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QSAR design of triazolopyridine mGlu2 receptor positive allosteric modulators

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ABSTRACT

Two QSAR approaches were applied to assist the design and to prioritise the synthesis of new active mGlu2 receptor positive allosteric modulators (PAMs). With the aim to explore a particular point of substitution the models successfully prioritised molecules originating from chemistry ideas and a large virtual library. The two methods, 3D topomer CoMFA and support vector machines with 2D ECFP6 fingerprints, delivered good correlation and success in this prospective application. Fourteen molecules with different substituent decoration were identified by the *in silico* models and synthesised. They were found to be highly active and their mGlu2 receptor PAM activity (pEC_{50}) was predicted within 0.3 and 0.4 log units of error with the two methods. The value of the molecules and the models for the future of the project is discussed.

1. Introduction

Glutamate is the major excitatory neurotransmitter in the central nervous system (CNS) and modulates synaptic responses by activating ionotropic glutamate (iGlu) receptors and G protein-coupled glutamate receptors termed metabotropic glutamate (mGlu) receptors [1]. mGlu2 and mGlu3 receptors reduce transmission at glutamatergic synapses in brain regions where excessive glutamate activity may be implicated in the pathophysiology of anxiety and schizophrenia [2]. There is substantial pre-clinical evidence that mGlu2 receptor activation may provide anxiolytic and/or antipsychotic effects [3–10]. Indeed, mGlu2/3 receptor agonists have been tested in clinic in schizophrenia and anxiety related studies [11–15]. Despite this progress, activation of mGlu2 receptor with agonists presents challenges of selectivity, receptor desensitisation and brain penetration. Therefore, positive allosteric modulators (PAMs) have emerged as an attractive alternative to activate the mGlu2 receptor [16]. Allosteric mGlu modulators generally have better selectivity (less amino acid conservation at allosteric sites compared to the orthosteric site) and improved central nervous system (CNS) penetration (not requiring amino-acid functionality and therefore being less polar). In addition allosteric modulators only exert their effects in the presence of endogenous glutamate leading to less liability of receptor desensitisation [17–19] whilst also responding to physiological glutamate fluctuations. Given these potential advantages, the number of reported selective mGlu2 receptor PAM chemical series continues to increase [20,21]. Extensive preclinical data [22–31] supports the value of this approach and to date two mGlu2 receptor PAMs have advanced to clinical trials (AZD8529 [32,33] and JNJ-40411813 [34–38] also known as ADX71149).

Within our internal mGlu2 receptor PAM program the triazolopyridine scaffold [31] (Fig. 1) was identified as a series of particular importance and subjected to thorough exploration. The scaffold is a SAR evolution of the imidazopyridine core which was discovered by scaffold hopping from a high throughput screen (HTS) pyridone hit series [29]. The initial exploration at R3 focussed on aromatic substituents but subsequent amine substitution led to promising advanced leads such as **1** (JNJ-42153605) with mGlu2 receptor PAM functional activity of pEC_{50} of 7.8 ($-\log EC_{50}$, 17 nM). Interestingly, the introduction of spirocyclic amines at the R3 position delivered highly potent compounds such as **2**, **3** and **4**. In order to gain further SAR understanding and determine the scope of amine substitution we began considering ideas containing structurally complex diamines such as **5** and **6**. As many of these complex diamines, including spiro ring fusion, stereocenters and aromatic decoration, are often commercially unavailable, further prioritisation was required to help assist design and focus synthesis efforts. Up to this time no previous applications of QSAR to the design of

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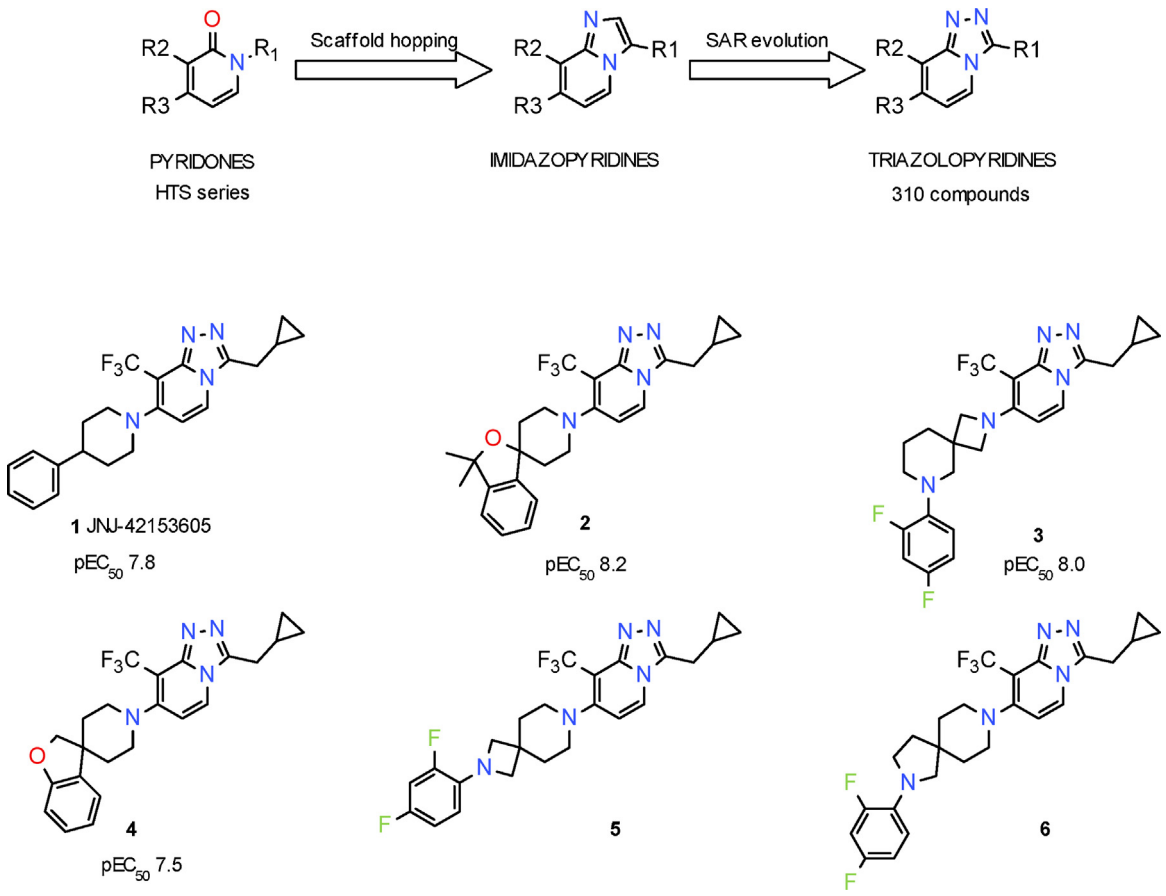

Fig. 1. (Top) Evolution of our mGlu2 receptor PAM lead series also showing the R1, R2 and R3 substitution. Triazolopyridine example active compounds **1** (JNJ-42153605) to **4**, and two molecules **5** and **6** shown as examples of the ideas being considered for synthesis in this study.

mGlu2 receptor allosteric modulators have been reported, and had not been attempted internally by us.

With a wealth of active triazolopyridines synthesised and no experimental receptor structure available, ligand based Quantitative Structure Activity Relationship (QSAR) approaches were applied with various considerations for suitable methods. The molecules in Fig. 1 had apparent 3D structural variation between linear and curved geometries and it was speculated that 3D descriptors might be relevant. Consistent SAR provided confidence to overlay molecules of the same chemical series permitting 3D QSAR. Despite this, it was clear that many of the training and target molecules had high 2D similarity, often containing small variations on piperidine and similar amine substituents. Therefore a QSAR methodology using 2D molecular fingerprints was included to contrast with the 3D approach. With hundreds of training set compounds and an application of the model to a large set of ideas and virtual compounds the modelling approaches needed minimal manual intervention.

Two approaches were used to build local QSAR models to search for new R3 substituents on the triazolopyridine mGlu2 receptor PAM scaffold. The methods, topomer CoMFA [39], and support vector machine (SVM) using ECFP6 fingerprint descriptors meet the considerations described above. Topomer CoMFA combines topomers [40] with the well-established Comparative Molecular Field Analysis (CoMFA) [41] approach to provide a new variant for 3D QSAR based on partial least squares (PLS) regression correlation of steric and electrostatic fields with activity. A topomer is a 3D conformation of a fragment generated by a rule-based approach from the 2D structure and independent of receptor or solvent. Whilst this appears unrealistic and possibly undesirable, the result could be beneficial for many applications of 3D QSAR although there are still few independent studies demonstrating this. In the triazolopyridine case here, full molecules were fragmented into a scaffold and R-groups according to Fig. 1. The R-group substituents were converted to topomers from which fields were calculated to correlate with and predict activity. The same R-group in two different molecules will have identical geometry and therefore CoMFA fields. This reduces noise going into the model and the difference in activity is rationalised in terms of only the difference in the parts of the molecule which have changed, thereby being a faithful 3D translation of the similarity principle [42]. In its original form support vector machine modelling is a supervised learning approach which constructs a hyperplane(s) best separating categories, here it is used for regression analysis [43]. Implemented in the R package within Pipeline Pilot and coupled with fingerprint descriptors, this approach offers a powerful and high performing QSAR method commonly used in contemporary applications [44]. SVM performance can be poor for large datasets (i.e. tens of thousands of molecules or more) but this was not considered to be a concern for this application. A more relevant issue in this study is the lack of easy interpretability of the predictions which could be detrimental for communication and decision making in a project team.

At the time of performing this study, a set of 310 triazolopyridines with measured pEC_{50} mGlu2 receptor PAM activities ranging from 4.3 (50 μM) to 8.8 (1.6 nM) was available. These molecules were used as input for local QSAR models built with the two approaches. The models were applied to the prospective design of new amine R3 substituents. Subsequent to this work the project continued and a further 353 more molecules were synthesised containing this scaffold, referred to as the 'future' set of

compounds. The performance of the models in both the prospective R3 substituent design study and for the future synthesised compounds is discussed. The QSAR approach proved to be successful for the identification of new active mGlu2 receptor PAM molecules in the prospective design phase. To the best of our knowledge this manuscript is amongst the first prospective applications of topomer CoMFA for the design, synthesis and identification of novel bioactive molecules. These molecules were of value to open new chemical space for future exploration. Although the 3D and 2D QSAR models had similar statistical performance, they did not deliver similar predicted activities over the large virtual library. Therefore the methods were capturing different features in the R3 substitution position of the mGlu2 receptor PAMs. The statistical value of the models declined as the project evolved.

2. Experimental details

2.1. [^{35}S]GTPγS binding assay

Measurement of mGlu2 positive allosteric modulatory activity of test compounds in membranes containing human mGlu2 was performed using frozen membranes that were thawed and briefly homogenised prior to pre-incubation in 96-well microplates (15 mg/assay well, 30 min, 30 °C) in assay buffer (50 mM HEPES pH 7.4, 100 mM NaCl, 3 mM $MgCl_2$, 50 μM GDP, 10 mg ml^{-1} saponin) with increasing concentrations of positive allosteric modulator (from 0.3 nM to 50 μM) and either a minimal pre-determined concentration of glutamate (PAM assay), or no added glutamate. For the PAM assay, membranes were pre-incubated with glutamate at EC_{25} concentration, i.e. a concentration that gives 25% of the maximal response glutamate. After addition of [^{35}S]GTPγS (0.1 nM, f.c.) to achieve a total reaction volume of 200 μl, microplates were shaken briefly and further incubated to allow [^{35}S]GTPγS incorporation on activation (30 min, 30 °C). The reaction was stopped by rapid vacuum filtration over glass-fibre filter plates (Unifilter 96-well GF/B filter plates, Perkin-Elmer, Downers Grove, USA) microplate using a 96-well plate cell harvester (Filtermate, Perkin-Elmer, USA), and then by washing three times with 300 μl of ice-cold wash buffer ($Na_2PO_4{\cdot}2H_2O$ 10 mM, $NaH_2PO_4{\cdot}H_2O$ 10 mM, pH = 7.4). Filters were then air-dried, and 40 ml of liquid scintillation cocktail (Microscint-O) was added to each well, and membrane-bound [^{35}S]GTPγS was measured in a 96-well scintillation plate reader (Top-Count, Perkin-Elmer, USA). Non-specific [^{35}S]GTPγS binding is determined in the presence of cold 10 mM GTP. Each curve was performed at least three times using duplicate sample per data point and at 11 concentrations. Many compounds are also measured in additional independent experiments on subsequent dates.

2.2. Molecule preparation and QSAR models

The QSAR model building was performed on the triazolopyridine chemical series, Fig. 1. There were 310 molecules matching the tri-substituted scaffold and having measured functional [^{35}S]GTPγS binding pEC_{50}'s ranging from 4.3 (50 μM) to 8.8 (1.6 nM). Molecules with unmeasurable pEC_{50}'s due to inactivity up to the maximum concentration cut-off were excluded. Molecules were prepared and descriptors calculated as suitable for the two different QSAR approaches.

For topomer CoMFA, molecule preparation and descriptor calculation is handled automatically by the software, within the Sybyl suite from Certara. The molecules are input in their full structures. The topomer fragments become the 3D-QSAR poses, with each training set ligand (as a "2D" structure) being cut apart at the three selected acyclic substituent bonds shown in Fig. 1 and topomers then being generated automatically from the resulting R-groups (monovalent fragments). Rather than the classical single CoMFA column, a completed topomer CoMFA table contains multiple "CoMFA columns", each column containing the set of comparable ligand R-groups. Molecules for training sets were imported from SD files. Hydrogens and charges were calculated automatically. The CoMFA lattice was automatically generated according to defaults as a 2 Angstrom grid whose *X*, *Y*, and *Z* coordinates extend 4 Angstroms beyond the maximum and minimum *X*, *Y*, and *Z* among all atomic coordinates. The number of model components that minimised the lowest leave-one-out cross-validation standard error of prediction (SDEP, $\sqrt{(\sum (pEC_{50}^{pred} - pEC_{50}^{expt})^2/n)}$) was chosen for the final model.

The support vector machine (SVM) QSAR models were built in Pipeline Pilot [45]. For the QSAR modelling in pipeline pilot molecules were prepared by removing salts, adding hydrogens and standardising molecules. Ionisation states were assigned using the ionise molecule component set at pH 7.4. Extended connectivity fingerprints (ECFP6) [46] were calculated also in Pipeline Pilot, folded to a fixed-length array of counts of 128 bits and used as descriptors for the SVM model. The SVM was set to learn the continuous range of bioactivity using an epsilon regression approach ensuring the residuals did not exceed 0.1 (epsilon). The SVM model parameters Gamma was set at 1/nx where nx is the number of descriptor properties. The cost variable associated with training set errors was set as either 1.0 or 2.0. Leave-one-out cross validation was used to provide comparison with the topomer CoMFA models. Whilst it is understood that more sophisticated cross validation or feature selection can improve statistical performance of QSAR models, in this case such approaches were not employed.

2.3. Testing QSAR model performance

To assess the performance and stability of the QSAR modelling approaches the available 310 molecules were partitioned into training and test sets. The dataset was randomly partitioned twenty times into 90% training and 10% test sets. Then, twenty separate models were built with both the topomer CoMFA and the SVM-ECFP6 methods. Model performance was inspected via the internal correlation r^2, the leave one out (LOO) cross validated correlation q^2 and the associated standard error of prediction (SDEP). Comparing parameters over all twenty models provides information about the stability of the QSAR approach for these molecules and the sensitivity to small changes in training sets. Y-scrambling is a valuable tool to check for the possibility of overtraining or possibility of identifying misleading artificial models [47]. This was performed here and no chance models, with coincidental correlation between descriptors and response, were found. For instance, a topomer CoMFA model built on Y-scrambled data of the best performing training set delivered an r^2 and LOO q^2 of 0.06 and −0.05 respectively.

2.4. Applying QSAR to propose new molecules

Two approaches were used to gather ideas for profiling with the QSAR models and ultimately consider for synthesis. Firstly, novel SAR derived ideas were proposed by project chemists. In addition, a virtual library (VL) was constructed to explore a much larger chemical space. The virtual library was built by enumerating the scaffold motif with decoration pre-installed in R1 and R2 positions as shown in Fig. 2. Commercially available primary and secondary amines were introduced at R3. Special treatment was assigned to deprotect and elaborate protected diamines as illustrated for piperazine in Fig. 2. The protecting group was removed and the open valence capped with hydrogen, phenyl and benzyl groups representing different plausible decoration. Products were

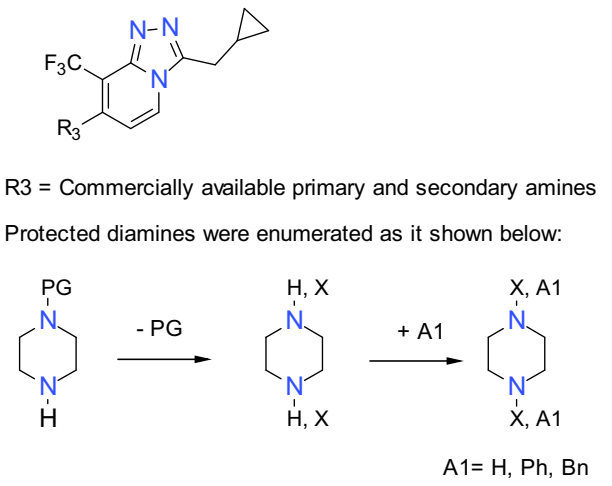

Fig. 2. Virtual library of triazolopyridine compounds used for profiling with the mGlu2 receptor PAM QSAR models and proposing for synthesis.

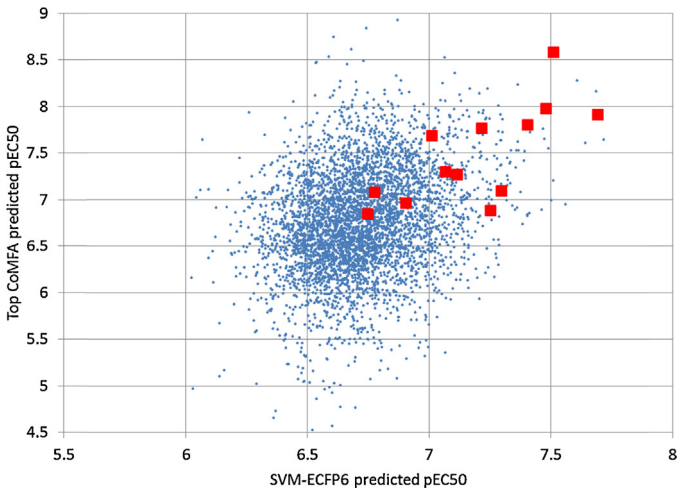

Fig. 3. Application of the topomer CoMFA and SVM-ECFP6 QSAR models to all idea and virtual compounds. The molecules selected for synthesis are highlighted as large red squares.

then enumerated and filtered using calculated physicochemical properties (removing MW > 500, *A* log P > 6, Si, P, nRB > 10, nHA > 8, nHD > 2, and PSA > 80), which reduced 10110 virtual products to 4900 unique molecules. The best performing topomer CoMFA and SVM-ECFP6 models with the lowest q^2 SDEP were then used to predict activities for all ideas and virtual compounds.

2.5. Visualising the chemical space

Visual analysis of the diversity of the combined chemical space was performed to provide a means to contextualise the similarity of the molecules. The 2D reduction was generated with stochastic proximity embedding (SPE). A conceptually simple approach, SPE iteratively refines a starting arrangement of objects by repeatedly selecting pairs at random, and adjusting their coordinates so that their distances on the map match more closely their respective proximities. The resulting Euclidean embedding optimally preserves the similarities between the related observations. SPE has been applied to both diversity visualisation and 3D conformer generation [48,49]. In the high-performance parallel implementation here it is coupled with in house developed Fingerprints, similar to ECFP4. The Tanimoto distance is then used as the similarity metric to create the SPE projections. This was performed with the in-house program ABCD [50].

3. Results

The input dataset was partitioned randomly into twenty different training and test sets as described in the experimental section. Both topomer CoMFA and SVM-ECFP6 QSAR models were built and applied for all training and test sets. Statistical properties are presented in Table 1. The columns describe the correlation between experimental mGlu2 receptor PAM functional activity and predicted activity firstly for the training set (r^2), secondly for the internal LOO cross validation (q^2) with the associated standard error of prediction (SDEP, as expected in log units of pEC_{50}), thirdly for the external test set (r^2 and SDEP) and finally the correlation r^2 and error SDEP for the prospective design of new compounds. The first two rows are from the QSAR building and testing phase and data are averaged over all twenty models, the standard deviation is given in parentheses for each property. The last two rows are the statistical properties for the single model from each method with the best performance as defined by largest r^2 on the external test set.

Immediately striking is the similar performance between the two methods. For instance, average cross validated q^2 and SDEP were 0.63 and 0.46 for topomer CoMFA models and 0.64 and 0.46 for SVM-ECFP6. There were small improvements for SVM-ECFP6 in SDEP applied to the external test sets when averaged over all models. Both methods had good performance and were capable of delivering predicted pEC_{50} activities within less than 0.5 log unit of error (for both cross validation and external test sets). The similarity in predictive ability between the cross validation and the external test sets was satisfactory and provides confidence in the value of the models for predicting mGlu2 receptor PAM activity on further new molecules. In general the standard deviations of the statistical properties averaged over the twenty training sets were small, suggesting little variation and sensitivity to training set composition, again offering confidence in the QSAR models and predictions for these datasets. The q^2 of the external test sets had the largest standard deviation, but the standard deviation in predicted activity (SDEP) was still relatively small and similar, 0.07 and 0.06, for topomer CoMFA and SVM-ECFP6 approaches respectively. Comparison of the chemical space coverage of the training and test sets for the best performing individual model did not produce concerns of a limited test set. On the contrary, the test set had good and even coverage of the chemical space occupied by the training set.

The best model from each method was then applied to the SAR based designed compounds and the virtual library to generate predicted pEC_{50}'s for over 5000 compounds, Fig. 3. The plot shows a broad range of predicted activity and interestingly there is no correlation between the 3D and 2D methods on this large set of virtual compounds, r^2 0.06. Compounds with high predicted activity by either or both methods, generally $pEC_{50} > 7$, were scrutinised further and amongst these a total of fourteen were selected and synthesised. Molecules were subsequently screened for mGlu2 receptor PAM activity in the functional [^{35}S]GTPγS binding assay. Excellent results were seen, actives were found displaying efficacious pEC_{50}'s in the range 6.1–8.5. The correlation of predicted to experimental activities is shown in Fig. 4. Reasonable correlation was seen with r^2 of 0.60 and 0.55 for topomer CoMFA and SVM-ECFP6 respectively. Reassuringly this was closely in line with the performance of the methods on the external test set when averaged over the twenty models. As these molecules span a smaller range in activity of less than 2.5 log units, the r^2 may not be an ideal metric for assessing the value of the predicted activities. In addition, the SDEP of just 0.27 and 0.41 demonstrate the accuracy of the model predictions and their high value for molecular design.

Table 1
Statistical parameters for QSAR models, see text for more details. Training and test set statistics are averaged over twenty different random separations of the total 310 input compounds (standard deviations of the properties are given in parenthesis). Prospective design was performed using the best performing single model.

	Training	LOO cross-validation		External test set		Prospective design	
	r^2	q^2	SDEP	r^2	SDEP	r^2	SDEP
Averaged over 20 training and test sets							
Top-CoMFA	0.89 (0.04)	0.63 (0.03)	0.46 (0.02)	0.60 (0.13)	0.45 (0.07)	N/A	N/A
SVM-ECFP6	0.96 (0.01)	0.64 (0.02)	0.46 (0.01)	0.65 (0.16)	0.31 (0.06)	N/A	N/A
Best individual model							
Top-CoMFA	0.93	0.64	0.45	0.80	0.31	0.60	0.27
SVM-ECFP6	0.95	0.61	0.48	0.83	0.23	0.55	0.41

The statistical performance of the methods should be placed in the context of the standard deviation of the pEC_{50} over the compounds in the training set and the error in the experimental assay pEC_{50} measurement. Although the range of pEC_{50} activity in the training set was large, ~4.5 log units, the standard deviation was in comparison quite small, 0.73. The rule of thumb that approximately two thirds of data in a normal distribution lay within ±1 standard deviation indicates that a majority of compounds cluster around the mean pEC_{50} of 6.9, such datasets can be a challenge for QSAR. Also, if the statistical performance of the models, as measured by the error in prediction of 0.27 and 0.41 for the CoMFA 3D and SVM-ECFP6 QSAR methods respectively, was superior to the error in the experiment itself then it is likely that the models suffer from the artefacts of over training. For this work the reported experimental mGlu2 receptor PAM functional pEC_{50}'s were measured in triplicate with eleven concentration response. Our mGlu2 receptor PAM drug discovery program ran for many years and active compounds from various chemical series have been assayed [20,21,27–31,35]. During this time, 1474 compounds from multiple chemical series with measurable pEC_{50}'s were tested at least twice in independent triplicate experiments, which could include new membrane preparations or new batches of compounds. The average experimental standard deviation for these repeat independent experiments was 0.1 log units of pEC_{50} whilst the average standard error was 0.07. Although the number of repeat experiments for each compound is often too small to assume normal distribution behaviour, these low values indicate small spread in experimental results for repeat testing. Therefore, the performance of the QSAR models is close to the accuracy limit of the experiment but is not outperforming it, a result which would have suggested model over training.

Molecules **5**–**9** are examples of the active molecules from the prospective QSAR application, design and synthesis and are shown in Fig. 5. All fourteen synthesised molecules contained the same scaffold, R1 and R2 groups, but presented new amine substituents at the R3 position. The amine groups are in some cases close analogues of previously seen sidechains but with specific decoration, such as **7**. Others, such as **5**, **6** and **9** were similar to **3** for example but were prioritised from a list of additional potential spirodiamines. The most active newly synthesised molecule identified overall, not shown in Fig. 5, was an analogue of **2**, proposed as one of various ideas from chemistry with alternative halogen decoration on the distal spiro-phenyl. As inferred from the SDEP, the agreement between predicted and experimental pEC_{50} mGlu2 receptor PAM activity was in most cases very good. Of those shown in Fig. 5, molecule **7** was the most active with mGlu2 receptor PAM pEC_{50} of 8.1. Molecule **7** is an intriguing close analogue of **1**. The molecule originated as a chemistry idea (not from the VL). In the canonical derivation of the 3D topomeric conformations the R3 substituents in **1** and **7** differ only in the introduction of the methyl group in the 3 position of the piperidine. As shown in Fig. 6 the two R3 groups are perfectly overlapped. Regardless of whether induced changes in the orientation of the distal 4-phenyl group may be expected by the presence of the 3-Me group, the topomer CoMFA philosophy is to correlate only the introduced structural change with the activity difference. The methyl reaches a preferred region of steric space and this contributes to an increased steric field contribution to the model prediction, 2.8 compared to 2.5 for R3 in **1**. Therefore, the use of the topomer approach with CoMFA, whilst greatly facilitating the model building process, does not permit full structural relaxation and behaves as a pseudo-3D or 2.5D methodology.

Four subsets of triazolopyridine compounds are discussed in this work: the training set used to build the QSAR models, the idea and VL molecules assessed with the QSAR models, the prospectively designed and synthesised molecules and finally the future set of compounds made after this work. The chemical diversity and clustering of the different sets was analysed by applying the SPE approach as described in the Method section. The resulting plot shown in Fig. 7 captures the similarity of molecules based

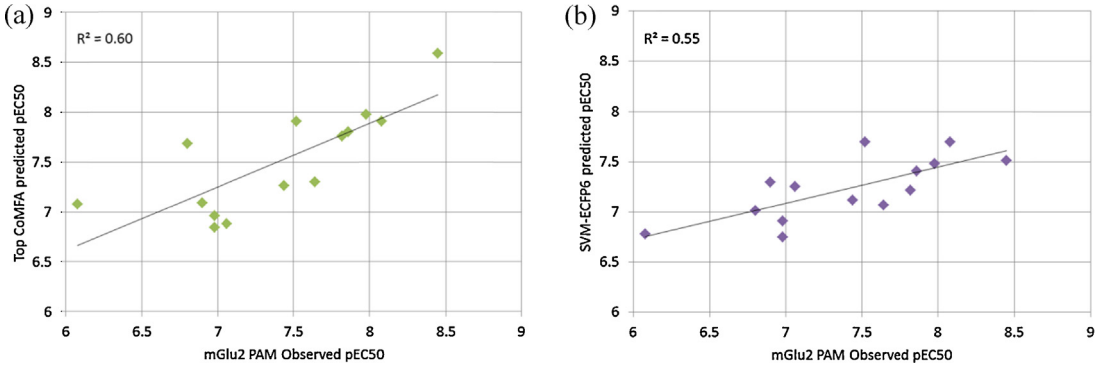

Fig. 4. Prospective design of 14 new mGlu2 receptor PAM molecules synthesised based on QSAR recommendations. Comparison of the predicted versus observed experimental mGlu2 receptor PAM activities calculated using the best topomer CoMFA (a) and SVM-ECFP6 (b) models. Compounds originated from the chemistry ideas and virtual library and were synthesised on the basis of high predicted activity.

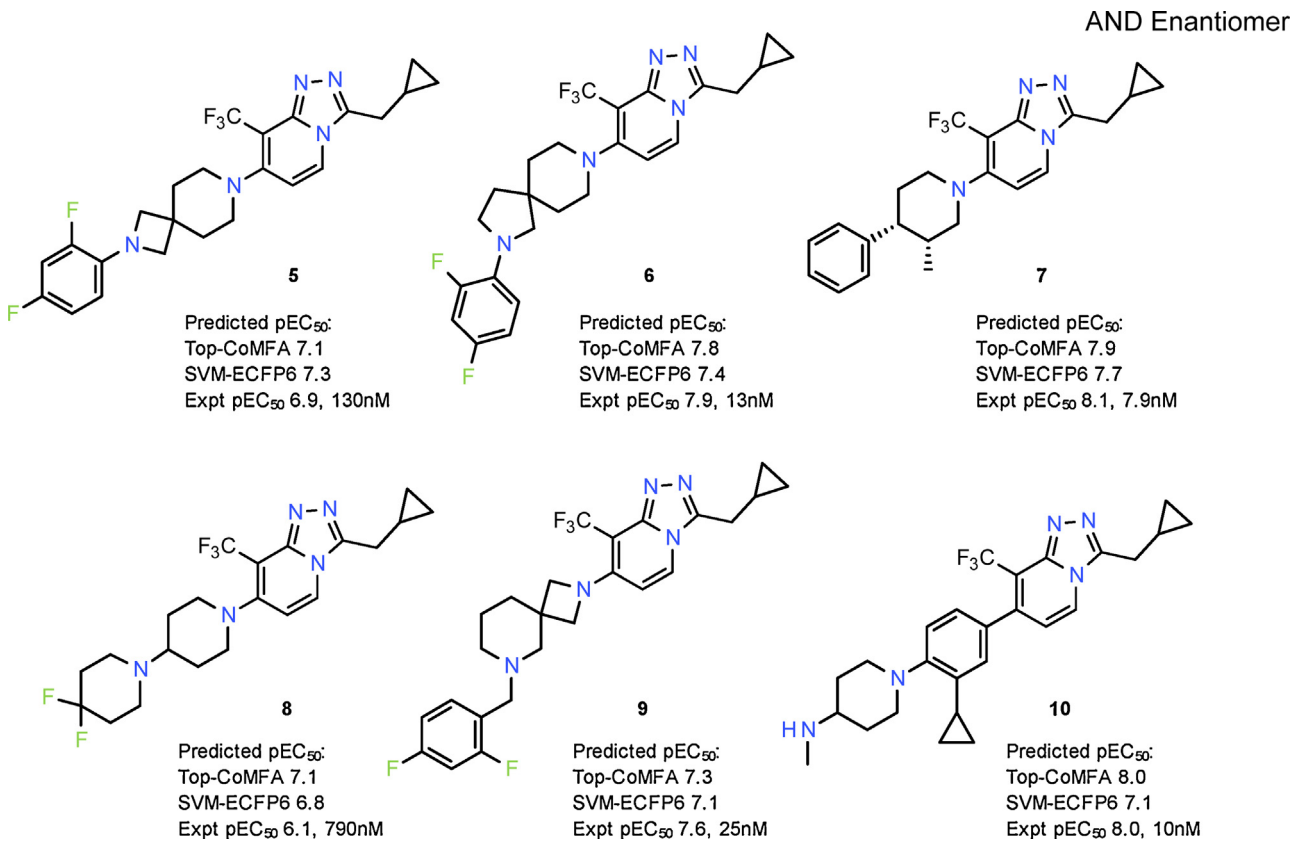

Fig. 5. Molecules **5**–**9** recommended for synthesis based on QSAR predictions by both CoMFA and SVM models. Molecule **10** is an example of a future compound well predicted by 3D topomer CoMFA approach.

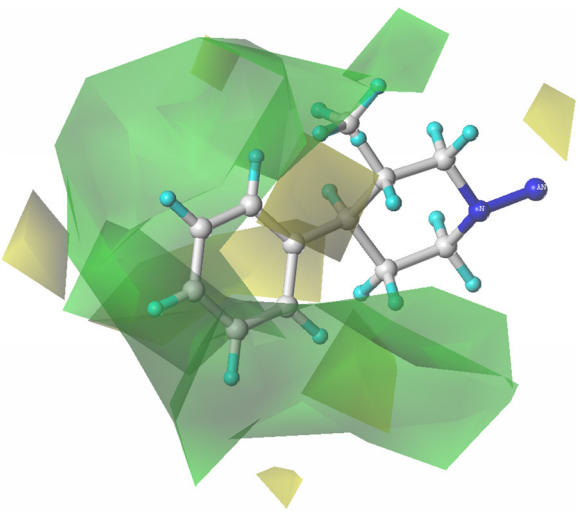

Fig. 6. Overlay of the topomer representation of the R3 substituent from molecules **1** and **7**. Green is favourable and yellow is unfavourable steric regions. The sidechains are perfectly overlapping and indistinguishable except the substituent from **7** contains a methyl group at the 3 position of the piperidine ring.

on the underlying chemical structural fingerprints. All molecules contained the same triazolopyridine scaffold with different R1, R2 and R3 substitution. The points on the plot represent individual molecules which when clustered closely together in the 2D dimensional reduction is indicative of higher structural similarity. The figure therefore provides a means to contextualise the similarity of the molecules. The overwhelming majority of molecules (4900) were from the VL. Whereas the training, prospective design, and future sets were much smaller: 310, 14 and 353 molecules respectively. Therefore, as expected the VL spans and defines the entire range of the chemical space. The mGlu2 receptor PAM active molecules are understandably clustered in small regions of bioactive chemical space and large areas of the VL are likely to contain only inactive molecules. The prospective designed compounds tend to move away from the majority of the training set but do not depart completely from training set chemical space. This likely explains the good performance of the model at predicting mGlu2 receptor PAM activity for these molecules. Some of the future set of compounds can be found in similar chemical space to the training set, however, there are clusters such as those in the top right and bottom left extremes of the plot which are dissimilar to the training set and do not overlap in chemical space.

4. Discussion

Topomer CoMFA and SVM-ECFP6 QSAR models have been applied to the design of new mGlu2 receptor PAMs exploring the R3 scaffold substitution position (Fig. 1). Brief comments were made of this application in a manuscript prepared during the time of conducting this study [51]. Here, details and a more thorough analysis, revealing strengths and weaknesses are provided for the first time along with discussion of the deeper relevance for the project.

The similarity in statistical performance between 3D and 2D QSAR models in Table 1 may lead to an incorrect assumption that the R3 substituent does not depend on 3D structure, or alternatively that the important 3D properties are captured in an implicit way by 2D descriptors. It was our understanding when performing this study, and proposing synthetic ideas such as **5** and **6**, that

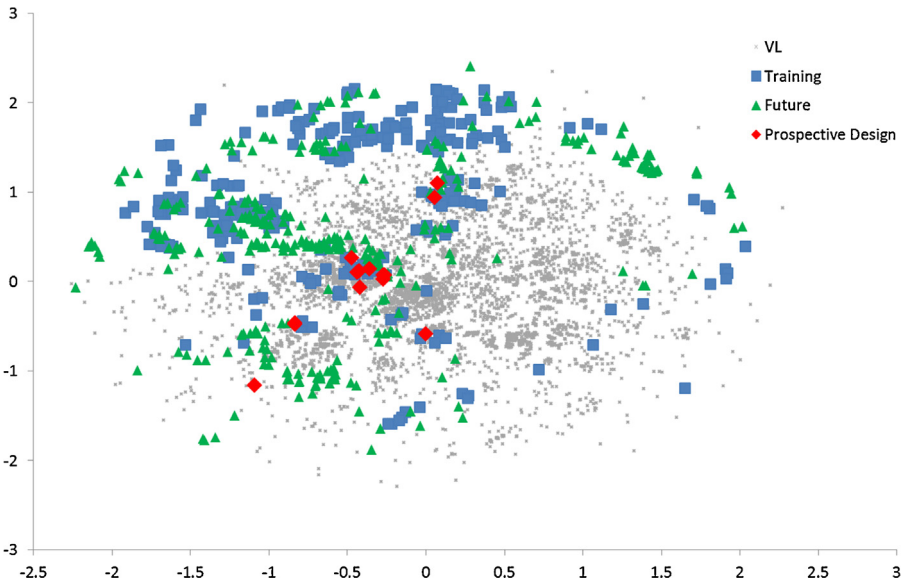

Fig. 7. Diversity map generated for triazolopyridine molecules studied: VL and ideas (small grey crosses), training set (blue squares), prospective designed and synthesised compounds (red diamonds), future synthesised molecules (green triangles). Axes are two dimensions generated with SPE using a fingerprint Tanimoto similarity metric.

consistent 3D conformations exist in this region of the molecules. It was known that the R3 position permitted quite broad structural variation for mGlu2 receptor PAMs, but existing SAR and previous overlay work all suggested preferred geometries and vectors were present [29]. As such it was a specific aim of this work to provide a quantitative assessment of how ideas such as **5** and **6** 'fit' in 3D with synthesised examples such as **1**, **2** and **3**. Possible explanations for the similar statistical properties are likely due to the dominant 2D similarity of the compounds in the training set. Known inactive molecules were omitted from the training set due to having unmeasurable pEC_{50}'s at the concentration cut-off. Hence a small number of molecules with significantly different 3D geometry at R3 were not included. This was likely detrimental for capturing the gross 3D effects. Another likely factor is that the canonical topomer conformations are highly dependent themselves on atom connectivity and do not provide unrestricted 3D energetic relaxations of the fragments. Also, this apparent similarity in 2D and 3D models was compounded by selecting molecules for synthesis in the prospective application which were close to the diagonal in Fig. 3 and had good predicted activity by both methods. Anyhow, despite the similar performance statistics the overall lack of correlation (r^2 0.06) between predicted activities in Fig. 3 confirms that the two models are capturing different effects. Further inspection of Fig. 3 and comparison with molecules synthesised after this study reveals different examples with good predicted activity by both one approach and not the other.

The QSAR models were firstly applied to a combined series of ideas arising from the project team medicinal chemists and a large virtual library and then subsequently to 'future' molecules synthesised after this study. Fig. 7 provides visual analysis of the diversity of the combined chemical space. The figure highlights the value of the QSAR prospective design. In general the molecules were distinct from the majority of the training set which tends to cluster on the left and top of the plot. The prospective design compounds were more centrally placed and occupy areas with only a few training set examples along with molecules subsequently synthesised in the future design set. This indicates how the molecules helped to direct and focus exploration on areas of chemical space which at the time of model building had a much lower degree of exploration. Eventually future compounds moved into newer areas of chemical space outside of both the training and prospective designed sets of compounds.

To consider the robustness of the 3D topomer CoMFA and 2D SVM ECFP6 QSAR models for continued use, and further understand their differences, they were applied to predict the activity of all compounds synthesised on the same chemical scaffold in the following months and years after this study. In total an additional 353 molecules were synthesised after this prospective QSAR design experiment was completed, referred to as the future set of molecules in the previous sections. CoMFA models are widely considered to be useful only for local QSAR modelling, suitable for design of close analogues to the training set. The correlation between predicted and observed experimental mGlu2 receptor PAM pEC_{50} activity was very poor when evaluated across the entire dataset, with r^2 being just 0.10 and 0.12 for topomer CoMFA and SVM-ECFP6 respectively. This full set of compounds has similar mean and standard deviation in pEC_{50} functional activity compared to the training set, 6.8 and 0.75 respectively. Whilst this indicates a relatively challenging spread of data for a typical QSAR application, the data structure is not inferior to the training set and therefore is not the source of the poor predictivity. The running 20 average predicted error in activity is shown in Fig. 8 for compounds in sequential order of registration in our internal J&J compound database. The topomer CoMFA method had a better performance for compounds synthesised in the period immediately after this work, with SDEP in the range of just 0.3 log units of pEC_{50}. There are then a group of compounds for which performance between the 3D and 2D approaches were similar, after which the SVM begins to perform substantially better. Closer inspection of the 150th to 250th compounds synthesised after this study provides an interesting explanation. In this period a series of molecules were pursued which introduced a methylene spacer at R3, directly between the scaffold and a cyclic tertiary amine substituent, i.e. molecules analogous to **1** but containing a CH_2 group between the scaffold and the piperidine [52]. Activity was maintained in a satisfactory range, typically with pEC_{50} between 6 and 7.5, and these molecules were extensively explored as they offered interesting profiles with improvements in solubility. Nevertheless, the relevance for this work is that such molecules are perceived by 2D fingerprint descriptors to be highly similar and are predicted

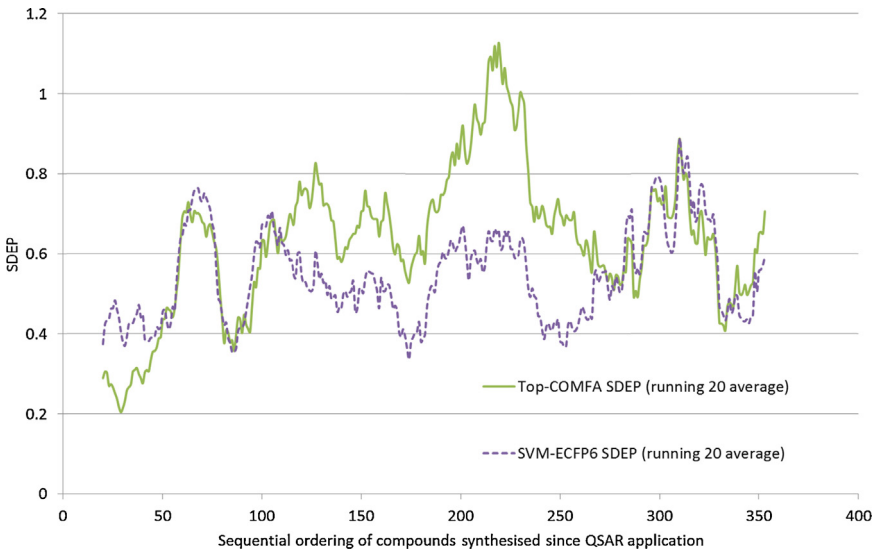

Fig. 8. Variation in SDEP (standard error of prediction) for QSAR models applied to compounds synthesised after the application in this study. Curves show the running 20 average of SDEP.

with SDEP in the range of 0.5–0.6 by the SVM-ECFP6 model. However, the methylene at the attachment point delivers a bent 3D conformation dissimilar to linear examples such as **1** for instance. Therefore the 3D topomer CoMFA model had difficulty to accurately predict the activity of these compounds with SDEP as high as 1.0 log unit as there were no such similar examples in the training set. Conversely, there were molecules with trisubstituted phenyl groups at R3 such as **10** (Fig. 5) which conserved a similar linear 3D geometry but have quite different underlying atom connectivity compared to previously discussed training set examples. As such the topomer CoMFA model predicted more accurately than the 2D SVM-ECFP6 approach. Finally, looking at compounds in the 300–350th range there is again closer agreement in mGlu2 receptor PAM predicted activity. Overall, the 2D SVM-ECFP6 model has less variation in SDEP across the entire range of compounds, 0.3–0.9, whereas the topomer CoMFA model SDEP is between 0.2 and 1.1. It should not be overlooked that the 2D bias of typical medicinal chemistry optimisation is very likely to influence this comparison. The tendency to introduce previously active and already known substructures whilst exploring a novel structural modification is common and justified for many reasons. It is evident here with the aforementioned homologated methylene containing compounds which provide a very different 3D conformation, but are often decorated with previously known motifs such as 4-phenylpiperidine and analogous groups. The analogue bias and this way of navigating chemical space favour 2D methods in retrospective comparisons of this type [53].

It is also worthy to note that this experiment set out to assess the ability of the QSAR methods for identifying new R3 substitution on the triazolopyridine scaffold originating from two sources: a VL of computationally generated compounds and sketched ideas originating from the project team. As discussed above, the selection for synthesis focussed on compounds predicted to be highly active by both methods (Fig. 3) and many compounds with high predicted potency were considered. Ultimately, compounds had to be prioritised for synthesis by team members and there was careful scrutiny amongst the highly ranked molecules, inevitably this imparted some bias and it is highly likely to have had positive effects on the success criteria and perceived model performance. Also, this experiment did not include negative controls, namely selection, synthesis and biological testing of compounds predicted to be inactive.

5. Conclusions

In this work the successful application of QSAR for the identification of new active triazolopyridine mGlu2 receptor PAMs with alternative R3 substitution has been described. The aims were met and the QSAR approach served for *in silico* profiling of a large virtual library and idea space. Overall, two approaches were used and robust models built and applied. This work provides an example of the prospective application and value of 3D CoMFA QSAR coupled with topomers for automated alignment, and is one of the first reported independent applications of the technology. Whilst the models had similar statistical performance they were capturing different properties, as shown by the lack of correlation between predicted activities by the two methods. New actives were found which moved into regions of structural diversity largely distinct from the majority of training set compounds. Model performance broke down over the time course of the project likely explained by the structural diversity of future molecules compared to the training set. The 2D QSAR method based on ECFP6 fingerprints was less unstable to structural variation in the future compounds than the 3D topomer CoMFA mode. The circumstances in this project were ideal for QSAR application and success. There was an abundance of SAR for many close analogues with a range of more than 3 log units of activity. Also, the molecules could be fragmented in an ideal way to permit topomeric alignment and therefore making efficient use of a large dataset for field based QSAR. Perhaps most importantly, there was a committed interest from a medicinal chemistry point of view to continue exploring the series and its substitution to find new actives.

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