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Evaluation of the EVA Descriptor for QSAR Studies: 3. The use of a Genetic Algorithm to Search for Models with Enhanced Predictive Properties (EVA_GA)

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Summary

The EVA structural descriptor, based upon calculated fundamental molecular vibrational frequencies, has proved to be an effective descriptor for both QSAR and database similarity calculations. The descriptor is sensitive to 3D structure but has an advantage over field-based 3D-QSAR methods inasmuch as structural superposition is not required. The original technique involves a standardisation method wherein uniform Gaussians of fixed standard deviation (σ) are used to smear out frequencies projected onto a linear scale. This smearing function permits the overlap of proximal frequencies and thence the extraction of a fixed dimensional descriptor regardless of the number and precise values of the frequencies. It is proposed here that there exist optimal localised values of σ in different spectral regions; that is, the overlap of frequencies using uniform Gaussians may, at certain points in the spectrum, either be insufficient to pick up relationships where they exist or mix up information to such an extent that significant correlations are obscured by noise. A genetic algorithm is used to search for optimal localised σ values using crossvalidated PLS regression scores as the fitness score to be optimised. The resultant models are then validated against a previously unseen test set of compounds. The performance of EVA_GA is compared to that of EVA and analogous CoMFA studies.

Introduction

EVA is a molecular descriptor that is derived from calculated fundamental infra-red (IR) and Raman range vibrational frequencies [1,2,3]. The descriptor has the advantage over popular 3D-QSAR methods such as CoMFA [4] in as much as it is invariant to rotation and translation of the structures concerned and it is therefore not necessary to superpose compounds in order to provide descriptors. Extensive studies [2,3] have indicated that EVA can successfully be used to develop QSAR models for a range of different structural classes, exhibiting various degrees of conformational freedom and with a variety of biological endpoints. These studies also found that EVA, like field-based 3D-QSAR, can perform well

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with heterogeneous sets of structures. In most cases the EVA models were found to be statistically entirely comparable to those obtained using CoMFA but without the difficulties associated with structural superposition. A detailed study with a benchmark steroid dataset [3] indicated that EVA can provide statistically robust QSAR models when this is judged by the scores from internal crossvalidation, random permutation tests and external test set prediction.

This paper describes a modification to the way in which the EVA descriptor is calculated that has been developed with a view to providing QSAR models with enhanced internal and external predictivity. The "classical" EVA descriptor (henceforth referred to as EVA) is derived by projecting normal mode frequencies (NMFs) onto a linear scale and then smearing them out using Gaussian kernels such that proximal frequencies are permitted to overlap. A fixed-dimensional standardised descriptor is then extracted for any chosen molecule, as described in more detail below. Previously, for a given analysis, EVA has been extracted using Gaussian kernels of fixed standard deviation (σ) across the spectrum. This is necessary because it means that each frequency (i.e., each part of the spectrum) is equally weighted prior to regression analysis. It has been found that the quality of the QSAR model very often is dependent upon the chosen σ and that the best σ to use can vary substantially [2,3]. The general approach [3] has been to generate many sets of EVA descriptors based upon a variety of σ and, on the basis of training set crossvalidation results, select a σ expansion term that is expected to provide an optimally predictive model for a previously unseen test set. The effectiveness of this model-selection method has been clearly demonstrated with a steroid dataset [3].

In the work described herein σ has been permitted to have localised values at different regions on the linear scale. This approach should permit the determination of an optimal or near-optimal overlap of kernels across the spectrum, where the quality of this overlap is judged by the scores from subsequent PLS regression using the derived descriptor matrix. The basis of this study is the postulate that there exist localised values of σ associated with different regions of the spectrum that provide improved internal and external predictivity relative to those obtained with any model based on a fixed σ term. At the same time there is a requirement to search for an optimal set of these σ values and, for reasons explained below, a genetic algorithm (GA) has been used to direct this search. PLS [5] crossvalidation regression scores are used as the fitness function to be optimised by the GA. The proposed technique is fundamentally different from more standard variable selection techniques [6-8] in as much as variables are not included/excluded by the procedure; rather, it is their

information content (i.e., the selection of frequencies contributing to a variable) that is altered through the adjustment of kernel overlap.

An incentive for the development of this new approach to EVA, referred to hereafter as EVA_GA, is that there are a number of datasets with which EVA has previously not performed well [2], either in absolute terms or relative to CoMFA – the reasons for this under-performance are not apparent. In addition to the potential for providing improved QSAR predictions, it may be the case that the use of localised σ improves the possibilities for interpretation of an EVA QSAR model (i.e., back-tracking to structure).

Methods

Notation

The following is an alphabetic list of abbreviations related to PLS analyses: A – number of PLS LVs; CV – crossvalidation; F – Fischer significance score; G – number of CV groups; LOO – leave-one-out CV (G = M); LNO – leave-n-out CV, where n > 1; LV_{opt} – optimum number of latent variables (LVs); M – number of training set molecules; pr^2 – test set predictive-r^2; PRESS – predictive residual sum of squares; q^2 – LOO CV r^2; r^2 – fitted model r^2; SE and SE_{CV} – Standard Error and CV-SE; TG – training set; X – matrix of descriptor variables; Y – dependent variable (bioactivity etc.)

For the EVA descriptor and EVA_GA the following abbreviations are used: BFS – linear bounded frequency scale; CONV_CRIT – difference between fitness scores of the least and most fit members of a GA population; H – Hamming threshold; LV_{max} – number of LVs evaluated by EVA_GA; MAX_CYCLES – maximum number of GA generations; N – number of atoms in a molecule; NBINS – number of bins into which BFS is divided; NMF – normal mode frequency; NPOP – number of GA chromosomes; R – set of r σ values from which elements of V are selected; V – a GA chromosome; V_{opt} – an optimal V.

Software and Hardware

All the work described herein was carried out using a multiprocessor Silicon Graphics Origin 200 R10000. The molecular modelling software used was Sybyl 6.3 [9]. The software required to run the GA, to perform the EVA standardisation process and to do the GA-related PLS analyses was custom-written in the C programming language.

Classical EVA

The EVA descriptor [1,2] is derived from fundamental molecular vibrational frequencies of which there are 3N-6 (or 3N-5 for a linear compound such as acetylene) for an N-atom structure. The frequency values are projected onto a linear bounded frequency scale covering the range 1 to 4,000 cm^{-1} and then smeared out, and therefore overlapped, through the application of Gaussian kernels to each and every frequency value. Finally, the BFS is sampled at fixed intervals of L cm^{-1}. The value of the EVA descriptor at a point, x, on the BFS is the sum of amplitudes of the overlapped kernels at that point:

$$EVA_x = \sum_{i=1}^{3N-6} \frac{1}{\sigma\sqrt{2p}} e^{-(x-f_i)^2/2\sigma^2}$$

where f_i is the i^{th} normal mode frequency of the compound concerned.

This process is repeated for each dataset structure, thus providing a descriptor of fixed dimension for all compounds. Typically a descriptor set may be derived using a σ of 10 cm^{-1} and an L of 5 cm^{-1} resulting in 800 (4,000/L) descriptor variables [1]. The number of variables is thus very much larger than the number of compounds in a standard QSAR dataset and the Partial least squares to Latent Structures (PLS) technique [5] has been used to provide a robust regression analysis. The purpose of the EVA smoothing procedure is not to simulate an experimental IR spectrum (transition dipole data is not used and, therefore, all kernels are of fixed maximum amplitude) but rather it is to apply a density function such that vibrations at slightly different frequencies in different compounds can be "overlapped" and thus compared with one another. The extent of this overlap is governed by σ and the proximity of vibrations on the BFS.

Localising σ

In classical EVA the kernels have a uniform fixed standard deviation (equal width, height and shape) for all frequencies in all compounds while, as stated above, σ is here permitted to have localised values in different spectral regions. The local values are to be selected so as to improve model predictivity and, as with the selection of a suitable fixed σ value, training set CV is used to select an optimal set of localised σ.

There are a number of ways in which the concept of a localised σ might be applied to EVA-descriptor generation. It is possible to associate each and every NMF in each and every compound of a dataset with its own localised σ value. Such a scheme would, however, only be appropriate were there not to be a requirement to make external test predictions, since there would be no way of assigning localised σ values for the test set compounds without

including them in the optimisation procedure. In addition, the number of adjustable parameters would be extremely large (M × (3N-6)) for typical QSAR datasets. It was, therefore, decided to divide the BFS into NBINS bins of equal width (w), with each of which a localised σ value is associated. The Gaussian kernel for any frequency in any structure whose value falls within a given bin (spectral sub-region) is thus expanded using the σ associated with that bin. NBINS (4,000/w) is thus independent of both M and the number of NMFs (proportional to N). A potential solution is thus a vector, V, consisting of NBINS elements:

$$V = \{ \sigma_1, \sigma_2, \sigma_3, \ldots \sigma_{NBINS-2}, \sigma_{NBINS-1}, \sigma_{NBINS} \}$$

Each of the NBINS sub-regions cannot be independently evaluated because the information content of descriptors located in adjacent bins is generally not independent: except where σ is very small indeed, kernels centred in adjacent bins tend to overlap one another thus adding additional signal (or noise) to the descriptors concerned. The extent of such overlap depends upon the relative frequency values and the local σ applied. Only the main spectral sub-regions (the fingerprint / functional group-stretching and hydrogen-stretching) are sufficiently far apart on the BFS such that there is no overlap unless σ were to be extremely large (Figure 1).

Without imposing constraints upon the values that local σ can assume the search space is huge; e.g., if only integer values in the range 1 to 50 cm^{-1} were to be permitted then full coverage of σ space would require a search of 50^{NBINS} permutations. Therefore, a restriction is placed on the values that local σ can assume and are taken from a user-defined r element vector (R) where:

$$R = \{ \sigma_i, \sigma_{ii}, \ldots, \sigma_{r-1}, \sigma_r \}$$

A suitable set of values for R may, for example, be {5, 10, 15, 20, 40} and a solution V has elements taken from R. The use of a representative set of discrete values such as these is justified since previous work has shown that for small changes in σ there tends to be little difference in the ensuing PLS scores [2,3]. For a particular dataset, R may be selected to reflect results obtained when using a range of fixed σ values; i.e., one may wish to bias the solution toward previously obtained results. The total number of permutations where r = 5 is thus 5^{NBINS} which, where w = 40 cm^{-1}, is equivalent to 5^{100} (~10^{70}). In practice there are substantial regions of the IR spectrum in which there tend to be no NMFs (Figure 1), particularly outside the skeletal region (~1,500–4,000 cm^{-1}). This feature means that, for the melatonin data set described below, and where w = 40 cm^{-1}, NBINS is reduced from 100 to

62. This significantly reduces the available permutations to 5^{62} (~10^{43}) but nonetheless remains a large search space. A σ value of zero has not been permitted here since this would allow NMFs to be omitted from consideration altogether, although this may form the basis of a variable selection procedure.

A second problem that arises with the use of localised σ values is that, without some form of scaling, there will be variance that is related solely to the chosen σ (i.e., kernel maximum amplitude differences) rather than to differences in frequency value location on the BFS. Therefore, all kernels are scaled to a maximum amplitude of unity prior to determining the local EVA descriptor values. This means that the kernels differ only in terms of their width, and to a lesser extent, shape (Figure 2) rather than height, shape and width.

Searching for an Optimal Solution (V_{opt}) ˜ EVA_GA

As stated previously the number of possible solutions to be explored is immense and all possible permutations of the elements of V cannot be evaluated systematically. Therefore, a technique is required that permits a sampling of the search space in as thorough a manner as possible without the requirement to cover that space in its entirety. Genetic algorithms [10,11] provide an obvious and convenient means to approach the stated problem. GAs are now a well-established stochastic technique for performing directed random searches of a problem space and have been widely applied to drug design and chemometric problems [12]. A wide variety of alternative formulations are available the selection of which are to some extent arbitrary; details of the chosen methods are given below while Figure 3 is a generalised overall schema for EVA_GA.

A. Chromosome encoding. In the current context a chromosome conveniently consists of the vector, V, described above. In order to ensure the diversity of the initial population a Hamming threshold (H) was applied such that at the outset each chromosome was permitted to have a maximum of H genes of identical value to those of any other chromosome. The minimum possible value of H depends upon NPOP, NBINS and the number of possible different values associated with each bin (r).

B. Chromosome fitness evaluation. The chromosome fitness function is the q^2 score from PLS CV based upon an EVA descriptor set derived using V; the higher the q^2 score the greater the chromosome fitness. Both LOO and LNO CV have been implemented ˜ the advantages and disadvantages of these two approaches are discussed below. The SAMPLS algorithm [13] provides a highly efficient implementation of PLS-1 and, for univariate Y only, gives identical results to the classical NIPALS [5] and SIMPLS [14] algorithms.

SAMPLS is based upon reduction of the X block data to an M-by-M covariance matrix of all the pair wise "distances" between each of M molecular descriptor vectors which is then used to fit all PLS LVs *independent* of the original number of variables. SAMPLS was custom-written so as to provide a very efficient implementation and full integration with the GA and EVA descriptor code; for example, with M = 21 and 1000 variables LOO CV using five LVs required only ~0.01 seconds.

C. Reproduction. The reproductive stage involves three steps; viz. parent selection, crossover and mutation. Parents are selected using the roulette wheel method whereby parents are selected in a probabilistic manner in which those with a higher fitness are more likely to be selected than those with lower fitness. However, an *élitist* model [15] also was implemented in which the best member of the current parent population is forced to be in next generation. Both single and double crossover points are permitted, the selection of which is done at random as are the points at which crossover takes place. Mutation is permitted at random points on a randomly selected chromosome – a chromosome may be selected for mutation (or crossover) more than once – and, while the σ at the mutated point is selected at random from R, the new value is forced to be different from the current value. Child population duplicates are mutated in the same way. The probability, P_m, of mutation is set to 0.05 (although this can be altered by a user) ˜ this is somewhat higher than a typical value of 0.01 and was chosen to encourage exploration of the large search space. The probability, P_c, of crossover is also user-definable but was fixed at 0.85 herein.

D. Evaluation of ultimate GA solution(s). The optimal solution(s) provided by the GA is (are) evaluated against a previously unseen set of compounds (the test set) where such is available. This enables one to test for over-fit to the training set and must be considered a crucial model validation procedure where, as here, a large number of adjustable parameters (σ) are involved. Training set random permutation tests also are applied to all V_{opt} and estimates made that the observed q^2 and r^2 scores could be chance effects; 1,000 permutations of the activity data were made in every case.

E. PLS model selection strategies. The selection of model-dimensionality (LV_{opt}) and thus the fitness score (q^2) of a particular chromosome during evolution of the GA requires careful consideration. Scoring on the basis of the first q^2 maximum (keyword: MAX_Q2) provides the most obvious method. However, in the interests of efficiency it is desirable to extract as few LVs as possible while at the same time model parsimony is, in general terms, considered to be desirable [16] when external predictivity is a criterion. Model parsimony

can be favoured by using, for example, a formula for calculating SE_{CV} that penalises additional LVs [16]:

$$SE_{CV} = \left(PRESS / (M - A - 1)\right)^{1/2}$$

Thus, models can be extracted on the basis of the *first* SE_{CV}-minimum (keyword: SECV_MIN). Alternatively, or additionally, a 5% rule (keyword: 5%_RULE) may be applied [16] wherein an additional LV is permitted only where it raises q^2 by ≥ 0.05 units. In general, but not always where M is small, the latter method is at least as parsimonious as the SECV_MIN approach. However, the purpose of the GA is to search for better solutions, the quality of which are judged by the q^2 scores. A "better" solution can be seen as any vector, V, that provides a higher q^2 than obtained previously. An q^2 improvement may be very small, and may require an additional LV in comparison to other models with slightly smaller q^2 but may provide an intermediary model in the progress toward a significantly better solution. It is, therefore, arguable as to whether MAX_Q2, SECV_MIN or the 5%_RULE should be the model selection criterion and various comparative tests are made using otherwise identical EVA_GA runs.

An upper bound to the value of LV_{opt} is that it should not exceed M/4 since the use of a ratio greater than this results in increased probability of chance correlation [17]. Finally, it is not acceptable to make predictions of the biological activity of structures to greater precision than the error in (reproducibility of) the original measurements. This factor has been directly addressed for the steroids [3] while the relevant information is not available for the melatonin compounds.

F. Default EVA_GA parameters. The following set of default GA parameters are defined: CONV_CRIT = 0.05 (i.e. there is no significant difference between the fitness scores of the most and least fit population members); MAX_CYCLES = 100; NBINS = 100 (i.e., $w = 40\ cm^{-1}$); NPOP = 100; PLS_MODEL_SELECTION = 5%_RULE. Crossvalidation can be LOO or LNO; unless otherwise stated, in the latter case G = 7 and CV is repeated 50 times and mean values reported for q^2 and SE_{CV}. Parameters such as R and LV_{max} are set according to the dataset involved and can be based upon examination of a range of results with EVA.

Datasets

The performance of EVA_GA was evaluated using datasets for which external test sets were available and consist of a benchmark steroid dataset [4,18,19] and a set of melatonin receptor ligands [20]. The use of test sets must be considered essential for validating an

EVA_GA QSAR model since the large number of adjustable parameters (NBINS) means a priori that there is great potential for training set overfit.

Steroids. The steroid set consists of 21 TG and 10 test set compounds (Table 1), originally investigated (in terms of 3D-QSAR analysis) by Cramer et al. [4]. This dataset has been described in detail previously together with both CoMFA and EVA analyses [3] and is not described further here; the activity data are measured corticosteroid-binding globulin affinities expressed as log [K]. The PLS results with EVA were good and it is of interest to determine whether or not EVA_GA can enhance this in any way. Whilst this dataset has been widely used as a benchmark for novel QSAR methods [18] it completely lacks any sort of experimental design; seven of the ten test set compounds have structural features not explicit in the training set. With this in mind statistical experimental design techniques [21,22] have been applied to these structures as described below. It is legitimate to make quite precise predictions of the steroid binding affinities – a lower bound to the SE of ~0.08 (equivalent to $r^2 > 0.995$) has previously been estimated [2].

Melatonin. The melatonin receptor ligands (Table 2 and Figure 4) consist of a TG of 44 structures and a test set of 9 structures taken from a 3D-QSAR investigation by Sicsic et al. [20]. This TG (analysis "J" in Ref. 20) provided the best CoMFA model selected from a range of different TGs having up to 48 compounds and should thus provide a stringent test of the relative performance of EVA/EVA_GA. The TG ("T" name prefix in Table 2) consists of five classes of structure, including 9 indole, 21 naphthalene, 2 tricyclic, 2 tetraline and 10 benzene-based compounds. The test set ("Z" name prefix) consists of 9 compounds, 7 of which belong to the benzene, naphthalene or tricyclic classes and which to some extent reproduce structural features present in the TG. However, there are no explicit TG examples of the m-ethoxy substituents of the test compounds Z55 and Z56, one of the test set compounds is a quinolinic structure (Z49) and compound (Z50) is structurally related to one of the naphthalene compounds. There are, therefore, four test set compounds which a priori might be expected to (but need not necessarily) provide predictive problems for a QSAR model.

Both the TG and test set compounds exhibit binding affinities (pKi) covering five orders of magnitude for chicken brain melatonin receptors (Table 2). The 44 TG compounds thoroughly and regularly span activity space (Figure 5). However, two of the test set compounds (Z49 and Z56) have lower activity than any of the 44 TG structures while only one of the TG compounds (T04) is less active than Z54. Not only is Z56 the least active compound overall but there is a gap of ~0.54 pKi units between it and the least active TG compound (T04), a much larger distance than exists elsewhere in "activity space". There is

once again, therefore, an expectation that there are likely to be predictive difficulties particularly with compound Z56 and, possibly, Z49. In the original CoMFA study [20] structure T47 has lower activity than Z56 but was excluded from the best CoMFA analysis since it was considered an outlier, a not unreasonable finding given that its pKi is ~0.6 units lower than that of T04.

Calculation of Normal Mode Frequencies (EVA)

Semiempirical. The steroid dataset was treated using the AM1 Hamiltonian of MOPAC 6.0 [23] with the parameters described previously [3]. The conformations used for the CoMFA analyses were adopted as the starting points for the MOPAC geometry optimisation of all structures. None of the 31 structures had imaginary ("negative") normal mode frequencies, indicating that the optimized geometries were at or very close to a stationary point

MM3 Molecular Mechanics. The melatonin ligands were geometry minimised using MM3(94) [24] molecular mechanics. As with the steroids, CoMFA conformations [20] were used as the starting points for the MM3 runs. The MM3 FULL_MATRIX option is required for a FORCE calculation to be done; all other MM3 parameters were left at their default values. Eleven of the structures had one imaginary NMF but the most negative of these was only -28.9 cm^{-1}. The calculations are in any case unreliable from -50 cm^{-1} to 50 cm^{-1} so NMFs within this range are not significant; imaginary NMFs are excluded from consideration when generating the EVA descriptor.

CoMFA Analyses

For both datasets CoMFA analyses were performed so as to provide benchmark values against which to judge the performance of EVA/EVA_GA. The steroid CoMFA analysis has been described in some detail previously [3]; the structures and conformations are those of Wagener et al. [19] and were aligned using an RMS fit of the 3, 5, 6, 13, 14 and 17 skeletal carbon atoms (Figure 6) with deoxycortisol (H11) as a template. For the melatonin ligands the superposed conformations were obtained directly from the original authors [20]. Most of the melatonin ligands have a highly flexible ethylamido side-chain (Figure 4) so the CoMFA alignments are based upon atom-based RMS fitting to the restrained tricyclic compounds (T33 and T34) using the alignment centres defined in that Figure.

CoMFA was undertaken using a 1 Å grid-spacing rather than the default 2 Å. There is considerable evidence to suggest that results with the latter spacing are likely to be unreliable [8] and, therefore, the CVR^2-GRS (Crossvalidated-r^2-Guided Region Selection) method [8] – an unsophisticated domain-based variable selection procedure – was applied to the analyses. In addition, the robustness of the models was assessed at both 2 and 1 Å grid-

spacing through reorientation tests, in which all compounds are reoriented as an aggregate rigid body within the bounding CoMFA 3D grid. This was done systematically, at fixed intervals of either 1° and 10° through 360° in each plane separately and in various combinations, and training and test set modelling and prediction performed for each orientation; this provides a means of estimating the stability and true statistical performance of the CoMFA PLS models. Evaluations such as this incorporating test set predictions have not been previously published.

Aside from the grid resolution all other CoMFA parameters were kept at the Sybyl default values. MOPAC 6.0 AM1 [23] charges were used for the steroid analysis while Sybyl [9] Gasteiger and Marsili charges were utilised with the melatonin receptor ligands per the original publication [20]. As with the EVA analyses LOO or LNO (steroids only) CV was used with a maximum of M/4 LVs depending on the dataset size. Analyses were done using steric and electrostatic fields combined and were performed for unscaled and blockscaled data. Sybyl PLS was used for CoMFA regression analysis and models were selected on the basis of the SECV_MIN rule noted above.

Results and Discussion

Steroid Dataset

CoMFA and EVA. As stated above the chosen steroid dataset previously has been investigated in some detail using both EVA and CoMFA [3] and only brief comments will be made here. With EVA the best models with fixed σ were obtained where σ = 3/4 cm^{-1} (Table 3). These models had a q^2 of 0.80 (two LVs) and a pr^2 of 0.69 or 0.76 (excluding an outlier (M31) with a fluorine substituent not explicit in the TG). Test set predictions for σ values other than 3/4 cm^{-1} rapidly become very poor (Figure 7) so there is a quite distinct, limited range of optimal fixed σ for this dataset. The melatonin dataset on the other hand has a much broader (contiguous) band of σ values over which pr^2 scores are relatively stable (see below). CoMFA modelling with this steroid dataset (Table 3) provides a very high q^2 score of 0.87 (two LVs) and an equally high test set pr^2 (0.84) where the fluorine outlier is excluded. However, the CoMFA model is extremely sensitive to M31 and, when it is included in the test set, the pr^2 score drops to 0.45. It has been suggested that this difference between CoMFA and EVA reflects the different information content of the vibrational and field-based descriptors. It is therefore of interest to determine whether or not optimisation of the EVA model using EVA_GA alters the sensitivity of the method to M31. It should also be noted that with a 1 Å CoMFA grid-spacing the PLS scores are quite stable under

aggregate reorientation [3] ˜ the test pr^2 scores show the greatest variation, ranging from 0.42 to 0.48 (all compounds) and 0.81 to 0.86 (M31 excluded).

EVA_GA To start with the default GA parameters noted above were used together with R = {1, 2, 3, 4, 5} and LV_{max} = 2 both chosen according to EVA results (Figure 7, Table 3). However, a wide variety of alternatives parameters were investigated also (Tables 4–7). The most obvious feature of most of the results obtained is that it is possible to enhance q^2 by up to 0.08 units (LOO CV) and 0.06 units (LNO CV) relative to the best EVA model (Table 3). The best predictive results are obtained where two or more LVs are available to EVA_GA and in general, but not exclusively, two LVs are optimal for both TG and test set predictions. Where two or more LVs are available test set pr^2 scores (0.70-0.75) are virtually identical or slightly smaller than that obtained with EVA. Again, provided at least two LVs are available, EVA_GA is not sensitive to the PLS model selection criteria and there is nothing to be gained from setting MAX_CYCLES > 100 (Table 5) although, where MAX_CYCLES = 50 the results over five runs of the GA show considerable variation. In addition EVA_GA is not sensitive to the alternative values of NBINS that were investigated (Table 6).

EVA_GA appears to be most sensitive to the choice of R set values (Table 7). For example, if relatively large σ such as 20 and 30 cm^{-1} are made available to the GA then, whether one, two or three LVs are used, LOO q^2 is enhanced (to 0.82. 0.86 and 0.90 respectively) while pr^2 scores are very poor where either M31 is included (0.47, 0.39 and 0.25) or excluded (0.59, 0.52 and 0.42); LNO-based searches provide only slightly better results in some cases. This is a similar finding to that with EVA where pr^2 scores (and TG CV scores) are poorer where σ is not very close to 4 cm^{-1} (Figure 4). Examination of the V_{opt} solutions for each of the five GA runs indicates that large σ are incorporated into the solutions when made available. Random permutation tests applied to two sets of results where R = {1, 2, 3, 4, 5} and {4, 8, 10, 20, 30} and where LV_{max} is two (Table 7 footnotes) indicate that (for LOO q^2) in the latter case the estimated probability of chance correlation (p) is 0.021 for LV1 (i.e., greater than 1%) and 0.0005 for LV2 (mean p over 5 GA runs) while in the former case p is 0.0007 or 0.0006 for one or two LVs respectively. Thus, it seems that the possibility for chance correlation is greatly increased where large σ are used. Where the fitted-r^2 is considered, in all cases $p < 2.7 \times 10^{-13}$. Thus, even in the absence of the poor test set predictions where R = {4, 8, 10, 20, 30}, the models based on the R set with smaller σ would be favoured for predictive purposes.

As noted above the steroid dataset lacks any sort of experimental design, statistical or otherwise, and in view of this the dataset as a whole was re-examined using PCA and PLS. It is acknowledged, however, that implicit in EVA_GA are changes to the descriptor space and that experimental design can be properly applied only where descriptor space is constant. However, we proceed on the assumption that some sort of design consideration is better than none at all. As an initial step PLS CV was applied to all 31 structures (σ = 4 cm^{-1}) giving a q^2 of 0.75 (two LVs) against which the scores from subsequent designed models can be compared; note that a total of 21.9% of the X block (i.e., EVA descriptor) variance is explained by these two LVs. A PCA (no scaling) was then applied to the 31-compound X matrix. However, 19 PCs are required to explain 90% of the variance in X* with the first seven PCs explaining (cumulatively) 15.1%, 25.4%, 34.5%, 41.8%, 47.5 %, 52.8% and 57.8% respectively – additional PCs explain < 5% further variance. The number of design points (compounds) required for a two-level factorial design (FD) with k variables (here, significant PCs) is 2^k and that for a fractional FD (FFD) is 2^{k-1} (ignoring centre-points). Thus even where k = 7 and a two-level FFD is applied there is a requirement for a minimum of 64 compounds. This is in any case an unsatisfactory summary of the univariate variance in X since 42.2% is left unexplained where only 7 PCs are considered. Therefore, further analysis was done so as to eliminate compounds that might be considered outliers: this can be done either in terms of the X space alone or in both X and Y space combined. Outliers in X space can be identified using Hotelling's T^2, a multivariate generalisation of Student's t-test, which provides an elliptical confidence region for the data when viewed as two-dimensional score plots. Using 0.01 as a confidence limit, and through examination of all score plot combinations up to 7, 19 (90 % of X explained) and 30 (100 % of X explained to three d.p.) PCs, then 0 compounds, 4 compounds (M1, L16, M27 and M31) and 10 compounds (previous four plus: H7, L13, H19, H20, M21, M24) respectively can be considered significant outliers. When these compounds are excluded and PCA repeated 16 or 14 PCs respectively are required to explain 90% of the variance in the reduced descriptor blocks. Even with a 0.05 confidence limit for T^2, using which threshold 21 compounds can be excluded, 7 PCs are required to explain 90% of the variance in X for the remaining 10 compounds; clearly too many design variables where only 10 compounds are available. Thus, even where the chemical justification for excluding compounds is ignored, it seems to be the case that experimental design in PC space is difficult if not impossible with these compounds and this descriptor.

In consequence of the difficulty of performing a PCA-based design it was decided to do a design in the PLS LV space which focuses attention upon the variance in X that is related to

* An equally large number of PCs is required to summarise the X matrix for the 53 melatonin ligands.

Y and is, therefore, a supervised or biased design. As noted above LOO CV using all 31 compounds provides LOO/LNO q^2 scores of 0.75 / 0.74 (2 LVs) – an additional LV does not improve q^2 any further. Clearly, a FD with only two significant variables requires only four data points. However, ten data points is generally considered to be the minimum required for PLS analysis and, therefore, further compounds were selected, including centre-points, so as to span the LV space thoroughly, giving a new TG consisting of L4, H6, H7, L9, L13, L18, H22, H23, M26, M27 and H30 (DESIGN_A). EVA analysis (Table 8) provided an optimal model where $\sigma = 4$ cm^{-1} with LOO/LNO q^2 scores of 0.55 / 0.54 (one LV) – which are somewhat less than all-compound CV – with an r^2 of 0.89 and a pr^2 of 0.51 (or 0.55 excluding M31). It is to be expected that CV using a sparse, designed set of compounds give a lower q^2 relative to instances where there is much redundancy. Application of EVA_GA to DESIGN_1 (Table 8) provided enhanced q^2 and r^2 scores (0.71 and 0.96 respectively) while the pr^2 score was, once again, not significantly altered whether or not M31 is included in the test set.

A second design was made (DESIGN_B) but this time the three largest outliers from all-compound CV (H22, M27, H31) were excluded entirely. With EVA this set of 28 compounds provided optimal LOO/LNO q^2 scores of 0.84 / 0.83 (2 LVs) where $\sigma = 4$ cm^{-1}. Ten compounds were picked from an LV score plot as before (Table 8) which provided an EVA model with LOO/LNO q^2 scores of 0.69 / 0.66 (2 LVs) and a pr^2 of 0.69; that is, both predictive scores are reasonably high and their values very similar indicating that here q^2 is good indication of model predictivity. The application of EVA_GA (Table 8) provided enhanced q^2 scores (0.81 with 2 LVs) and a slightly reduced pr^2 score (0.66, whether M31 is included or not). Thus, overall it appears that there is nothing to be gained or lost in terms of test compound predictivity through the application of EVA_GA with the various steroid training / test sets evaluated.

Melatonin Receptor Ligands

CoMFA Results. A CoMFA was performed using a set of aligned structures obtained directly from Sicsic et al. [20]; note that for reasons discussed above dataset "J" was selected from that paper. The results of our CoMFA are listed in Table 9 together with those obtained by the original authors. It is apparent that our results differ somewhat from those of Sicsic et al. despite ensuring as far as possible that the CoMFA parameters were identical. The reason for this is most likely that, as noted above [8], a 2 Å grid resolution usually adds a sampling error into the descriptors in as much as the results obtained depend upon the orientation of the structures as an aggregate body relative to the 3D grid. For this reason a 1 Å resolution has been recommended [8] since it is said to provide relatively orientation-

independent results. Indeed, the mean PLS scores (q^2, r^2, pr^2) of ~3,800 reorientations of the aggregate using a 2 Å resolution are almost identical to the single orientation 1 Å results (Table 9); this also applies where mean values from the same set of reorientations are assessed at a 1 Å grid-spacing. The range of PLS scores obtained is extremely wide at 2 Å, particularly for the test set predictions (>0.4 units), and the scores obtained by Sicsic et al. certainly fall within these limits. At a 1 Å resolution the PLS scores are more stable covering ~0.1 units for q^2 and r^2 while pr^2 scores again show the greatest variance ranging from 0.66 to 0.80 for all nine compounds and from 0.53 to 0.76 where Z55 and Z56 are excluded. What is more there is only a very low correlation between q^2 and pr^2 ($r = 0.15$) so choosing a suitable orientation on the basis of CV scores provides no indication as to what the true pr^2 may be. Overall, these results indicate that a 2 Å resolution is inadequate with this dataset and that test set scores can show significant variation even at a 1 Å resolution.

EVA Results. A large number of EVA descriptor sets derived using a range of different fixed Gaussian σ were evaluated on the basis of training and test set statistics. It is clear from the LOO CV results (Figure 8) that the best training set models are those where σ = 3–15 cm^{-1}, depending upon which LVs are considered. LV1 is maximal where σ < ~4 cm^{-1}, while the addition of LV2 and subsequent LVs results in progressively higher peaks where σ ≈ 10 cm^{-1}. Thus, where σ = 10 cm^{-1} (Table 10), if the 5%_RULE is applied q^2 is 0.46 (2 LVs), while a model based on SECV_MIN has a q^2 of 0.53 (5 LVs). If MAX_Q2 is the selection criterion then $q^2 = 0.58$ (8 LVs); this is in fact the highest observed q^2 for all models where a maximum of ten LVs are extracted. Thus, whatever criterion is used to select LV_{opt}, and thence the optimal σ to use, q^2 is not particularly high. Test set predictions where σ = 10 cm^{-1} are, on the other hand, somewhat better (Table 10) with the parsimonious models providing the best pr^2 scores of 0.66 for all nine compounds and ~0.81 (2 or 5 LVs) if the previously noted outliers (Z55/Z56) are excluded. Overall, with this data set, and in contrast to the steroid results, the selection of LV_{opt} and the best fixed σ is not clear cut. In comparison to CoMFA these EVA results are poorer, particularly where q^2 is considered while there are much smaller differences in pr^2 scores. The EVA predictions are quite sensitive to the presence of compounds Z55 and Z56 (~0.15 units difference) while this is less the case for CoMFA – 0.06 units difference where the mean values of aggregate reorientation at 1 Å resolution are considered (Table 9).

EVA_GA. As previously, an initial R set was chosen based upon σ values centred around the optimal EVA training set σ of 10 cm^{-1} (Figure 8); thus, $R_1 = \{3, 5, 8, 10, 12\}$. These results also suggest that LV_{max} should be two or three; the larger value was chosen since this permits the GA to select either dimensionality. However, the effect, if any, of alternative

choices of parameters are considered below. The PLS_MODEL_SELECTION method was the 5%_RULE since this provides the most straightforward selection of LV_{opt} from the optimal solutions produced by the GA.

If the results where $R = R_1$, as suggested by the EVA results, are considered (Table 11) it is apparent that it is always the cases that solutions can be obtained with EVA_GA that have substantially higher q^2 than EVA. It is also the case that this improvement does not require additional LVs. Indeed, the one-LV EVA_GA models have roughly the same LOO/LNO q^2 (~0.58/~0.53) as the eight-LV EVA model (Table 10) and test set predictivity that is equal to that of the optimal EVA models (pr^2 = ~0.65 or ~0.80, including and excluding Z55/Z56 respectively). If further LVs are made available to the GA then it is clear that two or three LV models provide the best test set scores (~0.75 / ~0.88) representing worthwhile improvements over the EVA scores. Even where five LVs are made available to the GA, LV_{opt} is indicated to be two or three provided that the more conservative LNO CV is used for fitness scoring during population evolution. The use of LOO CV for fitness scoring where $LV_{max} > 3$ produces the highest q^2 scores (up to 0.70 with LV_{opt} = 4 or 5) but the models begin to show signs of overfit to the TG (pr^2 = ~0.58 / ~0.79). If an alternative R set is considered (R_2 = {2, 4, 6, 8, 10}) the results (Table 11) are almost identical to those with R_1 as might be expected, the only substantive difference being the better pr^2 scores where $LV_{max} = 5$. If very much larger σ are made available to the GA (R_3 = {5, 10, 15, 20, 30}) q^2 scores can be enhanced to similar levels as with R_1 and R_2 while their is little or no improvement in pr^2 scores relative to EVA where $LV_{max} > 1$. Where only one LV is available q^2 and test set scores are poorer than when more LVs are available as was found also with sets R_1 and R_2. The findings with R_3 suggest that lower σ help to limit the possibilities for TG overfit – this was even more strongly indicated with the steroid results (Table 7). In any case the EVA results over a range of fixed σ (Figure 8) suggest that the use of large σ would not be useful. Note that none of the models listed (Table 11) are contraindicated by random permutation tests at any number of LVs.

Thus far the results described have been with default EVA_GA settings and a variety of R sets and LV_{max}. The results with alternative MAX_CYCLES (Table 12) suggest that 100 cycles is certainly adequate, where the other parameters are their default values, and there is clearly little or nothing to be gained from using more than 100 GA iterations. Where only 50 iterations are available the mean score values (over five GA runs) are similar to those where more runs are used but, as with the steroids, there is much greater variation in the scores over the five runs and 100 iterations is preferred. Where alternative bin widths are considered (Table 13) the best results in terms of prediction are obtained where NBINS is

100 or 200 while poorer scores are obtained where NBINS ≥ 400 despite the improvements to q^2. However, even where NBINS = 800 (~5^{425} available permutations where empty bins are excluded) and MAX_CYCLES = 1,000 (Table 13) test set predictions remain at least as good as those with EVA (Table 10).

Conclusion

A method has been described that explores an alternative formulation of the EVA QSAR technique (EVA_GA) incorporating the localisation of the values of the main EVA parameter, the Gaussian kernel width (σ). A genetic algorithm has been used to explore localised "σ space" using the scores from LOO or LNO PLS crossvalidation as the fitness to be maximised by the GA. When applied to a benchmark steroid dataset, for which really quite good results had already been obtained using classical EVA, the EVA_GA could always find improved training set models but for the most part test set predictivity was improved not at all. However, except with certain parameter choices (availability of high σ) contraindicated by both the classical EVA results and random permutation tests, test set predictivity was as good as that with EVA. Similar results were obtained where the training / test division of structures was modified using statistical experimental design criteria.

With a second relatively heterogeneous set of melatonin receptor ligands, representing five structural classes, the results obtained were much more encouraging. Again, it was always found that higher q^2 scores (typically, up to 0.25 units better) could be obtained with EVA_GA compared to fixed σ EVA. However, in contrast to the steroid results, test set predictive scores were also substantially enhanced in most cases. As with the steroid set the availability (and incorporation by EVA_GA into optimal solutions) of σ values larger than those suggested by the EVA results leads to indications of training set overfit. Where large numbers of latent variables are made available to EVA_GA the possibilities for overfit increase although, with this melatonin dataset, the use of the more conservative LNO PLS crossvalidation helps to control model dimensionality such that this avoided.

Overall, additional work is needed so as to verify that EVA_GA is an effective technique, to attempt to generalise these findings into a set of parameters that might be expected to be widely applicable, and to examine the obtained models in detail so as to look at what changes are being made by EVA_GA in descriptor space. Further development of EVA_GA might include the incorporation of some limited form of random permutation testing into the chromosome scoring function, perhaps simply to reject a chromosome entirely if it fails to meet certain criteria. Also being considered is combination of the method described with

more standard variable selection procedures in which variables may be removed from consideration entirely; i.e., permit σ to be zero.

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Figure Captions

Fig. 1. Histogram summarising the number of fundamental NMFs found in different regions of the IR spectrum (melatonin receptor ligand training dataset, bin widths (w) of 40 cm^{-1}).

Fig. 2. Example of the different kernel widths and shapes obtained after expansion with selected Gaussian standard deviation (σ) values (after scaling to unit maximum amplitude) for a single hypothetical frequency at 29 cm^{-1}.

Fig. 3. Overview of GA routine.

Fig. 4. Melatonin training and test set compounds with CoMFA superposition centres[a].

Fig. 5. Distribution of melatonin receptor ligands in activity space.

Fig. 6. Steroid skeleton.

Fig. 7. Steroid dataset: cumulative q^2 for successive PLS LVs for classical EVA models derived from a range of σ values.

Fig. 8. Cumulative LOO q^2 for successive PLS LVs for classical EVA models derived from a range of σ values: melatonin receptor ligands.

TABLE 1
STEROID CBG-BINDING AFFINITIES

	Compound	CBG Affinity
Training Set		log [K]
M1	Aldosterone	6.279
L2	Androstanediol	5.000
L3	Androstenediol	5.000
L4	Androstenedione	5.763
L5	Androsterone	5.613
H6	Corticosterone	7.881
H7	Cortisol	7.881
M8	Cortisone	6.892
L9	Dehydroepiandrosterone	5.000
H10	Deoxycorticosterone	7.653
H11	Deoxycortisol	7.881
M12	Dihydrotestosterone	5.919
L13	Estradiol	5.000
L14	Estriol	5.000
L15	Estrone	5.000
L16	Etiocholanolone	5.255
L17	Pregnenolone	5.255
L18	17-Hydroxypregnenolone	5.000
H19	Progesterone	7.380
H20	17-Hydroxyprogesterone	7.740
M21	Testosterone	6.724
Test Set		
H22	Prednisolone	7.512
H23	Cortisol 21-acetate	7.553
M24	4-Pregnene-3,11,20-trione	6.779
H25	Epicorticosterone	7.200
M26	19-Nortestosterone	6.144
M27	16α,17-Dihydroxy-4-pregnene-3,20-dione	6.247
H28	17-Methyl-4-pregnene-3,20-dione	7.120
M29	19-Norprogesterone	6.817
H30	11β,17,21-Trihydroxy-2α-methyl-4-pregnene-3,20-dione	7.688
M31	11β,17,21-Trihydroxy-2α-methyl-9α-fluoro-4-pregnene-3,20-dione	5.797

Structure numbers and activity group classification prefixes (but not the structures themselves) are those used by Good et al. [25]: H - high activity; M - medium; L - low.

TABLE 2
MELATONIN RECEPTOR LIGANDS BINDING AFFINITIES[a]

name	pKi	name	pKi	name	pKi	name	pKi	name	pKi
T01	9.17	T12	10.62	T25	8.66	T36	6.67	Z49	6.23
T02	10.49	T13	9.92	T26	7.71	T37	6.67	Z50	8.59
T03	6.80	T15	7.52	T27	9.26	T38	6.71	Z51	10.30
T04	6.31	T16	8.03	T28	8.45	T39	6.94	Z52	8.85
T05	9.85	T17	6.49	T29	8.23	T40	6.66	Z53	7.77
T06	8.60	T19	9.62	T30	8.97	T41	6.64	Z54	6.41
T07	8.60	T20	10.14	T31	7.92	T42	7.19	Z55	6.83
T08	8.17	T21	9.41	T32	7.25	T43	7.15	Z56	5.77
T09	7.66	T22	8.77	T33	7.46	T44	7.38	Z57	7.09
T10	9.27	T23	8.57	T34	8.22	T45	6.54		
T11	9.74	T24	9.17	T35	6.69	T46	6.60		

[a] Binding affinities for chicken brain melatonin receptors [20]. Training set compounds are prefixed by "T" while test set compounds are prefixed by "Z".

TABLE 3
CLASSICAL EVA AND COMFA PLS STATISTICS: STEROID DATASET

			CV		Fitted Model				Test Set pr^2	
Analysis	Parameters	LV_{opt}	q^2 LOO / LNO[a]	SE_{CV} LOO / LNO[a]	p[b]	r^2	SE	p[b]	With/Without M31	M31 residual
"Classical" EVA	σ = 4 cm^{-1}	2	0.80 / 0.79	0.55 / 0.57	0.001	0.96	0.24	0.0029	0.69 (0.74)	+0.67
CoMFA	See main text	2	0.87 / 0.84	0.45 / 0.49	0.0001	0.93	0.32	0.00002	0.45 (0.84)	+1.91

[a] Mean of 200 runs of LNO CV where G = 7.
[b] For both LOO q^2 and fitted r^2, p is an estimate of the probability of chance correlation based upon 1,000 random permutations of Y.

TABLE 4
EVA_GA PLS RESULTS: STEROID DATASET

GA Parameters[a]		CV[b]				Fit[b]	Test Set[b]
RULE[c]	LV_{max}	CV	RULE[d]	LV_{opt}	q^2	SE_{CV}	r^2	pr^2 All / No M31
5%_RULE	3	LOO	BOTH	2	0.84	0.49	0.98	0.68 / 0.72
		LNO	BOTH	2	0.82	0.53	0.98	0.66 / 0.70
	2	LOO	BOTH	2	0.86	0.47	0.98	0.68 / 0.73
		LNO	BOTH	2	0.84	0.50	0.98	0.67 / 0.71
	1	LOO	BOTH	1	0.83	0.50	0.96	0.65 / 0.70
		LNO	BOTH	1	0.80	0.53	0.95	0.65 / 0.70
SECV_MIN	3	LOO	SECV_MIN	2[e]	0.86	0.47	0.99	0.68 / 0.74
			5%_RULE	1[f]	0.83	0.51	0.96	0.67 / 0.71
		LNO	SECV_MIN	2	0.83	0.51	0.98	0.65 / 0.71
			5%_RULE	1	0.80	0.53	0.96	0.63 / 0.68
	2	LOO	SECV_MIN	2	0.86	0.47	0.99	0.64 / 0.70
			5%_RULE	1	0.83	0.50	0.97	0.63 / 0.68
		LNO	SECV_MIN	2	0.83	0.51	0.98	0.63 / 0.68
			5%_RULE	1	0.80	0.54	0.96	0.62 / 0.65
MAX_Q2	3	LOO	SECV_MIN	3[f]	0.86	0.48	0.99	0.63 / 0.69
			5%_RULE	1	0.80	0.53	0.96	0.62 / 0.66
		LNO	SECV_MIN	2	0.82	0.52	0.98	0.65 / 0.70
			5%_RULE	1	0.79	0.55	0.95	0.62 / 0.66
	2	LOO	SECV_MIN	2	0.86	0.46	0.98	0.65 / 0.71
			5%_RULE	1	0.83	0.49	0.96	0.63 / 0.68
		LNO	SECV_MIN	2	0.82	0.52	0.98	0.66 / 0.71
			5%_RULE	1	0.79	0.54	0.96	0.64 / 0.69

[a] Default GA parameters unless otherwise stated; R = {1, 2, 3, 4, 5}.
[b] All PLS statistics are for descriptors derived from V_{opt} and are mean values taken from 5 GA runs.
[c] Rule used to select LV_{opt} and thus the chromosome fitness score (q^2) during evolution of the GA.
[d] Rule used to select LV_{opt} and thus the final PLS statistics for V_{opt}.as distinct from [c].
[e] LV_{opt} = 3 for 2 runs.
[f] LV_{opt} = 2 for one of the five runs.

TABLE 5
EVA_GA PLS RESULTS: STEROID DATASET: EFFECT OF MAX_CYCLES[a]

GA Parameters			CV			Fit	Test Set
MAX_CYCLES[b]	LV_{max}	CV	LVopt	q^2	SE_{CV}	r^2	pr^2 All / No M31
50	2	LOO	2	0.84	0.50	0.97	0.66 / 0.70
100	2	LOO	2	0.86	0.47	0.98	0.68 / 0.73
200	2	LOO	2	0.87	0.46	0.98	0.66 / 0.70
400	2	LOO	2	0.85	0.48	0.98	0.69 / 0.73
1000	2	LOO	2	0.87	0.45	0.98	0.67 / 0.72

[a] See footnotes to Table 4 for further information; R = {1, 2, 3, 4, 5}.
[b] Maximum iterations of the GA - each run was started separately with a different random seed and CONV_CRIT set so that convergence was not reached in any run.

TABLE 6
EVA_GA PLS RESULTS: STEROID DATASET: ALTERNATIVE BIN WIDTHS[a]

GA Parameters			CV				Fit	Test Set
NBINS	w	LV_{max}	CV	LVopt	q^2	SE_{CV}	r^2	pr^2 All / No M31
50	80 cm^{-1}	2	LOO	2	0.83	0.51	0.97	0.68 / 0.73
			LNO	2	0.80	0.55	0.97	0.65 / 0.69
100	40 cm^{-1}	2	LOO	2	0.86	0.47	0.98	0.68 / 0.73
			LNO	2	0.84	0.50	0.98	0.67 / 0.71
200	20 cm^{-1}	2	LOO	2	0.85	0.48	0.98	0.63 / 0.69
			LNO	2	0.82	0.52	0.98	0.67 / 0.71
400	10 cm^{-1}	2	LOO	2	0.86	0.46	0.99	0.65 / 0.72
			LNO	2	0.83	0.51	0.98	0.67 / 0.74
400[b]	10 cm^{-1}	2	LOO	2	0.88	0.44	0.98	0.69 / 0.75
			LNO	2	0.85	0.49	0.98	0.66 / 0.72
800[c]	5 cm^{-1}	2	LOO	2	0.89	0.41	0.99	0.66 / 0.73
			LNO	2	0.85	0.48	0.98	0.69 / 0.75

[a] See footnotes to Table 4 for further information; R = {1, 2, 3, 4, 5}.
[b] MAX_CYCLES = 400.
[c] MAX_CYCLES = 500. All model converged prior to MAX_CYCLES of the GA.

TABLE 7
EVA_GA PLS RESULTS: STEROID DATASET: ALTERNATIVE R SETS[a]

GA Parameters		CV				Fit	Test Set
R	LV_{max}	CV	LVopt	q^2	SE_{CV}	r^2	pr^2 All / No M31
{1, 2, 3, 4, 5}	2	LOO	2	0.86[b]	0.47	0.98[c]	0.68 / 0.73
		LNO	2	0.84	0.50	0.98	0.67 / 0.71
{2, 3, 4, 5, 6}	2	LOO	2	0.84	0.50	0.97	0.67 / 0.72
		LNO	2	0.81	0.54	0.97	0.66 / 0.71
{2, 3, 4}	2	LOO	2	0.79	0.57	0.97	0.68 / 0.73
		LNO	2	0.79	0.57	0.98	0.68 / 0.72
	1	LOO	1	0.76	0.59	0.95	0.62 / 0.68
		LNO	1	0.77	0.57	0.95	0.64 / 0.69
{3, 4, 5, 8, 10}	3	LOO	2	0.85	0.47	0.97	0.61 / 0.65
		LNO	2	0.83	0.51	0.96	0.65 / 0.70
	2	LOO	2	0.86	0.47	0.97	0.64 / 0.68
		LNO	2	0.83	0.51	0.97	0.62 / 0.66
	1	LOO	1	0.79	0.54	0.93	0.62 / 0.68
		LNO	1	0.79	0.55	0.93	0.61 / 0.68
{4, 8, 10, 20, 30}	3	LOO	3	0.90	0.40	0.98	0.25 / 0.42
		LNO	3	0.87	0.45	0.98	0.27 / 0.41
	2	LOO	2	0.86[d]	0.45	0.95[e]	0.39 / 0.52
		LNO	2	0.85	0.48	0.95	0.45 / 0.60
	1	LOO	1	0.82	0.51	0.91	0.47 / 0.59
		LNO	1	0.80	0.54	0.91	0.49 / 0.59

[a] See footnotes to Table 4 for further information.
[b] Chance correlation estimates, p, for q^2 are 0.0007 (LV1) and 0.0006 (LV2) (mean values for the five GA runs).
[c] Chance correlation estimates, p, for r^2 are 0 (LV1 and LV2).
[d] Chance correlation estimates, p, for q^2 are 0.021 (LV1) and 0.0005 (LV2).
[e] Chance correlation estimates, p, for r^2 are 2.7×10^{-13} (LV1) and 0 (LV2).

TABLE 8
STEROIDS: ALTERNATIVE TRAINING AND TEST SET DESIGNS

		Classical EVA				EVA_GA[1]				
Design	σ_{opt}	LOO/LNO q^2	LV_{opt}	r^2	pr^2 All / No M31	LV_{max}	LOO q^2	LV_{opt}	r^2	pr^2 All / No M31
A[a]	4 cm^{-1}	0.55 / 0.54	1	0.89	0.51 / 0.57	2	0.71	1	0.96	0.50 / 0.55
B[b]	4 cm^{-1}	0.69 / 0.66	2	0.99	0.69 / 0.70	2	0.81	2	0.99	0.66 / 0.66

[1] Default GA parameters: models selected according to 5%_RULE; R = { 1, 2, 3, 4, 5}.
[a] ALL 31 compounds retained: TG = {L4, H6, H7, L9, L13, L18, H22, H23, M26, M27, H30}; M = 11; 20 Test compounds.
[b] H22, M27 and M31 excluded entirely: TG = {L4, H6, L9, H10, L13, L18, H23, M24, M26, H30}; M = 10; 18 Test compounds.

TABLE 9
MELATONIN RECEPTOR LIGANDS: DETAILED COMFA PLS RESULTS.

	CoMFA Settings		Training Set			Test Set pr^2	
Model	Grid Resolution	Scaling	LOO CV q^2	SE_{CV}	Fitted r^2	All	Excl. Z55/56
Literature	2Å	not known	0.80 (5)[a]	0.61	0.97	0.76	not known
Supplied	2Å	None	0.65 (3)	0.76	0.84	0.68	0.67
		Block	0.66 (3)	0.75	0.88	0.67	0.58
	1Å	None	0.69 (3)[b]	0.72	0.86[c]	0.72	0.71
		Block	0.64 (2)	0.76	0.79	0.68	0.64
CVQ^2-GRS [8]	q^2 cut-off = 0.1[d]	None	0.70 (3)	0.71	0.87	0.74	0.72
	q^2 cut-off = 0.3[d]	None	0.67 (3)	0.74	0.84	0.72	0.65
Aggregate Reorientation[e]	2Å	None	0.58 – 0.81[f] mean 0.69	nc[g]	0.72 – 0.97[f] mean 0.87	0.43 – 0.88[f] mean 0.70	0.13 – 0.88[f] 0.67
	1Å	None	0.67 – 0.76[f] mean 0.72	nc[g]	0.85 – 0.97[f] mean 0.93	0.66 – 0.80[f] mean 0.73	0.53 – 0.76[f] 0.67

[a] Model LV_{opt} picked on the basis of the (coincident) largest q^2 and smallest SE_{CV} [20].
[b] Random permutation: for LOO q^2, $p = 2.2 \times 10^{-5}$.
[c] Random permutation: for fitted r^2, $p = 2.2 \times 10^{-6}$.
[d] Cut-off value for q^2 using which sub-regions are excluded from the final CoMFA; a 1 Å grid resolution is used throughout.
[e] See main text for details for reorientations used.
[f] Minimum and maximum observed values.
[g] Mean and ranges for SE_{CV} not calculated.

TABLE 10
CLASSICAL EVA PLS STATISTICS: MELATONIN DATASET

			CV			Fitted Model			Test Set pr^2	
Parameters	RULE	LV_{opt}	q^2 LOO / LNO[a]	SE_{CV} LOO / LNO[a]	p^b	r^2	SE	p^b	All	Excl. Z55/56
σ = 10 cm^{-1}	5%_RULE	2	0.46 / 0.44	0.93 / 0.95	0.0005	0.79	0.58	0.0[e]	0.66	0.81
	SECV_MIN	5	0.54 / [c]	0.90 / [c]	0.0026	0.95	0.29	0.0[e]	0.66	0.82
	MAX_Q2[d]	8	0.58 / 0.53	0.90 / 0.94	0.0043	0.98	0.19	0.0[e]	0.43	0.60

[a] Mean of 200 runs of LNO CV where G = 7.
[b] For both LOO q^2 and fitted r^2 p is an estimate of the probability of chance correlation based upon 1,000 random permutations of Y.
[c] Two LVs were optimal using LNO CV.
[d] This also corresponds to the overall q^2 maximum where ten LVs are extracted.
[e] Normalised Z > 23.

TABLE 11
EVA_GA PLS RESULTS: MELATONIN DATASET: ALTERNATIVE R SETS[a]

GA Parameters			CV				Fit	Test Set
R	LV_{max}	CV	RULE[b]	LVopt	q^2	SE_{CV}	r^2	pr^2 All / No Z55/56
R_1 = {3, 5, 8, 10, 12}	5	LOO	SECV_MIN	5[d]	0.70	0.72	0.96	0.58 / 0.79
			5%_RULE	4[h]	0.69	0.73	0.95	0.58 / 0.80
		LNO	SECV_MIN	2[i]	0.61	0.80	0.88	0.74 / 0.87
			5%_RULE	2[g]	0.61	0.80	0.87	0.76 / 0.90
	3	LOO	BOTH	3[j]	0.65	0.76	0.90	0.72 / 0.89
		LNO	BOTH	2[f]	0.61	0.79	0.88	0.77 / 0.89
	2	LOO	BOTH	2	0.64	0.76	0.87	0.76 / 0.87
		LNO	BOTH	2	0.61	0.80	0.86	0.75 / 0.88
	1	LOO	n/a	1	0.58	0.82	0.75	0.65 / 0.83
		LNO	n/a	1	0.55	0.84	0.74	0.63 / 0.80
R_2 = {2, 4, 6, 8, 10}	5	LOO	SECV_MIN	5[c]	0.70	0.72	0.98	0.63 / 0.80
			5%_RULE	4[d]	0.69	0.73	0.95	0.66 / 0.83
		LNO	BOTH	2[e]	0.64	0.77	0.89	0.74 / 0.88
	3	LOO	BOTH	3[d]	0.67	0.74	0.92	0.76 / 0.89
		LNO	SECV_MIN	2[f]	0.62	0.79	0.89	0.72 / 0.86
			5%_RULE	2[g]	0.61	0.79	0.88	0.73 / 0.86
	2	LOO	BOTH	2	0.65	0.76	0.88	0.74 / 0.87
		LNO	BOTH	2	0.63	0.78	0.87	0.74 / 0.86
	1	LOO	n/a	1	0.59	0.80	0.76	0.67 / 0.80
		LNO	n/a	1	0.58	0.81	0.76	0.65 / 0.78
R_3 = {5, 10, 15, 20, 30}	3	LOO	BOTH	3	0.68	0.73	0.91	0.69 / 0.77
		LNO	BOTH	3	0.63	0.78	0.90	0.64 / 0.80
	2	LOO	BOTH	2	0.63	0.78	0.83	0.65 / 0.80
		LNO	BOTH	2	0.58	0.82	0.82	0.64 / 0.80
	1	LOO	n/a	1	0.55	0.85	0.70	0.50 / 0.74
		LNO	n/a	1	0.53	0.86	0.68	0.47 / 0.74

[a] See footnotes to Table 4 for further information.
[b] Rule used to select LV_{opt} and thus the final PLS statistics for V_{opt}.– not an EVA_GA parameter.
[c] For two solutions LV_{opt} = 4. [d] For one solution LV_{opt} = 2. [e] For one solution LV_{opt} = 4.
[f] For two solutions LV_{opt} = 3. [g] For one solution LV_{opt} = 3.
[h] For one solution LV_{opt} = 2; for one solution LV_{opt} = 5. [i] For one solution LV_{opt} = 5.
[j] For two solutions LV_{opt} = 2.

TABLE 12
EVA_GA PLS RESULTS: MELATONIN DATASET: EFFECT OF MAX_CYCLES[a]

GA Parameters			CV			Fit	Test Set
MAX_CYCLES[c]	LV_{max}	CV	LVopt	q^2	SE_{CV}	r^2	pr^2 All / No Z55/56
50	3	LOO	3[c]	0.64	0.77	0.90	0.74 / 0.89
100	3	LOO	3[c]	0.65	0.76	0.90	0.72 / 0.89
200	3	LOO	3[c]	0.67	0.74	0.91	0.74 / 0.90
400	3	LOO	3	0.68	0.73	0.92	0.73 / 0.90
1000	3	LOO	3	0.70	0.71	0.92	0.72 / 0.90

[a] See footnotes to Table 4 for further information; R = {3, 5, 8, 10, 12}.
[b] Maximum iterations of the GA – each run was started separately with a different random seed and the CONV_CRIT set so that convergence was not reached.
[c] For one solution $LV_{opt} = 2$.

TABLE 13
EVA_GA PLS RESULTS: MELATONIN DATASET: ALTERNATIVE BIN WIDTHS[a]

GA Parameters			CV				Fit	Test Set
NBINS	w	LV_{max}	CV	LVopt	q^2	SE_{CV}	r^2	pr^2 All / No Z55/56
50	80 cm^{-1}	3	LOO	3[b]	0.61	0.80	0.90	0.70 / 0.83
100	40 cm^{-1}	3	LOO	3[c]	0.65	0.76	0.90	0.72 / 0.89
200	20 cm^{-1}	3	LOO	3	0.71	0.70	0.95	0.72 / 0.88
400	10 cm^{-1}	3	LOO	3	0.75	0.65	0.96	0.73 / 0.81
800	5 cm^{-1}	3	LOO	3	0.80	0.58	0.98	0.68 / 0.86
800[d]	5 cm^{-1}	3	LOO	3	0.91	0.39	0.99	0.67 / 0.81

[a] See footnotes to Table 4 for further information; R = {3, 5, 8, 10, 12}.
[b] For one solution LV_{opt} = 2. [c] For two solutions LV_{opt} = 2.
[d] MAX_CYCLES = 1000; CONV_CRIT = 0.025. For three of the five EVA_GA runs the population converged (after between 965 and 972 cycles) while the other two runs were very close to convergence (both 0.039) at MAX_CYCLES.

Fig. 1. Histogram summarising the number of fundamental NMFs found in different regions of the IR spectrum (melatonin receptor ligand training dataset, bin widths (w) of 40 cm^{-1}).

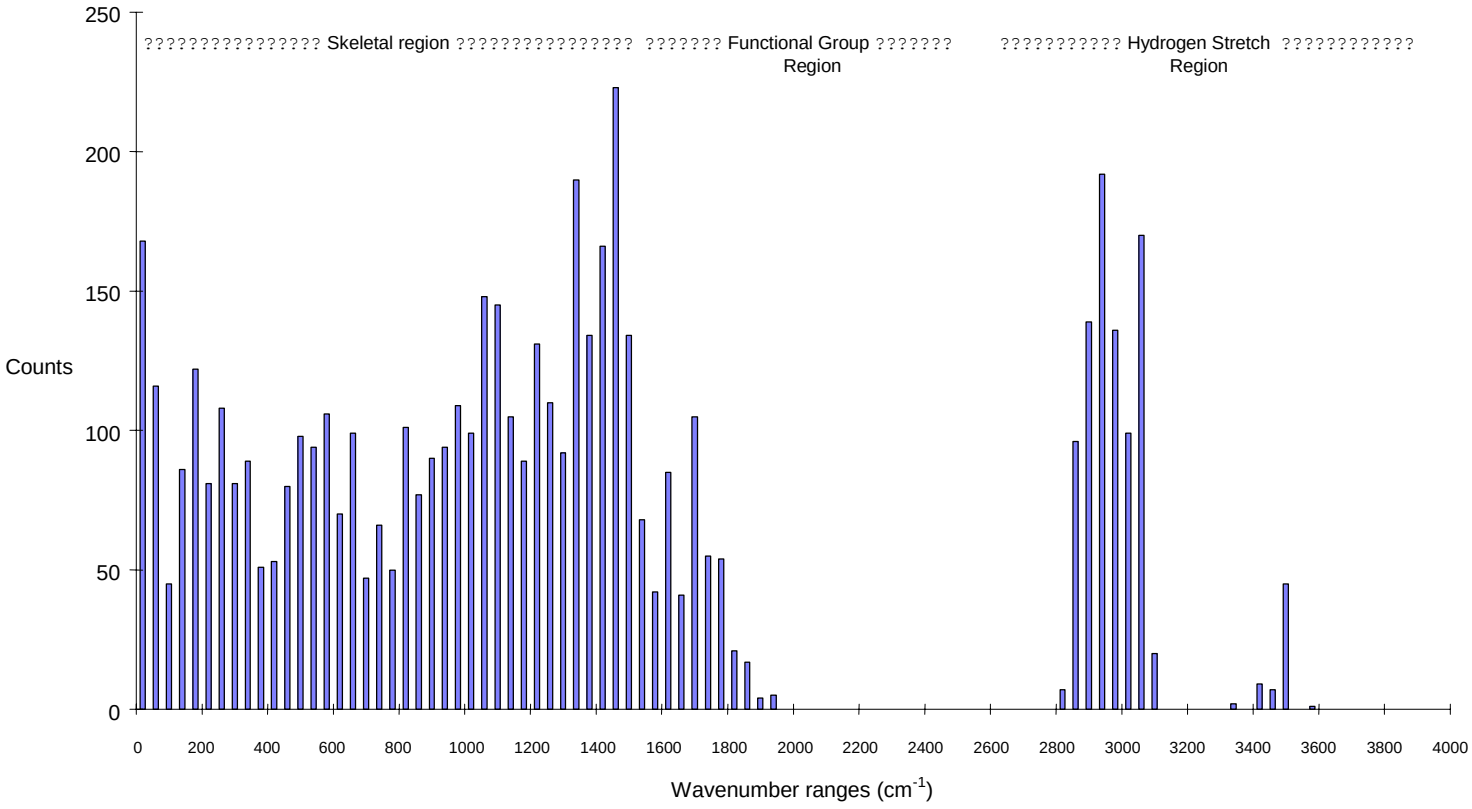


Fig. 2. Example of the different kernel widths and shapes obtained after expansion with selected Gaussian standard deviation (σ) values (after scaling to unit maximum amplitude) for a single hypothetical frequency at 29 cm^{-1}.

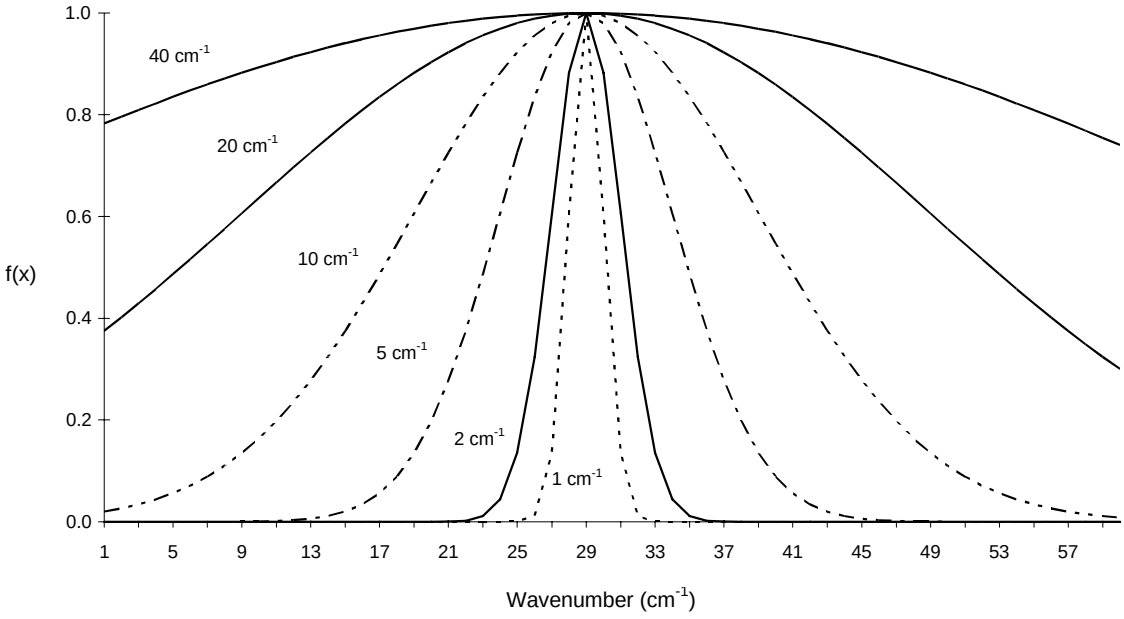


Fig. 3. Overview of GA routine.

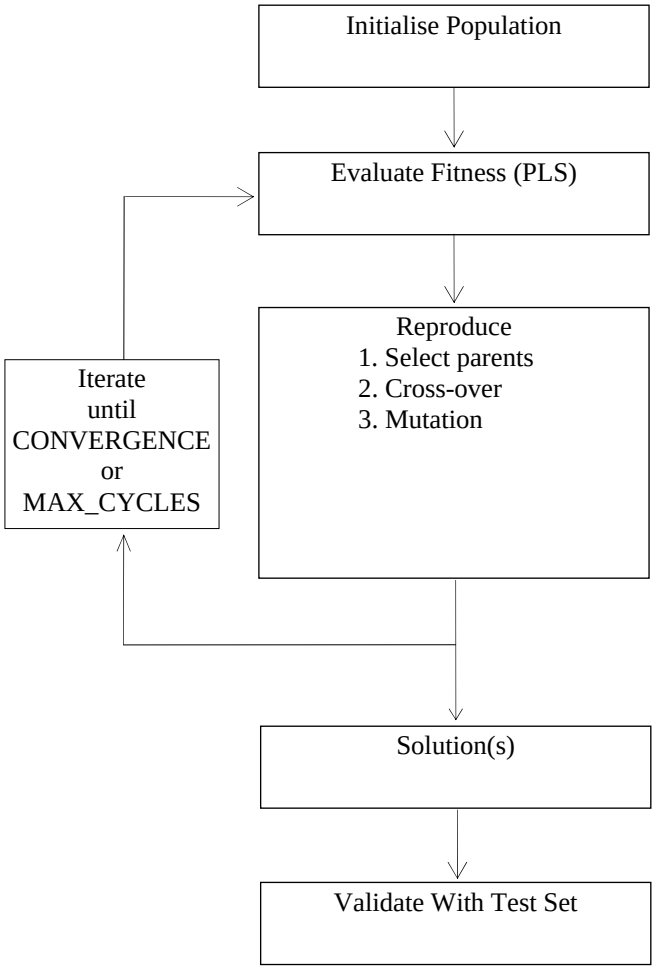
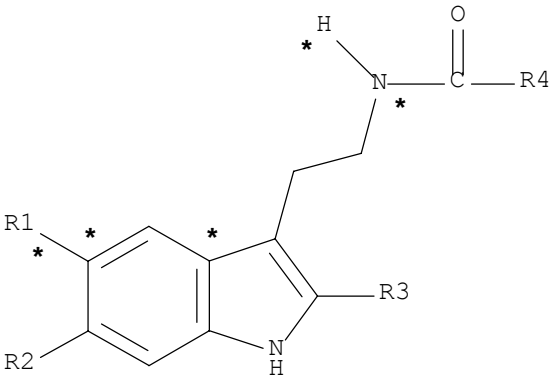
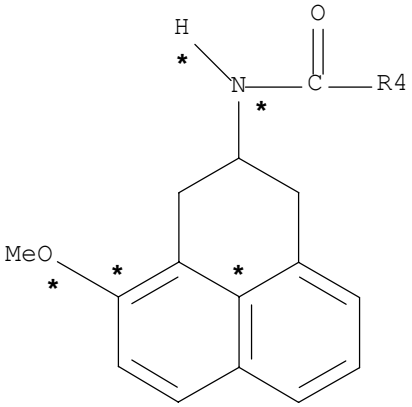


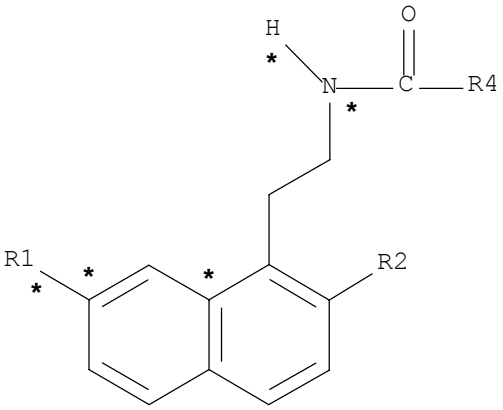
Fig. 4. Melatonin training and test set compounds with CoMFA superposition centres[a].



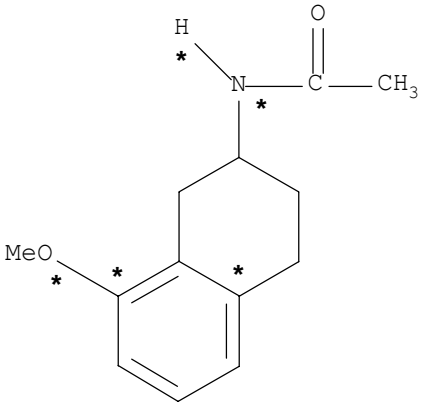
Indoles (T01-T09)



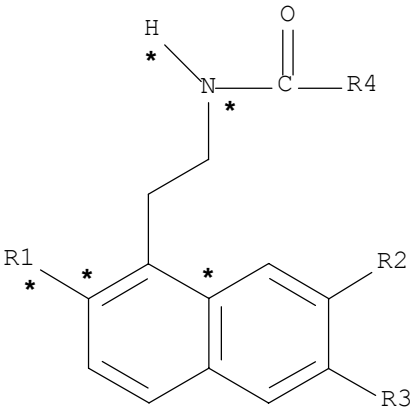
Tricyclics (T33-T34, Z52)



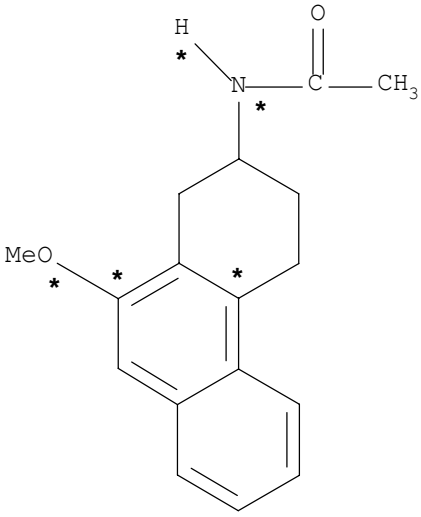
Naphthalenes: Type I (T10-T22)



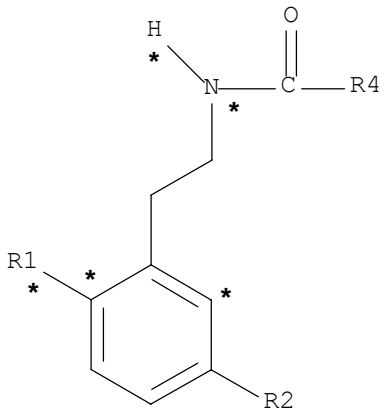
Tetraline (T35)



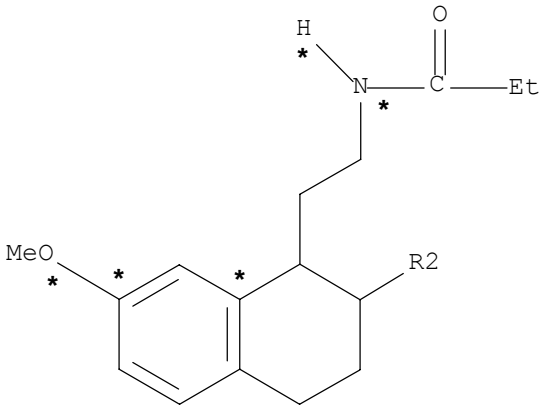
Naphthalenes: Type II (T23-T32, Z51)



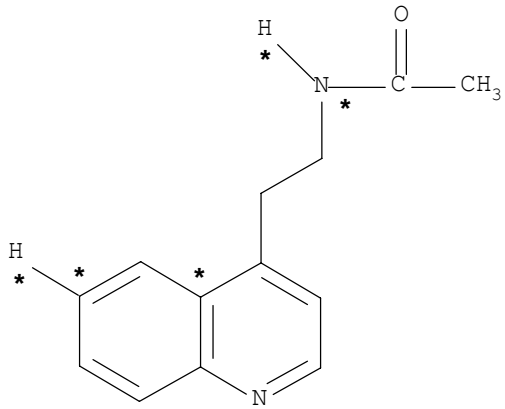
Tetraline (T36)



Benzenes
(T37-T46, Z53-Z57)



Naphthalene-like (Z50)



Quinolinic (Z49)

[a] TG and test set compounds prefixed with "T" and "Z" respectively.

Fig. 5. Distribution of melatonin receptor ligands in activity space.

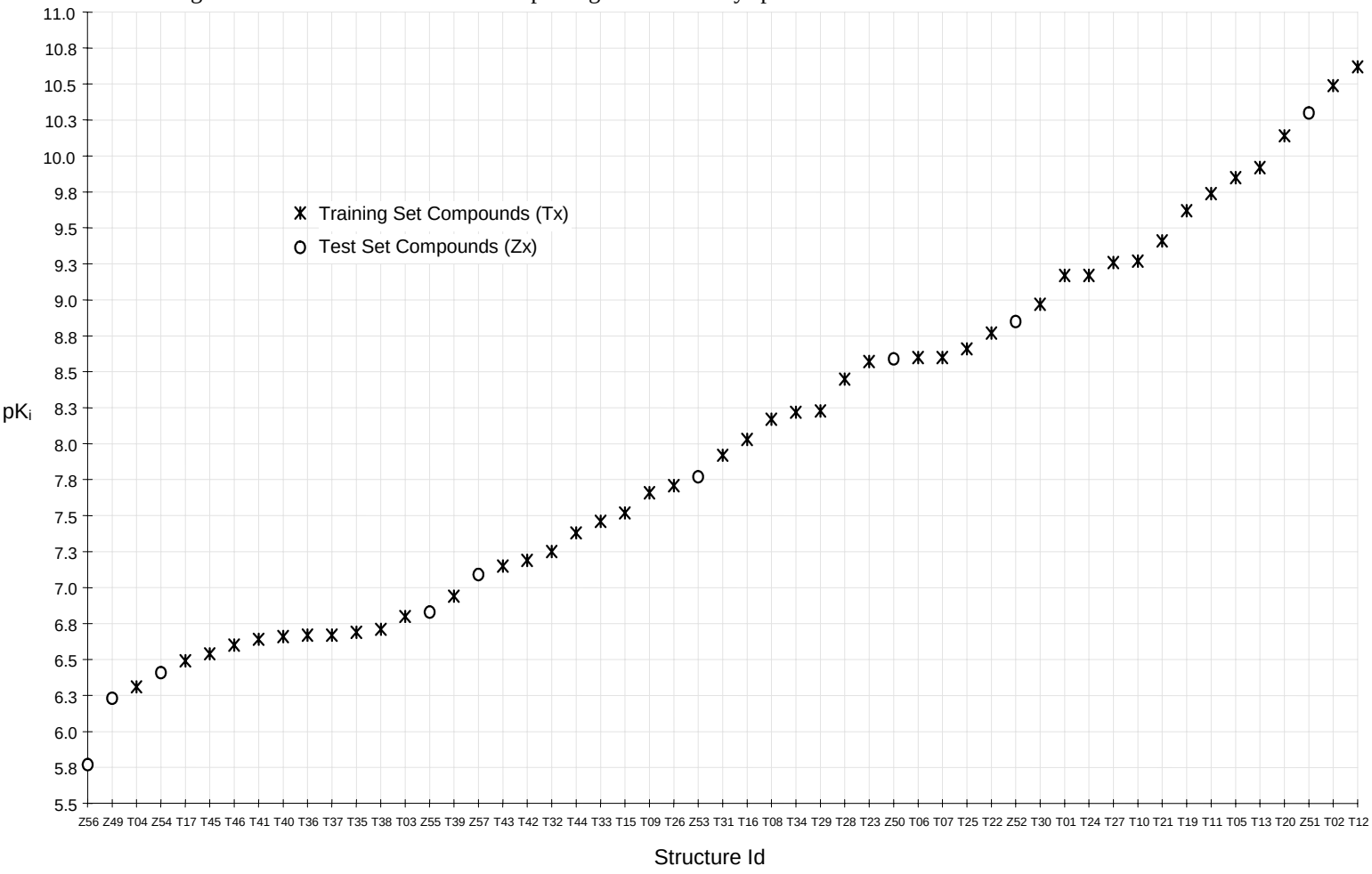


Fig. 6. Steroid skeleton.

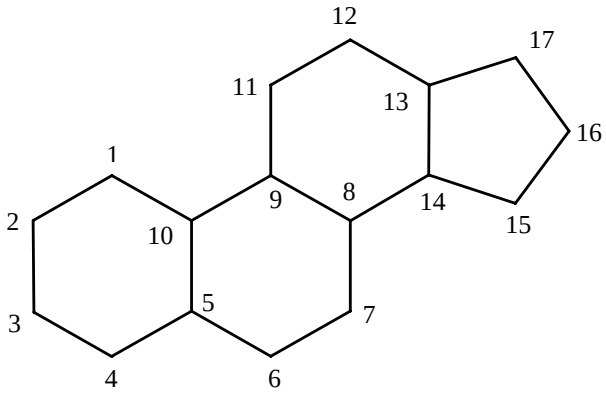


Fig. 7. Steroid dataset: cumulative q^2 for successive PLS LVs for classical EVA models derived from a range of σ values.

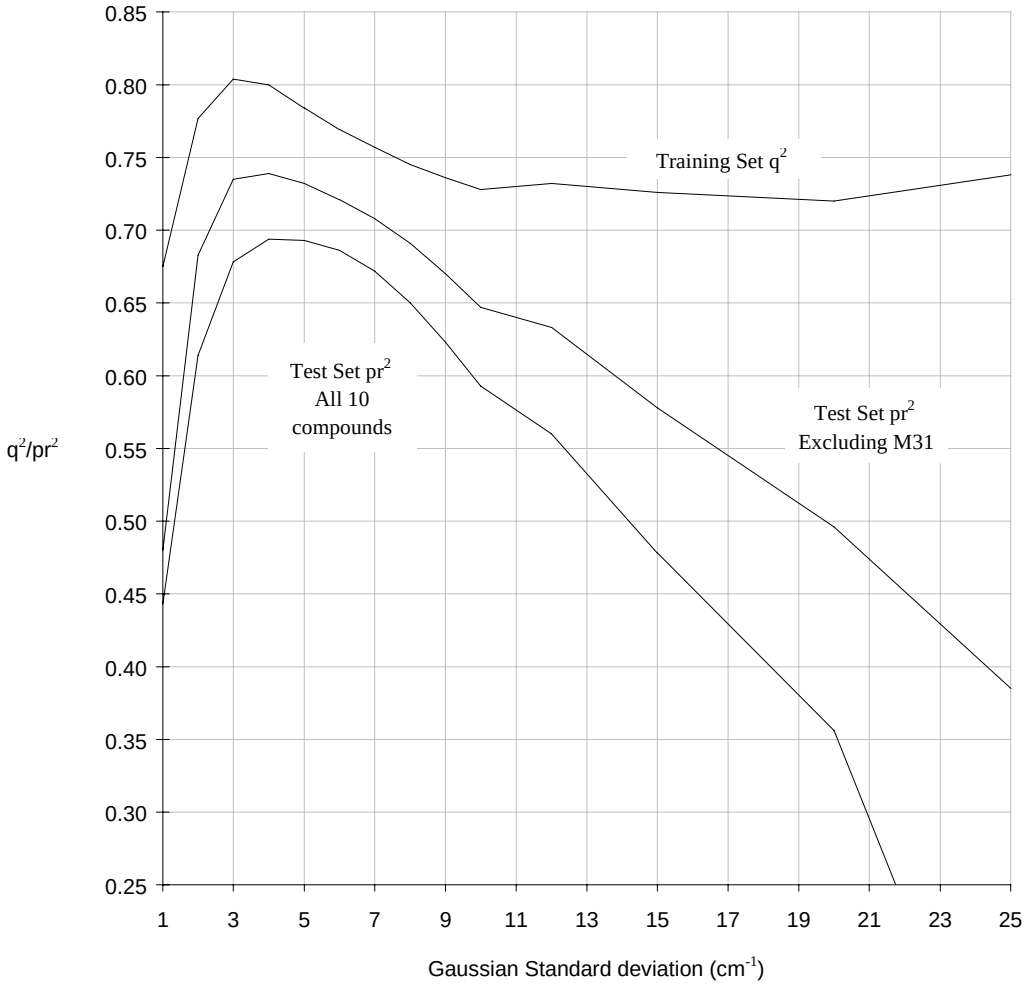


Fig. 8. Cumulative LOO q^2 for successive PLS LVs for classical EVA models derived from a range of σ values: melatonin receptor ligands.