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Waters, Laura J., Manchester, Kieran R., Maskell, Peter D., Haegeman, Caroline and Haider, Shozeb

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

- 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.

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

3
4 **Abstract**

5 The illicit market for new psychoactive substances is forever expanding. Benzodiazepines
6 and their derivatives are one of a number of groups of these substances and thus far their
7 number has grown year upon year. For both forensic and clinical purposes it is important to
8 be able to rapidly understand these emerging substances. However as a consequence of the
9 illicit nature of these compounds, there is a deficiency in the pharmacological data available
10 for these 'new' benzodiazepines. In order to further understand the pharmacology of 'new'
11 benzodiazepines we utilised a quantitative structure-activity relationship (QSAR) approach.
12 A set of 69 benzodiazepine-based compounds was analysed to develop a QSAR training set
13 with respect to published binding values to GABA_A receptors. The QSAR model returned an
14 R² value of 0.90. The most influential factors were found to be the positioning of two H-bond
15 acceptors, two aromatic rings and a hydrophobic group. A test set of nine random compounds
16 was then selected for internal validation to determine the predictive ability of the model and
17 gave an R² value of 0.86 when comparing the binding values with their experimental data.
18 The QSAR model was then used to predict the binding for 22 benzodiazepines that are
19 classed as new psychoactive substances. This model will allow rapid prediction of the
20 binding activity of emerging benzodiazepines in a rapid and economic way, compared with
21 lengthy and expensive *in vitro/in vivo* analysis. This will enable forensic chemists and
22 toxicologists to better understand both recently developed compounds and prediction of
23 substances likely to emerge in the future.

24
25 **Keywords:** benzodiazepines; QSAR; biological activity; prediction; new psychoactive
26 substances; GABA_A receptor

27 **Introduction**

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

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

51 prevalent issue and it is likely that it will become an even more worrying trend as their
52 misuse continues to rise.

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

57 Quantitative structure-activity relationship (QSAR) models attempt to correlate molecular
58 structure to biological activity, often using a variety of molecular descriptors such as
59 physiochemical, topological, electronic and steric properties [23]. Typically, a set of
60 compounds whose biological activity is known is used to create a ‘training’ dataset and a
61 model. This model can then be used to predict the unknown biological activity of compounds
62 with a similar structure or to explore the structural features that are important for the specific
63 biological activity in question. QSAR has been extensively used for a variety of reasons such
64 as compound development in the pharmaceutical industry and the pharmacological
65 interpretation of drug-related deaths [24-26]. In terms of applications towards new
66 psychoactive substances, the predictive power of QSAR has been mainly applied to
67 cannabinoid binding to the CB₁ and CB₂ receptors [27-29] but has also been used to examine
68 the biological activity of hallucinogenic phenylalkylamines [30], the binding of
69 phenylalkylamines, tryptamines and LSD to the 5-HT_{2A} receptor [31] and methcathinone
70 selectivity for dopamine (DAT), norepinephrine (NAT) and serotonin transporters (SERT)
71 [32]. Currently, the majority of novel benzodiazepines have not been analysed to determine
72 their physicochemical and biological properties as this would require a substantial investment
73 in both time and money. It is for this reason that a fast, yet economical method to predict their
74 properties is desirable.

75 QSAR has previously been applied to benzodiazepines to predict bioavailability, absorption
76 rate, clearance, half-life and volume of distribution for a group of benzodiazepines. This
77 study included phenazepam [33], a benzodiazepine that appeared as an NPS in 2007 [34].
78 Other benzodiazepines (such as etaziolam) only appeared as new psychoactive substances in
79 the years following the publication of this study. Furthermore, the application of a QSAR
80 methodology has been used for modelling post-mortem redistribution of benzodiazepines
81 where a good model was obtained ($R^2 = 0.98$) in which energy, ionisation and molecular size
82 were found to exert significant impact [35]. Quantitative structure-toxicity relationships
83 (QSTR) have been used to correlate the toxicity of benzodiazepines to their structure in an
84 attempt to predict the toxicity of these compounds [36]. More recently, a study reported the
85 use of QSTR whereby it was concluded that it is possible to identify structural fragments
86 responsible for toxicity (the presence of amine and hydrazone substitutions as well as
87 saturated heterocyclic ring systems resulted in a greater toxicity) and potentially use this
88 information to create new, less toxic benzodiazepines for medical use [37].

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

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

100 **Methods and Materials**

101 **Selection of the dataset**

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

113 **QSAR/Software and Data Analysis Method**

114 The 88 benzodiazepines were converted from SMILES to 3D structures based on Merck
115 Molecular Force Field (MMFF) atom type and force field optimisation. These compounds
116 were then aligned by common substructure and confirmation to Ro 05-306. Subsequently, the
117 aligned compounds were clustered by Atomic Property Fields (APF) to identify
118 benzodiazepines with poor alignment. The APF method, designed by MolSoft, uses the
119 assignment of a 3D pharmacophore potential on a continuously distributed grid using physio-
120 chemical properties of the selected compound(s) to classify or superimpose compounds.
121 These properties include: hydrogen bond donors, acceptors, Sp² hybridisation, lipophilicity,
122 size, electropositivity/negativity and charge [45, 46]. Poorly aligned benzodiazepines
123 identified by APF clustering were subjected to re-alignment using APF-based flexible

124 superimposition. At this point, 10 benzodiazepines with poor alignment were removed to
125 improve model accuracy. (Supplementary Information Table 1S).

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

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

142 Further analysis of the PLS model fragment contributions from the 69 compounds was
143 conducted using SPCI software. Here, a 2D QSAR model was built using the same PLS
144 methodology as above. Additionally, a consensus model was created from averaging the
145 predictions of PLS, gradient boosting, support vector machine and random forest modelling
146 methods. The compounds were then subjected to automatic fragmentation and contribution
147 calculations, which resulted in information on 11 key contributing groups [48]. Using Ligand

148 Scout with default settings, four ligand-based pharmacophore models were created using
149 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
150 Figure 3.

151 Ten benzodiazepines that had the highest predicted binding values were docked into a
152 modelled GABA_{A5} receptor using ICM software. The GABA_{A5} receptor model was generated
153 by homology modelling, using the crystal structure of a human GABA(A)R-beta3
154 homopentamer (PDB id 4COF) as a template. A pre-defined binding site containing co-
155 crystallised benzodiazepine is already present in the template, which was retained in the final
156 model. Modeller software was used to generate the homology models [49]. The final chosen
157 model was energy minimized using the ACEMD software [50]. The stereochemistry was
158 checked using Procheck and ProSA software [51, 52]. The benzodiazepine in the allosteric
159 binding site on the GABA_{A5} receptor was used as a chemical template to dock NPS-
160 benzodiazepines and the best-scoring conformations were analysed.

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

163

164 **Results and Discussion**

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

168 From the pharmacophore model visualised in Figure 3 for highly bound benzodiazepines (log
169 1/c of 8.0 – 9.0), it is evident that important binding features for the benzodiazepines were the
170 positioning of two H-bond acceptors, two aromatic rings and a hydrophobic group all with
171 weights of 1.0.

172 The predicted binding values are not presented here but are listed in Supplementary
173 Information (Table S1). They can be visualised in Figure 4 as a plot of the observed binding
174 value versus the predicted binding value.

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

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

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

185 The NPS-benzodiazepine with the highest predicted log 1/c value was flunitrazolam with
186 8.88, closely followed by clonazolam with 8.86. However, based upon experimental data,

187 meclonazepam with a log $1/c$ value of 8.92 (8.52 predicted) actually exhibited the greatest
188 binding affinity. Only two benzodiazepines in the training set experimental values had a log
189 $1/c$ value of 8.92; these were meclonazepam and brotizolam with the rest falling below this
190 point. In general, the limitations to this model are most likely caused by the small size of the
191 data set. It is widely reported that QSAR models have poorer predictive capabilities with
192 training sets under 100 compounds [54, 55]. Moreover, the diversity of substitutions within
193 the small set of training compounds, created difficulties with APF superimposition and
194 therefore may have reduced the accuracy of the model predictors. Secondary modelling with
195 SPCI highlighted these limitations and demonstrated the existing dataset was less suitable for
196 PLS 2D QSAR modelling [48]. However, the consensus from multiple modelling methods
197 improves the predictive power of the 2D QSAR model. Additionally, as experimental errors
198 in the training set are amplified both by the logarithmic scale and when calculating the
199 weighted contributions, consistency and accuracy in the initial experimental values are
200 essential for a strong QSAR model. Ideally, further improvements to the model could be
201 made by using a larger training dataset with lower diversity yet this cannot be achievable as a
202 consequence of limitations on literature data available.

203 From these docking studies with the modelled GABA_{A5} receptor it can be seen that they only
204 partially occupy the available volume at the allosteric binding site (exemplified in Figure 6
205 for flunitrazolam). From the ten compounds that had the greatest binding affinity, four had
206 non-bonded interactions with the T80 region within the receptor, two had non-bonded
207 interactions with the K182 and S231 regions respectively. There were also stacking
208 interactions with the Y96 region for four of the compounds. Therefore the possibility is that
209 the binding is not completely optimal for these benzodiazepines and that with a modified
210 chemical structure, a greater binding affinity could be theoretically possible. The reality

211 exists that a benzodiazepine with an optimised binding affinity could emerge onto the illicit
212 drugs market and could potentially (but not necessarily) exhibit a greater potency.

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

215 There are 35 benzodiazepines and their derivatives currently subject to international control,
216 30 of these compounds had binding values listed in the original source [44]. The average log
217 $1/c$ value for these 30 controlled compounds was 7.57. Out of these compounds, 43 % (13 out
218 of 30) had a log $1/c$ value that was greater than 8.00. The average log $1/c$ value for the whole
219 training dataset was 7.81 and 48 % of the compounds (33 out of 69) had a log $1/c$ value that
220 was greater than 8.00. These values are fairly similar, however when comparing the results of
221 the benzodiazepines that are new psychoactive substances, the average log $1/c$ value that was
222 predicted was 8.22 and 68 % of the compounds (15 out of 22) had a log $1/c$ value that was
223 greater than 8.00. From this it is appears that benzodiazepines that are appearing as new
224 psychoactive substances are more likely to have a greater binding affinity at the GABA_A
225 receptor. Whether this trend is deliberate is unclear.

226 A log $1/c$ value of 7.88 was obtained for 4-chlorodiazepam (Ro 5-4864). This suggests a
227 relatively high affinity for the GABA_A receptor when compared with the log $1/c$ values for
228 clinically-used benzodiazepines; the binding value for diazepam is 8.09 and 8.40 for
229 triazolam. However it has been reported that the experimental value for 4-chlorodiazepam
230 (Ro-4864) is actually 3.79 (i.e. an IC₅₀ value of 160,500 nM) in one dataset when compared
231 with a log $1/c$ of 7.80 for diazepam and 8.72 for triazolam in the same dataset [56]. There are
232 obvious impracticalities with comparing different datasets as a result of differences in
233 methods (e.g. the use of [³H]-diazepam versus [³H]-flunitrazepam as a radioligand), the
234 differences in the species used (rat vs. mouse) and the differences in GABA_A receptor
235 expression between different brain homogenates. Despite this it is clear that 4-

236 chlorodiazepam observes an extremely low affinity for GABA_A receptors and one that this
237 model did not accurately predict. This most likely results from the deficit of compounds in
238 the training dataset that had a similar substitution on the R_{4'} position of the phenyl ring.
239 Indeed, this model focused upon the ‘classical’ 1,4-benzodiazepine, triazolobenzodiazepine,
240 imidazobenzodiazepine and thienotriazolodiazepine substitutions. Substitutions on the R_{4'}
241 position of the phenyl ring are known to exhibit strong steric repulsion at the GABA_A
242 receptor interface and therefore compound binding is severely inhibited [40] [57]. 4-
243 chlorodiazepam is an outlier and atypical benzodiazepine as it does not act upon the GABA_A
244 receptor; instead exerting its pharmacological effects through the translocator protein 18 kDa
245 (TSPO), previously known as the peripheral benzodiazepine receptor [58, 59].

246

247 The oxazolobenzodiazepine flutazolam, a prescription drug in Japan, had a predicted log 1/c
248 binding value of 6.83 which seems extremely low compared with the other benzodiazepines
249 in this dataset. To the best of the authors’ knowledge there exists no experimental GABA_A
250 receptor binding data for flutazolam. However other oxazolobenzodiazepines have low
251 affinities for the GABA_A receptor such as ketazolam with a log 1/c value of 5.89 [60] and
252 oxazolam with a log 1/c value of 5.00 [61]. These log 1/c binding values are from additional
253 sources – the previous paragraph discusses the difficulties in comparing binding values from
254 different datasets. Nonetheless it is clear that oxazolobenzodiazepines exhibit a much lower
255 affinity for the GABA_A receptor. If the value for flutazolam is correct then this QSAR
256 model successfully predicted the low binding affinity of flutazolam despite having no
257 oxazolobenzodiazepines in the training dataset which serves as an indicator to the potential
258 strength of the model.

259 **Conclusions**

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

270

271 **References**

272

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Tables

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

Name	Substitutions				Log 1/c predicted	Basic structure
	R ₇	R ₁	R ₂ '	R ₃		
Diclazepam	Cl	CH ₃	Cl	-	8.39	
Desalkylflurazepam	Cl	-	F	-	8.44	
Meclonazepam	NO ₂	-	Cl	CH ₃	8.52	
Phenazepam	Br	-	Cl	-	8.12	
Desmethylflunitrazepam	NO ₂	-	F	-	8.46	
3-hydroxyphenazepam	Br	-	Cl	OH	8.42	
Flubromazepam	F	-	Br	-	8.37	
Nifoxipam	NO ₂	-	F	OH	8.63	
Cloniprazepam	NO ₂	-	Cl	C ₃ H ₅ CH ₃	7.83	
Nimetazepam	NO ₂	CH ₃	-	-	7.87	
4-chlorodiazepam ^a	Cl	CH ₃	-	-	7.88	

^a4-chlorodiazepam has a Cl substituted on the R₇ position of the phenyl ring

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499 **Table 2. Structural information and predicted binding values for triazolobenzodiazepines**

Name	Substitutions				Log 1/c predicted	Basic structure
	R ₈	R ₁	R ₂ '	R ₄		
Flubromazolam	Br	CH ₃	F	-	8.77	
Clonazolam	NO ₂	CH ₃	Cl	-	8.86	
Flunitrazolam	NO ₂	CH ₃	F	-	8.88	
Bromazolam	NO ₂	CH ₃	-	-	8.25	
Adinazolam	Cl	CH ₃ N(CH ₃) ₂	-	-	7.18	
Pyrazolam ^a	Br	CH ₃	-	-	7.79	
Nitrazolam	NO ₂	CH ₃	-	-	8.34	

^aPyrazolam has a 2-pyridyl ring at position 6 rather than a phenyl ring

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502 **Table 3. Structural information and predicted binding values for thienotriazolodiazepines**

Name	Substitutions			Log 1/c predicted	Basic structure
	R ₉	R ₂	R ₂ '		
Deschloroetizolam	CH ₃	CH ₂ CH ₃	-	7.96	
Etizolam	CH ₃	CH ₂ CH ₃	Cl	8.64	
Metizolam	-	CH ₂ CH ₃	Cl	8.34	

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504 **Table 4. Structural information and a predicted binding value for an oxazolobenzodiazepine**

Name	Substitutions			Log 1/c predicted	Basic Structure
	R ₁₀	R ₇	R _{2'}		
Flutazolam	Cl	CH ₂ CH ₂ OH	F	6.83	

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507 **Table 5. Observed and predicted binding values for new psychoactive substances**

Compound	Log 1/c observed	Log 1/c predicted	% (log 1/c obs.) / (log 1/c pred.)
Adinazolam	6.87	7.18	95.9 %
Desalkylflurazepam	8.70	8.44	103.1 %
Desmethylflunitrazepam (fonazepam)	8.82	8.46	104.3 %
Etizolam	8.51	8.64	98.5 %
Meclonazepam	8.92	8.52	104.7 %

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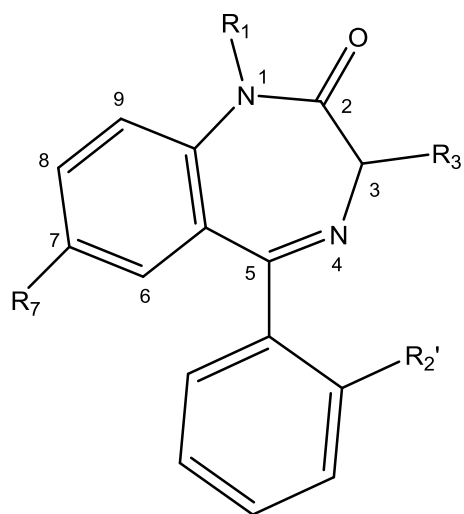
515 **Table 6.** Binding scores and molecular descriptors of the 10 compounds exhibiting the
 516 greatest binding affinity for the receptor

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Compound Name	Score	Number of Atoms in ligand	Number of rotatable torsions	Hydrogen Bond energy	hydrophobic energy in exposing a surface to water	van der Waals interaction energy	internal conformation energy of the ligand	desolvation of exposed h-bond donors and acceptors	solvation electrostatics energy change upon binding	potential of mean force score
Flunitrazolam	-17.9003	37	1	-1.55071	-6.12229	-27.3992	4.10324	10.7377	13.4407	-158.403
Clonazolam	-15.4617	37	1	-1.53992	-6.124	-27.9233	7.64508	11.6698	16.8309	-154.162
Flubromazolam	-18.2738	35	0	-1.61755	-6.89366	-25.8773	3.57746	11.0855	12.122	-151.357
Etizolam	-18.7025	38	1	-2.03733	-7.14073	-25.5154	7.89581	11.8052	11.0572	-101.516
Nifoxipam	-20.836	33	2	-5.90608	-4.9646	-22.352	6.0639	12.5432	13.905	-129.57
Meclonazepam	-13.4447	35	1	-2.27939	-5.98463	-21.8787	5.69717	10.6159	14.6192	-124.257
Desmethylflunitrazepam	-15.5192	32	2	-0.82246	-5.27009	-26.2114	2.37454	10.376	11.0938	-144.474
Desalkylflurazepam	-21.7837	30	0	-2.01574	-5.82939	-27.462	0.691701	9.53716	11.4106	-154.372
Diclazepam	-16.8002	33	0	-0.60989	-6.76567	-25.688	2.00693	10.3028	10.9647	-121.093
Metizolam	-13.7614	35	1	-1.78622	-6.65559	-24.7768	3.51234	14.5321	12.8708	-138.056

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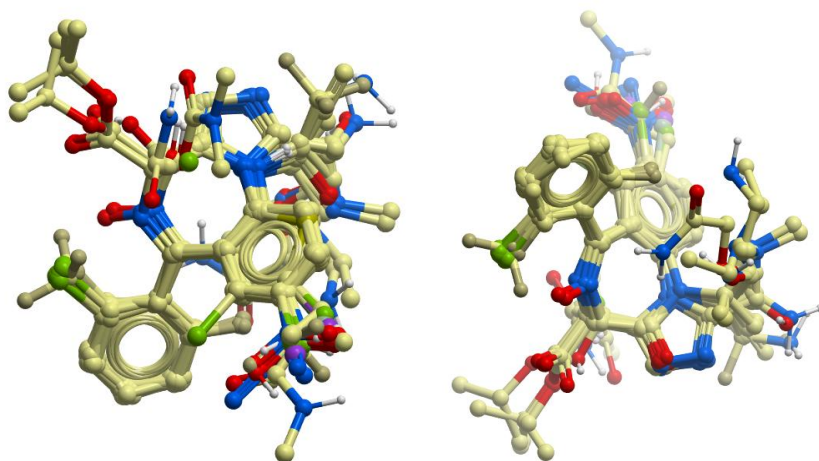
536 **Figures**
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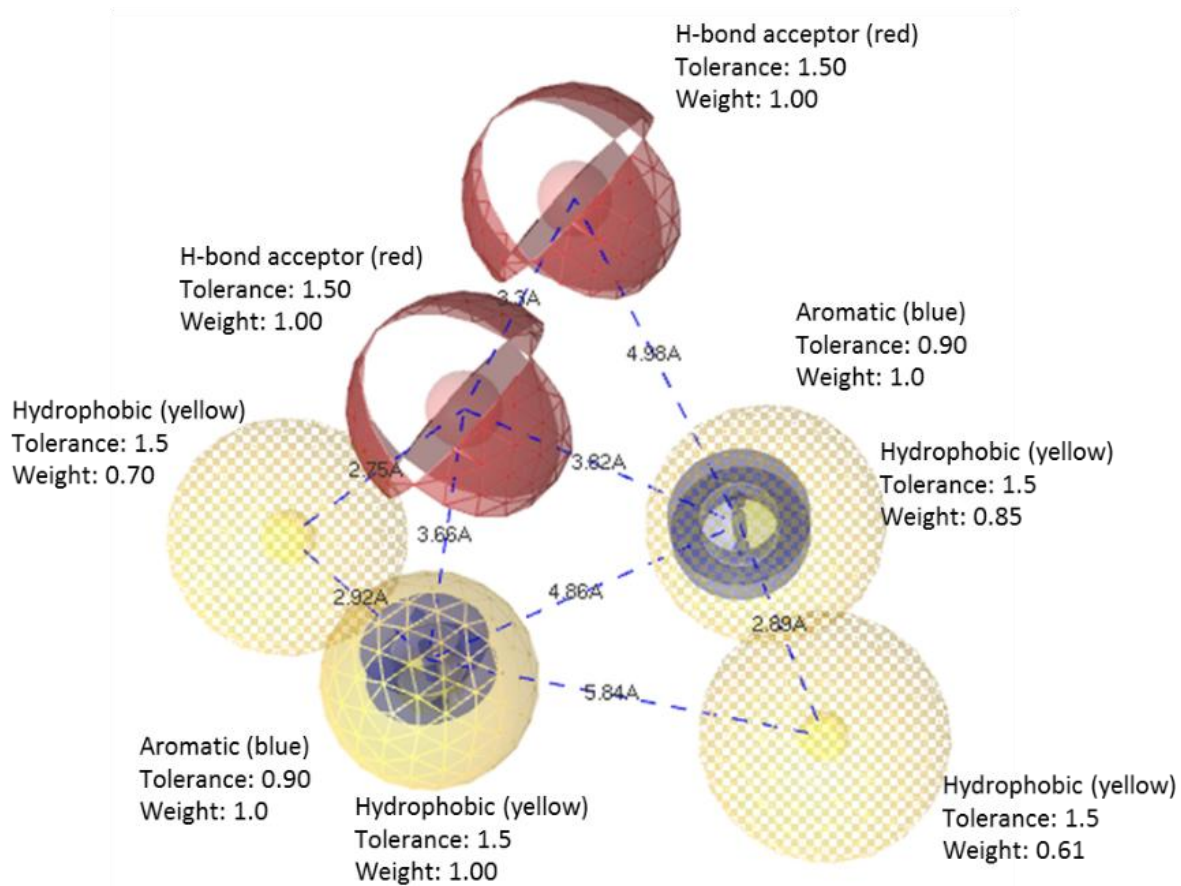
539 **Figure 1: The basic structural formula for benzodiazepines considered in this work**

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542 **Figure 2: Alignment of 69 training set benzodiazepines shown in two orientations.**

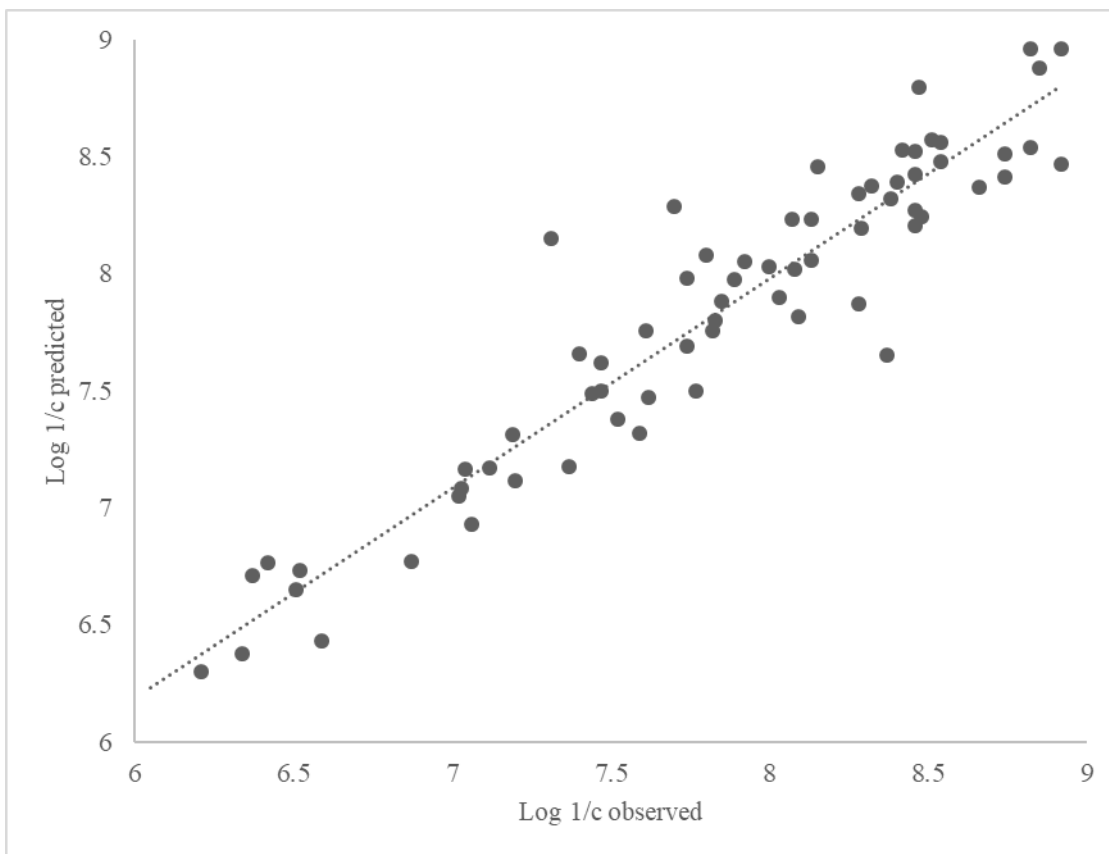


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544 **Figure 3: Pharmacophore model of 33 compounds with binding values 8.0-9.0**

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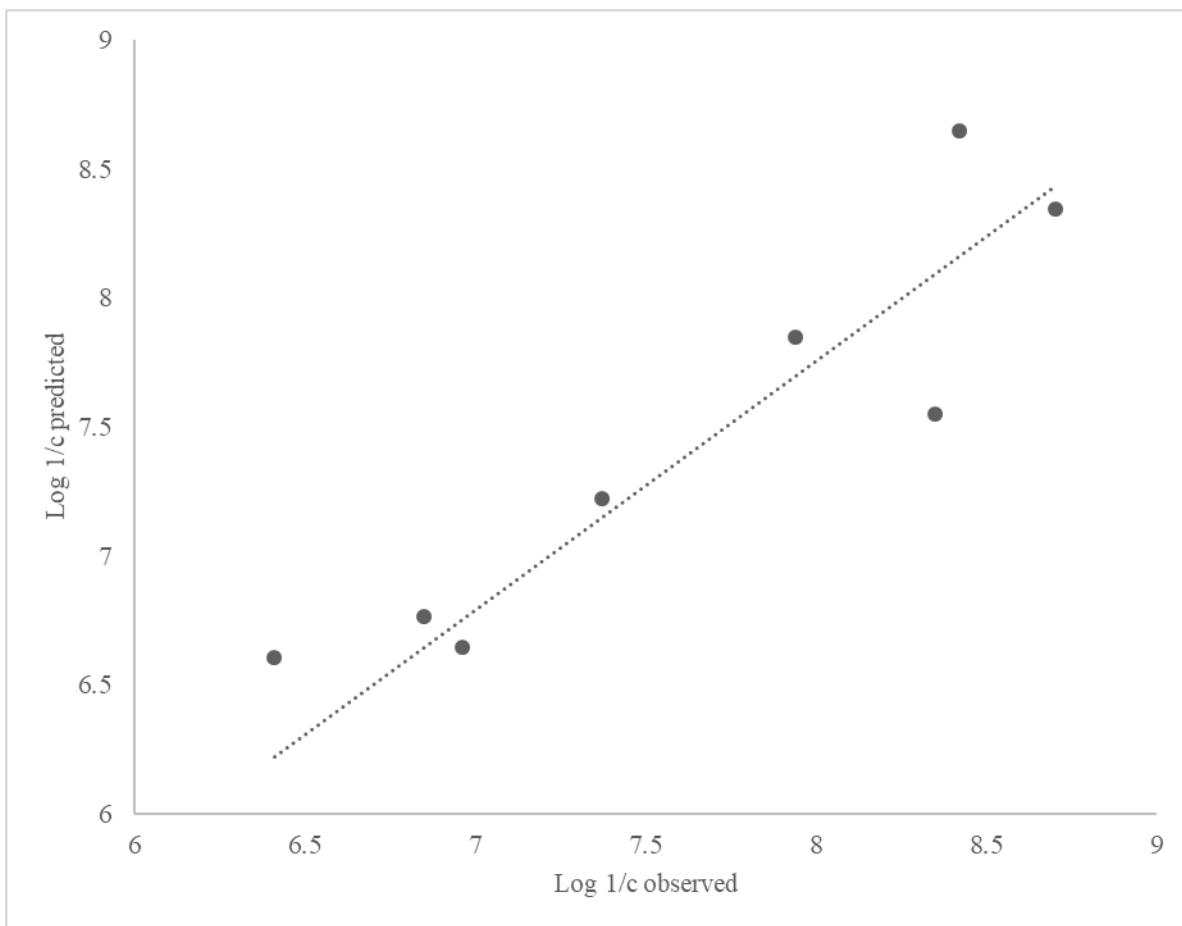
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548 **Figure 4:** Literature (i.e. observed) binding values (log 1/c) vs. QSAR predicted binding
549 values fit with a partial least squares (PLS) regression ($R^2 = 0.90$).

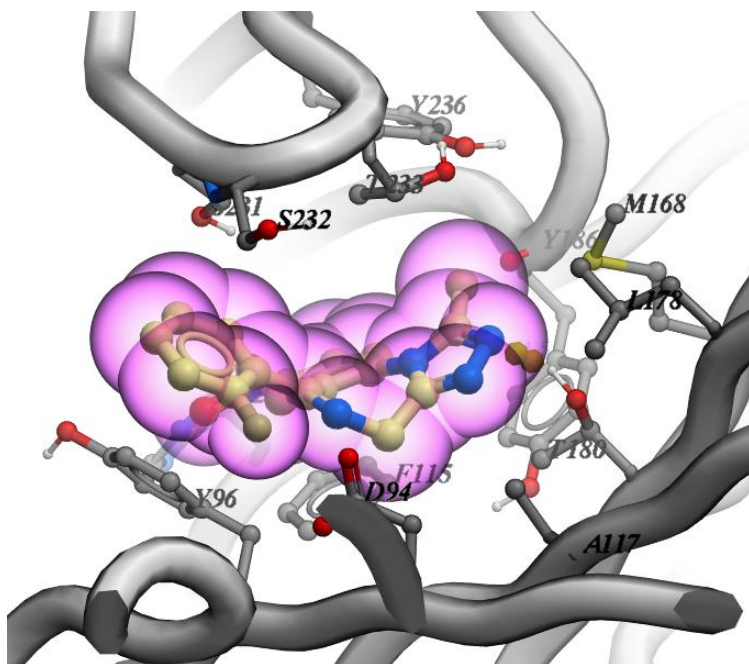
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552 **Figure 5:** Literature (i.e. observed) binding values (log 1/c) vs. QSAR predicted binding
553 values for 9 compounds randomly selected for internal validation ($R^2 = 0.86$).

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557 **Figure 6:** Visualisation of the NPS-benzodiazepine flunitrazolam binding to the allosteric
558 site of the GABA_{A5} receptor

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