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Scientific paper

Development of QSAR Model Based on Monte Carlo Optimization for Predicting $GABA_A$ Receptor Binding of Newly Emerging Benzodiazepines

Aleksandra Antović[1], Radovan Karadžić[1], Jelena V. Živković[2] and Aleksandar M. Veselinović[2,*]

[1] *Institute of Forensic Medicine, Faculty of Medicine, University of Niš, Bulevar Dr Zorana Đinđića 81, 18000 Niš, Serbia*

[2] *Department of Chemistry, Faculty of Medicine, University of Niš, Bulevar Dr Zorana Đinđića 81, 18000 Niš, Serbia*

** Corresponding author: E-mail: aveselinovic@medfak.ni.ac.rs*
Fax: +381 18 4238770; Phone: +381 18 4570029

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Abstract

The rising prevalence and appeal of designer benzodiazepines (DBZDs) pose a significant public health concern. To evaluate this threat, the biological activity/potency of DBZDs was examined through in silico studies. To gain a deeper understanding of their pharmacology, we employed the Monte Carlo optimization conformation-independent method as a tool for developing QSAR models. These models were built using optimal molecular descriptors derived from both SMILES notation and molecular graph representations. The resulting QSAR model demonstrated robustness and a high degree of predictability, proving to be very reliable. The newly discovered molecular fragments used in the computer-aided design of the new compounds were believed to have caused the increase and decrease of the studied activity. Molecular docking studies were used to make the final assessment of the designed inhibitors and excellent correlation with the results of QSAR modeling was observed. This discovery paves the way for the swift prediction of binding activity for emerging benzodiazepines, offering a faster and more cost-effective alternative to traditional in vitro/in vivo analyses.

Keywords: Benzodiazepines; QSAR; Monte Carlo optimization; New psychoactive substances; $GABA_A$ receptor

1. Introduction

Everyday prescriptions involve benzodiazepines, as well as their derivatives, in the form of anxiolytic, anti-insomnia and anti-convulsant drugs for the purpose of tackling a multitude of medical conditions by acting on the gamma-aminobutyric acid type A ($GABA_A$) receptor.[1] Gamma-aminobutyric acid (GABA) is the endogenous neurotransmitter for the $GABA_A$ receptor and its binding reduces cell excitability.[2] Much lower cellular excitability is effected by benzodiazepines that potentiates $GABA_A$ receptor's response to GABA. In physiological terms, this leads to relaxation and sedation.[1] In such instances, the medical benefit of benzodiazepines is visible, since their anxiolytic effects lessen agitation and stress in patients. Nevertheless, owing to the psychoactive effects of the mentioned, there is a long abuse history of benzodiazepines, and they are frequently illegally secured.[1–5] Recently, the black market has had a steady supply of benzodiazepines. They are either licensed as prescription drugs in countries which are not their original home country, or are newly-synthesized and they are called 'new psychoactive substances' (NPS).[6–9] Most of the benzodiazepines that have appeared in this manner have not been subjected to regular pharmaceutical trials. For this reason, their effects can vary greatly and their activity may prove to be extremely hazardous.[10] Even though the use of benzodiazepines is quite safe if they are taken as prescribed, simultaneous use of benzodiazepines and opioids (whether abused or prescribed) can cause respiratory depression and even lead to death.[11,12] Numerous side effects may occur if benzodiazepines are not carefully monitored and if they are not prescribed. Such side-effects include dependency and tolerance in the event that the medication is taken long-

term. Furthermore, sudden withdrawal can lead to medical problems, such as insomnia and anxiety.[13,14] A certain number of overdose cases, driving under the influence of drugs (DUID) and hospital admissions have already been reported with regard to the use of NPS benzodiazepines.[15–17] One of the most prominent issues is that illegal benzodiazepines are not controlled at all and represent a safety hazard. In addition, in the event that the trend of their abuse gets increasingly big, the situation might be even more worrying.

Benzodiazepines represent a diverse group of psychoactive compounds whose central structural component consists of a diazepine ring and a benzene ring. There is a multitude of derivatives, including imidazo-benzodiazepines, thienotriazolobenzodiazepines and triazolobenzodiazepines. Correlating molecular structure to biological activity is attempted through the use of the quantitative structure-activity relationship (QSAR), frequently with the use of a various molecular descriptors, such as electronic, topological, physiochemical and steric properties.[18] Commonly, a set of compounds with a known biological activity is used for the purpose of attaining a 'training' dataset and creating a model. Afterwards, the model may be utilized for predicting the unknown biological activity possessed by compounds having a similar structure or for exploring the key structural features for the relevant biological activity in question. There have been numerous reasons for the use of QSAR, such as the pharmacological interpretation of drug-related deaths and developing compounds in the pharmaceutical industry.[19–21] Over the recent years, an approach in which the studied activity is treated as a random event has showed promise in QSAR modeling: the Monte Carlo optimization method. The mentioned method relies on the approach which is of conformation-independent nature, where the optimal descriptors are based on topological molecular features and the molecules in the Simplified Molecular Input Line Entry System (SMILES) notation.[22–24] The simplicity and efficiency of the method described are the primary advantages over more commonly used methods. What is more, molecular fragments (calculated as SMILES notation descriptors) with an impact on studied activity and which can be associated with the studied compounds' chemical structures can also be determined with the use of this method. When it comes to the applications with regard to new psychoactive substances, the application of QSAR's predictive power has mainly been aimed at cannabinoid binding to CB1 and CB2 receptors.[25–27] However, its use has also been to examine the biological activity of hallucinogenic phenylalkylamines, as well as the binding of tryptamines, phenylalkylamines and LSD to the 5-$HT2_A$ receptor and the selectivity of methcathinone for norepinephrine (NAT), dopamine (DAT) and serotonin transporters (SERT).[28–30] At present, a great many novel benzodiazepines have not been analyzed, and their physicochemical and biological properties have not been determined, since this would entail making a considerable investment, both in terms of money and time. This is precisely why a quick and economical method is desirable for predicting their properties.

Predicting the absorption rate, clearance, bioavailability, half-life and distribution volume for a group of benzodiazepines has previously been the application of QSAR to benzodiazepines. This study included phenazepam, a benzodiazepine which appeared as an NPS in 2007.[31,32] Over the years, after the publication of this study, other benzodiazepines (such as etizolam) appeared solely as new psychoactive substances. Also, QSAR methodology has been applied for the purpose of modeling the post-mortem redistribution of benzodiazepines, in which case a good model was obtained ($R^2 = 0.98$), where energy, ionization and molecular size were discovered to have a significant impact.[33] In an attempt to predict how toxic these compounds are, the toxicity of benzodiazepines to their structure has been correlated with the use of quantitative structure-toxicity relationships (QSTR).[34] In recent years, a study concluded that identifying the structural fragments responsible for toxicity (the presence of hydrazone substitutions and amine, as well as saturated heterocyclic ring systems resulting in greater toxicity) was possible with the use of QSTR, and that the information could potentially be used in order to create new, less toxic benzodiazepines for medical purposes. Correlating the benzodiazepine structure to $GABA_A$ receptor binding and tearing apart the complex relationship between various substituents, as well as their effect on activity have been achieved with the use of different QSAR models, though no one has specifically attempted to predict the binding values for the benzodiazepines which represent new psychoactive substances.[35,36] The main aim of this study is the development of QSAR models for predicting $GABA_A$ receptor binding of newly emerging benzodiazepines.

2. Materials and Methods

The studied activity is expressed as the logarithm of the reciprocal of concentration (log 1/c), with "c" representing the molar inhibitory concentration (IC_{50}) required to displace 50% of [3H]-diazepam from synaptosomal preparations in the cerebral cortex of rats.[37,38] The primary objective of this study is to construct a QSAR model capable of predicting the potential biological activity of newly-appearing benzodiazepines. The ultimate aim is to enhance our comprehension of these substances and consequently reduce their potential harm more rapidly than through traditional in vitro/in vivo testing methods.

To establish relevant QSAR models, the initial step involved acquiring molecules from the literature sources.[37,38] These molecules were subsequently rendered as graphical representations using ACD/ChemSketch software v.11.0, and were then transformed into the SMILES

notation using the same software. The Supporting Information section provides the chemical structures of the compounds utilized in QSAR modeling, along with their corresponding SMILES notation. The dependent variable used for QSAR model was the relationship between GABAA receptor binding and the structure of characterized benzodiazepines, expressed as the logarithm of the reciprocal of concentration (log 1/c). The numerical values presented in Table S1 of the Supplementary material correspond to these data. After completing the construction of the appropriate database, it was divided into two sets through three different main molecule random splits. The first set was the training set, comprising 63 compounds (75%), while the second set was the test set, containing 21 compounds (25%). Subsequently, the distribution activity normality was assessed using the method outlined in published literature.[23,24] The CORAL (CORrelation and Logic, http://www.insilico.eu/coral) software was employed to create conformation-independent QSAR models using the Monte Carlo method and its algorithm, which treats the relevant activity as a random event. Two types of molecular descriptors, based on the SMILES notation and the molecular graph, were considered. Invariants were established as local graph invariants using the molecular graphs, specifically path numbers of length 2 and 3 (p2, p3), Morgan extended connectivity index of increasing order (EC0), the Code of Nearest Neighbors (NNCk) and the valence shells within the range of 2 and 3 (s2, s3). In recent years, the Simplified Molecular Input-Line Entry System (SMILES) notation, particularly in chemoinformatics, since the SMILES notation has emerged as the most convenient representation, especially in the field of chemoinformatics. In the realm of medicinal chemistry this is particularly advantageous, as establishing correlations between molecular fragments and descriptors based on the molecular graph can be quite challenging. In the realm of QSAR modeling, one can establish molecular optimal descriptors (DCW) by utilizing the SMILES notation, and these DCW descriptors can be computed as a result of applying Equation 1 to the SMILES notation.

$$DCW(T,N_{epoch})_{SMILES} = \Sigma CW(ATOMPAIR) + \Sigma CW(NOSP) + \Sigma CW(BOND) + \Sigma CW(HALO) + \Sigma CW(HARD) + \Sigma CW(S_k) + \Sigma CW(SS_k) + \Sigma CW(SSS_k) \quad (1)$$

This research employed SMILES notation-based descriptors, encompassing global, local, and HARD-index descriptors. An essential aspect of the developed QSAR model is the calculation of the correlation weight (CW) for each optimal descriptor used, which is accomplished through the application of the Monte Carlo method.[23,24] This process can be accomplished by generating suitable random numbers and observing how the distribution of these numbers adheres to specific properties or criteria. In this procedure, CW values are assigned randomly to all the optimal descriptors, including both SMILES notation-based descriptors and molecular graph-based ones, during each independent Monte Carlo run. Subsequently, the Monte Carlo optimization process is employed to compute the numerical data for correlation weights. These weights are instrumental in achieving the highest possible correlation coefficient between the optimal descriptors used and the target activity under study. The Monte Carlo method employs two parameters to attain this objective: the number of epochs (Nepoch) and the threshold (T). For the construction of QSAR models, a range of values was used, specifically 0 to 10 for T and 0 to 70 for Nepoch. The determination of the most effective combination of T and Nepoch, based on predictive performance, was conducted following the methodology outlined in published literature.[23,24]

The primary objective in any QSAR modeling process is to create a robust model capable of accurately, consistently, and objectively predicting the properties of new molecules. The effectiveness of the established QSAR models was assessed using the following methods: internal validation through the training set, external validation using the validation set, and data randomization through the Y-scrambling test. This was accomplished by utilizing various statistical parameters to assess the quality of the models. These parameters include the correlation coefficient (r2), cross-validated correlation coefficient (q2), mean absolute error (MAE), standard error of estimation (s), root-mean-square error (RMSE), the Fischer ratio (F), Rm2, and MAE-based metrics.[39–43] Recently, a new criterion called the Index of Ideality of Correlation (IIC) has been introduced to evaluate the predictive potential of QSAR models. The IIC takes into account both the correlation coefficient and the distribution of data points relative to the diagonal line in the coordinate space of observed versus calculated values of the studied endpoint. The IIC is calculated using Equations 2–5 as the final estimator for the QSAR model's performance.[44–46]

$$\Delta_k = observed_k - calculated_k \quad (2)$$

With data available for all Δ_k for the test set, in the test set, it is possible to calculate the sum of negative and positive values of Δ_k akin to the calculation of the mean absolute error (MAE):

$$^{-}MAE_{test} = \frac{1}{^{-}N}\sum_{k=1}^{^{-}N}|\Delta_k| \quad \Delta_k < 0, \quad ^{-}N \text{ is the number of } \Delta_k < 0 \quad (3)$$

$$^{+}MAE_{test} = \frac{1}{^{+}N}\sum_{k=1}^{^{+}N}|\Delta_k| \quad \Delta_k \geq 0, \quad ^{+}N \text{ is the number of } \Delta_k \geq 0 \quad (4)$$

$$IIC_{test} = r_{test} \times \frac{min(^{-}MAE_{test}, ^{+}MAE_{test})}{max(^{-}MAE_{test}, ^{+}MAE_{test})} \quad (5)$$

Molegro Virtual Docker (MVD) software was used to perform molecular docking studies on geometrically

optimized ligands using MMFf94 force field. The target of these docking studies was the CryoEM structure of human full-length alpha1beta3gamma2L GABA(A)R in complex with diazepam (Valium) (PDB: 6HUP). MVD uses a rigid receptor structure and a flexible ligand structure for docking studies. It accounts for both hydrophilic and hydrophobic interactions, with a particular focus on van der Waals and steric interactions. This includes the identification of hydrogen bonds between the amino acids in the studied ligands and the active site. These interactions can be quantified using scoring functions, which are calculated numerical values that correlate with relevant binding energies.[47] As a general rule, for most enzymes, the stronger the interaction between the receptor and the ligand, the higher the inhibition. Therefore, the numerical values obtained for scoring functions can be used to assess the potential inhibitory effect of the studied ligands.[24] To estimate inhibitory potential, the following scoring functions were calculated and used: Pose energy, MolDock, and Rerank Score. A published methodology was used to validate the entire molecular docking protocol.[48,49] Discovery Studio Client v20.1.0.19 was used to display two-dimensional representations of the interactions between the studied molecules and the amino acids in the dopamine transporter active site.

3. Results and Discussion

A pivotal aspect to consider is the applicability domain (AD), which is determined based on the criteria mentioned.[50,51] To establish the AD, we applied the methodology outlined in published literature and found that all the molecules encompassed by this study fell within the defined AD range, with no outliers detected.[23] Table S2 displays the values of statistical metrics used by the authors to assess the quality of the developed QSAR models for the studied activity. The results suggest that the method employed was effective in creating a QSAR model with strong reproducibility, as confirmed by the concordance correlation coefficient. The predictability of the established QSAR model was subsequently assessed using the values provided in Table S2, confirming the model's validity. Additionally, the model's validity was affirmed through the utilization of MAE-based metrics. The ultimate assessment of the developed QSAR models was carried out for both the test set and the training set, utilizing the Ideality of Correlation Index. The resulting values indicate that the developed QSAR models exhibit a strong predictive capability. Figure 1 displays the graphical representation of the best-developed QSAR model, which achieved the highest r^2 value across all three splits and was determined through the best Monte Carlo optimization run. Furthermore, a Y-randomization approach was implemented, involving the randomization of Y values in 1000 trials and across ten distinct runs, to assess the robustness of the developed QSAR models. Additionally, a Y-randomization procedure was employed, involving the randomization of Y values in 1000 trials and across ten separate runs to evaluate the robustness of the developed QSAR models. The values provided in Table S3 demonstrate that there was no chance correlation present in the developed models. In terms of the statistical results, the most favorable QSAR model was derived from the first split.

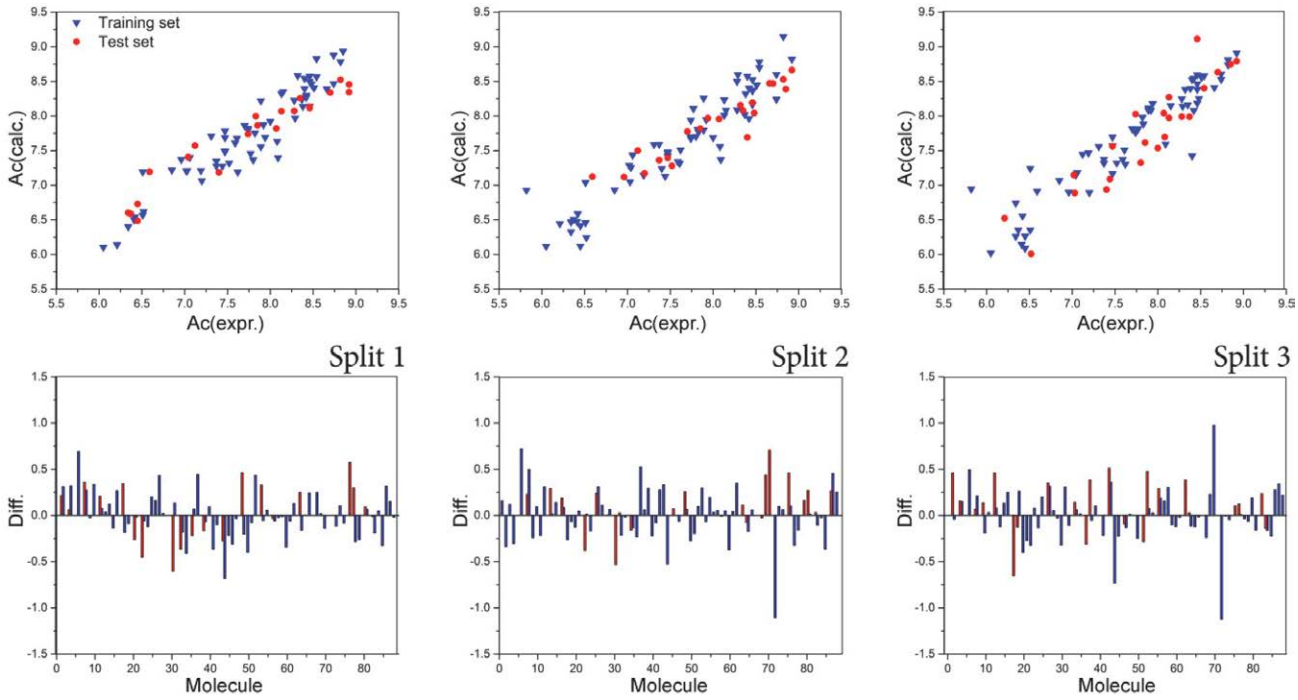

Figure 1. Graphical presentation of the best Monte Carlo optimization runs (the highest value for r^2) for the developed QSAR models.

Mathematical expressions for the best QSAR models, as determined by the test set r^2 values across all splits, are provided in Equations 6–8.

$$\text{Split 1: } \log(1/c) = 2.2950(\pm0.024) + 0.0484(\pm0.0002)\times DCW(1,12) \quad (6)$$

$$\text{Split 2: } \log(1/c) = 2.1642(\pm0.030) + 0.0504(\pm0.0002)\times DCW(1,20) \quad (7)$$

$$\text{Split 3: } \log(1/c) = -1.2010(\pm0.048) + 0.0700(\pm0.0004)\times DCW(1,20) \quad (8)$$

Equations 6–8 highlight that the optimal values for T and N_{epoch} for Split 1 are 1 and 12, respectively. Similarly, for Split 2, the preferred values for T and N_{epoch} are 1 and 20, respectively. Lastly, for Split 3, the recommended values for T and N_{epoch} are also 1 and 20, respectively.

The primary objective of this study is to create dependable QSAR models capable of predicting the correlation between $GABA_A$ receptor binding and the structure of characterized benzodiazepines, represented as the logarithm of the reciprocal of concentration (log 1/c). The quality of predictability is assessed through the application of a range of statistical parameters. The calculations for the conformation-independent models, constructed based on the optimal descriptors derived from SMILES notation invariants and a local graph, were executed using the Monte Carlo optimization method. The utilization of various statistical techniques enabled the evaluation of the resilience and predictive capability of the created QSAR models. The strong applicability of these models is evident from the numerical values employed to validate them. The molecular fragments employed in the QSAR modeling, categorized as SMILES notation fragments with either a positive or negative effect, were successfully identified using the Monte Carlo optimization method. These findings are detailed in Table S4 in the Supplementary material. An illustration of the calculation for both the summarized correlation weight (DCW) and the studied activity (pIC_{50}) of a molecule is provided in Table S5. For ease of interpretation, the molecular graph-based descriptors were excluded. Additionally, a graphical representation of the chosen molecular fragments is depicted in Figure 2.

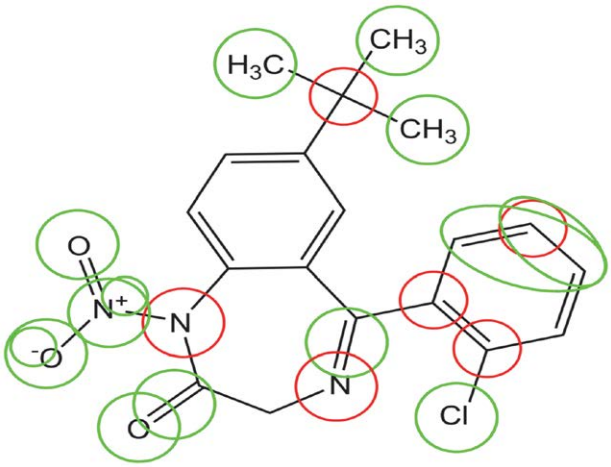

Figure 2. Contribution of Molecular Fragments to Benzodiazepines Binding Activity (Green – Increase, Red – Decrease).

Based on the results obtained from the QSAR modeling studies, the molecular fragments that exert an influence on the studied activity are: "O....." and "O...-....." – both a regular oxygen atom and an oxygen atom carrying a negative charge positively influence the studied activity. Moreover, fragments associated with a negative charge also contribute significantly to this impact, "-.....", also has positive impact on studied activity; "=.....", "O...=....." – the presence of a double bond, as well as a double bond on an oxygen atom, both exert a positive impact on the studied activity, but fragment "N...=....." associated to double bond on nitrogen atom has negative impact on the studied activity; While a regular nitrogen atom associated with the "N....." fragment has negative impact but nitrogen with positive charge, "N...+....." fragment, a nitrogen atom with a positive charge exerts a positive influence on the studied activity – "+....."; Molecular branching in the form of a simple molecular feature associated with the molecular fragment "(....." and molecular branching on a nitrogen atom, "N...(....," both have a negative impact. However, molecular branching on a carbon atom, "C...(....", has a positive impact on the studied activity; Furthermore, additional molecular branching on a carbon atom, defined as "(...C...(..." and "C...(...(...," has a positive impact. Likewise, a regular carbon atom or a methyl group, defined as "C.....", and two carbon atoms or an ethyl group, defined as "C...C.....", also have a positive impact on the studied activity; conversely, a single aromatic carbon atom, defined by the molecular fragment "c.....", negatively affects the activity. However, the presence of two or three connected aromatic carbon atoms, defined by the molecular fragments "c...c....." and "c...c...c...", positively influences the studied activity.

Obtained molecular fragment were further used for the Computer-Aided Design (CAD) of higher/lower activity compounds and summarized results are presented in Figure 3, where conformational-independent results in the CAD process generated the design of six novel potential inhibitors (structures presented in Figure 3). CAD process started with addition of methy group in ortho and para position which yield molecules A1 and A2, both having additional molecular fragment "C.....", SMILES notation descriptor, in comparison to molecule A. Additionally, molecules A1 and A2 have molecular branching on benzene ring with carbon atom involved, in comparison to molecule A, defined with molecular fragments – "c...(.....", "C...(.....", "c...c...(...", "c...C....." and "c...C...(...". These fragments have positive impact on studied activity so calculated values for pIC_{50} for molecules A1 and A2 were 7.4308 and 7.5591, respectively, both higher in comparison to

pIC_{50} for molecules A (7.2771). Molecules A3, A4, A5 and A6 have added hydroxyl group or chlorine atom in ortho and para position respectively. All molecules have added appropriate molecular fragments "O.....", "Cl.....", both with positive impact on studied activity. Like molecules A1 and A2, molecules A3, A4, A5 and A6 have molecular branching on benzene ring defined with "c...(.....". Addition of above stated fragments yield to the increase of calculated pIC_{50} for molecules A3, A4, A5 and A6 in comparison to molecule A.

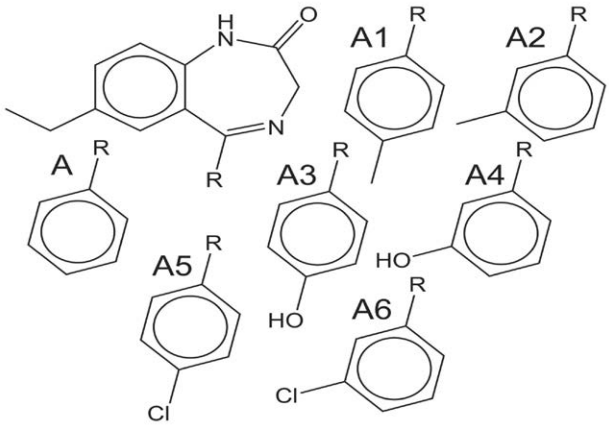

Figure 3. Chemical structures of designed molecules.

ing the highest energy. Overall, the results from the molecular docking studies, as represented by the scoring function values, correlate well with the QSAR modeling results. The Supplementary Information figures show all the interactions between the amino acids of the $GABA_A$ active site and the selected molecules. They also depict hydrogen bonds and hydrophilic and hydrophobic interactions within the binding pocket in two dimensions. Figure 3 shows the best-predicted poses of all the designed molecules within the active site of the $GABA_A$.

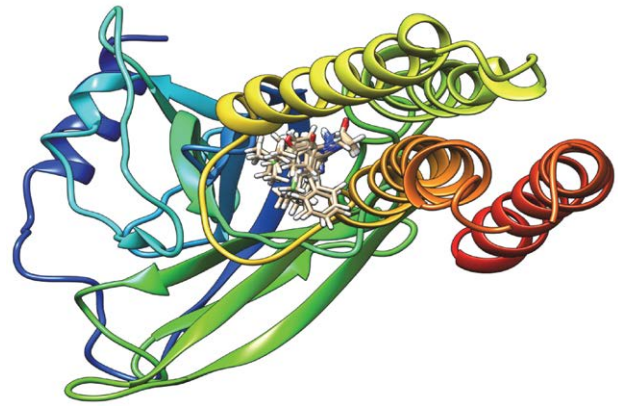

Figure 4. The best calculated poses for all the designed molecules within the active site of $GABA_A$.

Computational studies were performed using molecular docking to evaluate the binding affinities of all designed molecules and the template molecule A to the $GABA_A$. This was done to assess the predictive power of the developed QSAR models and to further validate them. Table 1 summarizes the calculated scoring functions for all molecules. Various scoring functions can be used to represent different ligand-amino acid interactions. Therefore, when assessing inhibitory potency, all scoring functions must be considered. The results from the MolDock and Re-Rank scoring functions show that all designed molecules have the potential to be more active than the template molecule A, with molecule A6 having the highest predicted activity. The energy scoring function results show that all designed molecules have higher interaction energies with the amino acids than molecule A, with molecule A6 also hav-

4. Conclusion

The effectiveness of the QSAR methodology, which relies on the Monte Carlo optimization in conjunction with molecular graph and SMILES notation descriptors, has been showcased in this study. It has proven to be a valuable approach for establishing the relationship between $GABA_A$ receptor binding and the structural characteristics of characterized benzodiazepines. To construct the conformation-independent QSAR models presented here, easily interpretable descriptors with a mechanistic interpretation were employed successfully. Additionally, this methodology has efficiently identified molecular fragments, characterized as SMILES notation fragments in QSAR modeling, that exhibit both positive and negative effects on the studied activity. Subsequently, the developed

Table 1. The list of all the designed molecules with their SMILES notation, calculated activities and score values (kcal/mol) for all computer-aided designed compounds

Molecule	SMILES notation	pIC50 (calc.)	Energy	MolDock Score	Rerank Score
A0	CCc1ccc2c(c1)C(=NCC(=O)N2)c1ccccc1	7.2771	−96.9452	−93.7531	−69.8862
A1	CCc1ccc2c(c1)C(=NCC(=O)N2)c1ccc(cc1)C	7.4308	−97.3104	−95.8847	−71.252
A2	CCc1ccc2c(c1)C(=NCC(=O)N2)c1cccc(c1)C	7.5591	−97.6046	−97.0659	−72.638
A3	CCc1ccc2c(c1)C(=NCC(=O)N2)c1ccc(cc1)O	7.5996	−97.3448	−95.7188	−71.8375
A4	CCc1ccc2c(c1)C(=NCC(=O)N2)c1cccc(c1)O	7.7038	−98.5056	−99.387	−62.6546
A5	CCc1ccc2c(c1)C(=NCC(=O)N2)c1ccc(cc1)Cl	8.0723	−97.1929	−95.606	−71.5423
A6	CCc1ccc2c(c1)C(=NCC(=O)N2)c1cccc(c1)Cl	8.3195	−100.576	−96.6462	−75.8107

QSAR models were used to design new compounds with higher pIC50 values. Molecular docking studies were then performed to validate the QSAR models and assess the potential activity of the designed molecules. A good correlation was observed between the calculated pIC50 values from the QSAR models and the calculated binding energies from the molecular docking studies. Notably, this approach facilitates a swift overview of the dataset without the need for complex calculations of molecular conformations. Consequently, it holds promise for future applications in rapidly and accurately assessing the relationship between $GABA_A$ receptor binding and the structure of novel benzodiazepines.

We have no conflict of interest to disclose.

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Povzetek

Naraščajoča razširjenost in uporaba benzodiazepinov na črnem trgu predstavlja pomembno javnozdravstveno skrb. V tem delu uporabljamo in silico tehnike, s katereimi ocenjujemo biološko aktivnost takšnih benzodiazepinov. Da bi poglobili razumevanje njihove farmakologije, smo uporabili metodo od konformacij neodvisne Monte Carlo optimizacije kot orodje za razvoj modelov QSAR. Ti modeli so bili zgrajeni z uporabo optimalnih molekulskih deskriptorjev, pridobljenih tako iz notacije SMILES kot tudi iz molekularnih grafov. Izdelani model QSAR je pokazal robustnost in visoko stopnjo napovedljivosti, kar kaže na njegovo zanesljivost. Novi molekularni fragmenti, odkriti pri računalniško podprtem načrtovanju novih spojin, povzročijo povečanje in zmanjšanje aktivnosti. Za končno oceno zasnovanih inhibitorjev smo uporabili orodja molekularnega sidranja, pri čemer smo opazili odlično ujemanje z rezultati modeliranja QSAR. Študija odpira pot hitremu napovedovanju vezavne aktivnosti za nove benzodiazepine ter ponuja hitrejšo in stroškovno učinkovito alternativo tradicionalnim analizam in vitro/in vivo.