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Identification of new analgesic candidates through virtual *in silico* screening and *in vivo* experimental test.

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Abstract: Currently, pain is closely linked to pathologies of high incidence worldwide. The *in silico* methods encompass all computer-aided techniques used in the design of compounds with desired properties, avoiding the high costs for the current tasks of synthesis and bioassays. In this sense, the fundamental objective of the present work is the identification of new analgesic candidates through virtual *in silico* screening using classification trees. For this purpose, a database of the literature is initially collected, and analgesic activity has been reported experimentally. Through the DRAGON software, a series of molecular descriptors were calculated and a Hierarchical Conglomerate Analysis (CAs) was performed in the STATISTICA software, allowing the separation of the initial database in training series and prediction series. Then we proceeded to obtain and validate the model used (Tree J48) through the WEKA software. Of these three compounds were evaluated experimentally *in vivo* with excellent results as analgesic drugs. In general, we can conclude that the use of these computational tools generates a great saving of resources with respect to traditional methods of analysis and also allows a rapid identification of compounds with a high probability that they are potential analgesics.

Keywords: in silico methods, potential analgesics, identification of new analgesic candidates, classification trees

1. Introduction

Pain is a problem not yet solved by medicine. According to the World Health Organization, 90 % of diseases are associated with pain. (1) The most common treatment for pain is the use of analgesics such as nonsteroidal anti-inflammatory drugs (NSAIDs) or opioids (OPs). Both families show quite serious secondary effects such as renal toxicity and gastrointestinal lesions or respiratory depression, tolerance and dependence.(2, 3)

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.(4) By this means, computational techniques are used to select a reduced number of potentially active compounds from large available chemical or combinatorial libraries. 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 tree classification that permits the classification of chemicals (analgesic and non-analgesic drug-like compounds) in a data set drawn from the literature, (2) to develop a virtual screening of some *in house* libraries in order to identify potential novel chemical and (3) to evaluate the *in vivo* analgesic activity of the best candidates *dry* selected by using in vivo assays.

2. Results and Discussion

In silico Studies

Cluster analysis. The structural diversity of the database is the first aspect that should be proved; following this aim, a hierarchical CA (k-NNCA) was carried out using the STATISTICA software.(5) All the variables showed *p*-levels<0.05 for the Fisher test. Both independent partitional clusterings were developed for the active compounds first and for the inactive ones later. After that, a *k*-MCAs was done, to assure the correct selection of the variety of the different chemical families in both training and prediction series. As can be observed in Figure 1, the *k*-MCAs partitioned the set of analgesic compounds into 11 clusters for the actives chemicals, and for the case of inactive ones (non-analgesic) 15 clusters were obtained. Later, a random selection was carried out to extract a representative compound of every family inside each active and inactive cluster. Besides, to assure a good splitting, we try to satisfy that members of the validation set be closer at least to one point of the training set. This criterion suggested in the international reports, was followed, permitting all subsystems to be represented simultaneously in the training and test sets

To this effect, 902 compounds were chosen for the training set; which includes 433 and 469 active and inactive compounds, respectively. In the same way the prediction series, for the validation of the model, included the remaining 288 chemicals, being 139 of them analgesics and the rest 149 inactive compounds with other clinical uses.

Tree Classification (Tree J48).

The data were used to obtain the model, able to classify compounds as analgesic-like (positive) or no analgesic-like (negative) through Tree Classification. For this purpose, the procedure of the statistic package WEKA (6) was fixed as a strategy for variable selection.

Virtual screening and identification of computational analgesic leads.

New analgesic compounds were selected using the obtained model for the virtual screening of new compounds and other preexisting substances. Out of the total compound screened 20 of them were chosen as analgesic candidates (eight isolated compounds and 12 products of the hydrolysis). Three of these substances were evaluated by using *in vivo* test.

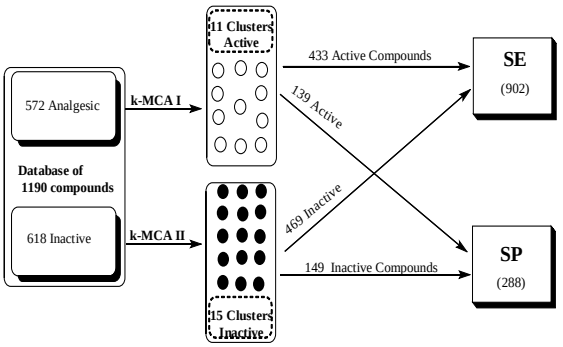

Figure 1. Partition scheme in Training and Test Set and Cluster Analysis.

Biological Assays:

As shown in the corresponding Table 1 there is an increase in the reaction time as time goes on, and in many cases comparable to morphine (reference drug).

Only when compound 3 and 4 were administered at 200 mg kg−1 was a significant increase ($P < 0.001$) seen in latency time at all observations. DMR 3 administration was more active and showed a dose-dependent response (Table 1). The animals treated with morphine gave a response latency period longer than 20 s, since it was established as the cut-off time for the protocol. The three compounds administered intraperitoneally to the mice showed anti-nociceptive activity in the Hot Plate test (Table 1). These results indicate a significant anti-nociceptive activity at both dose (100 and 200 mg kg−) levels studied.

Table 1. Effect of Compounds 1, 3 and 4 (50, 100 and 200 mg/kg, (i.p.) on Hot Plate reaction time in mice

Groups	Dose (mg.kg $^{-1}$)	Reaction time (time in s)		
		30	60	90
Morphine	10	>20*	>20*	>20*
Compound-1	50	9.56 ±4.2	9.48 ± 3.8	11.38 ± 4.7
	100	7.52 ± 3.1	10.94 ± 4.5	11.7 ± 4.8
	200	9.72 ± 4.1	10.6 ± 4.3	14.44 ± 4.7
Compound-3	50	11.12 ± 4.7	12.38 ± 5.2	13.14 ±5.5
	100	12.28±5.0	13.16±5.4	14.18±5.8
	200	12.5±5.1	18.26±5.4*	19.82±5.8*
Compound -4	50	9.04±3.7	10.82±4.4	11.38±4.6
	100	9.5±4.0	11.12±4.5	11.62±4.7
	200	9.54±3.9*	16.18±4.5*	18.9±4.8*

3. Materials and Methods

The database collected for our study consists of 1190 compounds in total. The active compounds in this set were 572, including representative of families with diverse structural patterns and analgesic action modes The others, 618 organic chemicals, having different clinical uses, were chosen as inactive compounds. In both cases (active and inactive ones) we considered the structural molecular variability as important goal to assure the quality. All these chemicals were taken from the Negwer Handbook(23) and Merck Index(24), where their names, synonyms and structural formulas can be found. The data stratification was done by using Cluster Analysis. This procedure permits to select compounds for the training and test sets, in a representative way.

Virtual Screening: the chemical library, with 20 compounds was evaluated using all the obtained models and the compounds with $\Delta P>0$ were classified as active. From these, three compounds were chosen for biological assays.

Biological Assays: For the biological evaluation of the three compounds (DMR1, DMR 3 and DMR 4), *in vivo* test (Hot Plate) was performed in order to assess the predicted analgesic activity and to clarify the mechanism of action of these compounds. The hot-plate test was used to measure the response latencies according to the method described previously at three doses levels: 50, 100 and 200 mg/kg. (9)

4. Conclusions

The results obtained in this work displayed a good correlation between the in silico and the experimental studies and highlight de potential of the WEKA software to predict the biological activity

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