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Exploring the therapeutic potential of Zonisamide derivatives through molecular docking and dynamic studies with GABARAP

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Computer based drug designing is an effective tool to shortlist promising drug molecules within short span of time. Designing drugs for neurological disorders is a necessity as it has affected millions of lives. Zonisamide is an effective sulfonamide drug administered to control seizures in epilepsy. This work aims to identify promising Zonisamide derivatives that form more stable complexes with inhibitory neurotransmitter GABARAP. We have designed 32 Zonisamide derivatives. These derivatives were optimised using Gaussian 09 and they were docked to the target GABARAP using PyRx software. The hydrophobic groups substituted at the benzene have lowered the binding energy of Zonisamide in most of the cases. Such derivatives were examined for their drug properties, oral activity and safety using ProTox-II, OSIRIS Property Explorer, and LogBB_Pred servers. The GABARAP residues engaged in the interaction with the derivatives were noted from LigPlot$^+$. The top three derivatives namely Z11 ((6-phenylbenzo[d]isoxazol-3-yl)methanesulfonamide), Z19 (5-(tert-butyl)benzo[d]isoxazol-3-yl) methanesulfonamide, and Z20 (5-phenylbenzo[d]isoxazol-3-yl)methanesulfonamide were finalised and the molecular dynamic simulation of their complexes were carried out using GROMACS 2020. Each of the finalised complexes was analysed for its stability, residue flexibility, compactness, solvent accessible surface area and energy. Among the three derivatives, we propose Z11 as the potential GABARAP modulator.

Keywords: Benzene, Hydrophobic, Modulator, Oral activity, Sulfonamide

Zonisamide (synthesised in 1972) is an efficient anticonvulsant medication which heals the symptoms associated with Parkinson's and Epilepsy like neurological illness[1]. Neurological disorders have affected the life quality of around one billion of the human world[2]. The nerve cells present in the brain interact with one another by passing on electric signals. A break or variation in this usual communication causes instant, repeated seizures. It varies between short periods and long times of intense shaking or shivering. It can even lead to unconsciousness[3].

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Suppl. Data available on respective page of NOPR

Abbreviations: B3LYP, Becke 3-parameter Lee Yang Parr; GABA, gamma-amino butyric acid; GABARAP, gamma-amino butyric acid receptor associated protein; LD_{50}, Lethal dose fifty percentage; MD, Molecular dynamics PDB, Protein data bank; RCSB, Research Collaboratory for Structural Bioinformatics; RMSD, Root mean square deviation; RMSF, Root mean square fluctuation; Rg, Radius of gyration; SASA, Solvent accessible surface area; SDF, Spatial Data File; TPSA, topological polar surface area

Zonisamide efficiently supplements the treatment of spasms, Lennox–Gastaut syndrome, partial-onset seizures, *etc*[4]. The very promising effects are observed when Zonisamide is used with Bupropion in the obesity treatment[5]. Medical practitioners at times depend on the drug Zonisamide for treating migraine, bipolar disorders, *etc*[6]. Zonisamide is usually accepted for monotherapy due to its long life (63-69hr) and wide-ranging actions. The bioavailability of this dug is very close to 100 % (unaffected by food intake). Some common side effects associated with Zonisamide intake include headache, nausea and somnolence[7]. In this work, computational studies have been carried on the drug Zonisamide. The interaction of Zonisamide and its derivatives were studied to the target Gamma (γ)-aminobutyric acid receptor-associated protein (GABARAP).

γ-aminobutyric acid, abbreviated as GABA, sustains the neurotransmitter inhibition and thus plays a crucial role by offsetting the uncontrolled excitation in the neuron. In normal conditions, this process always balances out. However, any variation in this

balance can lead to unrestricted excitation and epileptic seizures. γ-aminobutyric acid carries out this inhibition process by interconnecting with the GABAA receptor[8]. This receptor is an ion channel, and it puts down the creation of action potential in the next neuron. The studies support that the stability of these receptors in the neural junction was maintained by γ-aminobutyric acid receptor-associated protein, abbreviated as GABARAP. The anti-epileptic drugs trigger this inhibition process by maintaining the activities of the GABAA receptor and thereby regaining the excitation-inhibition balance[9].

The most crucial step in drug design is the theoretical evaluation of all possible drug-related aspects, including toxicity and stable complex formation with the target. This step helps to confidently identify potential derivatives and proceed with their synthesis. Computer-assisted drug design is highly necessary to avoid the time and financial losses that medicinal companies may face if low-potential derivatives are synthesized[10].

In the present study, we designed 32 derivatives (IUPAC names in Suppl. Table S1) by substituting hydrophobic or hydrophilic groups at the C atoms in Zonisamide and performed molecular docking with GABARAP. The conformers with lower binding energies compared to the parent drug could be obtained through docking. Thus, the derivatives capable of binding more spontaneously with the target could be identified[11]. In the next step, Molecular Dynamic studies (for 100 ns) of the GABARAP complexes of Zonisamide with top three derivatives were carried out. Molecular Dynamic studies could help to identify the stability of a complex involving derivatives and proteins. This study was carried out in a water system, where conditions like those in the body were created[12]. From the 32 derivatives, we have reached the top three derivatives after docking studies andextensive analysis of oral activity, drug properties, median lethal dose and toxic conditions. After the analysis, the plots of root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), solvent accessible surface area (SASA), and the energy of Zonisamide-GABARAP complex and the three GABARAP complexes of derivatives were studied. Through this analysis, a hit compound was proposed. These proposed compounds are better than Zonisamide for binding to GABARAP and have shown positive results in other analyses.

Materials and Methods

All Zonisamide derivatives (Z1-Z32) used in this work were optimised using the Gaussian 09 software with the 6-311G(d, p) basis set to obtain minimum energy stable structures for molecular docking[13,14].

Target for Docking

The target GABARAP was modelled with an I-TASSER server employing the protein data bank template 1KJT[15]. The modelled GABARAP was refined using the GalaxyWEB server[16]. This model was validated using the Errat plot[17], Ramachandran plot[18], and Verify3D[19]. A quality factor 99.02 was observed in the Errat plot of the downloaded model. Errat plot is provided in the Suppl. Information (Suppl. Fig. S1). In the Ramachandran plot (Suppl. Fig. S2), 95% of the GABARAP residues lay in the most favourable region. From Verify 3D results, the GABARAP model has 90.35 % amino acid residues possessed an average 3D - 1D score > = 0.2.

The PyRx software was used for the docking studies, and the grid box in the docking process was set up based on the active site determined from the Metapocket server[20,21]. The PyRx software provides nine conformers in each docking process, and the best conformer was identified based on the binding energy obtained. The ligands with binding energy lower than that of Zonisamide were selected for further analysis. The GABARAP residues that showed interaction with the derivatives were identified from the LigPlot Diagram[22]and Pymol[23].

Oral activity and drug-related properties

The oral activity of the Zonisamide derivatives was checked based on the five rules proposed by Lipinski. The five factors were obtained from the SwissADME server[24]. The small molecules with optimal Log P (below 5), optimal hydrogen donors (≤ 5) and acceptors (≤ 10) were identified as orally active molecules. The Molar refractivity range set up by Lipinski was between 40-130.

The drug-related properties of the derivatives were also checked using the SwissADME server. The factors like topological polar surface area, solubility, bioavailability score, and PAINS alert were analysed based on the method put forward by Ertl[25], Ali and co-workers[26], Martin and co-workers[27] and Baell and coworkers[28], respectively. A molecule that exhibits optimal flexibility is one with rotatable bonds lower than nine. The TPSA value should be in the range of 30-120 $Å^2$. Saturated molecules were identified from

the fraction Csp^3 values. The unsaturated molecule has a Csp^3 value lower than 0.25[29]. ESOL Model was used in the SwissADME server to evaluate the solubility[30].

Along with the results from the SwissADME server, LogBB_Pred[31] was also used to analyse the ability of the derivatives to cross the blood-brain-barrier. These factors were analysed to evaluate the drug-related properties of the derivatives.

Toxicity analysis

The rat LD_{50} (median lethal Dose) value of each derivative was analysed using a server to find out the toxicity class to which the compound belongs. It is the amount of orally administered molecule (mg) that kills fifty per cent of the total rats (kg) in the analysis. These studies were carried out with ProTox-II server[32]. The LD_{50} ranges in the toxicity class from 1 to 6 are $\leq$ 5 mg, 5 mg < LD50 $\leq$ 50 mg, 50 mg < LD50 $\leq$ 300 mg, 300 mg < LD50 $\leq$ 2000 mg, 2000 mg < LD50 $\leq$ 5000 mg, LD50> 5000 mg respectively. The similarity in the toxic dose range of derivatives and their parent compound was analysed.

Molecular Dynamic simulation

Zonisamide and its top three derivatives were subjected to Molecular Dynamic simulation (MD) studies for 100 ns. MD simulations were carried out with GROMACS 2020 software[33]. In the initial step, complex topology was prepared by combining the separately prepared ligand topology and the topology of the protein. The system was set up in a dodecahedron box, and the box contained water to ape the interaction in the living system. The protein in the complex, GABARAP, is charged, and as the system should be neutral, two chloride ions were added, and the solvated system was neutralised. The next step involved energy minimisation of the solvated complex. This was done using the steepest descent algorithm to obtain energetically favourable arrangements of atoms. After plotting the xvg file of potential energy in the origin software, the energy minimisation was confirmed. The next stage was equilibration. After restraining the derivative of the complex, the derivative-GABARAP complex was taken as a single unit to carry out temperature coupling using a Berendsen thermostat. Under canonical ensemble, the particle number, volume and temperature (NVT) were maintained constant, with temperature regulated at 300 K. In the isothermal-isobaric ensemble, the particle number, pressure, and temperature (NPT) were maintained constant, with pressure adjusted at 1 bar. In the next stage, the applied restrain to the derivative was withdrawn, and the system was run for 100 ns to evaluate the interaction of the derivative to the GABARAP at a similar condition in the body system. The stability of the complex was analysed through the plots of Root mean square deviation, Root mean square fluctuation, Radius of gyration, Solvent accessible surface area and Energy. All the figures were plotted using Origin[34].

Results and Discussion

Docking studies

Figure 1A & B depict the structures of the Zonisamide derivatives that were designed by substituting selected groups at any specified site from R_1-R_5. As detailed in Figure 1, the binding affinity (free energy of binding, ΔG) of the derivatives Z1, Z2, Z5, Z6, Z16, Z25, Z27, Z28, Z30, Z31, and Z32 were higher compared to the drug Zonisamide. NH_2 group at R_1, R_4 or R_5 have raised the binding affinity of Zonisamide. Similarly, the presence of the OH group at any of these three positions has also raised the binding energy of Zonisamide. Thus, the sites R_1, R_4 and R_5 may not be appropriate for substituting hydrophilic groups to increase the strength of target binding. The hydrophobic groups CH_3, CH_2CH_3, $CH(CH_3)_2$, $C(CH_3)_3$, C_6H_5, $CH_2C_6H_5$ and C_6H_{11} were chosen for the substitution to analyse the variation in the binding affinity. The substitution of the CH_3 group at any of the three specified sites, R_1, R_3, and R_4, has failed to raise the spontaneous binding of the drug Zonisamide. However, at R_5, only the substitution of the CH3 group succeeded in increasing the spontaneous binding. The hydrophobic group CH_2CH_3 at R_1 or R_5 was a failure to reduce the binding energy of Zonisamide. All the remaining substitutions have succeeded in reducing the target binding energy of Zonisamide. Thus, increasing the hydrophobicity of the hydrophobic region and hydrophilicity of the hydrophilic region has successfully raised the spontaneous binding of Zonisamide in most cases.

The following derivatives Z1, Z2, Z5, Z6, Z16, Z25, Z27, Z28, Z30, Z31, and Z32 were excluded because they do not bind to GABARAP more effectively than the drug Zonisamide.

The amino acids that had shown hydrophilic and hydrophobic interaction with the promising

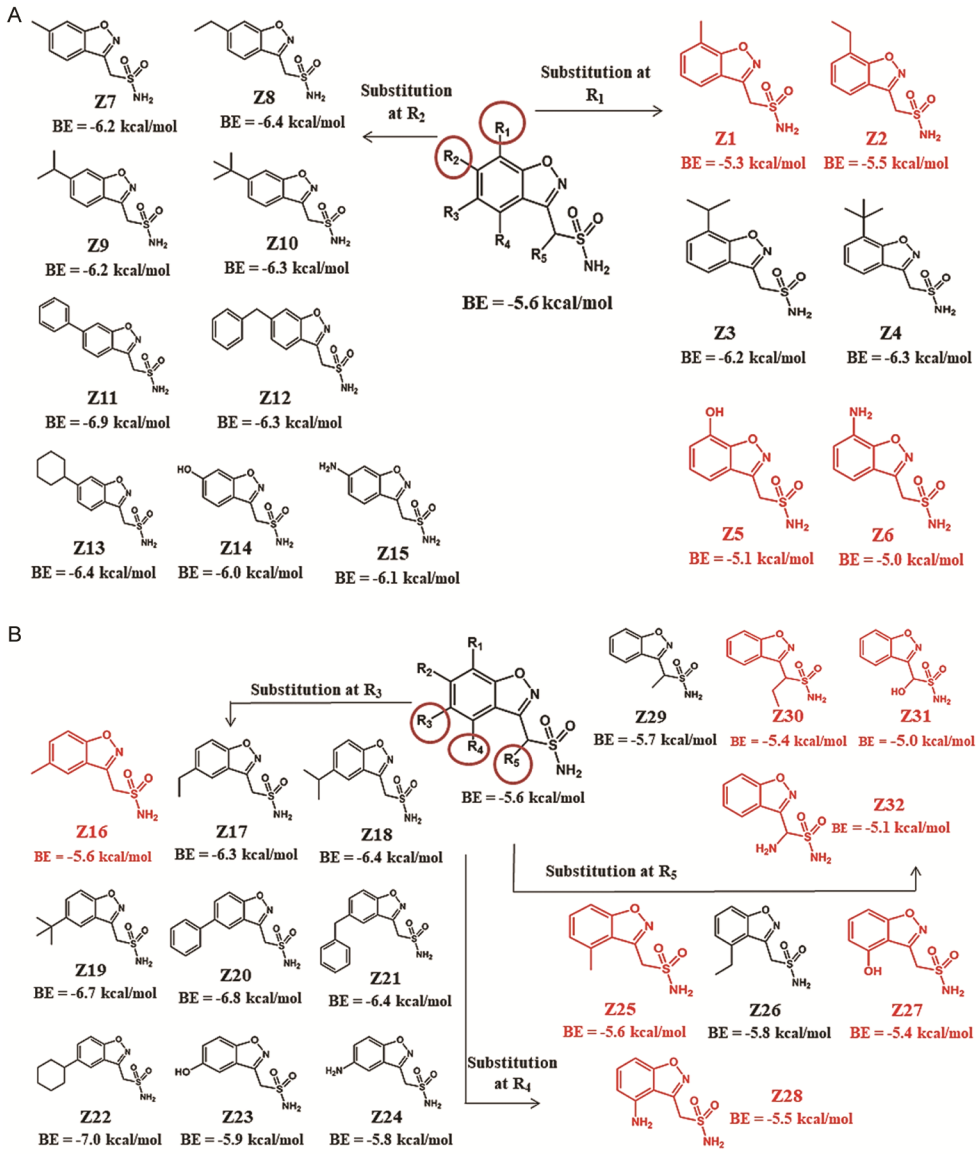

Fig. 1 — Binding energy (BE) and the structure of the Zonisamide derivatives obtained by the substitution of selected groups at (A) R_1 and R_2; and (B) R_3, R_4 and R_5

derivatives are listed in (Table 1). The amino acids Arg65, Ala75, Phe77, Thr87, Phe78, Leu76 and Tyr61 that had interacted with the Zonisamide were engaged in the hydrogen bonding or hydrophobic interaction with most of the derivatives. However, the derivatives Z26 and Z29 had shown interaction with amino acid residues that had not interacted with the drug Zonisamide.

Analysing the rat oral LD_{50} values, Zonisamide and all the designed molecules fell into toxicity class 4. This means these compounds can cause harm if taken orally in a dose between 300 mg-2000 mg (300 mg < $LD_{50} \leq$ 2000 mg). Thus, the derivatives and the drug Zonisamide had shown the same toxic range (Suppl. Table S2). The ProTox-II server has predicted these results with an accuracy of around 70%. As per the findings from the OSIRIS Property Explorer[35], the molecules Z15 and Z24 are tumerogenic. All other derivatives are inactive for toxic conditions like mutagenicity, cytotoxicity, irritation, hepatotoxicity, immunotoxicity, and reproductive effectiveness. The probability of inactivity shown in the Protox-II server for Hepatotoxicity is around 0.55 for all the derivatives. The probability score showing the inactivity of the derivatives for Mutagenicity and Cytotoxicity is between 0.50 and 0.75. Most of the

Table 1 — GABARAP residues engaged in the interaction with the designed derivatives of Zonisamide

ID	Amino acids engaged in Hydrogen bonds	Amino acids engaged in Hydrophobic interaction
Zonisamide	Arg65, Leu76, Tyr61	Ala75, Phe77, Thr87, Phe78, Leu76, Tyr61
Z3	Tyr61, Leu76, Ala72, Asp74, Glu73	Asp74, Glu73, Ala75, Tyr61, Phe77, Ile84, Thr87, Arg65
Z4	Tyr61, Leu76, Asp74, Ala72, Glu73	Glu73, Arg65, Asp74, Ala75, Leu76, Phe77, Thr87, Phe78, Tyr61, Ile84
Z7	Tyr61, Arg65, Leu76	Leu76, Ala75, Phe77, Phe78, Tyr61, Thr87, Phe78, Val57
Z8	Arg65, Leu76, Tyr61	Ala75, Leu76, Phe77, Thr87, Tyr61, Pro85, Val57, Ile84, Phe78
Z9	Arg65, Ala72	Leu76, Thr87, Arg65, Ala72, Leu76, Phe77
Z10	Arg65, Glu73, Ala72	Leu76, Ala75, Phe77, Thr87, Tyr61, Ile84, Phe78, Glu73, Arg65
Z11	Ala72, Asp74, Arg65	Glu73, Ala72, Ala75, Arg65, Phe77, Tyr61, Leu76, Val57, Pro85, Phe78, Ile84, Thr87
Z12	Thr87	Thr87, Pro96, Tyr61, Leu76, Phe78, Phe77
Z13	Arg65, Ala72	Tyr61, Phe77, Leu76, Thr87, Ile84, Phe78, Arg65, Ala72
Z14	Tyr61, Arg65, Leu76	Ala75, Phe77, Val57, Thr87, Phe78, Tyr61, Leu76
Z15	Arg65	Thr87, Arg65, Ala72, Leu76, Phe77, Phe78
Z17	Arg65, Gly58	Arg65, Gly58, Tyr61, Leu76, Phe77, Phe78, Thr87, Val57
Z18	Tyr61, Leu76, Arg65	Ala75, Phe77, Phe78, Thr87, Tyr61, Leu76
Z19	Leu76, Ala72, Tyr61	Ala75, Phe77, Phe78, Thr87, Ile84, Tyr61, Leu76
Z20	Tyr61, Arg65, Asp74, Ala72, Leu76	Thr87, Phe78, Phe77, Ile84, Ala75, Tyr61, Arg65, Asp74, Leu76
Z21	Gly58	Phe62, Gln59, Phe77, Tyr61, Phe78, Leu76, Thr87, Gly58
Z22	Tyr61, Ala72, Arg65, Asp74, Leu76	Ala75, Thr87, Phe78, Phe77, Ile84, Tyr61, Arg65, Asp74, Leu76
Z23	Phe78, Tyr61, Leu76, Arg65	Phe77, Thr87, Ile84, Phe78, Tyr61, Leu76, Arg65
Z24	Ala72, Tyr61	Ile84, Ala75, Tyr61, Arg65, Tyr61, Leu76
Z26	Glu112, Val114	Lys38, Asn82, Ser110, Phe79, Ala36, Pro37, Ala108, Met1, Glu112, Val114
Z29	Glu112, Val114	Lys38, Ala108, Met1, Ala36, Asn82, Ser110, Phe79, Val114, Glu112

derivatives showed 99% inactivity for Immunotoxicity. Thus, based on the toxic conditions analysed for the derivatives, two compounds, Z15 and Z24, are excluded.

Based on the Lipinski rule, the designed derivatives have favourable oral activity. This is because the factors like mass, hydrogen donors, acceptors, refractivity, and lipophilicity (Log P) of the derivatives fell in the optimal range. The bar graph representations of the five Lipinski factors of the derivatives are given in (Figs 2 & 3). Figure 2 depicts the mass and molar refractivity of the derivatives, whereas, (Fig. 3) provides the number of hydrogen acceptors, donors, and their Log P values.

The drug properties of the studied safe derivatives with lower binding energy than Zonisamide were evaluated. TPSA values of both Z14 and Z23 are 114.8 $Å^2$, and these two compounds have 69.34 % absorption. The TPSA value of Zonisamide and the remaining compounds is 94.57 $Å^2$ (Suppl. Fig. S3A). So, the remaining compounds have a higher percentage of absorption (76.37%). As the molecules are small and their log P values are well within limits, they showed high gastrointestinal absorption as well as permeability to the blood-brain barrier. The designed derivatives Z13 and Z22 were listed as the substrates of P-glycoprotein. As there can be a decrease in the bioavailability for these two derivatives, Z13 and Z22 cannot be listed as a promising derivative despite their lowest GABARAP binding energy.

The derivatives are flexible with rotatable bond number ≤ 4. Zonisamide is not a saturated molecule (Fraction Csp^3 lower than 0.25) whereas the derivatives Z3, Z4, Z8, Z9, Z10, Z13, Z17, Z18, Z19, Z22 and Z26 are saturated (Fraction $Csp^3 \geq 0.25$) (Suppl. Fig. S3B). The derivatives cannot have formulation problems as they are soluble in the

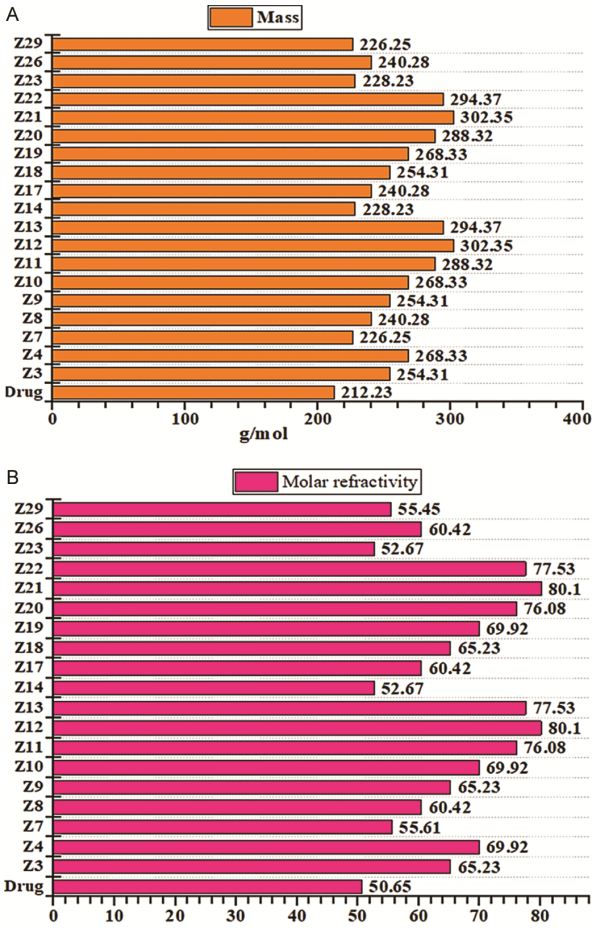

Fig. 2 — (A) Mass; and (B) molar refractivity of safe derivatives with lower target binding energy than Zonisamide

aqueous medium (Suppl. Table S3). The bioavailability value of 0.55 also reassures the oral activity of the derivatives. In total, the derivatives revealed good drug properties.

Based on these studies, the derivatives Z11, Z19 and Z20 were the top three molecules finalised for the Molecular Dynamic simulation analysis. The order of increase in the GABARAP binding energy of these three molecules is Z11 < Z20 <Z19.

Molecular dynamic studies

The best three derivatives were taken for 100 ns Molecular dynamic simulation.

Root mean square deviation

The stability of the Zonisamide-GABARAP complex and the complexes of GABARAP with Z11, Z20, and Z19 were analysed through RMSD plots (Fig. 4). RMSD plot of the c-alpha atoms of the protein in Zonisamide-GABARAP complex (Fig. 4A) was found to be very stable all along 100 ns.

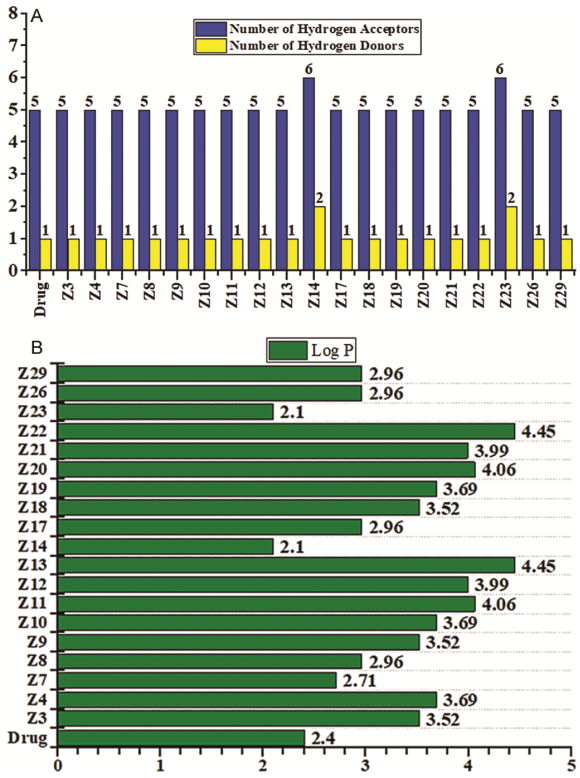

Fig. 3 — (A) Number of hydrogen donors, acceptors; and (B) Log P values of safe derivatives with lower target binding energy than Zonisamide

However, slight variations were observed in the first ns and also from 51 ns - 66 ns. The fluctuation at 65 ns has reached 0.217 nm. Except this, the whole RMSD plot of c-alpha atoms of the protein GABARAP existed below 0.195 nm. Flips in the RMSD plot of the drug Zonisamide at 2 ns, 6 ns, 10 ns, 30 ns, 35 ns, 42 ns, 67 ns, 84 ns, 94 ns and 98 ns indicated the jumping of ligand Zonisamide from the binding sites. This was confirmed by analysing interacting residues at each frame of the 100 ns trajectory. The RMSD plot of Zonisamide existed below 0.165 nm in the simulation.

After a 100 ns simulation, the drug Zonisamide interacted with Arg67, Tyr49, Leu63, Val51, and Phe60. The difference observed in the interacting residues before and after the simulation was due to the flip at 98 ns.

RMSD plot of the c-alpha atoms of the GABARAP in Z11-GABARAP complex has attained stability after 5ns (Fig. 4B). All along the 100 ns, RMSD plot of GABARAP and Z11 existed under 0.192 nm and 0.182 nm respectively. Two flips occurred in the first 15 ns of the RMSD plot of Z11, and in the second flip, ligand Z11 jumped to another binding site. From

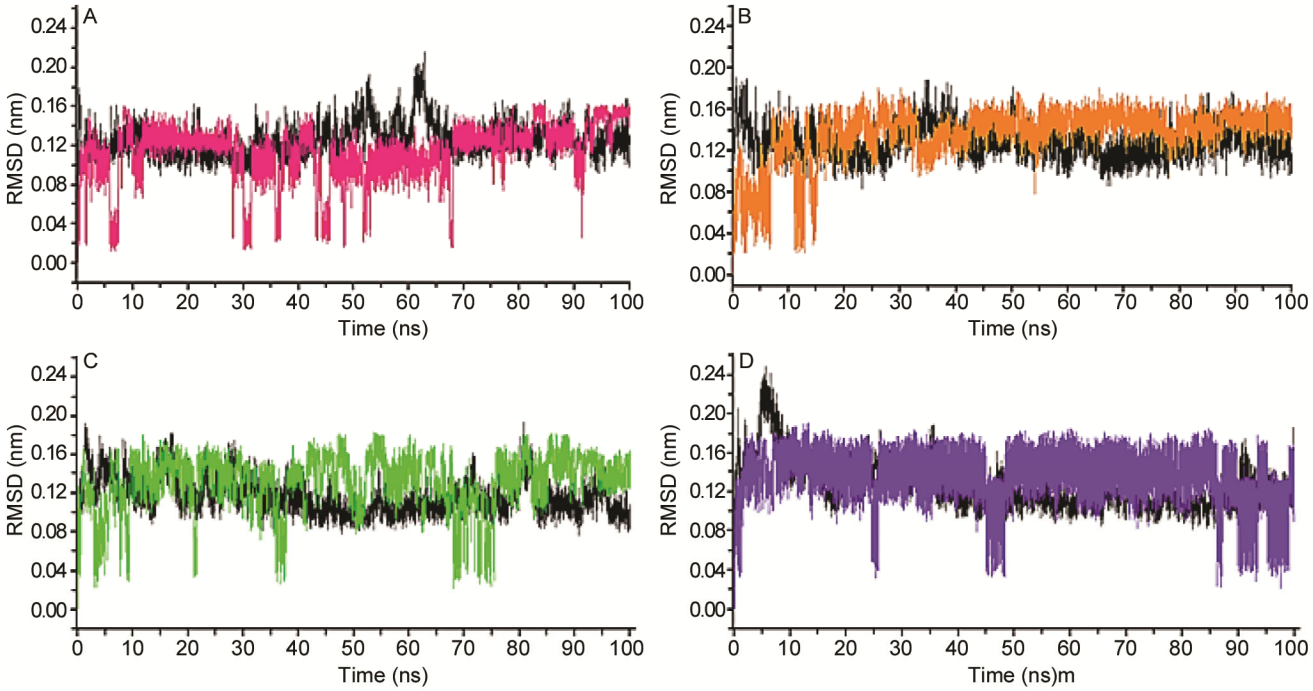

Fig. 4 — RMSD plot of four complexes (A) Zonisamide-GABARAP; (B) Z11-GABARAP; (C) Z20-GABARAP; and (D) Z19-GABARAP in the 100 ns Colour scheme: C-alpha atoms of GABARAP (black) Zonisamide (Pink), Z11 (Orange), Z20 (Green), Z19 (Violet)

around 15 ns to 100 ns, the interaction of Z11 with GABARAP was very stable, with the same amino acid residues around. After 100 ns simulation, Z11 has shown hydrophobic interaction to the residues Leu44, ASp43, Arg67, Ile64, Leu63, Leu50 and Tyr49. Thus, a stable Z11-GABARAP RMSD plot was observed after 15 ns.

RMSD plot of the c-alpha atoms of the GABARAP in Z20-GABARAP complex was very stable all along 100 ns, with RMSD values lesser than 0.195 nm (Fig. 4C). Slight negligible fluctuations were observed in the first 20 ns and around 80 ns. Although the RMSD plot of Z20 remained stable and stayed lower than 0.183 all along 100 ns, the ligand jumped out from the binding sites in the flips seen at 8 ns, 21 ns, 36 ns, 48 ns, 62 ns, 68 ns and 82 ns. This was confirmed from the trajectory frames at these respective nanoseconds. As a result, after a 100 ns simulation, Z20 formed hydrogen bonding to the residue His69 and hydrophobic interaction with the residues Lys66, Phe62, Leu70, Arg71, and His69. Excluding the flipped areas, the interactions of Z20 with GABARAP were stable.

In the Z19-GABARAP complex (Fig. 4D), the RMSD values of c-alpha atoms of GABARAP have varied to 0.25 nm in the first half of the first 20 ns. After 20 nanoseconds, it is observed that the RMSD values shown in the plot have stabilised, remaining below 0.190 nm. Although the RMSD of Z19 stayed lower than 0.192 nm all along 100 ns, flips at 26 ns, 45 ns, 85 ns - 98 ns have caused the ligand to jump out of the binding site in the docked complex. As a result, at 100 ns, Z19 has shown hydrogen bonding and hydrophobic interaction only with Glu12. Stable areas observed in the plot have indicated stable interaction between GABARAP and the ligand Z19.

Among the three complexes of derivatives, the least RMS deviation of the ligand has been observed in the GABARAP-Z11 complex. Thus, summing up the analysis of RMSD plots of the complexes and the snapshots of the frames at each nanosecond, the stable interaction of the protein has been observed with the derivative Z11. RMSD plots of the GABARAP-Z11 complex are more stable than the RMSD plots of the Zonisamide-GABARAP complex.

Radius of gyration

In order to analyse the compactness of GABARAP in the complexes with Zonisamide, Z11, Z20 and Z19, the Radius of gyration (R_g) of GABARAP in each of the four complexes was plotted (Fig. 5A & B). In all four complexes, stable Rg plots were observed with Rg score below 1.470 nm from 0 - 100000ps. Rg score of GABARAP in the GABARAP-Z20 complex

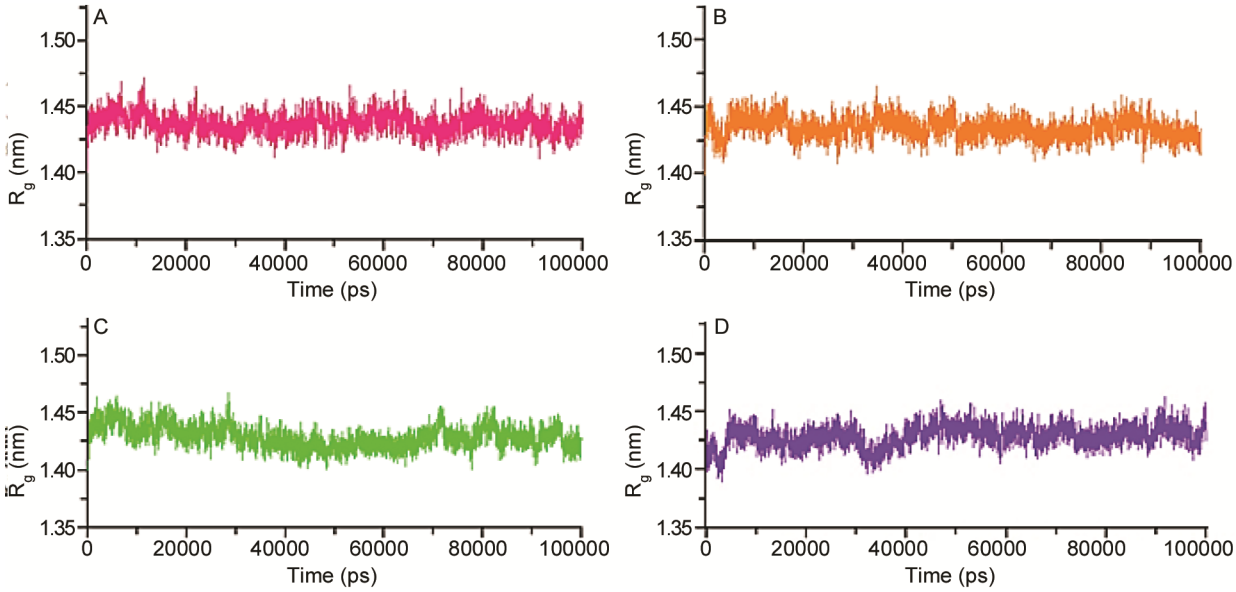

Fig. 5 — R_g plot of GABARAP in the complexes (A) Zonisamide-GABARAP (Pink); (B) Z11-GABARAP (Orange); (C) Z20-GABARAP (Green); and (D) Z19-GABARAP (Violet) in the 100 ns

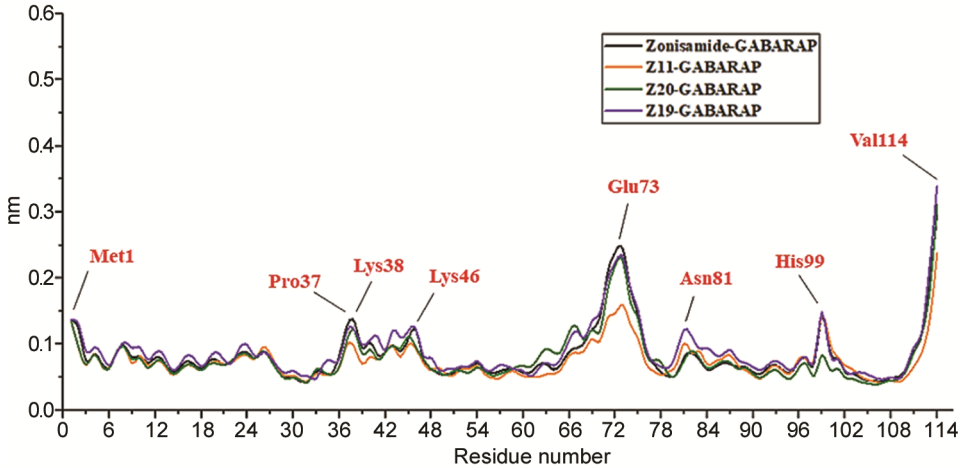

Fig. 6 — RMS fluctuations of the complexes (A) Zonisamide-GABARAP (Black); (B) Z11-GABARAP (Orange); (C) Z20-GABARAP (Green); and (D) Z19-GABARAP (Violet) in the 100 ns

has shown a slight decrease from 30000ps -70000ps (Fig. 5C). Similarly, in the GABARAP-Z19 complex, the Rg score has shifted below 1.40 nm in the first 5000ps and around 30000ps - 45000ps (Fig. 5D). The variations in these two complexes are very negligible. Thus, from the Rg plots, apart from the complex with Zonisamide, a high compactness of the GABARAP was observed during the complex formation with Z11.

Root means square fluctuation

The fluctuations in the GABARAP residues, when it has formed a complex with the drug Zonisamide and the selected best derivatives Z11, Z19 and Z20 are given in (Fig. 6). For all the complexes, notable fluctuations were observed only in the residues Pro37-Lys46, Ile68-Ala75, His99 and Glu112-Val114. In the Z19-GABARAP complex, fluctuations above 0.1 nm shown by the residues Met1 (0.1360 nm), Lys2 (0.1283 nm), Arg40 (0.1060 nm), Ile41 (0.1111 nm), Gly42 (0.0910 nm), Asp43 (0.1210 nm), Leu44 (0.1100 nm), Asp45 (0.1206 nm), Lys46 (0.1223 nm), Ile68 (0.1105 nm), His69 (0.1349 nm), Leu70 (0.1480 nm), Asp74 (0.1849 nm), Ala75 (0.1502 nm), Asn81 (0.1222 nm), Asn82 (0.1112 nm), His99 (0.1483 nm), Glu112 (0.1191 nm), Ser113 (0.2128 nm) and Val114 (0.3392 nm) are the maximum fluctuations when compared to the fluctuation of these residues in other complexes. In the GABARAP-Zonisamide complex,

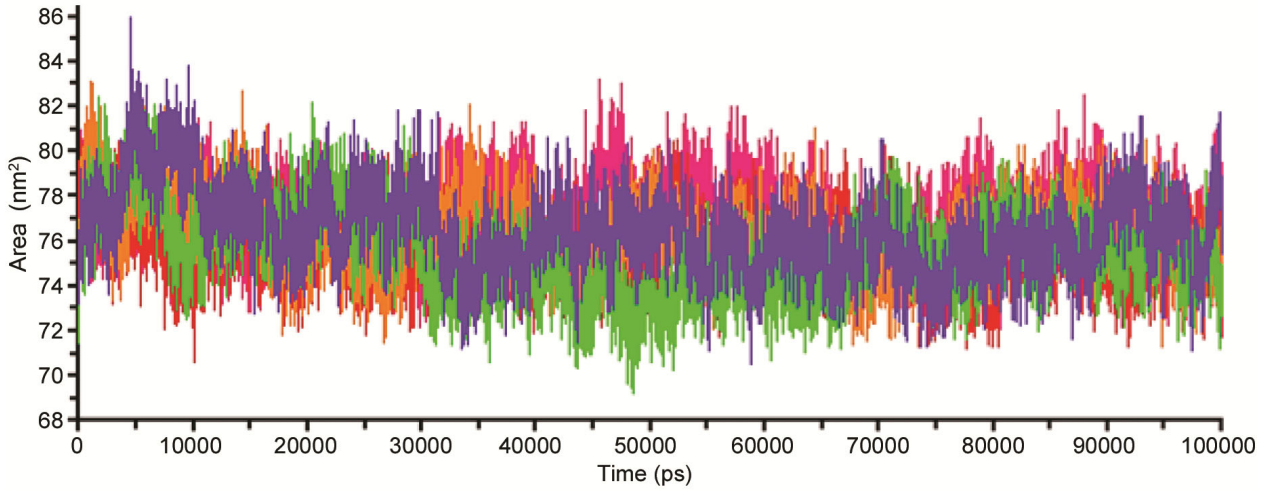

Fig. 7 — SASA plot of GABARAP in the complexes of Zonisamide-GABARAP (Pink), Z11-GABARAP (Orange), Z20-GABARAP (Green) and Z19-GABARAP (Violet) in the 100 ns

the residues Met1, Lys2, Pro37, Lys38, Arg40, Asp45, Lys46, Ile68, His69, Leu70, Arg71, Ala72, Glu73, Asp74, Ala75, His99, Glu112, Ser113 and Val114 have fluctuated above 0.1 nm. However, among these residues, only Pro37, Lys38, Ala72, Glu73, and Asp74 fluctuate more than the GABARAP residues in the Z19-GABARAP complex. Val114 is the residue that had shown the maximal fluctuation upon the ligand binding. The residue Glu100 has fluctuated to 0.1036 nm in the Z11-GABARAP complex. Fluctuations of this residue in other complexes are lower than 0.1 nm. The protein in the Z11-GABARAP complex has remained stable with comparatively less fluctuations than in other complexes Zonisamide-GABARAP, Z19-GABARAP and Z20-GABARAP.

Solvent accessible surface area

The area of the protein (in the complex) that the solvent could access during the simulation was analysed from the SASA plots (Fig. 7). The accessible protein area for the solvent varied between 72-83.3 nm^2, 71-83.2 nm^2, 69-82.5 nm^2 and 70-86 nm^2 for the complexes Zonisamide-GABARAP, Z11-GABARAP, Z20-GABARAP and Z19-GABARAP. Almost a similar SASA range could be seen for all the complexes. In the Zonisamide-GABARAP complex, a slight decrease in the solvent attainable protein area was seen between 25000ps - 30000ps, 60000ps - 80000ps and 95000ps - 1000000ps. For the Z11-GABARAP complex, the SASA of protein has shown a decrease from the initial area at 18000 - 25000ps, 40000 ps, 55000ps, 65000 – 80000 ps and in the final five thousand picoseconds. The SASA plot has shown a slight decrease in the first 30 ns for the Z19-GABARAP complex. The variations in the SASA plots for the above three complexes were negligible. But for the Z20-GABARAP, a notable decrease in the solvent accessible protein area was seen from 30000ps– 70000ps. In all these complexes, a decrease in the solvent interacting area may indicate the time at which the protein has interacted more strongly with the ligand. Energy plots of all the complexes are very stable (Suppl. Fig. S4).

Thus, in conclusion, in the MD simulation studies, the derivative Z11 has formed the most stable complex with GABARAP. The RMSD plots of the derivative Z11 are very stable[36-38]. The GABARAP interaction exhibited by Z11 is more stable than the interaction shown by the parent drug Zonisamide. Thus, Z11 is the hit compound among the 32 designed derivatives of Zonisamide[39]. The residues Thr87, Ile84, Phe78 and Tyr61 were engaged in the hydrophobic interaction with the groups substituted in the derivatives Z11, Z20 and Z19. In the derivatives Z20 and Z19, apart from these four residues, the residues Leu76 and Phe77 interacted with the substituted groups. However, in the case of Z11, Val57 and Pro85 were the extra residues engaged in the hydrophobic interaction[40,41].

The snapshots of the residues engaged in the interaction with Z11 at 0, 50 and 100 ns are given in (Fig. 8). The snapshots of GABARAP interactions with Z20 and Z19 are given in (Suppl. Fig.S5 and S6).

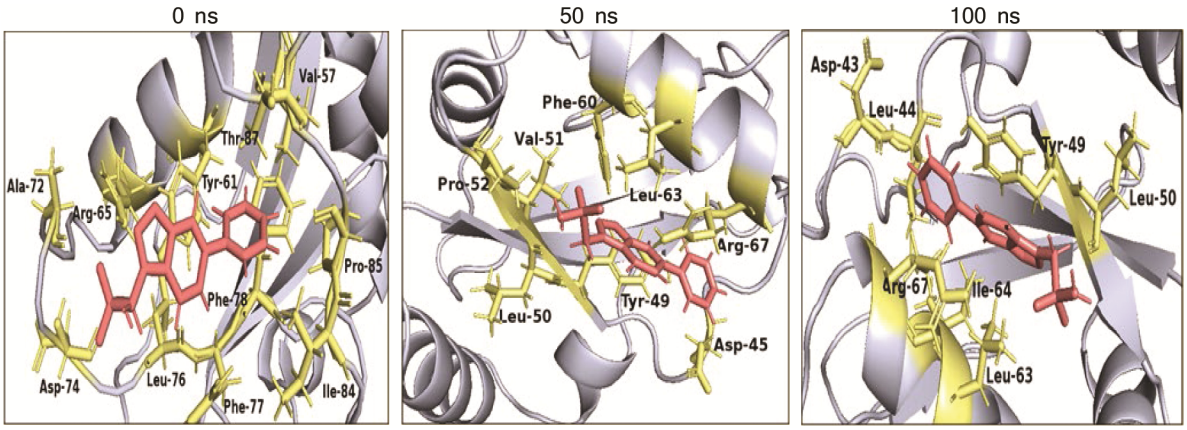

Fig. 8 — Snapshot showing the interacted residues of Z11 at 0, 50 and 100 ns

Conclusion

This work is a theoretical drug-designing approach to propose the best derivatives of Zonisamide. Extensive computational studies involving the analysis of oral activity (Lipinski rule of five), safety (Mutagenicity, Cytotoxicity, irritation, hepatotoxicity, Immunotoxicity and reproductive effectiveness), drug properties (Topological polar surface area, solubility, flexibility, intestinal absorption, saturation, *etc*.), binding energy and stability in GABARAP binding were carried out.

In this work, thirty-two derivatives (Z1-Z32) of Zonisamide were designed, and in most cases, the substitutions of the hydrophobic groups have elevated the spontaneous target binding of the drug. The derivatives Z1, Z2 Z5, Z6, Z16, Z25, Z27 Z28, Z30, Z31, Z32 were initially eliminated as their target binding energy was not lower than Zonisamide. After the analysis of drug properties, oral activity, and the safety of the derivatives, the tumerogenic compounds Z15 and Z24 were also eliminated. Z13 and Z22 have the probability of having less bioavailability as they are Pgp substrates. Evaluating all these factors, the molecules Z3, Z4, Z7-Z12, Z14, Z17-Z21, Z23, Z26 and Z29 were recognized as the best derivatives of Zonisamide. The stability of GABARAP complexes of Zonisamide and the GABARAP complexes of top three derivatives were evaluated critically through molecular dynamic simulation studies. The interaction time was 100 ns. Through this study, we propose Z11 as the hit compounds. It is because they have formed more stable complex with the protein, compared to Zonisamide and other derivatives. The residues of GABARAP had shown least fluctuations in the Z11-GABARAP complexes. The Rg plots showing the protein compactness were evaluated to conclude that GABARAP was very compact in the complexes of top three derivatives. But on comparison, Rg plot was slightly more stable for GABARAP in Z11-GABARAP complexes. Z11 formed most stable complex with GABARAP among the designed derivatives. Thus, through this work we propose Z11 as a promising derivative of Zonisamide.

The pharmacological effects and the target binding stability of the hit compound Z11 can be further studies through clinical studies. As the detailed evaluations of various drug-related aspects were carried out computationally, further clinical trials or research in this case will land up in promising derivatives.

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Conflict of interest

All authors declare no conflict of interest.

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