161.88	102.03
	TPSA (topological polar surface area)	164.37 Å²	164.37 Å²	123.00 Å²	189.66 Å²	201.00 Å²	160.07 Å²
Lipophilicity	Log $P_{o/w}$ (iLOGP)	4.19	4.53	3.07	2.16	4.48	1.71
	Log $P_{o/w}$ (XLOGP3)	1.24	1.24	4.32	4.80	4.18	0.73
	Log $P_{o/w}$ (WLOGP)	2.34	2.34	4.44	3.96	3.85	-0.07
	Log $_{Po/w}$ (MLOGP)	1.17	1.17	3.07	1.27	0.51	-0.87
	Log $_{Po/w}$ (SILICOS-IT)	1.28	1.28	5.12	3.46	3.21	0.00
	Consensus Log $_{Po/w}$	2.04	2.11	4.00	3.13	3.25	0.30
Solubility	Log S (SILICOS-IT)	-1.86	-1.86	-10.46	-6.59	-5.84	-1.02
	SILICOS-IT Solubility (mg/ml)	8.92e+00	8.92e+00	1.68e-08	1.38e-04	8.90e-04	3.90e+01
	SILICOS-IT Solubility (mol/l)	1.37e-02	1.37e-02	3.49e-11	2.56e-07	1.45e-06	9.59e-02
	Solubility Class	Soluble	Soluble	Insoluble	Poorly soluble	Moderately soluble	Soluble
Druglikeness	Druglikeness	3.42	3.42	-4.02	0.52	0.93	-6.63
	Drug-score	0.41	0.41	0.21	0.32	0.17	0.25

Table 7. Continue

ADME and Toxicity Analysis							
Properties		Drugs Approved by World but not FDA			Non-human Metabolites		
Pharmaco-kinetics	GI absorption	Low	Low	Low	Low	Low	Low
	BBB permeant	No	No	No	No	No	No
	P-gp substrate	Yes	Yes	Yes	No	No	No
	CYP1A2 inhibitor	No	No	No	No	No	No
	CYP2C19 inhibitor	No	No	Yes	No	No	No
	CYP2C9 inhibitor	No	No	Yes	Yes	No	No
	CYP2D6 inhibitor	No	No	No	No	No	No
	CYP3A4 inhibitor	No	No	Yes	No	No	No
Toxicity	Mutagenicity	No	No	No	No	Yes	No
	Tumorigenicity	No	No	No	No	No	No
	Irritant Effects	No	No	No	No	No	No
	Reproductive Effects	No	No	No	No	No	Yes

Conclusion

The GABAergic pathway is one of the most prominent pathways responsible for neuronal development and is managed through the interactions between γ-aminobutyric acid (GABA) which is the primary inhibitory neurotransmitter in CNS and its receptors which are $GABA_A$, $GABA_B$, or $GABA_C$ receptors. Among these receptors, the $GABA_A$ receptor has the highest expression level along with CNS and regulates neural cell migration, proliferation, growth, and synapse structures generation by performing phasic inhibition and tonic activation. To date, much evidence has revealed that defects in the GABAergic pathway might cause several serious diseases such as schizophrenia, epilepsy, anxiety, depression, insomnia, etc. Besides, it's recently been reported that anti-tumor immune response is suppressed through the inhibition of CD8 T cell activity by GABA metabolite secreted by B-cells. As such, the discovery of novel inhibitors targeting the GABAergic pathway and the repurposing of approved drugs are quite promising approaches for the treatment of regarding disorders from schizophrenia to cancer.

In this study, a molecular docking mediated virtual drug screening strategy targeting the allosteric site of the $GABA_A$ receptor has been carried out with a total of 8170 ligands consisting of 1609 FDA-Approved Drugs, 4254 Drugs Approved by World but not FDA, and 2307 Non-human Metabolites. Furthermore, the developed strategy has been validated with the re-docking of the Benzamidine ligand which is found in the crystal structure of the receptor, and the docking of 7 known GABA antagonists. Results demonstrate that two Digoxin derivatives which are ZINC000118915215 (DD1) and ZINC000118915217 (DD2) from the Drugs Approved by World but not FDA dataset have the highest binding affinity to the allosteric site of the $GABA_A$ receptor. Furthermore, Digoxin drug (ZINC000242548690) from the FDA-approved Drugs dataset has been analyzed as one with the highest binding affinity after Conivaptan drug (ZINC000012503187). In addition, ADME and toxicity studies have revealed that DD1 and DD2 have quite a drug potential since they are water-soluble, have no inhibitory effect on CYP isoforms, and have no possible undesired effects such as mutagenicity, tumorigenicity, irritant effects, and reproductive effects. While Digoxin is a molecule produced by Digitalis lanata, it's widely used in several cardiac diseases. Besides, Conivaptan is a Vasopressin receptors (V1a and V2) inhibitor and is also widely used in heart failure diseases. As such, the great potential of Digoxin and its derivatives (DD1 and DD2) in the treatment of GABAergic pathway-based disorders is declared in this report. In addition, Conivaptan has great potential, too, since it has a quite binding affinity to the allosteric site of the $GABA_A$ receptor. However, the findings of the report should be analyzed with molecular dynamics (MD) simulation studies, and the potential of the related drugs should be tested by in vitro and in vivo studies.

Funding Declaration

Any funding was not supported during the research.

Competing Declaration

The research have been carried out by only one author and the author has no relevant financial or non-financial interests to disclose.

Author Contribution Declaration

The research and submission have been carried out by only one author.

Data Availability Declaration

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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