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ORIGINAL ARTICLE

In silico analysis of the inhibitory activities of GABA derivatives on 4-aminobutyrate transaminase

Hira Iftikhar [a,1], Sidra Batool [a,1], Aakash Deep [b,*], Balasubramanian Narasimhan [b], Prabodh Chander Sharma [c], Manav Malhotra [d]

[a] *National Center for Bioinformatics, Quaid-i-Azam University, Islamabad, Pakistan*
[b] *Department of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak, India*
[c] *Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra, India*
[d] *Department of Pharmaceutical Chemistry, ISF College of Pharmacy, Ferozepur Road, Moga 142 001, India*

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Abstract Reduced levels of γ-aminobutyric acid (GABA) are cause of quite a many diseases, and it cannot be directly introduced into the body to enhance its level because of the blood–brain barrier. Thus the technique used for the purpose involves the inhibition of aminobutyrate transaminase (ABAT), the enzyme catalyzing its degradation. The structure of human ABAT is not currently known experimentally, thus, it was predicted by homology modeling using pig ABAT as template due to high level of sequence similarity and conservation. A series of new γ-aminobutyric acid (GABA) derivatives obtained from 4-(1,3-dioxoisoindolin-2-yl)butanoic acid are used in this study. These γ -aminobutyric acid (GABA) derivatives were used as ligand dockings against human ABAT as well as pig ABAT receptors.

1. Introduction

γ-Aminobutyric acid (GABA) is one of the most commonly occurring neurotransmitters and a regulator of neuronal activity in the brain (McGeer et al., 1983). Its degradation involves catalysis by aminobutyrate transaminase (ABAT, GABA transaminase, 4-aminobutyrate aminotransferase) during the first step. A fall in GABA levels, compared to the normal, has been elucidated as an effective symptom for a variety of neurological disorders (Gunne et al., 1984; Butterworth et al., 1983), such as Alzheimer's disease (Aoyagi et al., 1990), epilepsy (Bakay and Harris, 1981), Parkinson's disease (Nishino et al., 1988).

* Corresponding author. Address: Department of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak 124 001, Haryana, India.
E-mail addresses: aakashdeep82@gmail.com, aakashdeep1983@gmail.com (A. Deep).
[1] Equally contributing authors.

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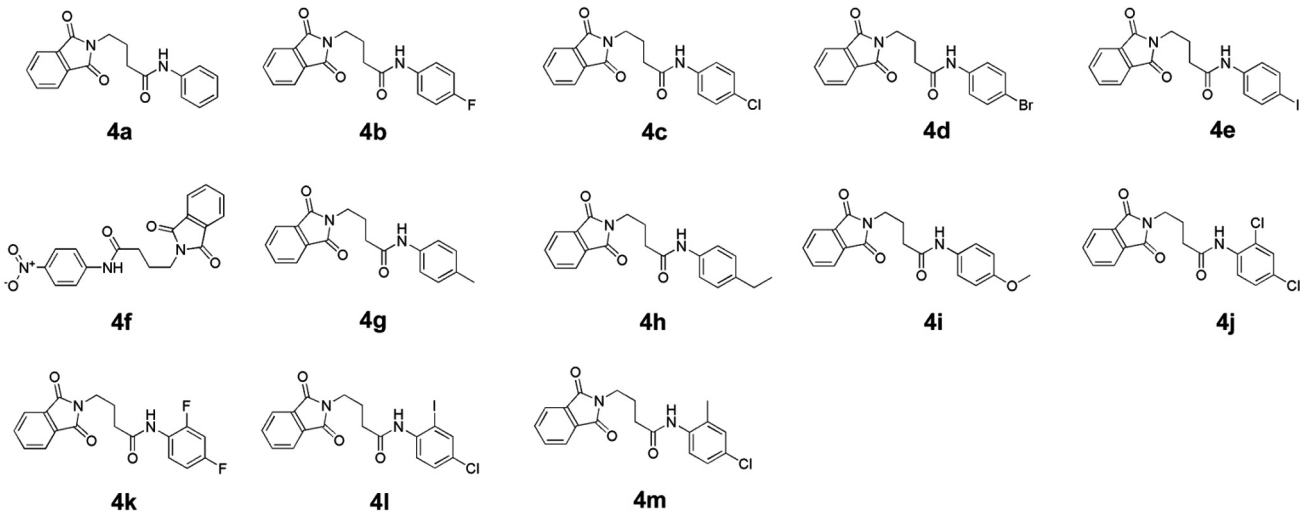

Figure 1 2D structures of GABA derivatives (**4a**–**4m**). (**4a**) 4-(1,3-Dioxoisoindolin-2-yl)-*N*-phenylbutanamide; (**4b**) 4-(1,3-Dioxoisoindolin-2-yl)-*N*-(4-fluorophenyl)butanamide; (**4c**) *N*-(4-Chlorophenyl)-4-(1,3-dioxoisoindolin-2-yl)butanamide; (**4d**) *N*-(4-Bromophenyl)-4-(1,3-dioxoisoindolin-2-yl)butanamide; (**4e**) 4-(1,3-Dioxoisoindolin-2-yl)-*N*-(4-iodophenyl)butanamide; (**4f**) 4-(1,3-Dioxoisoindolin-2-yl)-*N*-(4-nitrophenyl)butanamide; (**4g**) 4-(1,3-Dioxoisoindolin-2-yl)-*N*-p-tolylbutanamide; (**4h**) 4-(1,3-Dioxoisoindolin-2-yl)-*N*-(4-ethylphenyl)butanamide; (**4i**) 4-(1,3-Dioxoisoindolin-2-yl)-*N*-(4-methoxyphenyl)butanamide; (**4j**) *N*-(2,4-Dichlorophenyl)-4-(1,3-dioxoisoindolin-2-yl)butanamide; (**4k**) *N*-(2,4-Difluorophenyl)-4-(1,3-dioxoisoindolin-2-yl)butanamide; (**4l**) *N*-(4-Chloro-2-iodophenyl)-4-(1,3-dioxoisoindolin-2-yl)butanamide; (**4m**) *N*-(4-Chloro-2-methylphenyl)-4-(1,3-dioxoisoindolin-2-yl)butanamide.

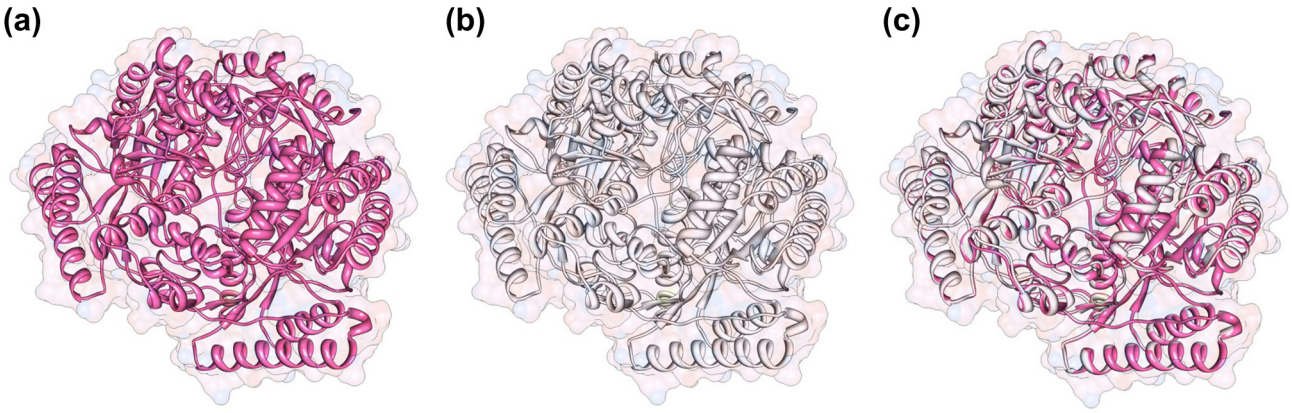

Figure 2 3D structures of template and target proteins, and their superimposition. (a) Structure of pig 4-aminobutyrate-aminotransferase used as template; (b) Structure of human 4-aminobutyrate transaminase obtained through homology modeling; (c) template-target superimposition with an RMSD of 0.2 Å.

Lower esophageal sphincter (LES) is also controlled by GABA (Cantu et al., 2003). Transient lower esophageal sphincter relaxations (TLESRs) cause an increase in frequency of reflux episodes (Dent et al., 1988) in people having disease linked ABAT alleles (Jirholt et al., 2011), are the principal motility factor in gastro-esophageal reflux disease (GERD; Holloway, 2000) and are reduced by signaling via GABA receptors (Lehmann, 2009; Beaumont et al., 2008). Mutations in ABAT, in relation to GERD, are expected to be located in non-coding regions (Ku et al., 2010). However, the protein ABAT is known to have some mutations that cause psychomotor retardation and early death (Medina-Kauwe et al., 1999; Jaeken et al., 1984).

In the case of ABAT inhibition, GABA-driven signaling via GABA receptors increases as a consequence of a rise in GABA levels in the synaptic junctions (Jung et al., 1977). Due to the fact that GABA cannot move across the blood–brain barrier without significant side effects (Toth et al., 1983), so far the most effective way to retain the levels of GABA is via inhibition of ABAT by targeting its active site.

This study was performed to analyze the inhibitory effects of certain experimentally known compounds on human ABAT, their interaction details and effectiveness. In this study we have used human ABAT 3D structure as receptor against a dataset of GABA derivatives obtained from 4-(1,3-dioxoisoindolin-2-yl) butanoic acid. As 3D structure of human ABAT was not known we have used homology modeling for structure prediction. Binding site of ABAT was identified and insilico analysis was carried out via performing docking studies of ABAT structures with GABA derivatives. Each docking

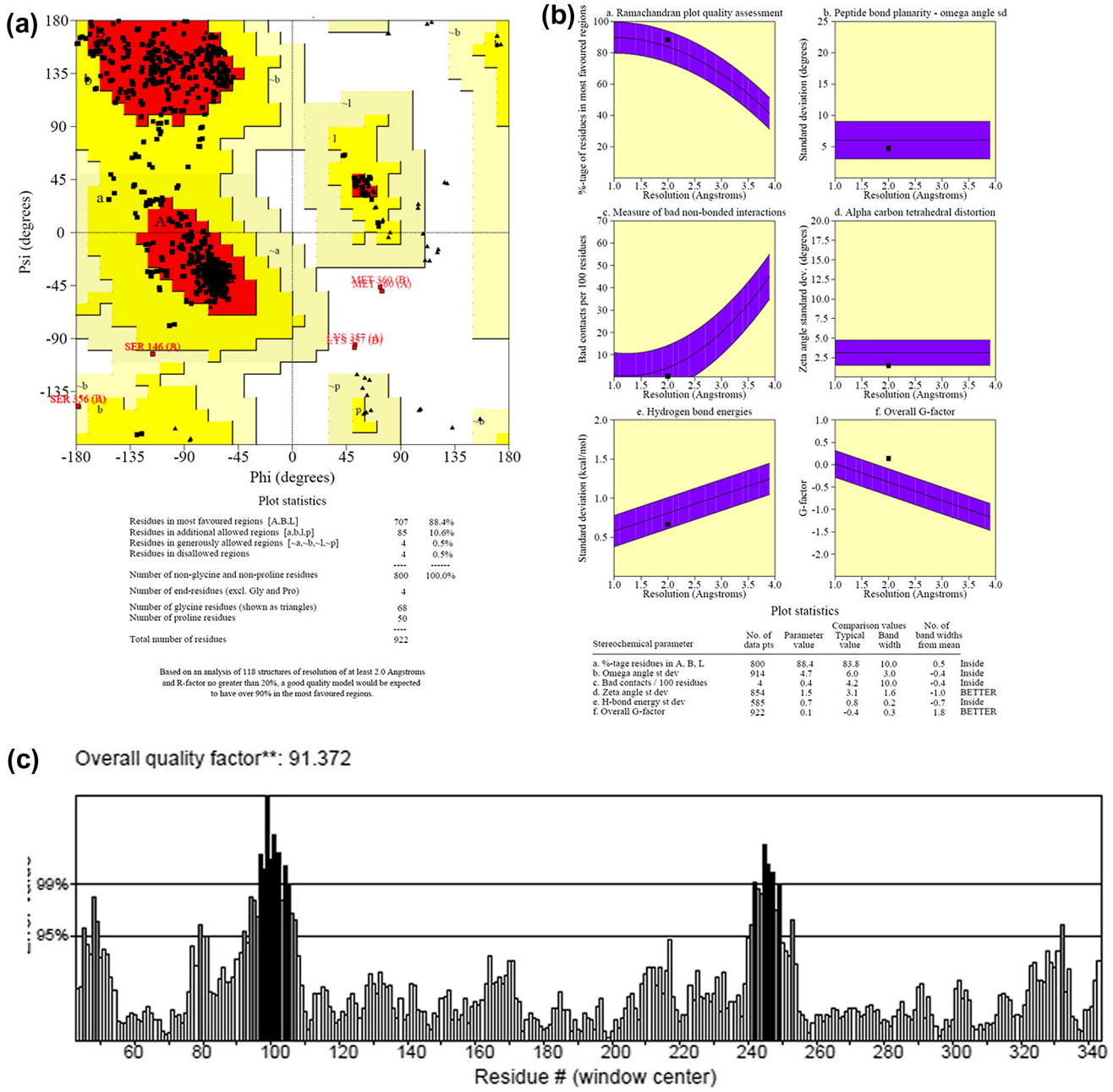

Figure 3 Structure validation of modeled protein, human ABAT. (a) Ramachandran plot showing 99% residues in the allowed region; (b) Procheck validation plot for main chain parameters; (c) Errat quality check with an overall quality factor of 91.372.

experiment was analyzed carefully for finding correct orientations of ligands with receptor binding site.

2. Materials and methods

2.1. Structure prediction

3D structure of human protein 4-aminobutyrate transaminase (from here-on referred to as target) is not known experimentally yet. For elucidation of the 3D structure of this protein, structure of pig 4-aminobutyrate transaminase (PDB ID: 1OHV; Storici et al., 2004; from here-on referred to as template) was used as a template with high sequence similarity. Protein structure prediction, carried out using Swiss-Model server (Arnold et al., 2006), was followed by model optimization and validation using Ramachandran plot (Kleywegt and Jones, 1996), Procheck, Errat (Colovos and Yeates, 1993), Verify3D (Luthy et al., 1992; Bowie et al., 1991) and WhatIF (Vriend, 1990) tools.

2.2. Ligand dataset

Yadav et al. reported a series of new γ-aminobutyric acid (GABA) derivatives obtained from 4-(1,3-dioxoisoindolin-2-

Table 1 Different energy values and *Ki* values after docking the compounds with receptor protein, human ABAT.

Ligands	Binding energy (kcal/mol)	*Ki* (μM)	Inter-molecular energy (kcal/mol)	vdW + Hbond + desolv Energy (kcal/mol)	Electrostatic energy (kcal/mol)	Final total internal energy (kcal/mol)	Torsional free energy (kcal/mol)	Unbound system's energy (kcal/mol)
4a	−7.13	5.89	−8.63	−8.44	−0.19	−0.29	1.49	−0.29
4b	−7.30	4.48	−8.79	−8.62	−0.17	0.02	1.49	0.02
4c	−7.87	1.71	−9.36	−9.18	−0.18	−0.16	1.49	−0.16
4d	−8.04	1.27	−9.54	−9.29	−0.25	−0.10	1.49	−0.10
4e	−7.59	2.74	−9.08	−8.77	−0.31	0.35	1.49	0.35
4f	−8.34	0.77	−10.13	−9.08	−1.06	−0.41	1.79	−0.41
4 g	−7.90	1.62	−9.39	−9.15	−0.24	1.39	1.49	1.39
4 h	−8.19	0.99	−9.98	−10.0	0.03	1.48	1.79	1.48
4i	−6.89	8.85	−8.68	−8.48	−0.21	−0.29	1.79	−0.29
4j	−7.83	1.81	−9.32	−9.16	−0.16	−0.51	1.49	−0.51
4 k	−7.68	2.35	−9.17	−8.81	−0.36	−0.42	1.49	−0.42
4 l	−7.91	1.60	−9.40	−9.32	−0.07	0.06	1.49	0.06
4 m	−8.25	0.90	−9.74	−9.47	−0.27	−0.46	1.49	−0.46

Table 2 Different energy values and Ki values after docking the compounds with receptor protein, pig ABAT.

Ligands	Binding energy (kcal/mol)	Ki (μM)	Inter-molecular energy (kcal/mol)	vdW + Hbond + desolv Energy (kcal/mol)	Electrostatic energy (kcal/mol)	Final total internal energy (kcal/mol)	Torsional Free Energy (kcal/mol)	Unbound System's Energy (kcal/mol)
4a	−7.35	4.09	−8.84	−8.71	−0.13	−0.34	1.49	−0.34
4b	−7.75	2.09	−9.24	−9.18	−0.06	−0.23	1.49	−0.23
4c	−8.67	0.44	−10.16	−10.25	0.09	1.08	1.49	1.08
4d	−9.29	0.16	−10.78	−10.64	−0.14	0.14	1.49	0.14
4e	−8.11	1.13	−9.61	−9.50	−0.11	−0.14	1.49	−0.14
4f	−8.92	0.29	−10.71	−9.99	−0.72	0.65	1.79	0.65
4 g	−9.15	0.20	−10.64	−10.38	−0.26	0.29	1.49	0.29
4 h	−8.69	0.43	−10.48	−10.41	−0.06	−0.29	1.79	−0.29
4i	−7.82	1.86	−9.61	−9.49	−0.12	−0.11	1.79	−0.11
4j	−7.79	1.94	−9.28	−9.23	−0.06	−0.25	1.49	−0.25
4 k	−7.11	6.14	−8.60	−8.53	−0.07	0.52	1.49	0.52
4 l	−8.26	0.89	−9.75	−9.55	−0.20	−0.09	1.49	−0.09
4 m	−8.10	1.16	−9.59	−9.27	−0.32	−0.44	1.49	−0.44

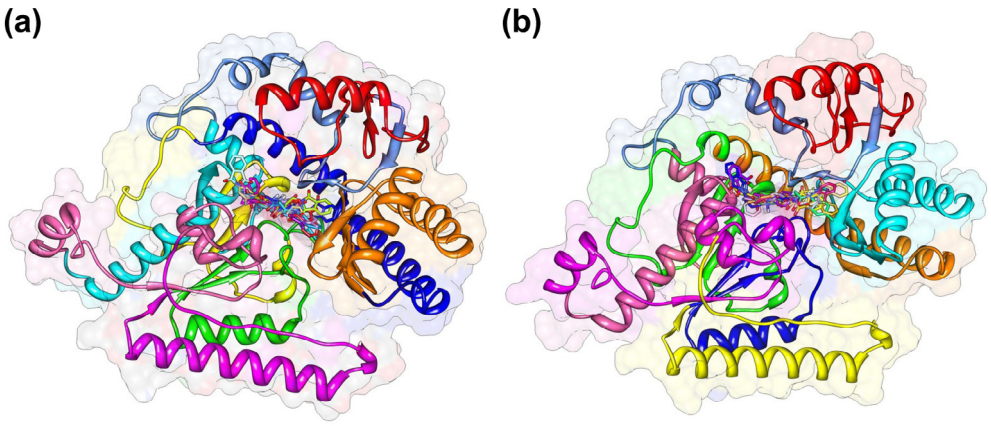

Figure 4 Binding of all GABA derivatives in (a) template binding site pig ABAT and (b) target binding site human ABAT. Ribbon represents the receptors while ligands are shown in sticks encapsulated in a mesh.

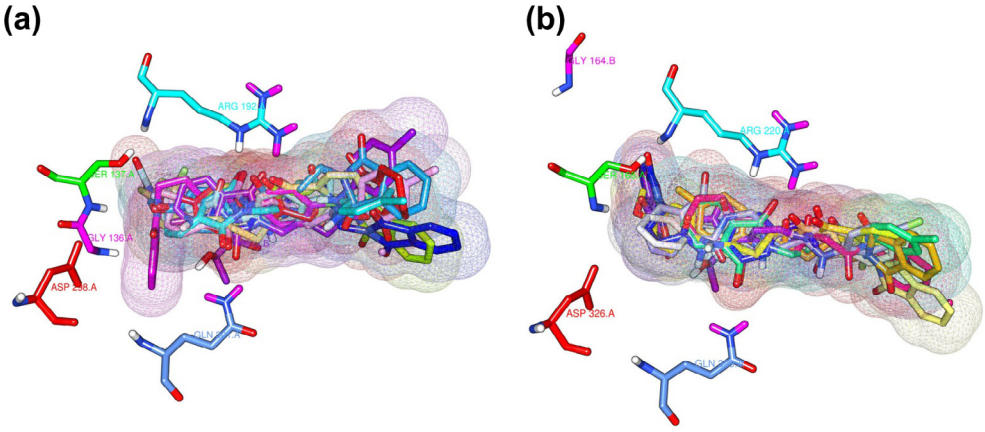

Figure 5 Residues involved in binding for (a) template and (b) target. All the ligands are shown in sticks enveloped by the mesh surface. Receptor residues are shown in sticks.

Table 3 Human ABAT active site residues involved in docking interactions with the compounds.

Compounds	Binding residues						
	Gly164	Ser165	Arg220	Asp326	Val328	Gln329	**Thr381**
4a	✓	✓	✓		✓		✓
4b	✓	✓	✓		✓	✓	✓
4c	✓	✓	✓	✓	✓		✓
4d	✓	✓	✓	✓	✓		✓
4e	✓	✓	✓		✓	✓	✓
4f	✓	✓	✓	✓			✓
4g		✓	✓	✓	✓	✓	✓
4h	✓	✓	✓	✓	✓	✓	✓
4i	✓	✓	✓	✓	✓		✓
4j		✓	✓	✓		✓	✓
4k	✓	✓	✓		✓	✓	✓
4l		✓	✓	✓	✓	✓	✓
4m		✓	✓	✓	✓	✓	✓

yl) butanoic acid by coupling it with various substituted amines by using DCC as a coupling reagent, which was used in docking against the target and its template. Fig. 1 shows 2D structures of this series designated as compounds **4a**–**4m** (Yadav et al., 2012).

2.3. Molecular docking studies

Using the tool AutoDock 4.2 (Sanner, 1999), automated dockings were performed for finding the conformations of selected ligands (compounds) binding to the active site of 4-aminobutyrate

Table 4 Atoms of the compounds and target protein residues involved in making hydrogen bonds and bond distance.

Compounds	Binding residue	Hydrogen bond	Distance (Å)
4a	Arg220	O–HN	2.35
		O–HN	2.35
	Thr381	NH–O	1.82
4b	Arg220	O–HN	1.78
		O–HN	1.95
4c	Arg220	O–HN	2.09
		O–HN	2.45
	Thr381	NH–O	1.84
4d	Arg220	O–HN	2.05
4e	Ser165	O–HN	2.11
		O–H	2.49
	Thr381	NH–O	2.13
4f	Arg220	O–HN	1.90
	Thr381	O–HN	1.83
4g	Arg220	O–HN	2.07
4h	Ser165	O–H	2.03
	Thr381	NH–O	1.91
4i	Ser165	O–HN	2.01
4j	Arg220	O–HN	1.79
4k	Ser165	O–H	2.12
		N–H	3.10
	Thr381	O–HN	2.24
4l	Arg220	O–HN	2.01
		O–HN	2.26
4m	Arg220	O–HN	1.78
		O–HN	2.13

transaminase. Polar hydrogen atoms were added to the receptor proteins and Kollman charges were alloted. Non-polar hydrogen atoms, in ligands, were merged and Gasteiger partial charges were assigned. Also, rotations were allowed for ligands' bonds, and random orientations and torsions were used during the procedure. AutoGrid was then used to generate grid maps by locating an $80 \times 80 \times 80$ Å3 grid around each receptor model, separated by 0.375 Å. The Lamarckian genetic search algorithm was used by setting the program to 100 runs, 150 population size, 2.5×10^6 energy evaluations, 27,000 maximum iterations, 0.02 mutation rate, 0.80 crossover rate and an RMS tolerance of 1.0 Å for cluster analysis. The most occurring docked conformation with the lowest binding energy was selected for that particular ligand.

2.4. Binding residues

From PDB (ID: 1OHV), the active site residues of the template protein were extracted followed by inference of target protein residues by alignment of the two proteins.

Template: Gly136A, Asp298A, Thr353B, Gln301A, Ser137A, Arg192A, Val300A.

Target: Gly164, Asp326, Thr381, Gln329, Ser165, Arg220, Val328.

3. Results and discussion

3.1. 3D structure prediction of human 4-aminobutyrate transaminase

Crystal structure of pig 4-aminobutyrate-aminotransferase (PDB ID: 1OHV; resolution 2.30 Å) was used as template for the prediction of 3D model for human 4-aminobutyrate transaminase. MSA analysis indicated 80% sequence identity between target and template sequences and an RMSD of 0.2 Å between the two models. Fig. 2 shows the protein structures of template (Fig. 2a) and target protein (Fig. 2b) along with superimposed pose of both (Fig. 2c).

3.2. Structure validation

Ramachandran plot indicated 99% residues in the allowed regions and none of the active site residues in the disallowed region. Moreover, parameters like peptide bond planarity, non-bonded interactions, Cα tetrahedral distortion, main chain H-bond energy and overall G factor for the predicted structure lie within the favorable range.

The homology models were further verified using Errat, Verify3D and WhatIF tools. Errat scored the overall quality factor for non-bonded atomic interactions very much above the accepted range giving a score of 91.3, which represent the high quality of our predicted model. Fig. 3 shows the structure validation by Ramachandran plot, Procheck and Errat programs.

Main chain parameters in terms of conformation of the predicted model were checked for deviations from the template using Procheck (Fig. 3b). Verify3D produced averaged data points with 96.97% residues exhibiting a score above 0.2, assuring a considerable high model quality. In the case of human 4-aminobutyrate transaminase, none of the scores for any residue given by WhatIF was found less than −5.0 indicating structure reliability. These data indicated that the predicted model is of good quality to allow further study.

3.3. Molecular dockings

Molecular docking studies were carried out with both target and template 4-aminobutyrate transaminase. All the compounds were found to strongly inhibit by completely occupying the active sites in the case of both the target protein, and its template model. All inhibitors showed low energy values as indicated in Table 1 for target and for template in Table 2. The inhibition constant values indicated the efficiency of the compounds to inhibit the protein, especially compounds **4f**, **4h**, and **4m** in the case of target and **4c**, **4d**, **4f**, **4g**, **4h**, and **4l** in the case of the template. These exceptionally low *K*i values and low energy values prove drug efficiency for these compounds.

For target protein, binding energy values range from −6.89 to −8.34 kcal/mol while for template protein, binding energy values range from −7.11 to −9.29 kcal/mol. Along with these, intermolecular energy values, vdW + Hbond + desolv energy values, electrostatic energy values, internal energy values and system's unbound energy also lie in a favorable range for both target and template as clearly indicated in Tables 1 and 2.

3.4. Binding mode of GABA derivatives

All GABA derivatives bind efficiently with binding sites of pig and human 4-aminobutyrate transaminase. Fig. 4a and b shows the binding mode of GABA derivatives with pig 4-aminobutyrate transaminase and human 4-aminobutyrate

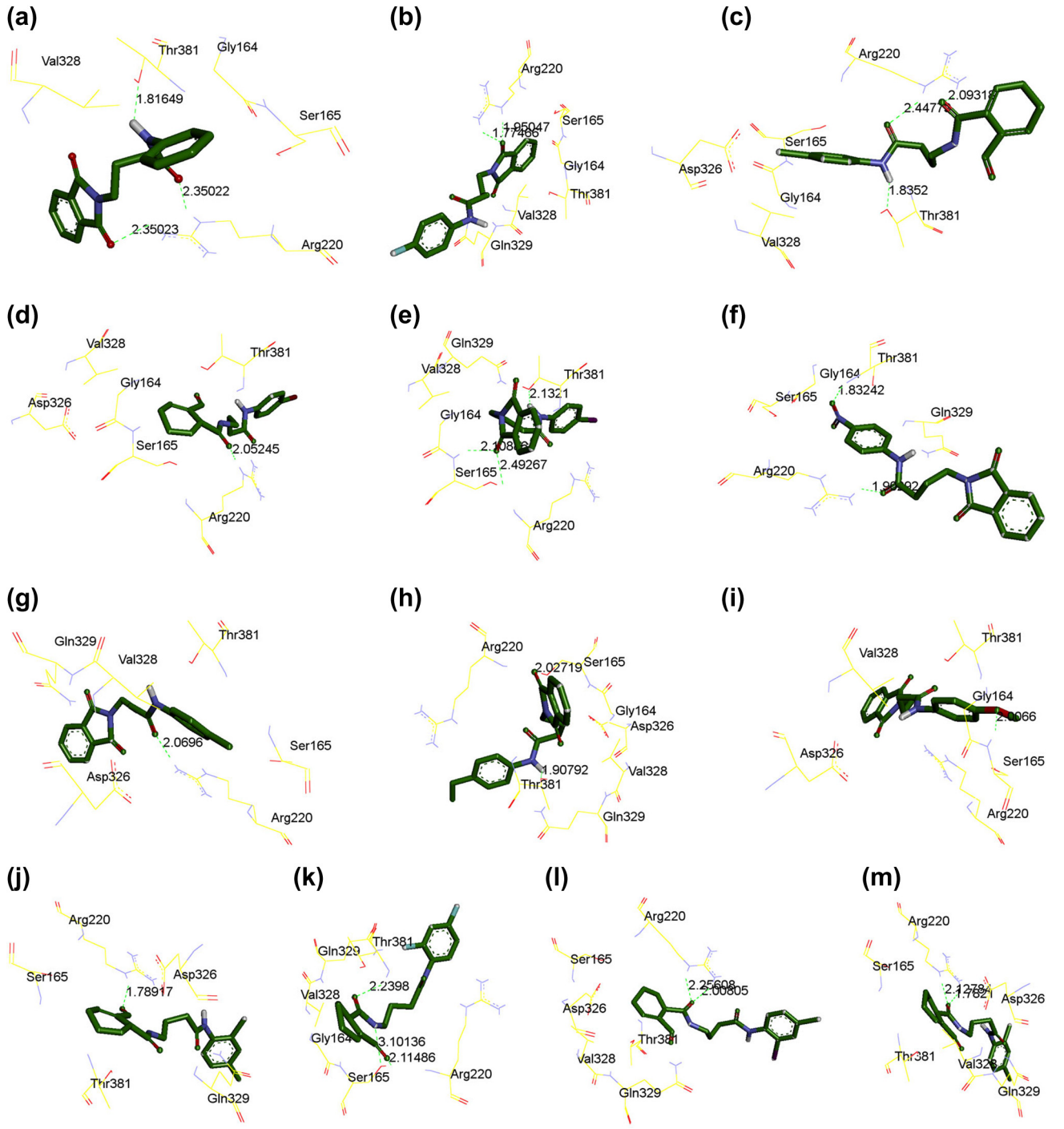

Figure 6 Interacting residues involved in docking of inhibitors at active site of human ABAT protein (H-atoms are colored gray, O-atoms red, N-atoms blue, I-atoms purple, C-atoms of inhibitors are colored green while C-atoms of receptor residues are colored yellow, and hydrogen bonds are shown with green dashed line labeled with distances in Å). (a–m) Compounds **4a**–**4m** mode of interaction with the human ABAT protein binding site.

transaminase respectively. It is clearly shown that all compounds bind in the same cavity for both template and target.

Fig. 5 further shows the residues involved in binding with both template and target receptors. Fig. 5a shows that Gly136, Asp298, Thr353, Gln301, Ser137A and Arg192 residues of template are involved in interaction while Fig. 5b shows that Gly164, Asp326, Thr381, Gln329, Ser165, and Arg220 residues of target are involved in interactions.

Human ABAT interaction with the compounds **4a**–**4m** was analyzed further in detail. Table 3 shows the binding residues' information for compounds **4a**–**4m**, we observed that all active

site residues for Human ABAT were involved in docking with nearly all the compounds.

Strong inhibitor binding is also reflected by the frequency of hydrogen bonds as shown in Table 4 and Fig. 6. Compounds **4a**, **4c**, **4e** and **4k** each made three hydrogen bonds with two residues. Ser165, Arg220 and Thr381 of human ABAT protein were mostly seen in making hydrogen bonds with these compounds, however, Arg220 was the most frequently occurring residue in hydrogen bonding. The details of hydrogen bonds formed by the compounds with binding residues, atoms involved in the bonds and distance are given in Table 4.

Fig. 6 shows the interacting residues involved in docking of inhibitors at the active site of human ABAT protein. Compound **4a** forms two hydrogen bonds with Arg220 (2.35 Å and 2.35 Å) and a single hydrogen bond with Thr381 (1.82 Å), while it interacts hydrophobically with Gly164, Ser165 and Val328 as shown in Fig. 6a. Compound **4b** makes hydrogen bonds with Arg220 (1.78 Å and 1.95 Å) and forms hydrophobic interactions with Gly164, Ser165, Val328, Gln329 and Thr381 (Fig. 6b). Compound **4c** forms hydrogen bonds with Arg220 (2.09 Å and 2.45 Å) and Thr381 (1.84 Å), while hydrophobic interactions are observed with Gly164, Ser165, Asp326 and Val328 (Fig. 6c). Compound **4d** forms a single hydrogen bond with Arg220 (2.05 Å), while Gly164, Ser165, Asp326, Val328 and Thr381 are shown to form hydrophobic interactions as shown in Fig. 6d. Compound **4e** shows hydrogen bonding with Ser165 (2.11 Å and 2.49 Å) and Thr381 (2.13 Å), and hydrophobic interactions with Gly164, Arg220, Val328 and Gln329 (Fig. 6e). Compound **4f** is shown to be involved in hydrogen bonding with Arg220 (1.90 Å) and Thr381 (1.83 Å), and in hydrophobic interactions with Gly164, Ser165, and Gln329 (Fig. 6f). Compound **4g** hydrogen bonds with Arg220 (2.07 Å) and forms hydrophobic interactions with Ser165, Asp326, Val328, Gln329 and Thr381 (Fig. 6g). Compound **4h** is shown to form hydrogen bonds with Ser165 (2.03 Å) and Thr381 (1.91 Å) and hydrophobic interactions with Gly164, Arg220, Asp326, Val328 and Gln329 (Fig. 6h). Compound **4i** forms a hydrogen bond with Ser165 (2.01 Å), and hydrophobic interactions with Gly164, Arg220, Asp326, Val328 and Thr381 (Fig. 6i). Compound **4j** interacts by forming a hydrogen bond with Arg220 (1.79 Å), and hydrophobically with Ser165, Asp326, Gln329 and Thr381 as shown in Fig. 6j. Compound **4k** makes hydrogen bonds with Ser165 (2.12 Å and 3.10 Å) and Thr381 (2.24 Å), and hydrophobic interactions with Gly164, Arg220, Val328 and Gln329 as shown in Fig. 6k. Compound **4l** is shown to have two hydrogen bonds with Arg220 (2.01 Å and 2.26 Å), and hydrophobic interactions with Ser165, Asp326, Val328, Gln329 and Thr381 (Fig. 6l). Compound **4m** forms hydrogen bonds with Arg220 (1.78 Å and 2.13 Å), while the residues Ser165, Asp326, Val328, Gln329 and Thr381 interact in a hydrophobic manner with the compound (Fig. 6m).

4. Conclusion

This study supported that the anticonvulsant compounds reported by Yadav et al. (2012) prove their *in silico* inhibitory activity on human protein 4-aminobutyrate aminotransferase that can be used in the treatment of epilepsy and some other diseases. As human ABAT protein structure was not known experimentally the homology modeling technique was used to predict the 3D structure using the known pig ABAT structure, keeping in view the fact that this protein has high sequence similarity and conservation in both human and pig. Inhibition of the active site residues with high activity, strong binding, low energy values and inhibition constant showed that these compounds can be used in drug design against certain diseases that can somehow be linked to the protein ABAT.

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