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The use of a quantitative structure-activity relationship (QSAR) model to predict GABA-A receptor binding of newly emerging benzodiazepines
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Abstract

The illicit market for new psychoactive substances is forever expanding. Benzodiazepines and their derivatives are one of a number of groups of these substances and thus far their number has grown year upon year. For both forensic and clinical purposes it is important to be able to rapidly understand these emerging substances. However as a consequence of the illicit nature of these compounds, there is a deficiency in the pharmacological data available for these ‘new’ benzodiazepines. In order to further understand the pharmacology of ‘new’ benzodiazepines we utilised a quantitative structure-activity relationship (QSAR) approach. A set of 69 benzodiazepine-based compounds was analysed to develop a QSAR training set with respect to published binding values to GABA_A receptors. The QSAR model returned an $R^2$ value of 0.90. The most influential factors were found to be the positioning of two H-bond acceptors, two aromatic rings and a hydrophobic group. A test set of nine random compounds was then selected for internal validation to determine the predictive ability of the model and gave an $R^2$ value of 0.86 when comparing the binding values with their experimental data. The QSAR model was then used to predict the binding for 22 benzodiazepines that are classed as new psychoactive substances. This model will allow rapid prediction of the binding activity of emerging benzodiazepines in a rapid and economic way, compared with lengthy and expensive in vitro/in vivo analysis. This will enable forensic chemists and toxicologists to better understand both recently developed compounds and prediction of substances likely to emerge in the future.

Keywords: benzodiazepines; QSAR; biological activity; prediction; new psychoactive substances; GABA_A receptor
Introduction

Benzodiazepines and their derivatives are routinely prescribed for a variety of medical conditions as anxiolytic, anti-insomnia and anti-convulsant drugs, acting on the gamma-aminobutyric acid type A (GABA<sub>A</sub>) receptor [1, 2]. The endogenous neurotransmitter for the GABA<sub>A</sub> receptor is gamma-aminobutyric acid (GABA), the binding of which reduces the excitability of the cell [3]. Benzodiazepines potentiate the response of the GABA<sub>A</sub> receptor to GABA which results in far less cellular excitability which, in physiological terms, results in sedation and relaxation [1].

In these circumstances benzodiazepines are medically beneficial by alleviating stress and agitation in patients through their anxiolytic effects. However, as a result of their psychoactive effects, benzodiazepines have a long history of abuse and are often illicitly obtained [4-6]. In more recent years a steady stream of benzodiazepines have appeared on the illicit market that have either been newly-synthesised or are licensed as prescription drugs in another country but not in the home country [7-10]. These are termed ‘new psychoactive substances’ (NPS) [11, 12]. The majority of these emerging benzodiazepines have not undergone standard pharmaceutical trials and can be quite variant in their effects and potentially dangerous in their activity [13]. Although relatively safe when used as medically prescribed, concurrent use of benzodiazepines and opioids (either prescribed or abused) can lead to respiratory depression and death [4, 14, 15]. When benzodiazepines are not carefully prescribed and monitored, they can cause a variety of side effects including tolerance and dependency if taken long-term and sudden withdrawal can cause medical problems including anxiety and insomnia [16-18]. These NPS benzodiazepines have already been reported in a number of overdose cases, driving under the influence of drugs (DUID) cases and hospital admissions [8, 19-22]. The lack of control and safety over these illicit benzodiazepines is a
prevalent issue and it is likely that it will become an even more worrying trend as their misuse continues to rise.

Benzodiazepines are a diverse group of psychoactive compounds with a central structural component consisting of a benzene ring and a diazepine ring (Figure 1). A whole host of derivatives exist which include triazolobenzodiazepines, thienotriazolobenzodiazepines and imidazobenzodiazepines (see Supplementary Information Figure S1 and Table S1).

Quantitative structure-activity relationship (QSAR) models attempt to correlate molecular structure to biological activity, often using a variety of molecular descriptors such as physiochemical, topological, electronic and steric properties [23]. Typically, a set of compounds whose biological activity is known is used to create a ‘training’ dataset and a model. This model can then be used to predict the unknown biological activity of compounds with a similar structure or to explore the structural features that are important for the specific biological activity in question. QSAR has been extensively used for a variety of reasons such as compound development in the pharmaceutical industry and the pharmacological interpretation of drug-related deaths [24-26]. In terms of applications towards new psychoactive substances, the predictive power of QSAR has been mainly applied to cannabinoid binding to the CB₁ and CB₂ receptors [27-29] but has also been used to examine the biological activity of hallucinogenic phenylalkylamines [30], the binding of phenylalkylamines, tryptamines and LSD to the 5-HT₂A receptor [31] and methcathinone selectivity for dopamine (DAT), norepinephrine (NAT) and serotonin transporters (SERT) [32]. Currently, the majority of novel benzodiazepines have not been analysed to determine their physicochemical and biological properties as this would require a substantial investment in both time and money. It is for this reason that a fast, yet economical method to predict their properties is desirable.
QSAR has previously been applied to benzodiazepines to predict bioavailability, absorption rate, clearance, half-life and volume of distribution for a group of benzodiazepines. This study included phenazepam [33], a benzodiazepine that appeared as an NPS in 2007 [34]. Other benzodiazepines (such as etazolam) only appeared as new psychoactive substances in the years following the publication of this study. Furthermore, the application of a QSAR methodology has been used for modelling post-mortem redistribution of benzodiazepines where a good model was obtained ($R^2 = 0.98$) in which energy, ionisation and molecular size were found to exert significant impact [35]. Quantitative structure-toxicity relationships (QSTR) have been used to correlate the toxicity of benzodiazepines to their structure in an attempt to predict the toxicity of these compounds [36]. More recently, a study reported the use of QSTR whereby it was concluded that it is possible to identify structural fragments responsible for toxicity (the presence of amine and hydrazone substitutions as well as saturated heterocyclic ring systems resulted in a greater toxicity) and potentially use this information to create new, less toxic benzodiazepines for medical use [37].

Various QSAR models have been used to correlate benzodiazepine structure to GABA$_A$ receptor binding and tease apart the complex relationship between various substituents and their effect on activity [38-43] although none have specifically attempted to predict binding values for benzodiazepines that are new psychoactive substances.

In this study we focus on the relationship between the structure of characterised benzodiazepines and GABA$_A$ receptor binding, expressed as the logarithm of the reciprocal of concentration ($\log 1/c$) where $c$ is the molar inhibitory concentration ($IC_{50}$) required to displace 50 % of [3H]-diazepam from rat cerebral cortex synaptosomal preparations [41]. The purpose of this work is to create a QSAR model that can be used to predict the potential biological activity of the newly-emerging benzodiazepines to help understand, and therefore minimise their harmful potential in a faster time scale compared with \textit{in vitro} \textit{in vivo} testing.
Methods and Materials

Selection of the dataset

The binding data for the benzodiazepines was used as obtained from the literature, experimentally determined using spectrometric measurements of [3H]-diazepam displacement [44]. Benzodiazepines were selected from four categories; 1,4-benzodiazepines, triazolobenzodiazepines, imidazobenzodiazepines and thienotriazolobenzodiazepines. Benzodiazepines that did not have definitive binding values (i.e. listed values were simply stated as >1000 or >5000) were excluded. For simplicity benzodiazepines with atypical atoms or substituents (e.g. Ro 07-9238 which contained a sodium atom and Ro 05-5065 which contained a naphthalene ring) were also excluded. Benzodiazepines that also had atypical substitutions (i.e. positions R6, R8 and R9 from Figure 1 which are not found in medically-used benzodiazepines or indeed those that are new psychoactive substances) were also excluded. In total, 88 benzodiazepines were selected for the training dataset.

QSAR/Software and Data Analysis Method

The 88 benzodiazepines were converted from SMILES to 3D structures based on Merck Molecular Force Field (MMFF) atom type and force field optimisation. These compounds were then aligned by common substructure and confirmation to Ro 05-306. Subsequently, the aligned compounds were clustered by Atomic Property Fields (APF) to identify benzodiazepines with poor alignment. The APF method, designed by MolSoft, uses the assignment of a 3D pharmacophore potential on a continuously distributed grid using physicochemical properties of the selected compound(s) to classify or superimpose compounds. These properties include: hydrogen bond donors, acceptors, Sp2 hybridisation, lipophilicity, size, electropositivity/negativity and charge [45, 46]. Poorly aligned benzodiazepines identified by APF clustering were subjected to re-alignment using APF-based flexible
superimposition. At this point, 10 benzodiazepines with poor alignment were removed to improve model accuracy. (Supplementary Information Table 1S).

From the remaining 78 aligned compounds, 9 compounds were selected using a random number generator based on atmospheric noise. These compounds were removed from the training set and used for final model validation. The residual 69 compounds were used as the training set to build a 3D QSAR model, as shown in Figure 2.

The APF 3D QSAR method was used where, for each of the 69 aligned compounds, the seven physicochemical properties were calculated and pooled together. Based on the activity data obtained from literature and the 3D aligned structures for the known compounds, weighted contributions for each APF component were obtained to allow quantitative activity predictions for unknown compounds. The optimal weight distributions were assigned by partial least-squares (PLS) methodology, where the optimal number of latent vectors for PLS was established by leave-one-out cross-validation on the training set. Then the weighted contributions were added together. The 9 compounds for validation and unknown compounds were assigned predicted binding values by calculating their fit within the combined QSAR APF. Any unknown benzodiazepines were subjected to the conversion and alignment protocol before predicted binding data was obtained. The above steps were conducted using Molsoft’s ICM Pro software [47].

Further analysis of the PLS model fragment contributions from the 69 compounds was conducted using SPCI software. Here, a 2D QSAR model was built using the same PLS methodology as above. Additionally, a consensus model was created from averaging the predictions of PLS, gradient boosting, support vector machine and random forest modelling methods. The compounds were then subjected to automatic fragmentation and contribution calculations, which resulted in information on 11 key contributing groups [48]. Using Ligand
Scout with default settings, four ligand-based pharmacophore models were created using compounds with binding values of 6.0–9.0, 7.0–9.0, 8.0–9.0 and 8.5–9.0, as exemplified in Figure 3.

Ten benzodiazepines that had the highest predicted binding values were docked into a modelled GABA_A5 receptor using ICM software. The GABA_A5 receptor model was generated by homology modelling, using the crystal structure of a human GABA(A)R-beta3 homopentamer (PDB id 4COF) as a template. A pre-defined binding site containing co-crystallised benzodiazepine is already present in the template, which was retained in the final model. Modeller software was used to generate the homology models [49]. The final chosen model was energy minimized using the ACEMD software [50]. The stereochemistry was checked using Procheck and ProSA software [51, 52]. The benzodiazepine in the allosteric binding site on the GABA_A5 receptor was used as a chemical template to dock NPS-benzodiazepines and the best-scoring conformations were analysed.

The distances between principle physiochemical properties and their weights in the pharmacophore model were calculated using the software LigandScout [53].

**Results and Discussion**

The data that was used to create the QSAR model (i.e. benzodiazepine structural substitutions and experimentally-observed binding values) is provided in the Supplementary Information (Table S1).

From the pharmacophore model visualised in Figure 3 for highly bound benzodiazepines (log 1/c of 8.0 – 9.0), it is evident that important binding features for the benzodiazepines were the positioning of two H-bond acceptors, two aromatic rings and a hydrophobic group all with weights of 1.0.
The predicted binding values are not presented here but are listed in Supplementary Information (Table S1). They can be visualised in Figure 4 as a plot of the observed binding value versus the predicted binding value.

Nine compounds were selected at random from the QSAR training set and their binding values estimated using the model as a system of internal validation. These estimated values were then compared to the experimental binding values (Figure 5).

The QSAR model was then used to predict the binding for 22 benzodiazepines that are classed as new psychoactive substances. The results are divided into four categories depending upon the nature of the substitutions, as shown in Tables 1, 2, 3 and 4.

Five compounds were present in the training dataset but have also appeared as new psychoactive substances; adinazolam, desalkylflurazepam, desmethylflunitrazepam (fonazepam), etizolam and meclonazepam. The experimental binding values from the literature and the predicted binding values are displayed in Table 5.

The NPS-benzodiazepine with the highest predicted log 1/c value was flunitrazolam with 8.88, closely followed by clonazolam with 8.86. However, based upon experimental data, meclonazepam with a log 1/c value of 8.92 (8.52 predicted) actually exhibited the greatest binding affinity. Only two benzodiazepines in the training set experimental values had a log 1/c value of 8.92; these were meclonazepam and brotizolam with the rest falling below this point. In general, the limitations to this model are most likely caused by the small size of the data set. It is widely reported that QSAR models have poorer predictive capabilities with training sets under 100 compounds [54, 55]. Moreover, the diversity of substitutions within the small set of training compounds, created difficulties with APF superimposition and therefore may have reduced the accuracy of the model predictors. Secondary modelling with SPCI highlighted these limitations and demonstrated the existing dataset was less suitable for
PLS 2D QSAR modelling [48]. However, the consensus from multiple modelling methods improves the predictive power of the 2D QSAR model. Additionally, as experimental errors in the training set are amplified both by the logarithmic scale and when calculating the weighted contributions, consistency and accuracy in the initial experimental values are essential for a strong QSAR model. Ideally, further improvements to the model could be made by using a larger training dataset with lower diversity yet this cannot be achievable as a consequence of limitations on literature data available.

From these docking studies with the modelled GABA_A5 receptor it can be seen that they only partially occupy the available volume at the allosteric binding site (exemplified in Figure 6 for flunitrazolam). From the ten compounds that had the greatest binding affinity, four had non-bonded interactions with the T80 region within the receptor, two had non-bonded interactions with the K182 and S231 regions respectively. There were also stacking interactions with the Y96 region for four of the compounds. Therefore the possibility is that the binding is not completely optimal for these benzodiazepines and that with a modified chemical structure, a greater binding affinity could be theoretically possible. The reality exists that a benzodiazepine with an optimised binding affinity could emerge onto the illicit drugs market and could potentially (but not necessarily) exhibit a greater potency.

The 10 compounds with the greatest binding affinity for the receptor are listed in Table 6 (lower scores indicate a greater binding effect).

There are 35 benzodiazepines and their derivatives currently subject to international control, 30 of these compounds had binding values listed in the original source [44]. The average log 1/c value for these 30 controlled compounds was 7.57. Out of these compounds, 43 % (13 out of 30) had a log 1/c value that was greater than 8.00. The average log 1/c value for the whole training dataset was 7.81 and 48 % of the compounds (33 out of 69) had a log 1/c value that was greater than 8.00. These values are fairly similar, however when comparing the results of
the benzodiazepines that are new psychoactive substances, the average log 1/c value that was predicted was 8.22 and 68% of the compounds (15 out of 22) had a log 1/c value that was greater than 8.00. From this it is appears that benzodiazepines that are appearing as new psychoactive substances are more likely to have a greater binding affinity at the GABA_A receptor. Whether this trend is deliberate is unclear.

A log 1/c value of 7.88 was obtained for 4-chlorodiazepam (Ro 5-4864). This suggests a relatively high affinity for the GABA_A receptor when compared with the log 1/c values for clinically-used benzodiazepines; the binding value for diazepam is 8.09 and 8.40 for triazolam. However it has been reported that the experimental value for 4-chlorodiazepam (Ro-4864) is actually 3.79 (i.e. an IC_{50} value of 160,500 nM) in one dataset when compared with a log 1/c of 7.80 for diazepam and 8.72 for triazolam in the same dataset [56]. There are obvious impracticalities with comparing different datasets as a result of differences in methods (e.g. the use of [^{3}H]-diazepam versus [^{3}H]-flunitrazepam as a radioligand), the differences in the species used (rat vs. mouse) and the differences in GABA_A receptor expression between different brain homogenates. Despite this it is clear that 4-chlorodiazepam observes an extremely low affinity for GABA_A receptors and one that this model did not accurately predict. This most likely results from the deficit of compounds in the training dataset that had a similar substitution on the R_{4'} position of the phenyl ring. Indeed, this model focused upon the ‘classical’ 1,4-benzodiazepine, triazolobenzodiazepine, imidazobenzodiazepine and thienotriazolodiazepine substitutions. Substitutions on the R_{4'} position of the phenyl ring are known to exhibit strong steric repulsion at the GABA_A receptor interface and therefore compound binding is severely inhibited [40] [57]. 4-chlorodiazepam is an outlier and atypical benzodiazepine as it does not act upon the GABA_A receptor; instead exerting its pharmacological effects through the translocator protein 18 kDa (TSPO), previously known as the peripheral benzodiazepine receptor [58, 59].
The oxazolobenzodiazepine flutazolam, a prescription drug in Japan, had a predicted log 1/c binding value of 6.83 which seems extremely low compared with the other benzodiazepines in this dataset. To the best of the authors’ knowledge there exists no experimental GABA\textsubscript{A} receptor binding data for flutazolam. However other oxazolobenzodiazepines have low affinities for the GABA\textsubscript{A} receptor such as ketazolam with a log 1/c value of 5.89 [60] and oxazolam with a log 1/c value of 5.00 [61]. These log 1/c binding values are from additional sources – the previous paragraph discusses the difficulties in comparing binding values from different datasets. Nonetheless it is clear that oxazolobenzodiazepines exhibit a much lower affinity for the GABA\textsubscript{A} receptor. If the value for flutazolam is correct then this QSAR model successfully predicted the low binding affinity of flutazolam despite having no oxazolobenzodiazepines in the training dataset which serves as an indicator to the potential strength of the model.

Conclusions

The emergence of benzodiazepines and their derivatives as new psychoactive substances necessitates the investigation of their pharmacological attributes. The use of a QSAR model is ideal to gain an understanding into the binding properties of these substances. In this work a QSAR model has been successfully developed to predict the binding data for NPS-benzodiazepines. Benzodiazepines that have emerged as new psychoactive substances appear to have a greater binding affinity to GABA\textsubscript{A} receptors than those benzodiazepines that are used medically and are under international control. Whether this trend will continue is uncertain. Further in vitro work would allow the compilation of more data to improve the accuracy of this model. However, this model does allow a rapid estimation of the binding affinity of emerging benzodiazepines before more detailed studies can be carried out.
References

1. M. Lader, Benzodiazepines Revisited-Will We Ever Learn?, Addiction. 106 (2011) 2086-2109
8. M. Łukasik-Głębocka, K. Sommerfeld, A. Teżyk, et al., Flubromazolam—a New Life-Threatening Designer Benzodiazepine, Clinical Toxicology. 54 (2016) 66-68
15. J.A. Gudin, S. Mogali, J.D. Jones, S.D. Comer, Risks, Management, and Monitoring of Combination Opioid, Benzodiazepines, and/or Alcohol Use, Postgraduate Medicine. 125 (2013) 115-130


25. A.G. Leach, Predicting the Activity and Toxicity of New Psychoactive Substances: A Pharmaceutical Industry Perspective, Drug Testing and Analysis. 6 (2013) 739-745


on Hallucinogenic Substances, Molecular Informatics. 18 (1999) 548-560
38. P.A. Borea, De Novo Analysis of Receptor Binding Affinity Data of Benzodiazepines, Arzneimittelforschung. 33 (1983) 1086-1088
40. A.K. Ghose, G.M. Crippen, Modeling the Benzodiazepine Receptor Binding Site by the General Three-Dimensional Structure-Directed Quantitative Structure-Activity Relationship
Method Remotedisc., Molecular Pharmacology. 37 (1990) 725-734


53. G. Wolber, T. Langer, Ligandscout: 3-D Pharmacophores Derived from Protein-Bound Ligands and Their Use as Virtual Screening Filters, Journal of Chemical Information and Modeling. 45 (2005) 160-169


55. A. Golbraikh, E. Muratov, D. Fourches, A. Tropsha, Data Set Modelability by Qsar, Journal of Chemical Information and Modeling. 54 (2014) 1-4


### Tables

#### Table 1. Structural information and predicted binding values for 1,4-benzodiazepines

<table>
<thead>
<tr>
<th>Name</th>
<th>Substitutions</th>
<th>Log 1/c predicted</th>
<th>Basic structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diclozapam</td>
<td>Cl CH₃ Cl-</td>
<td>8.39</td>
<td><img src="image" alt="Basic structure image" /></td>
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<tr>
<td>Desalkylflurazepam</td>
<td>Cl- F-</td>
<td>8.44</td>
<td></td>
</tr>
<tr>
<td>Meclonazepam</td>
<td>NO₂- Cl CH₃</td>
<td>8.52</td>
<td></td>
</tr>
<tr>
<td>Phenazepam</td>
<td>Br- Cl-</td>
<td>8.12</td>
<td></td>
</tr>
<tr>
<td>Desmethylflunitrazepam</td>
<td>NO₂- F-</td>
<td>8.46</td>
<td></td>
</tr>
<tr>
<td>3-hydroxyphenazepam</td>
<td>Br- Cl OH-</td>
<td>8.42</td>
<td></td>
</tr>
<tr>
<td>Flubromazepam</td>
<td>F- Br-</td>
<td>8.37</td>
<td></td>
</tr>
<tr>
<td>Nifoxipam</td>
<td>NO₂- F OH-</td>
<td>8.63</td>
<td></td>
</tr>
<tr>
<td>Cloniprazepam</td>
<td>NO₂- Cl C₂H₃</td>
<td>7.83</td>
<td></td>
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<tr>
<td>Nimetzepam</td>
<td>NO₂ CH₃-</td>
<td>7.87</td>
<td></td>
</tr>
<tr>
<td>4-chlorodiazepam</td>
<td>Cl CH₃-</td>
<td>7.88</td>
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</table>

*4-chlorodiazepam has a Cl substituted on the R₄ position of the phenyl ring*
Table 2. Structural information and predicted binding values for triazolobenzodiazepines

<table>
<thead>
<tr>
<th>Name</th>
<th>Substitutions</th>
<th>Log 1/c predicted</th>
<th>Basic structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flubromazolam</td>
<td>Br CH₃ F -</td>
<td>8.77</td>
<td>![Basic Structure Image]</td>
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<tr>
<td>Clonazolam</td>
<td>NO₂ CH₃ Cl -</td>
<td>8.86</td>
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</tr>
<tr>
<td>Flunitrazolam</td>
<td>NO₂ CH₃ F -</td>
<td>8.88</td>
<td></td>
</tr>
<tr>
<td>Bromazolam</td>
<td>NO₂ CH₃ - -</td>
<td>8.25</td>
<td></td>
</tr>
<tr>
<td>Adinazolam</td>
<td>Cl CH₃N(CH₃)₂ -</td>
<td>7.18</td>
<td></td>
</tr>
<tr>
<td>Pyrazolam*</td>
<td>Br CH₃ - -</td>
<td>7.79</td>
<td></td>
</tr>
<tr>
<td>Nitrazolam</td>
<td>NO₂ CH₃ - -</td>
<td>8.34</td>
<td></td>
</tr>
</tbody>
</table>

*Pyrazolam has a 2-pyridyl ring at position 6 rather than a phenyl ring.
Table 3. Structural information and predicted binding values for thienotriazolodiazepines

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<th>Substitutions</th>
<th>Log 1/c predicted</th>
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</thead>
<tbody>
<tr>
<td>Deschloroetizolam</td>
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<td>7.96</td>
<td>![Basic structure of Deschloroetizolam]</td>
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<tr>
<td>Etizolam</td>
<td>CH₃ CH₂CH₃  Cl</td>
<td>8.64</td>
<td>![Basic structure of Etizolam]</td>
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<td>Metizolam</td>
<td>-  CH₂CH₃  Cl</td>
<td>8.34</td>
<td>![Basic structure of Metizolam]</td>
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Table 4. Structural information and a predicted binding value for an oxazolobenzodiazepine

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<th>Substitutions</th>
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<th>Basic Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flutazolam</td>
<td>Cl CH₂CH₂OH  F</td>
<td>6.83</td>
<td>![Basic structure of Flutazolam]</td>
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Table 5. Observed and predicted binding values for new psychoactive substances

<table>
<thead>
<tr>
<th>Compound</th>
<th>Log 1/c observed</th>
<th>Log 1/c predicted</th>
<th>% (log 1/c obs.) / (log 1/c pred.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adinazolam</td>
<td>6.87</td>
<td>7.18</td>
<td>95.9 %</td>
</tr>
<tr>
<td>Desalkylflurazepam</td>
<td>8.70</td>
<td>8.44</td>
<td>103.1 %</td>
</tr>
<tr>
<td>Desmethyflumitrazepam (fonazepam)</td>
<td>8.82</td>
<td>8.46</td>
<td>104.3 %</td>
</tr>
<tr>
<td>Etizolam</td>
<td>8.51</td>
<td>8.64</td>
<td>98.5 %</td>
</tr>
<tr>
<td>Meclonazepam</td>
<td>8.92</td>
<td>8.52</td>
<td>104.7 %</td>
</tr>
</tbody>
</table>
Table 6 Binding scores and molecular descriptors of the 10 compounds exhibiting the greatest binding affinity for the receptor

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Score</th>
<th>Number of Atoms in ligand</th>
<th>Number of rotatable torsions</th>
<th>Hydrogen Bond energy</th>
<th>Hydrophobic energy in exposing a surface to water</th>
<th>van der Waals interaction energy</th>
<th>Internal conformation energy of the ligand</th>
<th>Desolvation of exposed H-bond donors and acceptors</th>
<th>Solvation electrostatics energy change upon binding</th>
<th>Potential of mean force score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluunitrazolam</td>
<td>-17.9003</td>
<td>37</td>
<td>1</td>
<td>-1.55071</td>
<td>-6.12229</td>
<td>4.10324</td>
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Figures

Figure 1: The basic structural formula for benzodiazepines considered in this work.

Figure 2: Alignment of 69 training set benzodiazepines shown in two orientations.
Figure 3: Pharmacophore model of 33 compounds with binding values 8.0-9.0
Figure 4: Literature (i.e. observed) binding values (log 1/c) vs. QSAR predicted binding values fit with a partial least squares (PLS) regression ($R^2 = 0.90$).
Figure 5: Literature (i.e. observed) binding values (log 1/c) vs. QSAR predicted binding values for 9 compounds randomly selected for internal validation ($R^2 = 0.86$).
Figure 6: Visualisation of the NPS-benzodiazepine flunitrazolam binding to the allosteric site of the GABA_{A5} receptor
Highlights

- There is a deficiency in the pharmacological data available for new benzodiazepines.

- 69 benzodiazepines were used to develop a quantitative structure-activity relationship (QSAR).

- The resultant QSAR model returned an $R^2$ value of 0.90.

- This model will allow rapid prediction of the pharmacology of emerging benzodiazepines.