Determining the Influence of Alcohol on the Pharmacological Effect of Benzodiazepines by Molecular Docking Tehnique

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Benzodiazepines represents a large category of medications that were originally developed to treat anxiety disorders or issues with anxiety, seizures, and issues with sleeping. The most common drugs abused along with benzodiazepines are other benzodiazepines, prescription pain medications and alcohol. Alcohol and benzodiazepine have a synergistic depressant effect on the central nervous system. Combining alcohol with benzodiazepines can be dangerous practice even if it is engaged in only occasionally. In the present study, using molecular docking technique we followed the binding energy of benzodiazepines with benzodiazepine receptor and efficacy of the flumazenil antidote against benzodiazepine in the presence and absence of alcohol. We realized correlation study of molecular descriptors value of benzodiazepines with benzodiazepine-$GABA_A$ complex binding energy.

Keyword: molecular docking, molecular descriptors, benzodiazepines, procedural sedation, neuroleptanalgesia

Benzodiazepines are a class of drugs with psychoactive effect, being known as minor tranquilizers [1]. Benzodiazepines improve the effect of the neurotransmitter gamma-aminobutyric acid (GABA) at $GABA_A$ receptor level, having sedative, hypnotic, anxiolytic, anticonvulsant, and muscle relaxant properties [2]. These characteristics make benzodiazepines useful in procedural sedation and analgesia, neuroleptanalgesia, crush induction, treating anxiety, insomnia, agitation, seizures, muscle spasms, alcohol withdrawal and as a premedication for medical or dental procedures [3]. Benzodiazepines can be taken in overdoses and can cause dangerous deep unconsciousness but fortunately death rarely results when a benzodiazepine is the only drug taken. When combined with other central nervous system depressants such as alcoholic drinks and opioids, the potential for toxicity and fatal overdose increases [4]. Alcohol increases activity of GABA and glycine as well as decreases the activity of excitatory neurotransmitters such as NMDA (N-methyl-D-aspirate).

Statistic data collected indicates a steady rise in hospital emergency department admissions associated with the misuse of benzodiazepines and alcohol from 2007 through 2011. Mixing two drugs with the same mechanism of action results in the enhancement of the effects of both drugs [5]. This means that the effects of both drugs are increased significantly compared to the use of either drug alone, a phenomenon known as potency synergism. An overdose on either drug can have serious and even fatal ramifications, including significant organ or brain damage due to a lack of oxygen as both drugs are respiratory depressants. When an individual drinks alcohol, the person's system metabolizes the alcohol before metabolizing any other substances [6]. This means that drugs like benzodiazepines remain in the individual's system longer if they consume these drugs with alcohol, the drug reaching higher plasma concentrations than normal. A person who drinks alcohol and continues to take benzodiazepines may develop extremely dangerous levels of benzodiazepines in their system [7]. Fumazenil is known as a benzodiazepine antagonist and acts like an antidote for pharmacological and toxic effects of benzodiazepines. The antagonistic properties of this drug are thought to be specifically mediated by competitive interaction at the central-type benzodiazepine receptors [8].

In the present study, using molecular docking technique we followed the binding energy of benzodiazepines with benzodiazepine receptor in the presence and absence of alcohol. We also wanted to see the efficacy of the flumazenil antidote against benzodiazepine in the presence and absence of alcohol. We have also highlighted the correlation of the molecular descriptors values (dipole moment and molecular energy) with the binding energy.

Experimental part

Materials and methods

We used the Gaussian program suite at DFT/B3LYP/6-311G for benzodiazepines optimization. The X-ray crystal structure of $GABA_A$ receptor (with 4COF code and X-ray diffraction at 2.97 Å resolution) was taken from the Protein Data Bank [9] and and refined with a Modrefiner program [10].

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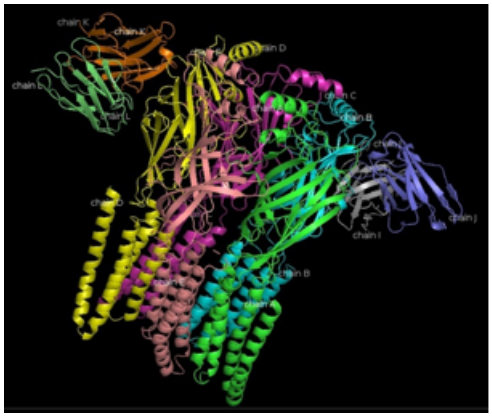


Image 1 $GABA_A$ receptor

The molecular docking analysis was performed using the Autodock 4.2.6 software together with the AutoDockTools [11], (a molecular viewer and graphical support for setup and analysis of docking runs). The preparation of receptor molecule involves adding polar hydrogens, computing the Gasteiger charge; the grid box was created using Autogrid 4 with 100×100×100 Å in x, y and z directions with 0.9 Å spacing. All the calculations were performed in vacuum [12].

For the docking process we chose the Lamarckian genetic algorithm (Genetic Algorithm combined with a local search), with a population size of 150 and a number of 20 runs. We exported all Autodock results in Discovery studio visualizer [13]. For all benzodiazepines we performed molecular quantum calculations of molecular geometries using the MOPAC 2016 program. The output data contains physico-chemical information about selected molecules [14].

We also realized correlation between molecular descriptors and estimated binding energy, using Regression Excel function from Microsoft Office package [15].

Results and discussions

Benzodiazepines are a class of psychoactive drugs whose core chemical structure is the fusion of a benzene ring and a diazepine ring. Benzodiazepine drugs are substituted 1,4-benzodiazepines, many of the pharmacologically active *classical* benzodiazepine drugs contain the 5-phenyl-1*H*-benzo[*e*] [1,4]diazepin-2(3*H*)-one substructure [16]. Dipole moment reflects the partial separation of electric charge in the molecule, being a predictor of the chemical reactivity of the molecules [17].

Table 1

MOLECULAR DESCRIPTORS FOR BENZODIAZEPINES

Benzodiazepines	Dipole moment (debye)	Total energy (hartree)
alprazolam	4.2155	-1389
clonazepam	2.96	-1397
chlordiazepoxide	3.63	-1347.49
prazepam	2.79	-1355.65
oxazepam	2.92	-1315.49
flunitrazepam	3.39	-1446.27
lorazepam	4.42	-1267.06
midazolam	2.09	-1328.3
diazepam	2.98	-1462.22

Energy (hartree) of the molecule is an important parameter, a more negative value representing a more stable molecule [18]. The highest molecular dipole is encountered for lorazepam, which contains two chlorine substituents and a hydroxyl group and the highest energy stability is held by diazepam and flunitrazepam (table 1).

Alcohol interacts with $GABA_A$, making a conventional hydrogen bond with alanine E:119, van der waals asparagine E:120, phenylamine A: 105, histidine A: 107, glycine A: 108, lysine E: 118, valine A: 109 (fig. 1). The binding free energy $GABA_A$-alcohol is -2.50 kcal/mol.

The most stable complex benzodiazepine-$GABA_A$, in the absence of alcohol is found in association with flunitrazepam. From the molecular docking calculation there is no clear increase or decrease in binding energy for the benzodiazepine-$GABA_A$ complex when administered together with alcohol. Even if for alprazolam, clonazepam, flunitrazepam and diazepam the binding energy GABAA-benzodiazepine decrease in the presence of alcohol, the overall pharmacological effect is stronger because it adds the SNC depressant effect of alcohol [19] (table 2).

We establish that exist a good regression between estimated free binding energy and the two molecular descriptors.

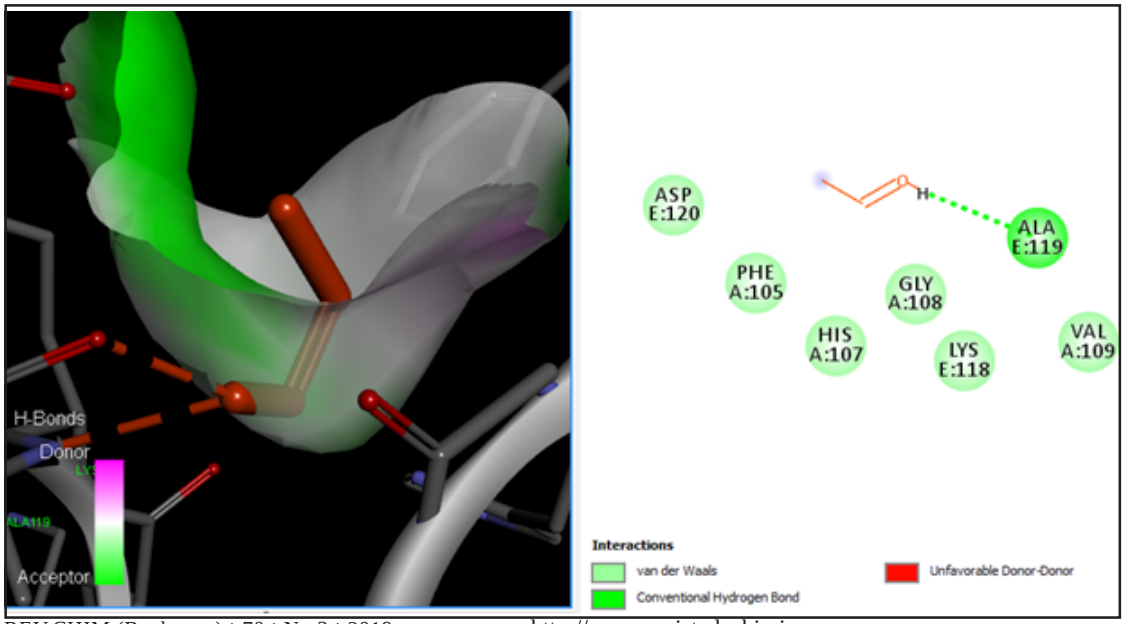


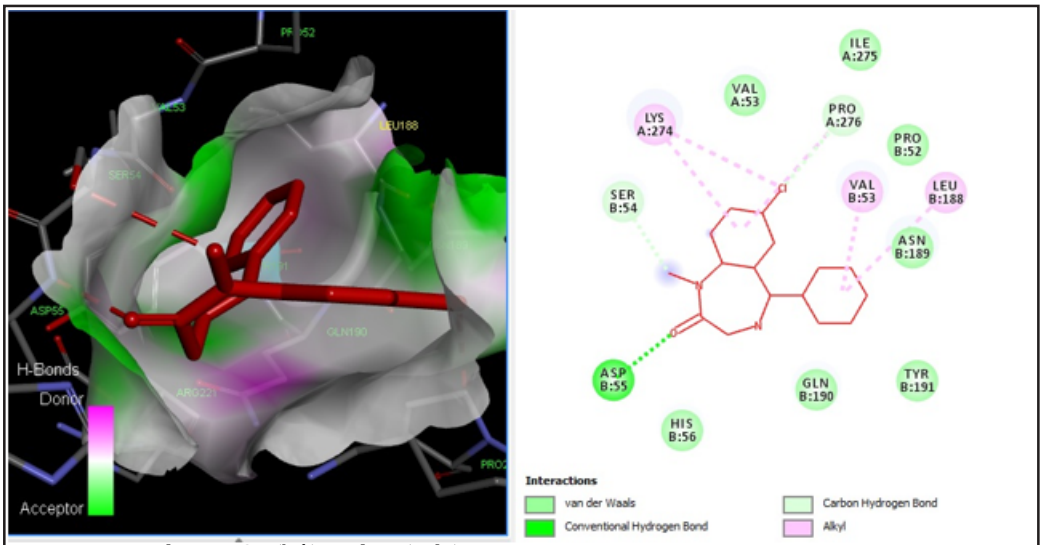
Fig. 1 $GABA_A$-alcohol interaction

Benzodiazepines	$GABA_A$ + benzodiazepines (kcal/mol)	$GABA_A$ + benzodiazepines (in the presence of alcohol) (kcal/mol)
alprazolam	-7.21	-6.67
clonazepam	-7.29	-7.14
chlordiazepoxide	-6.7	-6.85
prazepam	-6.89	-7.51
oxazepam	-5.97	-7.03
flunitrazepam	-7.82	-7.49
lorazepam	-5.69	-6.75
midazolam	-6.19	-6.76
diazepam	-7.84	-6.97

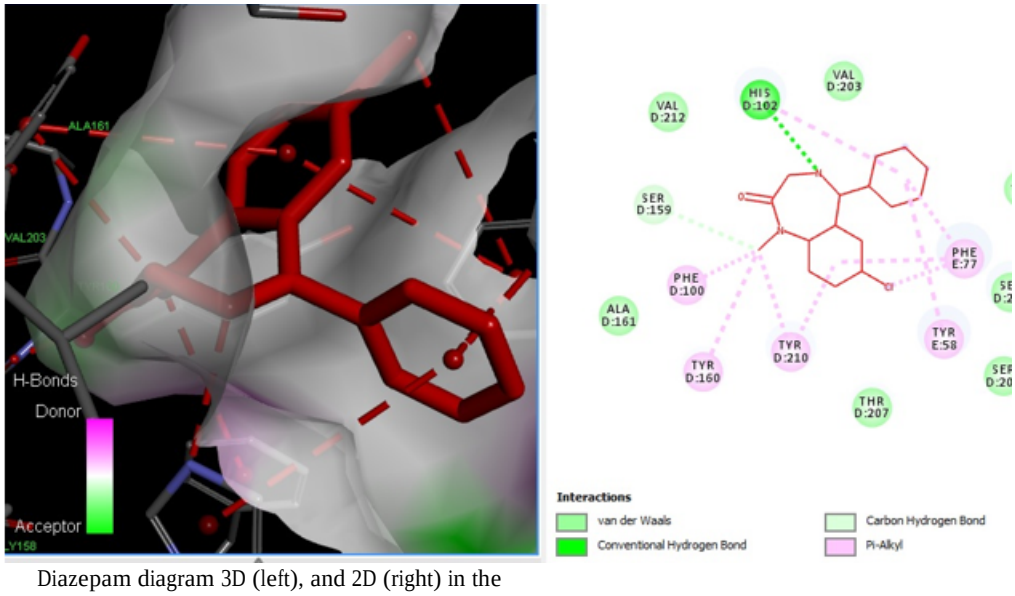
Table 2
ESTIMATED FREE BINDING ENERGY FOR $GABA_A$ + BENZODIAZEPINES WITH AND WITHOUT ALCOHOL

Estimated free binding energy= 10.38817-0.10336*Dipole moment+0.012353*Total energy (R^2=0.985).

From the 2D diagram we can see the ligand-protein interaction type and the pocket atoms from the active site.

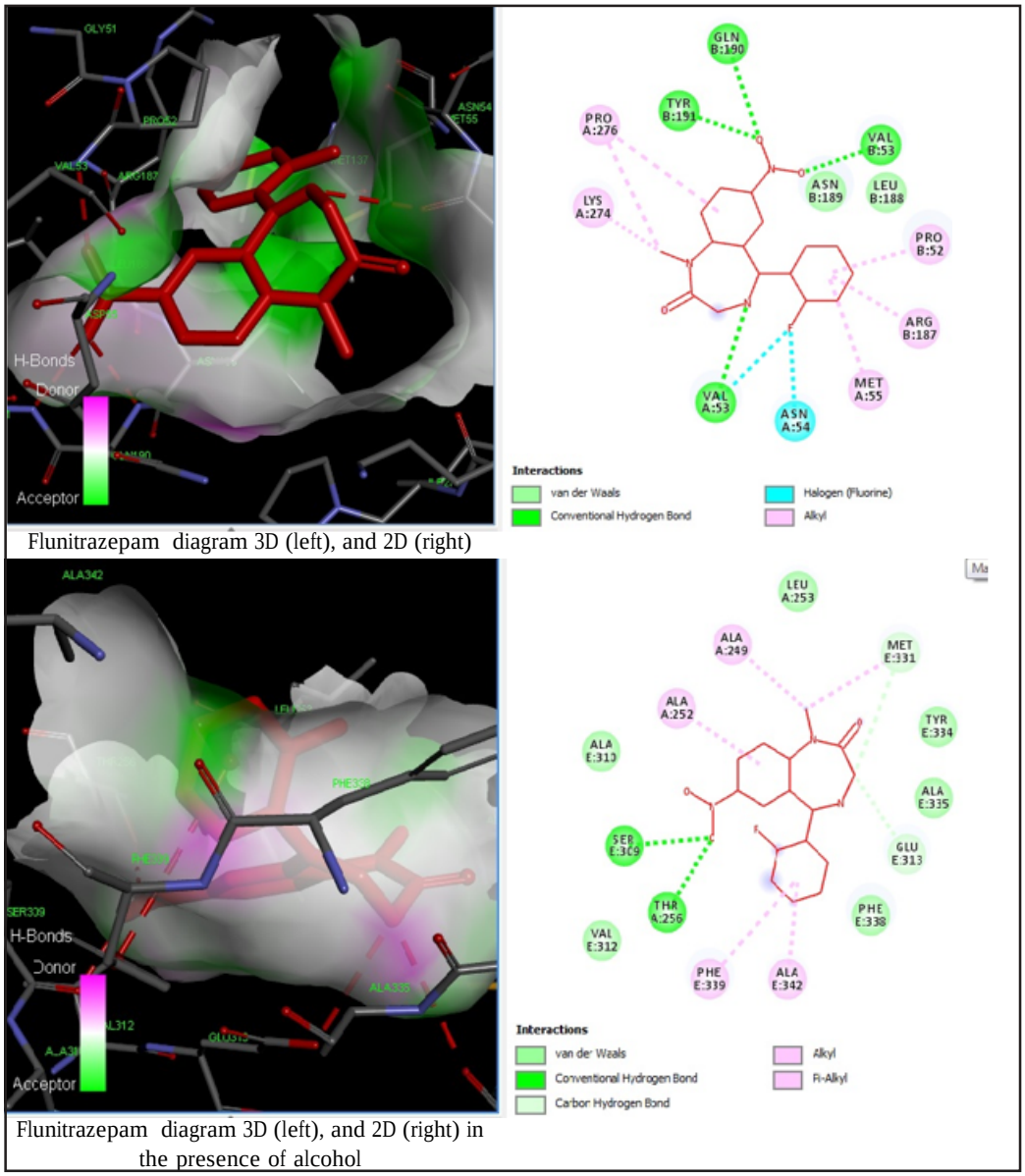


Diazepam diagram 3D (left), and 2D (right)



Diazepam diagram 3D (left), and 2D (right) in the presence of alcohol

Fig.2 Diazepam 3D and 2D diagram interaction with $GABA_A$



Flunitrazepam diagram 3D (left), and 2D (right)

Flunitrazepam diagram 3D (left), and 2D (right) in the presence of alcohol

Fig. 2. Flunitrazepam 3D and 2D diagram interaction with $GABA_A$

Diazepam realize a hydrogen bond with asparagine B:55, carbon hydrogen bond interaction with serine B:54 and proline A:276, alkyl interaction with valine B:53, leucine B:188, lysine A:274, and van der waals interaction with histidine B:56, glycine B:190, tyrosine B:191, asparagine B:189, proline B:52, isoleucine A:275, valine A:53.

Flunitrazepam binds with a hydrogen bond with valine A: 53, tyrosine B: 191, glycine B: 190, valine B: 53, asparagine halogen link A: 54, alkylation with proline A: 276, lysine A: 274 , methionine A: 55, arginine B: 187, proline B: 52, van der waals interaction with asparagine B: 189 and leucine B: 188.

We can see how alcohol consumption modifies the benzodiazepine binding site and the type of interaction with the $GABA_A$ receptor.

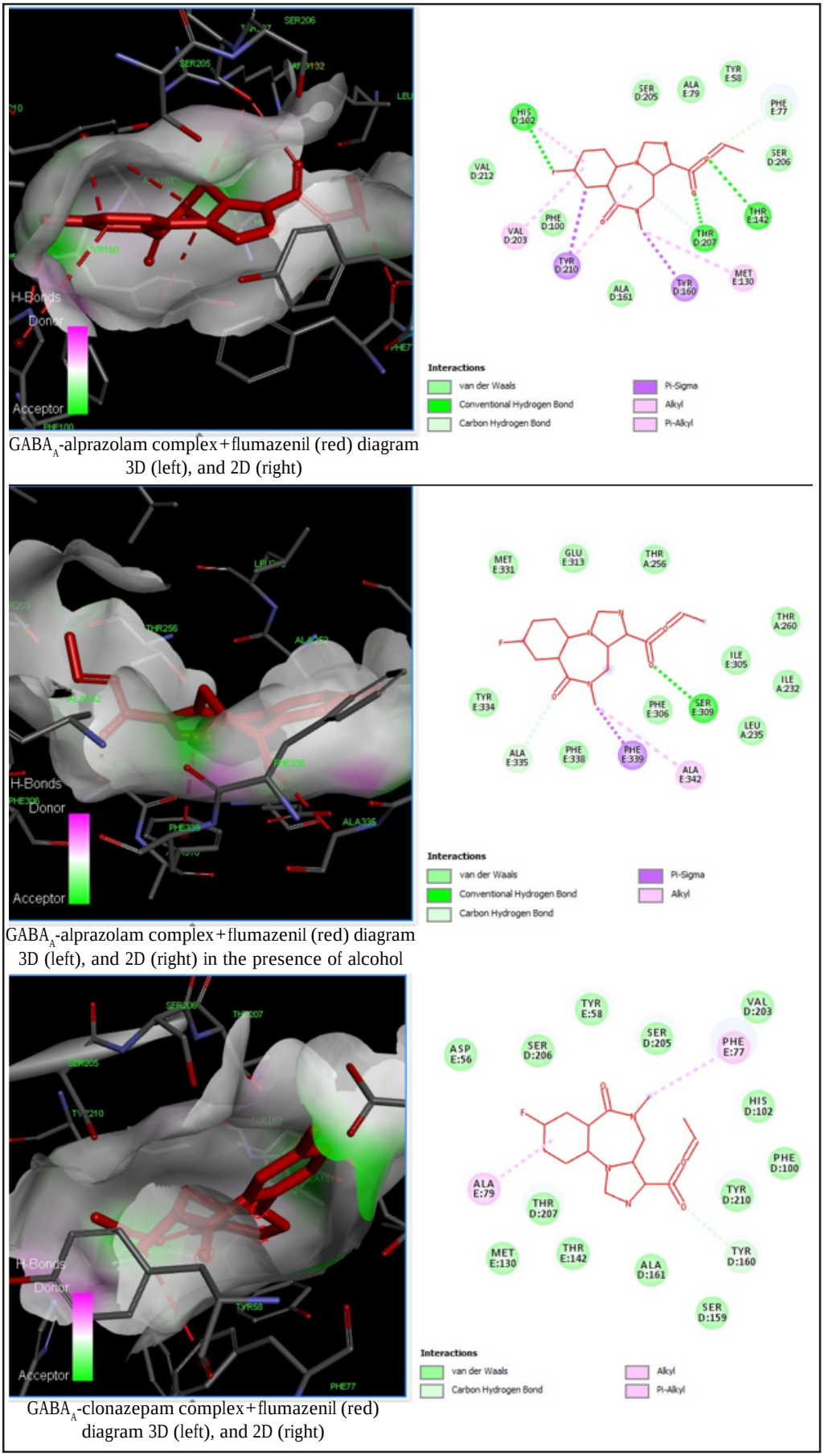
After alcohol consumption, diazepam binds with hydrogen bonds with histidine D: 102, via carbon bond hydrogen with serine D: 159, via pi-alkyl linkage with tyrosine D: 160, tyrosine D: 210, phenylamine D: 100, tyrosine E : 58, phenylamine E: 77, and van der waals interaction with valine D: 212, valine D: 203, asparagine E: 60, serine D: 205, serine D: 206, tyrosine D: 207, alanine D: 161.

Also the binding site of flunitrazepam and the interactive type changed, having hydrogen bonds with serine E: 309, tyrosine A: 256, carbon hydrogen bond with methionine E: 331, glutamic acid E: 313, alanine A: 249, alanine A: 252, phenylamine E: 339, alanine E: 342, interactive van der waals with leucine A: 253, tyrosine E: 334, alanine E: 335, phenylamine E: 310, valine E: 312 (fig. 2).

Table 3
ESTIMATED FREE BINDING ENERGY FOR FLUMAZENIL WITH $GABA_A$ - BENZODIAZEPINES COMPLEX WITH AND WITHOUT ALCOHOL

Benzodiazepines	$GABA_A$ - benzodiazepines complex+flumazenil (kcal/mol)	$GABA_A$ - benzodiazepines complex+flumazenil (in the presence of alcohol) (kcal/mol)
alprazolam	-6.47	-5.39
clonazepam	-6.65	-5.54
chlordiazepoxide	-5.61	-5.51
prazepam	-5.55	-6.81
oxazepam	-5.66	-5.43
flunitrazepam	-5.57	-6.77
lorazepam	-6.30	-6.95
midazolam	-5.49	-5.85
diazepam	-5.51	-5.71

The highest efficacy of flumazenil as a competitive antidote is in the case of previous administration of clonazepam and alprazolam (-6.65 kcal/mol and -6.47 kcal/mol). For both two benzodiazepines the effectiveness of flumazenil is reduced by alcohol consumption (fig. 3).

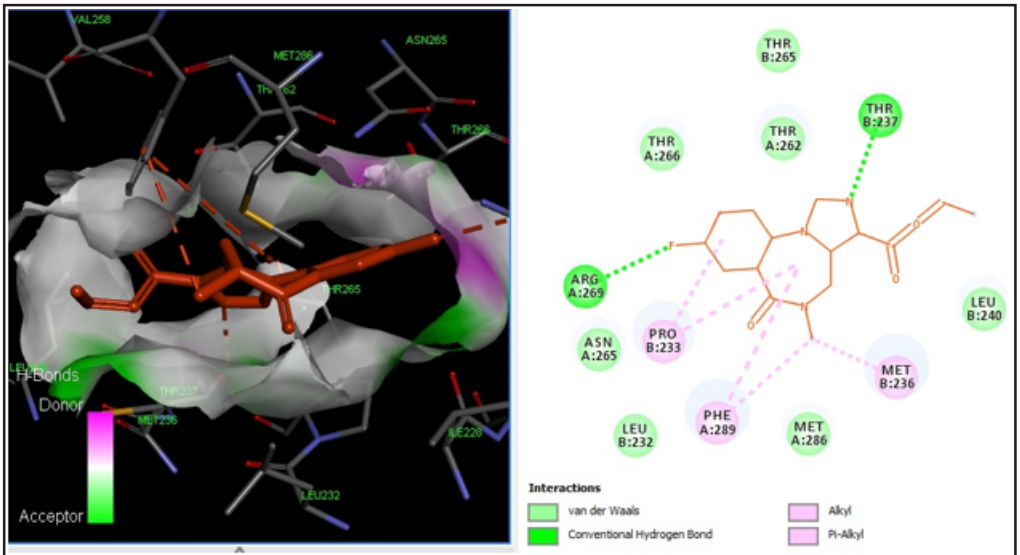


GABA$_A$-alprazolam complex+flumazenil (red) diagram 3D (left), and 2D (right)

GABA$_A$-alprazolam complex+flumazenil (red) diagram 3D (left), and 2D (right) in the presence of alcohol

GABA$_A$-clonazepam complex+flumazenil (red) diagram 3D (left), and 2D (right)

Fig.3 Alprazolam and clonazepam 3D and 2D diagram interaction with GABA$_A$ with and without alcohol



GABA$_A$-clonazepam complex+flumazenil (red) diagram 3D (left), and 2D (right) in the presence of alcohol

Fig. 3.

Conclusions

There are important reasons that the warnings on the instruction labels of benzodiazepines strongly advise against drinking alcohol with these drugs.

Combining alcohol with benzodiazepines can be dangerous practice even if it is engaged in only occasionally. Chronic abuse of these two drugs together can result in a number of serious short-term and long-term effects.

The highest molecular dipole is encountered for lorazepam, which contains two chlorine substituents and a hydroxyl group and the highest energy stability is held by diazepam and flunitrazepam.

From the molecular docking calculation there is no clear increase or decrease in binding energy for the benzodiazepine-GABA$_A$ complex when administered together with alcohol, but the overall pharmacological effect is stronger because it adds the SNC depressant effect of alcohol (-2.5 kcal/mol).

We establish that exist a good regression between estimated free binding energy and the two molecular descriptors: dipole moment and molecule energy.

The highest efficacy of flumazenil as a competitive antidote is in the case of previous administration of clonazepam and alprazolam, being reduced by alcohol consumption.

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