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CONCISE ARTICLE

Fragment pharmacophore-based *in silico* screening: A powerful approach for efficient lead discovery†

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Received 00th xxxxxxx 20xx,
Accepted 00th xxxxxxx 20xx

DOI: 10.1039/x0xx00000x

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Through a process of fragmentation, functionalization, and recombination of market approved molecules for cosmetic usage, we customized an *in-house* virtual library comprising molecules ideally suited for virtual screening. A computational pharmacophore-based screening of this virtual library followed by a 3 month optimization phase led to the identification of an optimized lead with all expected properties in hands to be developed as a candidate for skin benefits in cosmetic application. The success of this pilot project paves the way for other cosmetic targets of interest.

Introduction

Pharmacophore-based virtual screening and activity profiling has become one of the most popular *in silico* techniques for supporting medicinal chemists in their hit finding, hit expansion, hit to lead and lead optimization programs.[1-6] One of the key success factors on the long road from *in silico* screening to pre-clinical candidate is the relevance of the initial virtual library. In the present study, an *in-house* library of 92,000 molecules was specially designed with an optimal skin benefits profile and used for fragment pharmacophore-based *in silico* screening.

Focused library design

Whereas pharmacophore-based virtual screening is one of the most popular virtual techniques used to support medicinal chemistry in hit and lead discovery,[1-6] it still faces challenges, among them developing screening libraries that ensure optimal coverage of chemical space, physico-chemical properties and chemical tractability.[7] Moreover, safety-related problems continue being one of the major causes of drug attrition in preclinical and clinical development, forcing chemists to consider the toxicity issue as early as possible in the research process.[8] In this study, our objective was to quickly get access to an active, safe and easily accessible candidate molecule for development, through an innovative *in vitro* process to select new compounds for topical cosmetic applications. Of note, daily experiences from pharmacology show that an early detected activity can hardly forecast an *in vivo* efficacy, in real life conditions. Same holds true in cosmetic applications where, inversely to oral route, the notion of efficient topical concentrations (amount applied, frequency of usage, elimination by skin or washes...) is indeed much more difficult to predict and assess. Hence, we decided to design our own virtual screening library by using a process of fragmentation-recombination of used and approved molecules with skin beneficial properties in cosmetic application. Indeed, toxicological endpoints such as skin irritation or skin sensitization often result from presence of reactive functional groups in the molecules. Moreover, starting from already "cosmetic" molecules will allow ending up with new entities exhibiting suitable physico-chemical properties (e. g. water solubility, log P, ...) for cosmetic formulation.

A Fragmentation-recombination process

Starting from a collection of 274 structurally diverse parent molecules approved for various cosmetic applications (safety profile, good water solubility, favourable physico-chemical and pharmacokinetic properties, etc.), a process of smart fragmentation led to a set of 542 unique fragment molecules.

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† The authors declare no competing interests.

The fragmentation was performed considering either the chemical groups or the moieties that could be essential for the activity as well as the predictable physico-chemical properties. This phase was performed manually, molecule by molecule, based on our expertise in medicinal chemistry (Fig. 1).

The second step consisted in functionalizing - if necessary - the previously generated fragment molecules to prepare them for the recombination phase. Once again, this job was done manually taking into account toxicity concerns, green chemistry compatibility, as well as good chemical accessibility. In the cosmetic industry, constraints of environmental safety and production costs are major factors to prioritize for further development. At the end of the functionalization phase, only the 333 commercially available compounds were retained. In Fig. 1, the example of fragmentation - functionalization of 5,7-dihydroxy-2-phenyl-chromen-4-one (Chrysin), used in eye contour care products, is presented. Finally, the two-by-two assembly of the 333 fragments was performed using

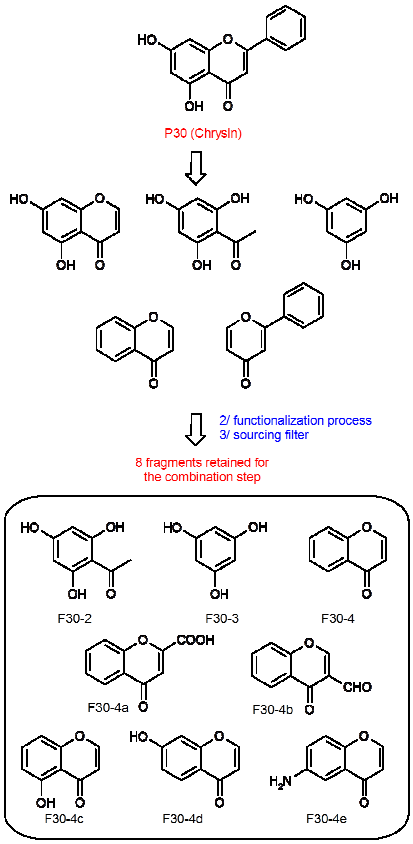

Fig. 1 Example of fragmentation - functionalization processes of 5,7-dihydroxy-2-phenyl-chromen-4-one (Chrysin).

Combichem® software (Accord For Excel® module), based on eight reactions compatible with green chemistry (e.g. esterification, peptidic coupling, …) leading to the generation of a virtual library comprising 92,000 unique molecules. Since each of the fragments was often polyfunctionalized it could undergo several different connection reactions, thus being represented in many different end products (Fig. 2).

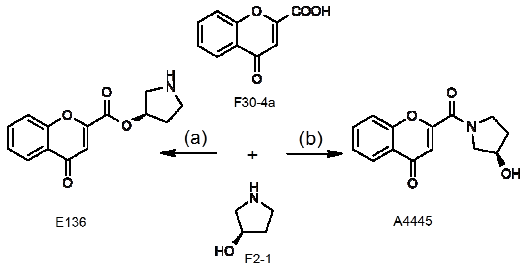

Fig. 2 Example of fragment recombination: (a) esterification; (b) amidation.

B Virtual library properties

Among the customized library of 92,000 molecules, only 1.5% were found to be commercially available (the search was done using e-molecules® interface), thus attesting the high level of originality of our virtual library. However, the compounds should be easy to prepare since they all originate from a combination of two commercially available fragments using standard and simple chemical reactions.

Whereas 70% of the parent molecules have a molecular weight (MW) below 300 Da, 85% of the virtual molecules have a MW between 150 and 450 Da and 60% below 300 Da (Fig. 3A). The histograms of the cLogP[9] distribution are identical for the parent library and the descendant virtual library with 80% of them below 2.5 (Fig. 3B). These parameters are in complete accordance with lead discovery processes[10-11] and parent cosmetic ingredient profiles.

Regarding the other physico-chemical properties (in particular: water solubility, skin bioavailability and environmental requirements), a same distribution profile was observed between the parent molecules set and the virtual compounds (results not shown). This result was quite satisfying as our aim was to get access to new derivatives that would keep the approved properties of the original molecules.

Fragment pharmacophore-based lead discovery

Our cosmetic-oriented virtual library in hands, we wanted to establish the proof of concept of our approach on a novel skin protein as target of interest, using Virtual Screening. It is noteworthy to underline that neither any parent molecule nor their corresponding fragments used in the generation of the virtual library were claimed to be active molecules on this specific biological target.

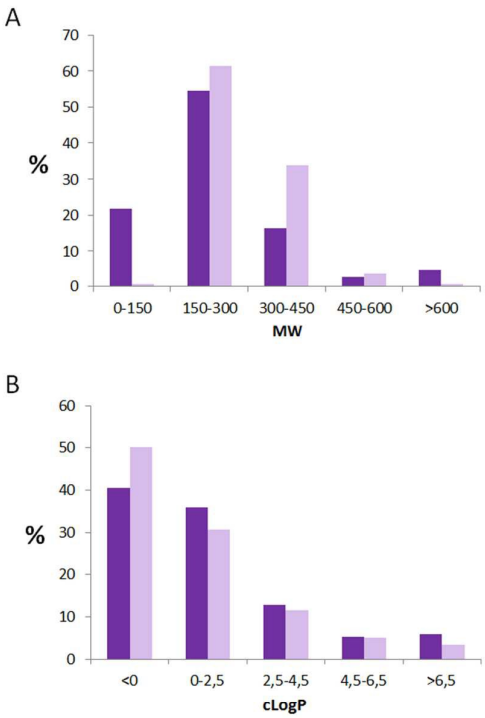

Fig. 3 Distribution of calculated physico-chemical properties (A molecular weight; B cLogP) of the parent molecules (dark purple) and the virtual descendant library (light purple).

A Pharmacophore-based virtual screening

Virtual Screening (VS) represents a fast and cost effective tool for medicinal chemists to reduce the enormous virtual space of chemical compounds to a more manageable number for further synthesis and screening against biological targets, which could lead to potential active compounds. This strategy is now largely employed in computer aided drug design and hit discovery processes.[12-15] Two main approaches are used in VS, depending on available data: Ligand-Based Virtual Screening (LBVS) and Structure-Based Virtual Screening (SBVS). LBVS uses 2D or 3D similarity searches from known active molecules. It has to play with their flexibility without knowing their active conformation.[16] SBVS applies different modelling techniques to mimic the binding interaction of a ligand to a biomolecular target. Hence, SBVS requires structural information on the target, usually obtained from X-ray crystallography or nuclear magnetic resonance (NMR).

The present study focused on the discovery of novel inhibitors of an enzyme of interest (undisclosed) for which the catalytic site was described. A recent X-ray structure of the protein co-crystallized with a ligand gave us the opportunity to develop a Structure-Based Virtual Screening.

To define and analyze the ligand protein interactions we used the 3D-pharmacophore approach.[3-4,6,9] Using LigandScout® software (Inte:ligand),[9] we generated 3D-pharmacophore models that point out all the putative interactions between the ligand and the protein. After optimization processes, we obtained one global pharmacophore model, representative of these specific interactions,[1-3] and suitable for Virtual Screening (Fig. 4).

However, the discrepancy between the length of the pharmacophore model (ca. 15 Å) and the size of the virtual molecules led us naturally to consider a computational fragment-pharmacophore-based screening approach.

The original pharmacophore model was partitioned into three zones giving rise to two pharmacophore models: PSB1 (zones 1+2) and PSB2 (zones 2+3) (Fig. 4). Virtual screenings of the *in-house* virtual library on models PSB1 and PSB2 led to the identification of 352 and 148 virtual hits, respectively.

Pharmacophore model refinement followed by the study of further parameters (nature and strength of interactions, pharmacophore fit score, scaffold diversity, molecule design, novelty) led to a pre-selection of 43 virtual hits.

B First hit discovery

The 43 virtual hits were synthesized and evaluated (Fig. 5). It is noteworthy that compounds could be prepared in two steps on average and that 85% of them had a water solubility higher than 1 mM which confirms the relevance of our customized virtual library. Among the 43 tested molecules, three hits with target activity in the millimolar range (PCI 9301, PCI 9240 and PCI 9298), belonging to two chemical series, were identified.

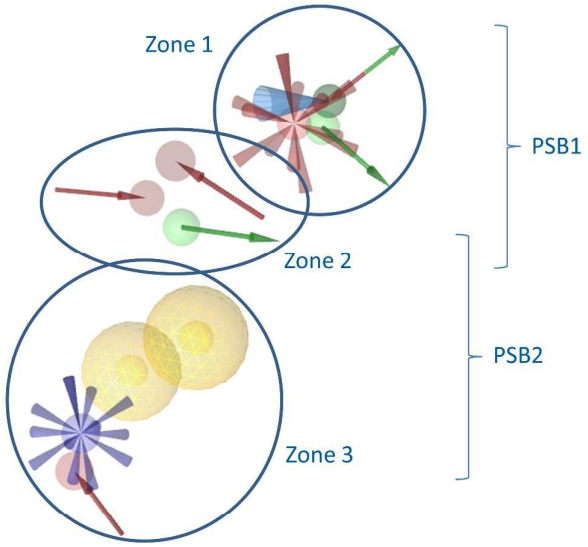

Fig. 4 Global pharmacophore model fragmented in three distinct zones. Ligand-protein interactions are depicted as follows: red arrow (H-bond acceptor), green arrow (H-bond donor), yellow spheres (hydrophobic), blue star (positive ionisable), red star (negative ionisable), blue cone (metal binding location).

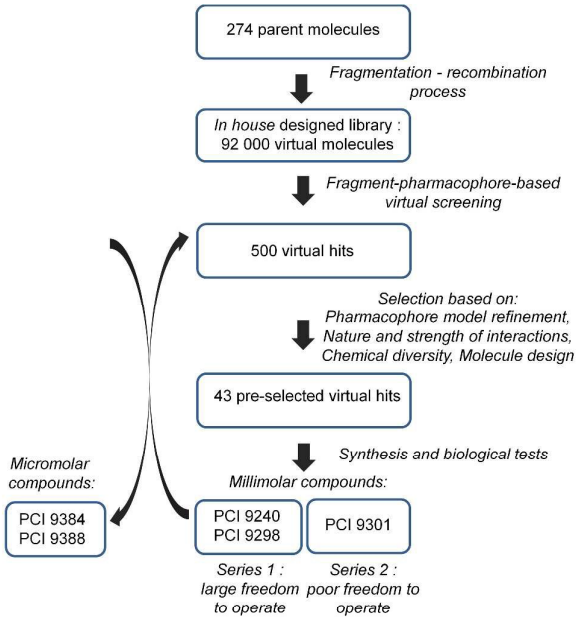

Fig. 5 Hit discovery workflow: from virtual library design to novel micromolar hits.

For intellectual property (IP) reasons, we focused on series 1 for a hit follow-up / hit validation process. Eight related analogues were selected from the virtual hit list and synthesized; two of them, PCI 9384 and PCI 9388, were found to be active on the selected enzymatic target in the micromolar range (< 500 µM), validating series 1 for the hit-to-lead phase (Fig. 5 and 6).

C Hit expansion – Lead discovery

In only three months, with two medicinal chemistry FTEs, tightly supported by molecular modelling, efficient hit expansion was achieved to target additional interactions in the zone 1 of the pharmacophore (Fig. 4). This optimization phase led to the discovery of PCI 9714 with low micromolar activity on the enzymatic target (IC_{50} < 10 µM). This level of efficacy is compatible with a cosmetic use. For example, L'Oréal marketed Pro-Xylane™ is a successful anti-aging product which exhibits an efficacy between 500 µM and 3 mM for glycosaminoglycans biosynthesis activation.[17]

As desired, PCI 9714 was a high-quality lead compound with already satisfying physico-chemical properties for cosmetic application (e.g. MW < 350 Da, water solubility > 5 mg/mL, very good calculated skin bioavailability,[18] good chemical stability in hydroalcoholic solution for 2 months at 45 °C). Environmental profile was favourable, with no ecotoxicity detected on alguae, daphniae and fish larvae.[19] The compound was not suspected to be classified PBT-vPvB ((very) Persistent, (very) Bioaccumulative and/or Toxic) according to our *in silico* prediction model.[19] No human toxicity issues were seen in our first sets of *in vitro* models (skin sensitization,[20-21] mutagenicity,[22] genotoxicity,[23] phototoxicity,[24] skin tolerance)[25]. The selectivity was studied over 11 enzymes belonging to the target sub-family (Fig. 7) and showed a great profile at 10 µM.

Moreover, the molecule presents a full free IP position and can be prepared in large scale (up to 1 kg) in 3 steps from low cost, industrially available starting materials.

Hence, the combination of two fragments (F159-3 and F137-1a) led to the discovery of PCI 9298, a small molecule with inhibitory activity in the high micromolar range. This hit underwent a hit-to-lead (H2L) optimization process to produce our lead PCI 9714 with single digit micromolar activity on the target. PCI 9714 can also be seen as the assembly of 3 fragments (F159-3, P14 and F17-2) belonging to the 333 fragment list followed by a single functionalization step (Fig. 8). This observation encourages us to extend the *in-house* virtual library by assembling more than two fragments in order

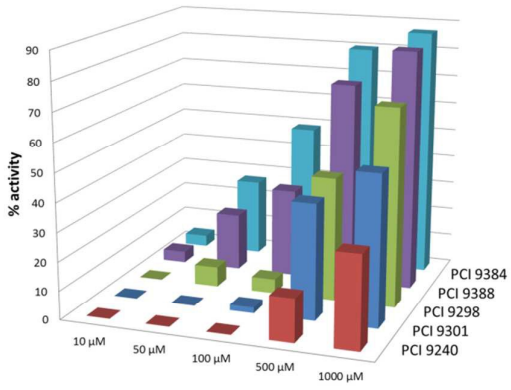

Fig. 6 % activity of selected compounds at various concentrations.

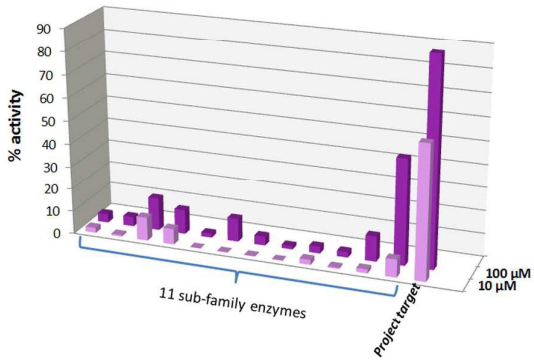

Fig. 7 % activity of PCI 9714 on 11 sub-family enzymes compared to the targeted enzyme.

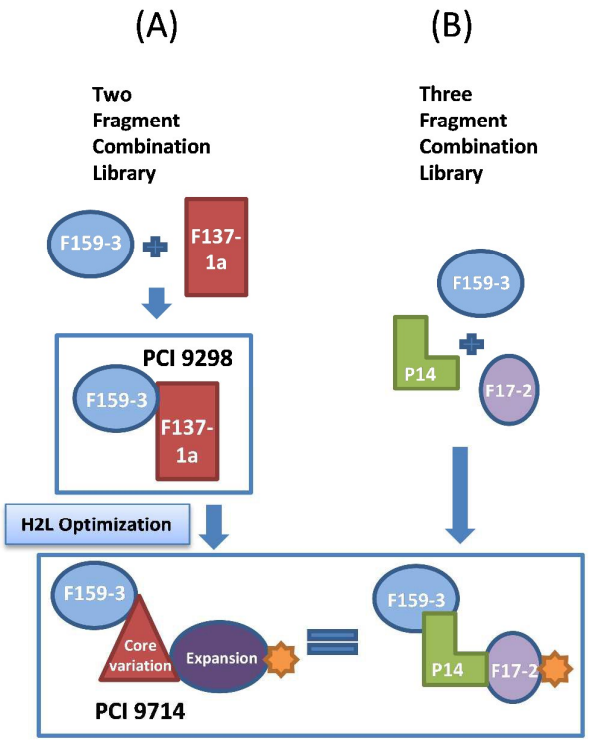

Fig. 8 Schematic representation of PCI 9714 discovery starting from our *in house* fragment library: (A) Two fragment assembly followed by Hit-to-Lead process. (B) Combination of three fragments, followed by a single functionalization step.

to develop a larger and more diverse virtual library, optimal for screening on a large panel of enzymes and other biological targets of interest to cosmetic applications. With that three-component library in hands, we would have been able to directly discover the development candidate without any lead optimization process.

Conclusions

The fragmentation of a smart library of market approved molecules in cosmetic applications into a selection of optimized fragments, and recombining these fragments following specific criteria, allowed us to produce an original *in-house* virtual library of 92,000 compounds with suitable properties for cosmetic use. Virtual screening of the library, using a fragment-based pharmacophore approach, followed by a short and oriented Hit-to-Lead optimization process, led to the discovery of novel development candidates having intrinsically the suitable properties for specific skin benefits in cosmetic applications. This is the first report of such a fragment-based tailor-made approach for cosmetics. Our unique virtual library proved being of valuable interest for a skin target, and thus could serve as a tool box for other targets. Beyond skin, with simple adjustment of the virtual screening specifications, this fragmentation-recombination strategy also paves the way for other cosmetic targets.

Acknowledgments

We would like to thank the L'Oréal Advanced Research teams who made a significant contribution to this work: Lucie Simonetti from Dominique Bernard's team for biological evaluation, Daniel Duché's team for human safety assessment, Marc Léonard's team for environmental profiling, Dominique Jullien's group for analytical and chemical stability studies, as well as Anne-Claude Dublanchet's team for industrial feasibility assessment, and especially Michel Navet for the scale-up work.

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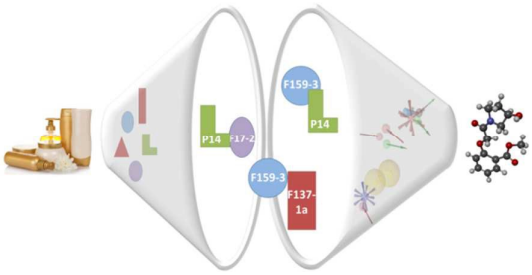

Fragment pharmacophore-based *in silico* screening: A powerful approach for efficient lead discovery

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Fragmentation-recombination process coupled with fragment-based pharmacophore *in silico* screening: a tailor-made approach for the discovery of new cosmetics.