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QSAR Models and Virtual Screening for Discovery of New Analgesic Leads

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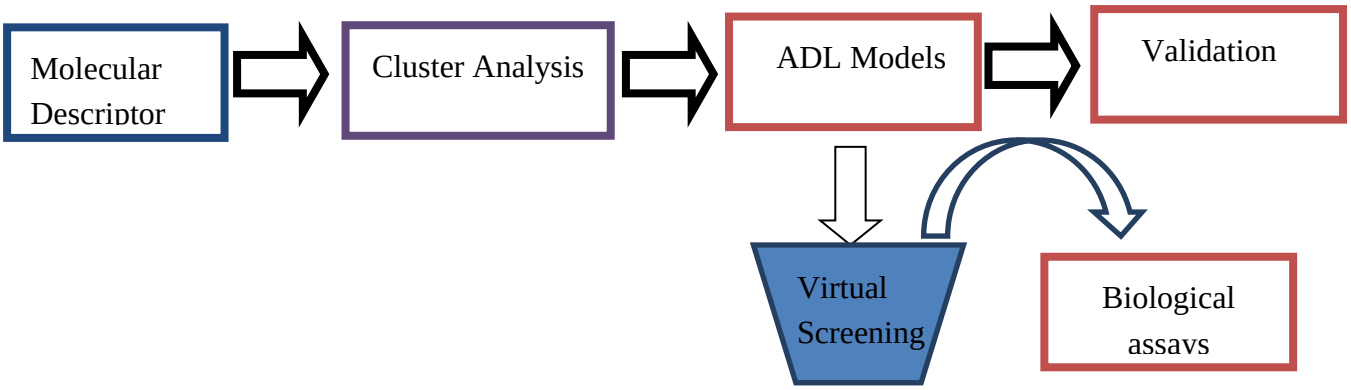
Abstract: The search for new selective pharmacological agents with no significant side-effects is an increasing requirement for the development of new drugs to be used in the treatment of acute and chronic pain. In the present study, a new series of compounds (VAM 1, 6, 10, 11, 12, 2–4) has been screening in QSAR–LDA mathematic models and pharmacologically evaluated. The antinociceptive properties of the new analgesic candidates obtained of virtual screening have been investigated *in vitro* tests. The pre-treatment with the compounds VAM 1, 2-4, 6, 10, 11, 12, showed a potent inhibition of IL-6 on RAW cells. The blocking efficacy of nineteen compounds on several isoforms of voltage-dependent sodium channels, expressed in *Xenopus laevis* oocytes, was tested (Nav1.3, Nav1.5, Nav1.6, Nav1.7, and Nav1.8). An exception was Nav1.6, where VAM 2–4 compound to result in substantial block indicating that acts specifically at this peculiar isoform. Compounds VAM 10 and VAM 2-4 are the most potent antinociceptive agents. These results indicate the potential of the compound VAM 2-4 to treat pain conditions.

Keywords: *In silico* Study, *TOMOCOMD-CARDD* Software, Non-Stochastic and Stochastic Linear Indices, Classification Model, Learning Machine-based QSAR, Analgesic Activity

Graphical Abstract:



Introduction:

The most frequent way to treat pain is by using analgesic drugs. Among the analgesics in use nowadays, those of reference continue to be acetylsalicylic acid and morphine, both isolated in the 19th century. In fact, the classical therapies for pain relief consist mainly in the use of Non-Steroidal Anti-inflamatory Drugs (NSAIDs) and opioids (OP). The first class, whose effects are mediated by the peripheral inhibition of cyclooxygenase (COX) is generally used in the treatment of mild to moderate pain [1]. OP drugs, are used in moderate to severe pain [2]. Both families show quite serious secondary effects such as renal toxicity and gastrointestinal lesions in the case of the NSAIDs or respiratory depression, tolerance and dependence in the case of opioids [3-4].

Current research in pain therapy looks at the discovery of new potent drugs devoid of the limiting side effects of the above-mentioned classes. In light of this virtual (computational) screening of chemical libraries has emerged as a complementary approach to techniques using the classical -*trial and error*- screenings [5-6]. By this means, computational techniques are used to select a reduced number of potentially active compounds from large available chemical or combinatorial libraries [7-9]. This *in silico* procedure will be used here in order to find predictive models that permit us the -rational-selection/identification as well as the design of new analgesics with the required properties.

Taking into consideration the arguments mentioned above, the aims of the present paper are: (1) to use a novel molecular descriptor family, atom-based non-stochastic and stochastic linear indices, in the generation of discriminant functions by linear discriminant analysis (LDA) that permits the classification of chemicals (analgesic and non-analgesic drug-like compounds) in a data set drawn from the literature, (2) to assess the 'biosilico' models by the use of different validation tests, (3) to develop a virtual screening of some *in house* libraries in order to identify potential novel chemical entities (NCEs) with analgesic activity and, (4) to examine the expression of the Nav1.3, Nav1.6, Nav1.7, Nav1.8, and Nav1.9 sodium channels and the electrophysiological properties of the drugs by studying their effects on the inward sodium current (I_{Na}) in *vitro*. (5) to determinate the effects of series of compounds over IL-6 on RAW cells.

Materials and Methods:

The database collected for our study consists of 1190 compounds in total. The active compounds in this set were 572 and 618 organic chemicals, having different clinical uses, were chosen as inactive compounds. In both cases (we consider the structural molecular variability as important goal to assure the quality (from *application domain* point of view) of our QSAR study.

The data stratification was done by using Cluster Analysis. From these chemicals, 902 were chosen to form the training set, being 433 of them active and 469 inactive ones. The great structural variability of the selected training data

makes possible the discovery of lead compounds, not only with determinated mechanisms of analgesic activity, but also with novel modes of action. The remaining subseries, consisting of 139 analgesics and 149 non-analgesics, were prepared as test sets for the external validation of the models (see Figure 1 for more details).

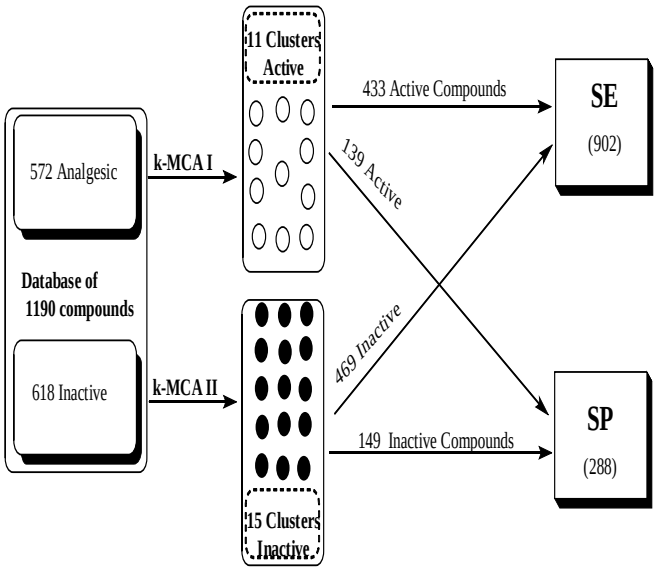


Figure 1. Partition scheme in Training and Test Set

In the present report, we used the *TOMOCOMD-CARDD* software [10]. We performed a hierarchical cluster analysis of the active and inactive series using statistical software package STATISTICA [11]. This procedure permits to select compounds for the training and test sets, in a representative way. This 'rational' design of the training and predicting series allowed us to design both sets, representative of the whole 'experimental universe. LDA was developed to classify compounds as analgesic-like (positive) or no analgesic-like (negative) through LDA, by using non-stochastic and stochastic linear indices as independent variables.

A chemical library, with 145 compounds was evaluated using all the obtained models. From these, nineteen compounds were chosen for biological assays (considered its structural diversity). An electrophysiological experiments *in vitro* was performed to study the effect of different compounds on sodium channels, the whole cell patch-clamp technique was used. The compounds were evaluated at 1, 10 and 100 μM concentrations. Also we carried out the determination of IL-6. The compounds evaluated ion this assay were VAM1, 2, 6, 10, 11, and 12: also at 1, 10 and 100 μM concentrations

Results and Discussion

The main classification-based QSAR equation derived by using forward stepwise LDA and all set of total and local atom-based linear indices computed is shown below:

$$\begin{aligned} \boldsymbol{AA} = & -1.334 + 0.001\ {}^{P}\boldsymbol{f}_{6}^{H}(\bar{x}) - 0.013\ {}^{G}\boldsymbol{f}_{3L}(\bar{x}_{E}) \\ & - 0.278\ {}^{P}\boldsymbol{f}_{3L}^{H}(\bar{x}_{E\text{-}H}) + 0.025\ {}^{K}\boldsymbol{f}_{2L}(\bar{x}_{E}) \\ & - 0.066\ {}^{G}\boldsymbol{f}_{0}^{H}(\bar{x}) - 3.133 \times 10^{-7}\ {}^{M}\boldsymbol{f}_{10}^{H}(\bar{x}) \\ & + 0.021\ {}^{P}\boldsymbol{f}_{5L}^{H}(\bar{x}_{E\text{-}H}) - 0.164\ {}^{P}\boldsymbol{f}_{2}(\bar{x}) \\ & + 0.015\ {}^{V}\boldsymbol{f}_{1}(\bar{x}) + 0.031\ {}^{P}\boldsymbol{f}_{3}(\bar{x}) \qquad \textbf{(1)} \end{aligned}$$

$\lambda = 0.40$	$F = 5.95$	$D^2 = 147.88$	$p<0.001$
$C = 0.84$	$Q_{total} = 91.82$	Spec = 89.61	
Sen = 93.05	*fpr* = 6.14		

where, ***AA*** refers to Analgesic Activity. $\mathbf{Q_{total}}$ to Accuracy, **Spec** to Specificity, **Sen** to Sensitivity and ***fpr*** refers to false positive rate.

The best of the 13 models obtained is showed in Equation 1. However, their real power and final aim reside at the ability to predict the biological properties of new compounds. Therefore, the use of a test set is essential to assess such a predictive power. The results of this model for the test set were Q_{total} = 88.77, Spec = 87.05, Sen = 89.63 and *fpr* = 9.59.

New analgesic leads were selected using the obtained models for the virtual screening of several databases, only the results of the virtual screening of quinoxalin chemical library will be showed.

Using heterologous expression in *X. laevis* oocytes, we investigated the potency of different compounds for sodium channels. The compounds VAM1, 2-4 and 10 evidence activity over the sodium channels. In the IL-6 determination assay, the compounds that evidence the best dose-answer relationship were VAM 10, 11 and 12. The rest showed good inhibition with 100 uM but the effect was smaller with the other ones. The inhibitory effects is dose dependent in all the cases. The more potent compounds were VAM 10 and VAM11.

Conclusions: Since the models obtained had a wide range application domain they could be useful in the selection of analgesic candidates. In some cases these models are better than those reported by the literature. Most powerful derivates could be obtained from the VAM 2-4 compound by using structure-activity relationship and molecular similitude studies. The compounds VAM-1, VAM-6, VAM-10, VAM-11 and VAM-12 showing *in vitro* analgesic activity, must be considered for further research in order to clarify their mechanism of action possibly related to IL-6.

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