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# New and mild method for the synthesis of alprazolam and diazepam and computational study of their binding mode to GABA<sub>A</sub> receptor

Ahmad R. Massah<sup>1,2</sup> · Sajjad Gharaghani<sup>3</sup> · Hamid Ardeshtari Lordejani<sup>1</sup> · Nahad Asakere<sup>1</sup>

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**Abstract** A new method for the synthesis of 8-chloro-1-methyl-6-phenyl-4H-benzo[f][1,2,4]triazolo[4,3-a][1,4]diazepine (alprazolam) and 7-chloro-1-methyl-5-phenyl-1H-benzo[e][1,4]diazepin-2(3H)-one (diazepam) from 2-amino-5-chloro benzophenone was described under mild conditions. Most of the synthetic steps were carried out under solvent-free conditions, and the products were obtained in high yield and purity. The products were characterized by comparison of physical properties with authentic samples and also by IR, <sup>1</sup>H NMR and <sup>13</sup>C NMR. Three-dimensional (3D) model of GABA<sub>A</sub> was constructed using X-ray crystal structure of *homopentameric caenorhabditis elegans glutamate-gated chloride channel (GluCl)* (3RHW) at 3.3 Å as the template based on sequence comparison and homology modeling method. The homology modeling and MD simulation studies predicted the 3D structure of receptor in a water environment. The resulted conformation of the receptor was used for docking of the alprazolam and diazepam. Docking studies indicated many important interactions of the drugs with the receptor. Furthermore, the complex of GABA with drugs was used in MD simulation to realize the conformation changes of the complex.

**Keywords** Alprazolam · Diazepam · GABA · Homology modeling · Molecular docking · Molecular dynamics simulation

## Introduction

Benzodiazepines are a well-known class of compounds with a wide range of central nervous system-related activities (Griffin *et al.*, 2013). Benzodiazepines showed different types of biological activity such as antitumoral and anticonvulsive activities (Doss *et al.*, 2000).

Aminobenzophenones are intermediates in the synthesis of benzodiazepine derivatives (Cortez-Maya *et al.*, 2012). A representative of this compounds are alprazolam and diazepam which contains a 1,2,4-triazole fused to the benzodiazepine core (Baumann *et al.*, 2011). The alprazolam and diazepam are clinically popular anxiolytic agents which possesses an atypical clinical profile relative to other benzodiazepines. In addition to anxiolytic properties, alprazolam and diazepam are efficacious in treatment of panic disorders and agoraphobia (Bolli *et al.*, 2004). Clinical evidence indicates that alprazolam is also beneficial for treatment of depression (Mendonça Júnior *et al.*, 2015). Generally, numerous compounds having favorable tranquilizing and toxic properties possess the 1,4-benzodiazepine skeleton (Liou *et al.*, 2004). This group of active substances is accessible by ring enlargement of quinazoline derivatives, by ring contraction of benzoxadiazocines, and by synthesis from 2-aminobenzophenones (Albaugh *et al.*, 2002).

γ-Amino butyric acid (GABA), the principal inhibitory neurotransmitter in the mammalian central nervous system (Granger *et al.*, 2005), exerts its physiological effects by binding to the ionotropic (GABA<sub>A</sub>, GABAc) and the

✉ Ahmad R. Massah  
Massah@iaush.ac.ir

<sup>1</sup> Department of Chemistry, Shahreza Branch, Islamic Azad University, Shahreza, Isfahan 86145-311, Iran

<sup>2</sup> Razi Chemistry Research Center, Islamic Azad University, Shahreza Branch, Shahreza, Isfahan 86145-311, Iran

<sup>3</sup> Department of Bioinformatics, Institute of Biochemistry and Biophysics (IBB), University of Tehran, Tehran, Iran

metabotropic (GABA<sub>B</sub>) receptors. The GABA<sub>A</sub> receptors are composed of five subunits arranged pseudo-symmetrically around the integral anion channel (Nayeem *et al.*, 1994). The most ubiquitous subtype, which accounts for approximately 30 % of GABA<sub>A</sub> receptors in the mammalian brain (Farrar *et al.*, 1999), contains two  $\alpha_1$ -, two  $\beta_2$ - and a single  $\gamma_2$ -subunit (Wieland *et al.*, 1992).

Benzophenones are a popularly prescribed sedative-hypnotic used in the treatment of insomnia. Unlike classical benzodiazepines (BZDs), alprazolam binds with high affinity to the BZ site, present at the  $\alpha_1/\gamma_2$  subunit interface of the GABA<sub>A</sub> receptor and exhibits relatively low affinity to GABA<sub>A</sub> containing the  $\alpha_2/\alpha_3$  subunits, and no significant affinity for the  $\alpha_5$  subtype (Renard *et al.*, 1999).

Several studies have been reported on molecular modeling of GABA<sub>A</sub>. Vijayan *et al.* (2012) have constructed GABA<sub>A</sub> models to explore the residues crucial for the interaction between GABA<sub>A</sub> and Zolpidem. In another study, Mokrab *et al.* (2007) constructed a 3D model of GABA type A to exploring ligand recognition and ion flow in GABA<sub>A</sub>. A common deficiency of the above-mentioned studies is that the authors predicted 3D structure of GABA<sub>A</sub> with the low identity template structure.

In this study a new, mild and green method was introduced for the synthesis of alprazolam and diazepam. Also, modeling and simulation approaches were utilized to find the 3D structure of GABA<sub>A</sub> and possible interaction with alprazolam and diazepam. Homology modeling and molecular dynamics simulation was performed on the constructed GABA<sub>A</sub> receptor model inserted in a water box. Furthermore, alprazolam and diazepam were docked into the final structure extracted from the MD trajectories of GABA<sub>A</sub>.

## Materials and methods

### Chemistry

#### General

All reagents were purchased from Merck Co. and used without further purification. Melting points were determined by using Amstead Electro Thermal 9200. The <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were recorded on a Bruker, DRX-400ADVANCE 400 MHz instrument using CDCl<sub>3</sub> as solvents and TMS as an internal standard. Chemical shifts are expressed as  $\delta$  (ppm). The IR spectra were recorded in KBr disks using an AnalystDate PerkinElmer FTIR spectrometer.

**Synthesis of 7-chloro-5-phenyl-1H benzo[e][1,4]diazepin-2(3H)-one (nordiazepam) (1)** To a vigorously stirred of 2-amino-5-chloro benzophenone (0.232 g, 1 mmol), chloro

acetyl chloride (1.2 mL, 2 mmol) was added drop wise at room temperature under solvent-free conditions during 30 min and the progress of reaction was monitored by TLC. After completion of the reaction, NH<sub>4</sub>OAc (0.23 g, 3 mmol) and K<sub>2</sub>CO<sub>3</sub> (0.42 g, 3 mmol) were added to the mixture at room temperature under solvent-free conditions and stirred for 2.5 h. When the reaction was completed, as it was shown by TLC, the water (30 mL) was added and the product was filtered off, washed with more water (2  $\times$  100 mL) and dried. The product was obtained in high yield and purity (94 % yield) and was used in the next step without any purification. m.p = 212–214 °C [217–218 °C (Barthel *et al.*, 2009)]; IR (KBr, cm<sup>-1</sup>): 701, 1640, 1685, 2939, 2978, 3272; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  4.22 (s, 2H), 7.56 (d, 4H, *J* = 11.6 Hz), 7.66 (d, 1H, *J* = 6.8 Hz), 7.75 (d, 2H, *J* = 6.4 Hz), 8.62 (d, 1H, *J* = 8.8), 11.5 (s, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  43.1, 123.1, 125.6, 128.4, 128.6, 130.0, 132.7, 133.2, 133.8, 137.6, 137.6, 165.4, 197.9.

**Synthesis of 1-acetyl-7-chloro-5-phenyl-1H-benzo[e][1,4]-diazepin-2(3H)-one (2)** To a vigorously stirred mixture of nordiazepam (1) (0.27 g, 1 mmol), a powder mixture of K<sub>2</sub>CO<sub>3</sub> (0.55 g, 4 mmol)/KOH (0.22 g, 4 mmol) and acetic anhydride (0.18 mL, 2 mmol) was added. The progress of the reaction was monitored by TLC. After completion of the reaction (3 h), water (3  $\times$  10 mL) was added and the 1-acetyl-7-chloro-5-phenyl-1H-benzo[e][1,4]diazepin-2(3H)-one (2) was easily isolated by a simple filtration in more than 80 % yield with high purity. The product was used in the next step without any purification. m.p = 163–165 °C [162–163 °C (Usui *et al.*, 1970)]; IR (KBr, cm<sup>-1</sup>): 706, 1672, 1690, 1766, 2923, 2854; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  2.2 (s, 3H), 7.25 (d, 1H, *J* = 2.4), 7.35 (m, 3H), 7.44 (d, 2H, *J* = 8.8), 7.49 (m, 3H), 7.57 (m, 2H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  20.3, 117.7, 121.2, 126.1, 128.7, 128.8, 129.1, 130.4, 131.5, 134.9, 138.2, 141.1, 158.7, 168.7.

**Synthesis of 8-chloro-1-methyl-6-phenyl-4H-benzo[f][1,2,4]triazolo[4,3-a][1,4]diazepine (alprazolam) (3)** A solution of 1 mmol (0.31 g) of 1-acetyl-7-chloro-5-phenyl-1H-benzo[e][1,4]diazepin-2(3H)-one (2), N<sub>2</sub>H<sub>5</sub>OH (4 mmol, 0.2 mL) and NaOAc (4 mmol, 0.2 mL) in 25 ml of AcOH was refluxed for 12 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the solution was cooled and the product was filtered, washed with water, dried, and crystallized from EtOH. (75 % yield); m.p = 228–230 °C [226–234 °C (Jackson and Hester, 1980)]; IR (KBr, cm<sup>-1</sup>): 701, 1643, 1661, 1697, 2896, 2922; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  2.4 (s, 3H), 7.46 (d, 1H, *J* = 2.8), 7.3 (m, 3H), 7.54 (d, 2H, *J* = 10.8), 7.68 (m, 3H), 7.77 (m, 2H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  22.3, 117.6, 122.1, 124.3, 126.3, 128.2, 129.4, 131.5, 133.7, 134.3, 136.5, 139.2, 156.9, 166.8.

**Synthesis of 7-chloro-1-methyl-5-phenyl-1H-benzo[e][1,4]diazepin-2(3H)-one (diazepam) (4)** To a vigorously stirred mixture of nordiazepam (1) (0.27 g, 1 mmol) and powder of  $K_2CO_3$  (0.42 g, 3 mmol), dimethyl sulfate (0.37 mL, 4 mmol) was added. The progress of the reaction was monitored by TLC. After completion of the reaction (2 h), water ( $3 \times 10$  mL) was added and the 7-chloro-1-methyl-5-phenyl-1H-benzo[e][1,4]diazepin-2(3H)-one (diazepam) was easily isolated by a simple filtration in 85 % yield and high purity. m.p = 130–132 °C [129–132 °C (Grant *et al.*, 2010)]; IR (KBr,  $cm^{-1}$ ): 701, 1653, 1675, 2939, 2978;  $^1H$  NMR( $CDCl_3$ , 400 MHz):  $\delta$  4.34 (s, 2H), 5.22 (s, 3H), 7.43 (d, 4H,  $J = 11.4$  Hz), 7.56 (d, 1H,  $J = 6.9$  Hz), 7.77 (d, 2H,  $J = 6.7$  Hz), 8.52 (d, 1H,  $J = 8.2$ ).

### Homology modeling

When the crystallographic structure of a protein target is not available the homology modeling could be a potential method to build its tertiary structure. To use the atomic coordinates of a crystallographic structure (template), the sequences of protein target and template need to share at least 30 % of identity.

The subunit composition we used for the GABA<sub>A</sub> receptor model has the stoichiometry  $(\alpha_1)_2(\beta_2)_2\gamma_2$ . This is the most common receptor subtype in the human central nervous system (Barnard *et al.*, 1998; Pirker *et al.*, 2000). The amino acid sequences of the  $\alpha_1$ - (NCBI reference sequence: NP-001121120.1),  $\beta_2$ - (NCBI reference sequence: NP-068711.1) and  $\gamma_2$ - (NCBI reference sequence: NP-944493.2) subunits of the human GABA<sub>A</sub> receptor were taken from the NCBI (Altschul *et al.*, 1997).

The crystal structure of *homopentameric caenorhabditis elegans glutamate-gated chloride channel (GluCl)* (3RHW-A) at 3.3 Å resolution was obtained as the modeling template of GABA<sub>A</sub> from the protein data bank (Berman *et al.*, 2000).

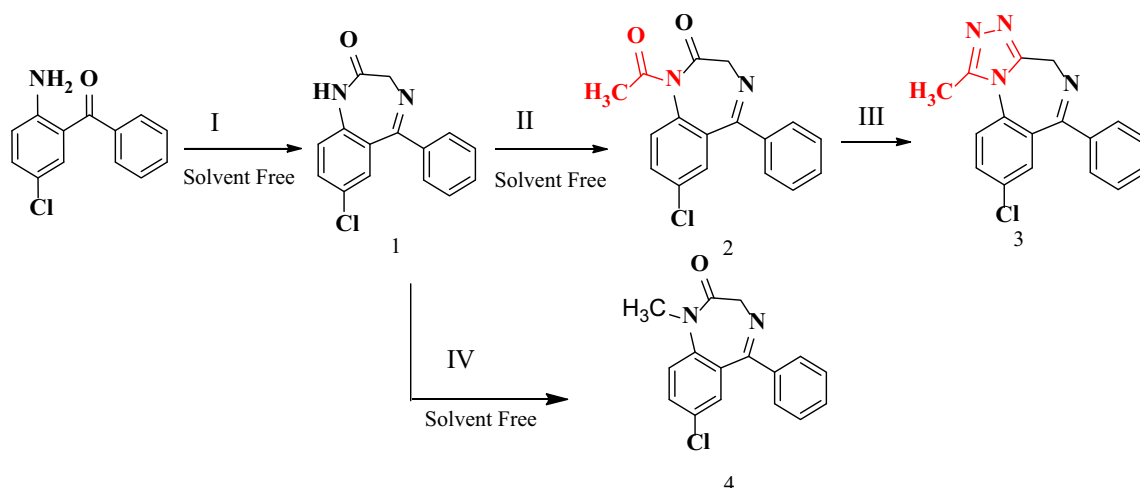
MODELLER (Sali and Blundell, 1993) version 9.11 was used to build homology models of GABA<sub>A</sub> from crystallographic structure *homopentameric caenorhabditis elegans glutamate-gated chloride channel (GluCl)*. From the alignments, 3D models containing all non-hydrogen atoms were obtained automatically using the methods implemented in MODELLER. From the 5 model generated with MODELLER for each alignment, the one corresponding to the lowest value of the DOPE potential and fewest restraints violations was selected for further analysis. The overall stereochemical quality of the final developed model for GABA<sub>A</sub> model was assessed by the RAMPAGE server (<http://www.cryst.bioc.cam.ac.uk/rampage>).

### Molecular dynamics simulations

The MD simulations were performed in two parts using the GROMACS 4.5.1 package (Berendsen *et al.*, 1995; Zhu and Vaughn 2005; Lindahl *et al.*, 2001; Van der Spoel *et al.*, 2005).

In the first part, the minimum energy conformation of predicted GABA<sub>A</sub> obtained from homology modeling process was used as the starting structure for MD simulation.

The topology parameters of GABA<sub>A</sub> were created. The interaction parameters were computed using the OPLS force field (Van Gunsteren *et al.*, 1996), with the intermolecular (nonbonded) potential represented as a sum of Lennard–Jones (LJ) force and pairwise Coulomb



**Scheme 1** I (1) chloro acetyl chloride, (2)  $NH_4OH/K_2CO_3$ , r.t, 3 h; II  $AC_2O$ ,  $K_2CO_3/KOH$ , r.t, 3 h; III  $N_2H_4OH/NaOAc$ , reflux, 12 h; IV Dimethylsulfate,  $K_2CO_3$ , r.t, 2 h

**Fig. 1** Multiple sequence alignment of GABA<sub>A</sub> with 3RHW

	10	20	30	40	50	60	
3rhw_A	-----SD-----					SKILAHLFTSGYDFVRPPTDNGGPV	
GABA	MRKSPGLSDCLWAWILLSTLTGRSYGQPSLQDELKDNTTVFTRILDRLLD					-GYDNRLRPLGLG-ERVT	
		**				** * *** * *	
	70	80	90	100	110	120	130
3rhw_A	VVSVNMLLRITISKIDVVNMEYSAQLTLRESWIDKRLSYGVKGDGQPDFVILTV					-GHQIWMPDTFFPNE	
GABA	EVKTDIFVTSFGPVSDHMEYTDVFFRQSWKDERLK--FKGP-MTVLRLNNLMASKIRTPDTFFHNG						
	*		***	* ** * **	**		* ***** *
	140	150	160	170	180	190	200
3rhw_A	KQAYKHTIDKPNVLIRIHNDGTVLYSVRISLVLSCPMYLQYYPMDVQQCSIDLASYAYTTKDIEYLWK						
GABA	KKSAVHNMTMPNKLRLITEDGTLTYMRLTVRAECPMHLEDFPMDAHACPLKFGSYAYTRADEVVYEW						
	*	*	** * **	*** ** *	*** *	*** *	***** * *
	210	220	230	240	250	260	270
3rhw_A	EHSPLQLKV-GLSSSLPSFQLTNTSTTYCTSVTNTGIYSCLRTTIQLKREFSYLLQLYIPSCMLVIV						
GABA	REPARSVVVAEDGSRLNQYDLLGQTVDSGIVQSSTGEYVVMTHFHLKRKIGYFVIQTYLPCIMTVIL						
		*	* *	*	** *	* ***	* * * * *
	280	290	300	310	320	330	340
3rhw_A	SWVSFWFDRTAIPARVTLGVTLLTMTAQSAAGINSQLPPVSYIKAIDVWIGACMTFIFCALLEFALVN						
GABA	SQVSFWLNRESVPARTVFGVTTVLTMTLSISARNSLPKVAYATAMDWFIACVAFVFSALIEFATVN						
	* **** *	***	**** *	**** *	* ** *	* * *	* * * * *
	350	360	370	380	390	400	
3rhw_A	HIANAG-----					TTE-----	
GABA	YFTKRGYAWDGKSVVPEKPKVKDPLIKNNNTYAPTATSYPNLRGDPGLATIAKSATIEPKVEVKPE						
	*						* *
	410	420	430	440	450	460	470
3rhw_A	-----WDISKRVDLISRALFPVLFVFNILYWS-----					R--FG---	
GABA	TKPPEPKKTFNSVSK-IDRLSRIAPLLFGIFNLVYWATYLNREPQLKAPTQHQMWRVRKRGYFGIWS						
		*	** *	* ** *	** *		* **

interaction and the long-range electrostatic force determined by the particle mesh Ewald (PME) method (Darden *et al.*, 1993; Essmann *et al.*, 1995). The solvated system was neutralized by adding chloride ions in the simulation, and the entire system was composed of protein, 59 Cl<sup>-</sup> counter ions, and 235497 solvent atoms. The energy was minimized using the steepest descent method of 1000 steps with a cutoff of 9 Å for van der Waals and Coulomb forces. MD simulation studies consist of equilibration and production phases. In the

first stage of equilibration, the solute (protein and counter ion) was fixed and the position-restrained dynamics simulation of the system, in which the atom positions of GABA<sub>A</sub> were restrained at 310 K. The water and the counter ion permitted to relax about the protein. The relaxation time of water was 20 ps. Finally, the full system was subjected to 20 ns MD at 310 K temperature and 1 bar pressure. The periodic boundary condition was used and the motion equations were integrated by applying the leap-frog algorithm with a time step of 2 fs.

**Fig. 1** continued

	480	490	500	510	520	530	540
3rhw_A	-----H/S--DSKILAHLFTS-----GYDFRVRPPTDNGGPVVSVNMLLRTISKIDVVNME						
GABA	FPLIIAAVCAQ-SVNDPNMSLVKETVDRLLKGYDIRLRPDFG-GPPVAVGMNIDIASIDMVSEVNMD						
		* *		*** * *	* * * *	*	***
	550	560	570	580	590	600	610
3rhw_A	YSAQLTLRESWIDKRLSYGVKGDGQPDFVILTVGHQIWPDPDFFPNEKQAYKHTIDKPNVLIRIHNDG						
GABA	YTTLTYFQQAWRDKRLSYNVIPLNLT--LDNRVADQLWVPDITYFLNDKKSFVHGVTVKNRMIRLHPDG						
	*	* * * * *		* * * * *	* * *	*	* * * *
	620	630	640	650	660	670	680
3rhw_A	TVLYSVRISLVLSCPMYLQYYPMDVQQCSIDLASYAYTTKDIEYLWKEHSPLQLKVLSSSLPSFQLT						
GABA	TVLYGLRITTTAACMMDLRRYPLDEQNCTLEIESGYTTDDIEFYWRGDDNAVTVG-TKIELPQFSIV						
	**** *	* * *	* * * *	* * * * *	*	*	* * *
	690	700	710	720	730	740	
3rhw_A	NTSTTYCTSVTNTGIYSCLRTTIQLKREFSFYLLQLYIPSCMLVIVSVWFWFDRTAIPARVTLGVTT						
GABA	DYKLITKKVVFSTGSYPRLSLSFKLRNIGYFILQTYMPSILITILSWVFWINWDASAARVALGITT						
		* * * *	***	* * * *	* * * * *	*	* * * * *
	750	760	770	780	790	800	810
3rhw_A	LLTMTAQSAAGINSQLPPVSYIKAIQVWIGACMTFIFCALFEFALVNHI-----ANA-						
GABA	VLTMTTINTHLRETLPKIPYVKAIDMYLMGCFVFMALLEYALVNYIFFGRGPQRQKAAEKAASAN						
	****	**	* * * *	* * * * *	* * * *	*	* *
	820	830	840	850	860	870	880
3rhw_A	-----G---TT---EWNDISKR-----						
GABA	NEKMRLDVNKMDPHENILLSTLEIKNEMATSEAVMGLGDPSTMLAYDASSIQYRKAGLPRHSFGRNA						
				*	*	* *	
	890	900	910	920	930	940	950
3rhw_A	-----VDLISRALFPVLFFVFVNILYWS-----R--FGH/SD-						
GABA	LERHVAQKKSRRLRRASQLKITIPDLTDVNAIDRWSRIFFPVVFSFFNIVWLYYVNMRSKSPGL-SDC						
				* * *	* * *	*	* * *

The atom coordinates were recorded every 1 ps during the simulation for latter analysis.

In the second phase, the obtained conformation of GABA<sub>A</sub> from the first phase of MD as well as the docked alprazolam/diazepam was employed in the MD simulation process. These processes were performed the same as phase 1. Finally, the simulation results of the Drug–protein complex were analyzed. The MD

simulation and results analysis were performed on the open SUSE 11.3 Linux. Visualization of protein–ligand complexes and MD trajectory analysis was carried out with the VMD software package (Humphrey *et al.*, 1996). 3D representations of the docking results were produced using MOLEGRO MOLECULAR VIEWER software (<http://www.molegro.com>) (Wallace *et al.*, 1995).

Fig. 1 continued

	960	970	980	990	1000	1010	1020
3rhw_A	-----SKILAHLF TSGYDFRVRPPTDNGGFPVVSVNMLLRT						
GABA	LWAWILLSTLTGRSYGQPSLQDELKDNTTVFTRILDRLLD-GYDNRRLRPLG-ERVTEVKTDIFVTS						
				** *	*** * *		*
	1030	1040	1050	1060	1070	1080	
3rhw_A	ISKIDVVNMEYSAQLTLRESWIDKRLSYGVKGDGQPDFVILTV-GHQIWMPTDFFPNEKQAYKHTIDK						
GABA	FGPVSDHDMEYITIDVFFRQSWKDERLK--FKGP-MTVLRLNNLMASKIRTPDTFFHNGKKSVAHNMTM						
	***	* ** * **	**		*	***** * *	*
	1090	1100	1110	1120	1130	1140	1150
3rhw_A	PNVLIRIHNDGTVLYSVRISLVLSCPMYLQYYPMDVQQCSIDLASYAYTTKDIEYLWKEHSPLQLKV-						
GABA	PNKLLRITEDGTLTYMRLTVRAECPMHLEDFPMDAHACPLKFGSYAYTRAEVVYEWTPREPARSVVVA						
	** * **	*** ** *		*** *	*** *	*****	* * *
	1160	1170	1180	1190	1200	1210	1220
3rhw_A	GLSSSLPSFQLTNTSTTYCTSVTNTGIYSCLRTTIQLKREFSFYLLQLYIPSCMLVIVSVWSFWFDRT						
GABA	EDGSRLNQYDLLGQTVDSGIVQSSTGEYVVMTTTFHLKRKIGYFVIQTYLPCIMTVILSQVSWFLNRE						
	* *	*		** *	*	***	* * * * * * * *
	1230	1240	1250	1260	1270	1280	1290
3rhw_A	AIPARVTLGVTTLLTMTAQSAQSAGINSQLPPVSYIKAIDVWIGACMTFIFCALLEFALVNHIANAG----						
GABA	SVPARTVFGVTTVLTMTTSLISARNSLPKVAYATAMDWFIACVAFVFSALIEFATVNYFTKRGYAWD						
	***	**** *		** * *	* * *	* * *	* * *
	1300	1310	1320	1330	1340	1350	1360
3rhw_A	-----TTE-----W						
GABA	GKSVVPEKPKVKDPLIKNNNTYAPTATSYTPNLARGDPGLATIAKSATIEPKVKPETKPEPKKTF						
						* *	
	1370	1380	1390	1400	1410	1420	
3rhw_A	NDISKRVDLISRALFPVLFVFNILYWSRF-----G/SDSKILAHLF						
GABA	NSVSK-IDRLSRIFPLFLGIFNLVYWATYLNREPQLKAPTPHQMWVRVKRGYFGIW-SFPLIIAAVC						
	* ** *	*** ** *	** *				* ** *

## Docking protocol

The structures of molecules were drawn with Hyper Chem (Hyper Cube Inc., Gainesville, FL) and structure-optimizing calculation was at the 6-31G\*\* level by employing the Becke three-parameter Lee–Yang–Parr (B3LYP) hybrid density functional theory using the quantum chemistry software Gaussian 09 (Frisch *et al.*, 2003). Docking was performed by Auto Dock Vina program (Trott and Olson, 2010).

## Results and discussion

### Chemistry

The synthesis pathway leading to the alprazolam and diazepam is depicted in Scheme 1. 2-amino-5-chloro benzophenone was acylated with chloro acetyl chloride under solvent-free conditions. The product was reacted in situ

Fig. 1 continued

	1430	1440	1450	1460	1470	1480	1490
3rhw_A	TS-----GYDFVRPPTDNGGPVVSVNMLLRTISKIDVVMMEYSAQLTLRES						
GABA	AQSVNDPSNMSLVKETVDRLLKGYDIRLRPDFG-GPPVAVGMNIDIASIDMVSEVNMDYTLTMYFQQA						
			*** * *	* * * *	*	*** *	
	1500	1510	1520	1530	1540	1550	1560
3rhw_A	WIDKRLSYGVKGDGQPDFVILTUGHQIWMPTDFFPNEKQAYKHTIDKPNVLRIRHNDGTVLYSVRISL						
GABA	WRDKRLSYNVIPLNLT--LDNRVADQLWVPDITYFLNDKKSFEVHGVTVKNRMRIRLHPDGTVLYGLRITT						
	* * * * *		* * * * *	* * *	*	* * *	* * *
	1570	1580	1590	1600	1610	1620	1630
3rhw_A	VLSCPMYLQYYPMDVQQCSIDLASYAYTTKDIEYLWKEHSPLQLKVLSSSLPSFQLTNTSTTYCTSV						
GABA	TAACMMDLRRYPLDEQNCTLEIESYGYTTDDIEFYWRGDDNAVTVG-TKIELPQFSIVDYKLITKKVV						
	* * *	* * *	* * *	* * *	*	* * *	*
	1640	1650	1660	1670	1680	1690	1700
3rhw_A	TNTGIYSCLRTTIQLKREFSFYLLQLYIPSCMLVIVSWVSFWFDRTAIPARVTLGVTTLLTMTAQSAG						
GABA	FSTGSYPRLSLSFKLKRNIQYFILQTYMPSILITILSWVSFWINYDASAARVALGITTTLTMTTINTH						
	* * *	***	* * *	* * * *	*	* * *	* * *
	1710	1720	1730	1740	1750	1760	
3rhw_A	INSQLPPVSYIKAIDVWIGACMTFIFCALLEFALVNHI-----ANA-----						
GABA	LRETLPKIPYVKAIIDMYLMGCFVVFVFMALLEYALVNYIFFGRGPQRQKAAEKAASANNEKMRLDVNK						
	**	* * *	* * *	* * *		* *	
	1770	1780	1790	1800	1810	1820	1830
3rhw_A	-----G---TT---EWNDISKR-----						
GABA	MDPHENILLSTLEIKNEMATSEAVMGLGDPSTMLAYDASSIQYRKAGLPRHSFGRNALERHVAQKKS						
				* *	* *		
	1840	1850	1860	1870	1880	1890	1900
3rhw_A	-----VDLISRALFPVLFVFNILYWSRF-----GH/S-----D						
GABA	RLRRRASQLKITIPDLTDVNAIDRWSRIFFPVVFSFFNIVYWLYYVNMSSPNIWSTGS-SVYSTPVFS						
			* * *	* * *		* *	

with ammonium acetate in the presence of  $K_2CO_3$  under solvent-free conditions to afford 7-chloro-5-phenyl-1H-benzo[e][1,4]diazepin-2(3H)-one (nordiazepam) (**1**) in high yield and purity. Then nordiazepam was reacted with acetic anhydride in the presence of  $K_2CO_3/KOH$  to furnish 1-acetyl-7-chloro-5-phenyl-1H-benzo[e][1,4]diazepin-2(3H)-one (**2**) in the absence of any solvent at room temperature. Finally, from the reaction of compound **2** with hydrazine hydrate, alprazolam (**3**) was obtained in high yield and

purity. Also, as it was shown in Scheme 1, for the synthesis of diazepam (**4**), nordiazepam was methylated with dimethyl sulfate in the presence of  $K_2CO_3$  at room temperature and under solvent-free conditions. The product in each step was obtained in high purity and used in the next step without any purification. All of the products were characterized by comparing the physical data with those of known samples and also using the  $^1H$ -NMR,  $^{13}C$ -NMR and IR methods.



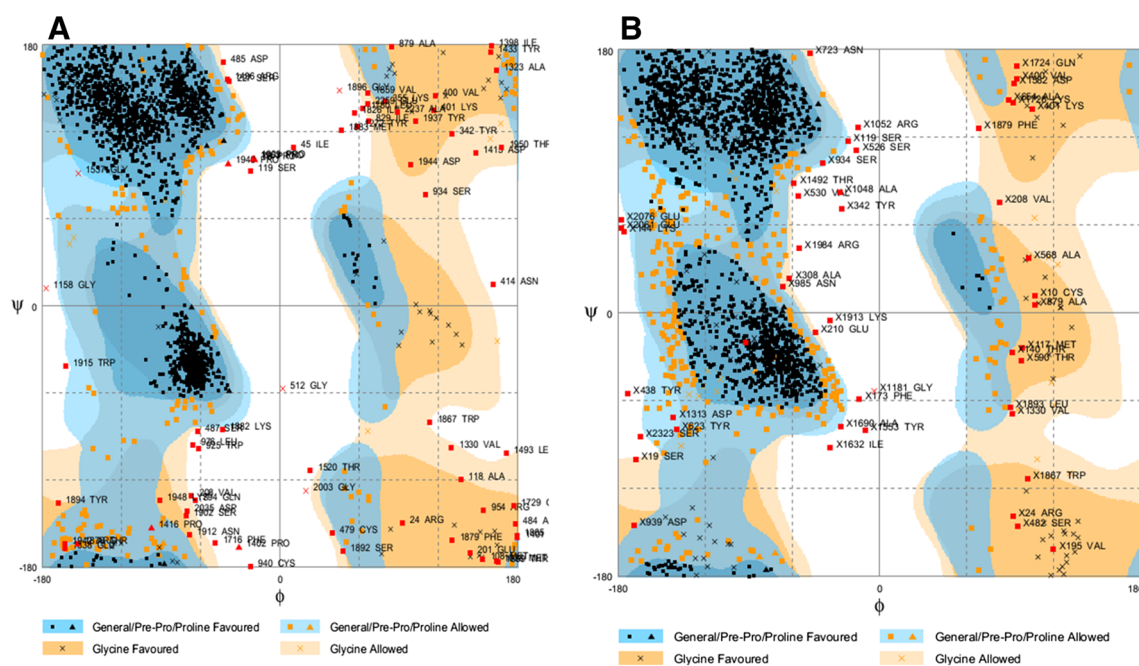
Fig. 1 continued

	1910	1920	1930	1940	1950	1960	1970
3rhw_A	SK----	ILAHL-----	FTS-----	GY-DFR-----	VRPPT----	D-----	N---G-----G--
GABA	QKMTVWILLLLSLYPGFTSQKSDDDYEDYASNKTWVLT	PKVPEGDVT	VILNNLLEGYDNKLRPDIGVK				
	*	** *	***	* *	* *	*	* *
	1980	1990	2000	2010	2020	2030	2040
3rhw_A	PVVSVNMLLRTISKIDVNMESYQLTLRESWIDKRLSYG	VGKDGQPDFVILT	VGHIWMPDTFFPN				
GABA	PTLIHTDMYVNSIGPVNAINMEYTI	DIFFAQMWYDRRLKFNSTIK	VLRLNSNMVG	KIWI	PDTFFRN		
	*	*	*	****	* * *		** ** ***** *
	2050	2060	2070	2080	2090	2100	
3rhw_A	EKQAYKHTIDKPNVLIRIHNDGTVL	YSVRISLVLS	SCPMYQYYPMDVQ	QCSIDL	ASYAYTTKDIEYLW		
GABA	SKKADAHWITTPNRMLRIW	NDGRVLYSLRLTIDAECQL	QLHNFPMDEHSCPLE	FSSYGYPRE	EIVYQW		
	* * *	* *	** *** ***** *	*	* *** *	** *	* * *
	2110	2120	2130	2140	2150	2160	2170
3rhw_A	KEHSPLQLKVGLSSSLPSFQLTNTSTTYCTSV	TNTGIYSCLRTTIQLKREFS	FYLLQLYIPSCMLVIV				
GABA	KR-SSVEVG	DTRSWRLYQFSFVGLRNTTEVVK	TSGDYVVM	SVYFDLSRRMG	YFTIQT	YIPCTLIVL	
	* *	* *	*	* * *	* *	* *** *	
	2180	2190	2200	2210	2220	2230	2240
3rhw_A	SWVSFWFDR	TAIPARVTLGV	TLLTMTAQ	SAGINSQLPPV	SYIKAIDVWIGAC	MTFIFCALLEFALVN	
GABA	SWVSFWINKDAVPARTSLG	ITTVLTM	TTLSTIARKSLPKVSYVTAM	DLFVSVC	FIFVFSALVEY	GT	LH
	*****	* ***	** ** ***** *	** *** *	*	* * * * *	
	2250	2260	2270	2280	2290	2300	2310
3rhw_A	HIANA-----						
GABA	YFVSNRKPSKDKDKKKNPAPTIDIRPRS	ATIQMNNATHLQERDEEYGYECLDGKDCASFFCCFEDCR					
				*	*		
	2320	2330	2340	2350			
3rhw_A	-----						
GABA	TGAWRHGRIHIRIAKMDSYARIF	FPTAFCLFNLVYVWSYLYL					
			*	*	** *	** *	

### Homology models construction and evaluation

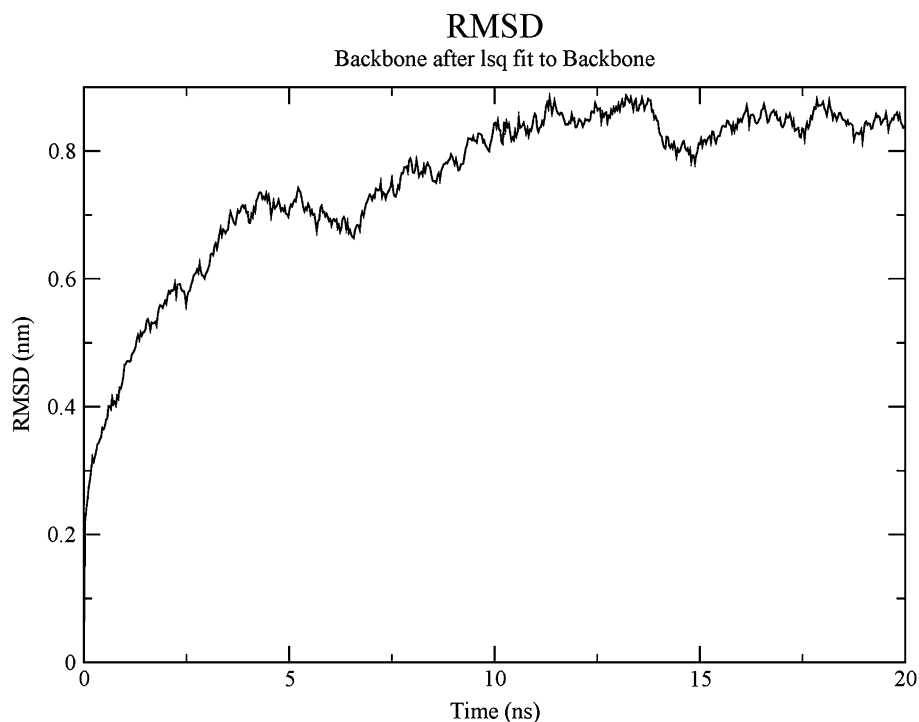
From BLAST search for GABA<sub>A</sub> ([www.ncbi.nlm.nih.gov/blast](http://www.ncbi.nlm.nih.gov/blast)), X-ray crystal structure of homopentameric *caenorhabditis elegans* glutamate-gated chloride channel (GluCl) with the PDB code of 3RHW was obtained as potential template for GABA<sub>A</sub> with the sequence identity of 45 %. Figure 1 shows multiple sequence alignment of GABA<sub>A</sub> with 3RHW.

The Ramachandran map for GABA<sub>A</sub> model was represented in Fig. 2 by RAMPAGE server (<http://www-cryst.bioc.cam.ac.uk/rampage>). In the Ramachandran plot analysis, the residues were classified according to their regions in the quadrangle. Predicted GABA<sub>A</sub> model indicates that more than 89 % of residue  $\phi$ - $\psi$  angles are in the favored or additional allowed regions of Ramachandran plot. It indicates that the final obtained 3D model of GABA<sub>A</sub> receptor is satisfactory.



**Fig. 2** Ramachandran plot of the  $\phi$ - $\psi$  distribution of GABA produced by RAMPAGE server **a** after homology modeling and, **b** after MD simulation part II

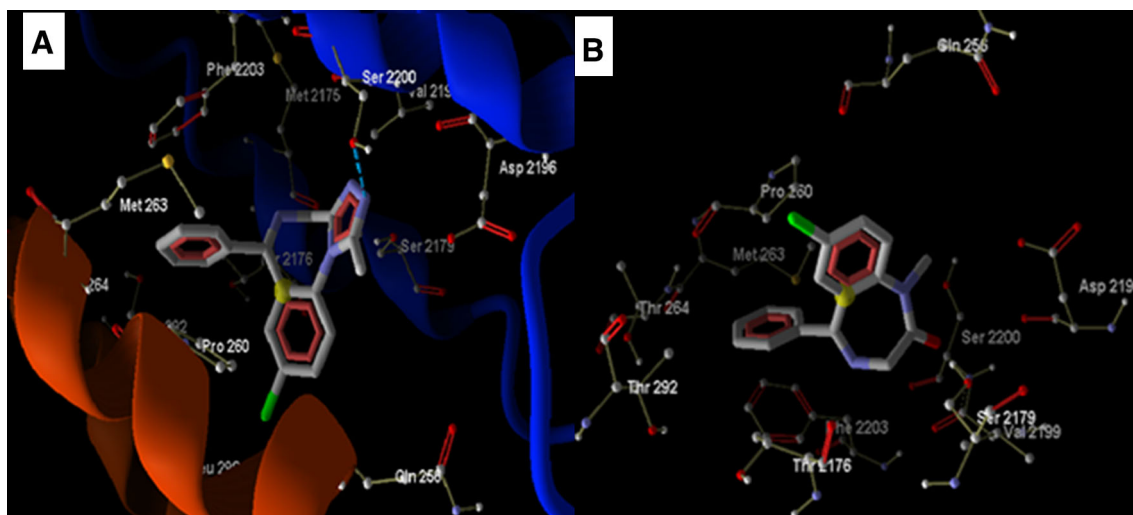
**Fig. 3** Time dependence of the RMSD (Å) from homology model of GABA for the backbone atoms in the 20 ns MD simulation of part I



### Molecular dynamic simulation of GABA<sub>A</sub>

In order to obtain conformation of GABA<sub>A</sub> in a water environment, a 20 ns MD simulation was performed on

GABA<sub>A</sub> in a water box. The stability of the receptor, water and ions was examined by the calculation of root-mean-square deviations (RMSD) of the enzyme with respect to its initial structure. Figure 3 shows the time history of the



**Fig. 4** 3D scheme representation of interactions between alprazolam (a) and diazepam (b) with the GABA<sub>A</sub>

RMSD for GABA<sub>A</sub> conformation in a water environment relative to the starting structure. Analysis of this figure indicates that the RMSD of GABA<sub>A</sub> reaches equilibration and oscillates around an average value after 10 ns simulation time. The RMSD value of protein backbone was calculated from 10 to 20 ns trajectory.

### Molecular docking studies

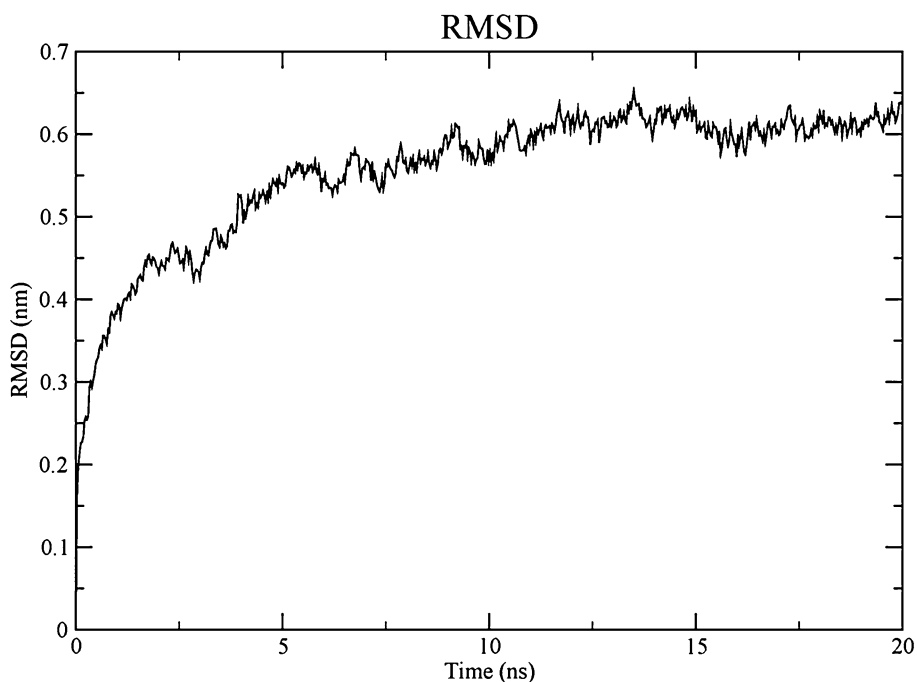
Figure 4 shows the best docked pose of alprazolam and diazepam in the binding site of the GABA<sub>A</sub> model. With respect to the docking results, the *N* moiety of triazole

cycle in alprazolam forms hydrogen bond with SER 2200 and docking score was  $-2.5$  kJ/mol.

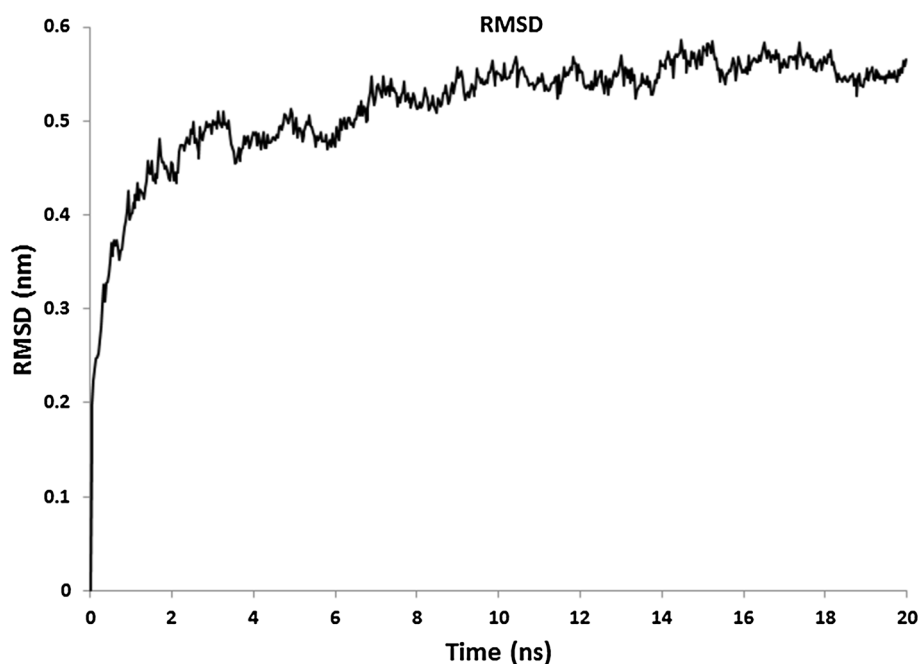
In addition, it can be seen clearly in the Fig. 4a, the alprazolam can form hydrophobic interactions with the side chain atoms of Gln 256, Thr 2176, Asp 2196, Pro 260, Met 263 and Thr 292. The phenyl rings interacts with the Thr 297, Thr 264, Pro 260. The docking score of alprazolam was  $-9.2$  kJ/mol.

The diazepam can form hydrophobic interactions with the side chain atoms of Phe 2203, Thr 292, Val 2199, Pro 260, Met 263 and Thr 267 (Fig. 4b). The phenyl rings interacts with the Phe 2203, Thr 2176, Pro 260, Met 263,

**Fig. 5** All atoms RMSD in the 20 ns MD simulation of phase II (alprazolam–GABA<sub>A</sub> complex)



**Fig. 6** All atoms RMSD in the 20 ns MD simulation of phase II (diazepam–GABA<sub>A</sub> complex)

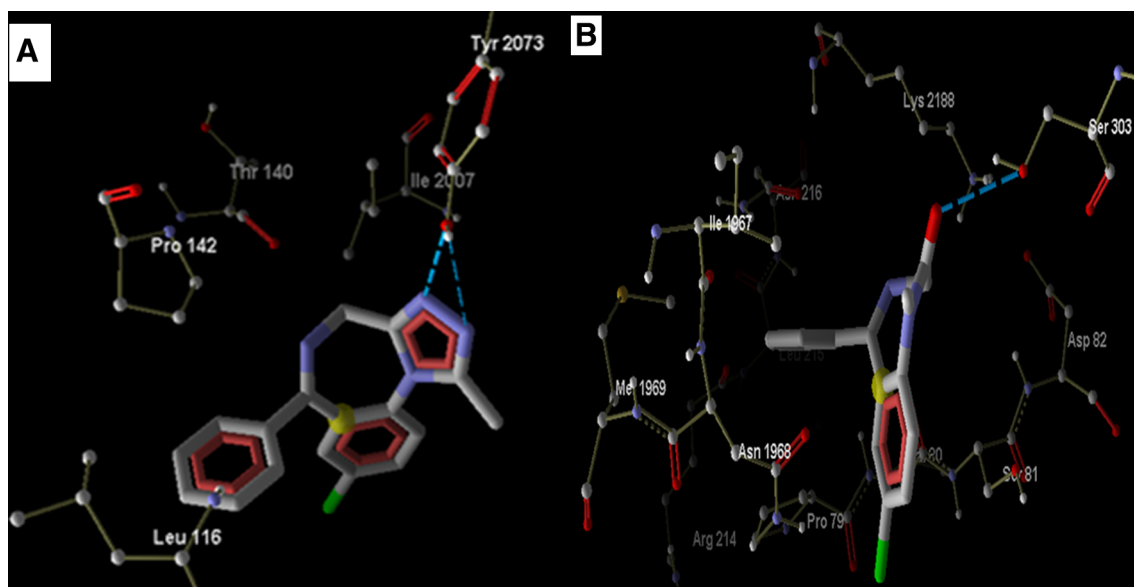


Thr 292 and CH<sub>2</sub> interact with the Val 2199. The docking score of diazepam was  $-8.0$  kJ/mol.

#### Molecular dynamics simulation on alprazolam–GABA<sub>A</sub> complex

In order to investigate the effects of inhibitor on the enzyme conformation and the stability of the drugs in active site, we

decided to perform a MD simulation on the alprazolam–GABA<sub>A</sub> complex. The MD simulation procedure was performed on alprazolam–GABA<sub>A</sub> complex in a water box. The trajectory stability was examined and corroborated by the analysis of RMSD value, and the results are shown in Fig. 5. The results reveals that the RMSD values for the complex has a rising in the first 10 ns and then stay stable in the rest of the simulation time.



**Fig. 7** 3D scheme representation of interactions between alprazolam (a) and diazepam (b) with the GABA<sub>A</sub> after molecular dynamics simulation

## Molecular dynamics simulation on diazepam–GABA<sub>A</sub> complex

The trajectory stability of diazepam–GABA<sub>A</sub> complex was examined and corroborated by the analysis of RMSD value, and the results are shown in Fig. 6. The results reveals that the RMSD values for the complex has a rising in the first 7 ns and then stay stable in the rest of the simulation time.

At the end of MD simulation, position and orientation of alprazolam and diazepam in the introduced binding site was changed (Fig. 7a). The results showed that, except for Pro 142, Ile 2007, Thr 140 and Leu 116, the rest of residues of active site determined by docking were changed and some new residues such as Pro142, Leu116 and Asn115 are positioned in proximity of alprazolam and could participate in the interaction. Also, at the end of MD simulation, new two hydrogen bonding was found to exist between alprazolam and Tyr2073 of GABA<sub>A</sub> model. The new hydrogen bond was formed between the nitrogen atom of 1,2,4-triazole ring of alprazolam as acceptor and carboxylic group of Tyr2073 as donor of hydrogen binding. These results revealed that MD simulation obligates alprazolam for maximum interaction with GABA<sub>A</sub>.

In the case of diazepam MD simulation, results showed that at the end of MD simulation new one hydrogen bonding was found to exist between diazepam and Ser 303 of GABA<sub>A</sub> model (Fig. 7a). This hydrogen bond was formed between the oxygen atom of carbonyl from diazepam as acceptor and amide group of Ser 303 as donor of hydrogen binding.

## Conclusion

In this work, alprazolam and diazepam were synthesized in high yield and purity under mild conditions. Then homology modeling, MD simulation, and molecular docking methods were performed to recognize the types of the interaction between the receptor and drugs. The homology modeling and MD simulation studies predicted the 3D structure of receptor in a water environment. The resulted conformation of the receptor was used for docking of the alprazolam and diazepam. Docking studies indicated many important interactions of the drugs with the receptor. Furthermore, the complex of GABA<sub>A</sub> with drugs was used in MD simulation to realize the stability of drugs in the binding site of GABA<sub>A</sub>.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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