Streamlining Anti-Inflammatory Lead Discovery by Aligning *in silico* and *in vivo* Screening. Prediction, Synthesis, and Biological Assay of Novel Metal Based Chelates

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Inflammation: a major life-threatening human condition Inflammatory responses can be excessive and may therefore result in pain, tissue damage, or chronic inflammation when not properly resolved.

Both acute and chronic inflammatory conditions such as osteoarthritis, inflammatory bowel disease and rheumatoid arthritis affect million people worldwide.

Anti-inflammatory drugs (A.I.Ds) have an important clinical role in the control of a response and resolution of excessive acute or chronic inflammatory processes. However these drugs exhibit severe undesired side effects among other limitations.

computer-aided molecular design.

> 587 molecules selected for the

antiinflammatory agents covering

mechanisms of action known so

model

the

far.

elaboration

broadest antiinflammatory

Molecular entities

Training set, Test set

and NMEs.



Ligand based virtual screening will allow us

to select new A.I. chemical entities from a

library prior to evaluate their antiinflammatory

In vivo testing

of high-scoring

molecules

New anti-inflammatory

chemotypes

properties in vivo in Zebrafish larvae.

formatic

Curgent need for new and safer AIDs

Strategy for the discovery of new antiinflammatory chemotypes

Sreening algorithm

Inactive

The ToMoCoMD-CARDD (TOpological MOlecular COMputer Design-

Computer Aided Rational Drug Design) approach has been introduced

for the classification and design of antiinflammatory agents using

Selection of structurally diverse antiinflammatory drugs

were

Zebrafish model for antiinflammatory evaluation

observed at 5dpf after 24h exposure to the tested drugs.

>10 larvae from 4 dpf per group. Complete tail transection and 10 µM LPS caused leukocyte

migration to the wound. Fixed larvae were

The zebrafish is a well-characterized model organism used in the screening of potential drug. The biological evaluation was assayed by using an in vivo model of acute inflammation in transgenic zebrafish *fli*-1: EGFP larvae. MTC: 10 larvae per well were placed using 10 μM, 30 μM and

100 µM as concentration in the medium. Characteristics of the larvae were

stained post 20h incubation and leukocytesnumbered (in triplicate).

= anti-inflammatory



On average one lead series can be developed from testing approximately 350,000 diverse compounds in a typical HTS screen.

Integration of chemoinformatics in drug discovery



Chemoinformatics is the combination of chemical synthesis, biological screening and data analysis to guide drug discovery and development



0000

000 Inactive

k-MAC II

The main data mining approaches used in cheminformatics are descriptor computations, structural similarity matrices, classification algorithms.
The ToMoCOMD-CARDD (TOpological MOlecular COMputer Design-Computer Aided Rational Drug Design) was used in this research

Training set

919 Compounds

587

Compounds

Chemical Database

626 Non-

anti-inflammator

compounds

Test set

294 Compounds

General algorithm used for designing the

k-MAC I Anti-inflammatory

ToMoCoMD-CARDD for the discovery of new antiinflammatory chemotypes

> Non stochastic and stochastic atom-based quadratic fingerprinting were used to codify the antiinflammatory-related chemical structure information from a comprehensive data set of 1213 organic compounds having a great structural variability,.

The two ligand-based antiinflammatory -activity classification models obtained by using Linear Discriminant Analysis resulted in a total 13 QSAR models classifying correctly 90.73% and 92.47%. respectively, of 919 chemicals the training set. These models showed moderate-tohigh Matthews correlation coefficients (MCC of 0.75 and 0.77) as well as a very good accuracy, sensitivity, specificity and false alarm rate.

➤These models were able to classify correctly 92.16% and 87.56% of 294 compounds in an external text set

compounds in an external test set. training and test sets throughout *k*-MCA ➤ The *ToMoCoMD*-CARDD models were best in predicting antiinflammatory activity when compared with six of the most recent models reported so far indicating that this

when compared with six of the most recent models reported so far indicating that this approach could be very useful to identify (design and/or select) new A.I. agents.

Discovery of a new antiinflammatory chemotype

➢ Finally, the fusion model was used for the identification of novel antiinflammatory compounds using virtual screening of 200 molecules available in our in-house library. Of these, six metal-based complexes were selected, synthesized and tested in an *in vivo* anti-inflammatory test using *Danio rerio* (zebrafish) larvae.



➤ Magnesium-valproic acid complexes (5 and 6) exhibit the best *in vivo* antiinflammatory properties with 77% and 79% reductions in relative leukocyte migration to the damage zone (RLM) scores, respectively, a result analogous to indometacin used as control. Valproic acid and sodium valproate, a leading antiepileptic drug, proved inactive in this paradigm.

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Conclusion & perspectives

> These results offer an attractive possibility to obtain new anti-inflammatory compounds by using ensemble LDA-assisted QSAR-classifier models, thereby significantly reducing the number of synthesized and tested compounds.

> The present study indicates that magnesium-valproic acid complexes may represent an important therapeutic alternative for the treatment of inflammatory conditions.