Boosting of retinol activity using novel lecithin:retinol acyltransferase inhibitors

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Introduction:

Retinol (ROH) and its esters are well accepted as efficacious skin anti-aging ingredients [1]. However, there is an ongoing desire to boost the efficacy of ROH in order to be able to reduce the concentration in topical compositions mitigating its irritative effects or simply to maximise its efficacy. In the skin, ROH is metabolized to retinoic acid by several metabolic steps (Figure 1A). Thus, activation or inhibition of these enzymes offers routes to improve the efficacy. ROH is mostly converted to retinyl esters in skin to become inactive storage reserves and thus its esterification limits active levels [2]. It has been shown that lecithin:retinol acyltransferase (LRAT) is a main enzyme responsible for ROH esterification [3]. The enzyme transfers the sn-1 fatty acyl group, largely linoleic acid, from phosphatidylcholine to ROH [4]. Thus, inhibition of LRAT would allow more ROH to be available for subsequent conversion to retinoic acid. Reactions by LRAT are facilitated by a catalytic diad consisting of His60 and Cys161 and take place in two distinct steps (Figure 1B). First, the ester group phosphatidylcholine substrate is attacked by the thiolate and covalently attached to the catalytic Cys residue. In a next step, ROH attacks this newly formed thioester, resulting in the ROH ester product [4]. In contrast to other acyltransferases, LRAT does not require a coenzyme A intermediate. We identified novel LRAT inhibitors and demonstrated their retinoid boosting effects on collagen III synthesis. Further, we deduced structure-activity relationships of the inhibitors to explore observed activity differences with AI driven computational methods.

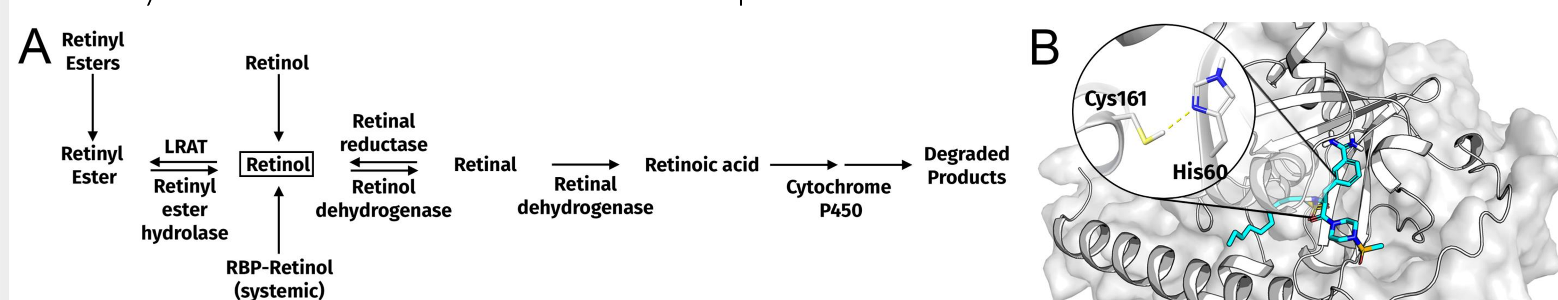


Figure 1: The role and functionality of LRAT. (A) Generalised metabolism of ROH and its esters to RA. (B) Structural overview of LRAT and catalytic diad.

Materials & Methods:

In vitro assay. Selected ingredients were assayed *in vitro* for their LRAT inhibitory potential in a phosphate buffer containing retinol, dilaurylphosphatidylcholine, dithioerythritol and bovine serum albumin (pH 7.0) for 60 min at 37°C. The reaction was quenched with ethanol before extraction with pentane and the resulting extracted retinoids were determined by reverse phase high performance liquid chromatography.

Ex vivo assay. As a proof-of-concept study to underline the promising potential based on LRAT inhibition we performed an *ex vivo* study on human skin via topical application. The inhibitors were applied 3 times (at day 0, 1, 4, 6) and the skin was harvested for histologic analysis at day 8. The collagen III level was assessed by immunohistologic staining and % stained surface of papillary dermis was quantified by image analysis using CellSense software. For each condition nine samples were stained and analysed (n=9). Statistics was calculated using unpaired student t-test.

Molecular modeling. Homology models of LRAT were generated using the SWISS-MODEL2 webserver using the input sequence in FASTA format derived from the UniProt database (Accession code: O95237). Models were constructed using the top-3 ranked template structures (PDB IDs: 4Q95, 2KYT, and 4DPZ) according to the global quality estimation score [5]. Furthermore, an alternative structure was obtained from the AlphaFold Protein Structure Database [6]. The protein structures were preprocessed using the Protein Preparation Wizard [7] within the Maestro Small-Molecule Drug Discovery Suite. Ligands were preprocessed using the LigPrep routine on default parameters. Docking was conducted using the Glide standard precision (SP) [8] and AutoDock Vina [9] docking engines. The centroid for these docking procedures was defined based on the mass center of the cocrystallized ligand in the homologous HRASLS3/LRAT chimeric protein (PDB ID: 4Q95). We visually inspected the binding modes regarding the proximity of the reactive centers (sn-1 ester carbonyl carbon and cysteine thiolate) involved in catalysis. The combination of the structural model obtained from AlphaFold together with the AutoDock Vina docking engine produced the most consistent results. Using the validated model, the stereoisomers of inhibitors 1 and 2 were docked to the active site of LRAT. Based on the introduced characteristics of binding modes of different lecithin derivatives, we selected poses of the inhibitors in accordance with this rationale by visual inspection.

Establishing a structural model I

- No wild-type crystal structure
- Solutions:
  - Classical homology modeling: SWISS-MODEL
  - Artificial intelligence: ALPHA-FOLD 2

Catalytic mechanism

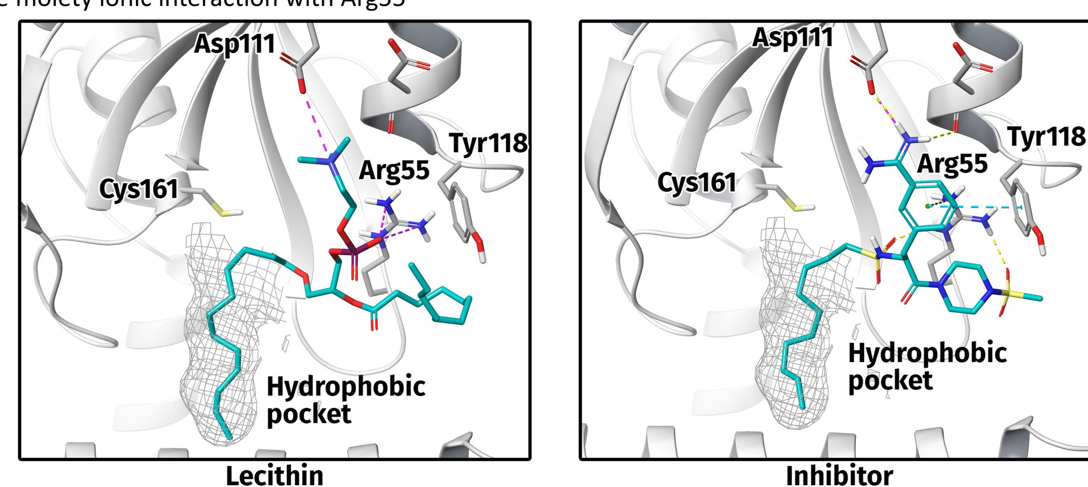
sn-1, sn-2, PC, lyso-PC, LRAT, all-trans-retinol acyl acceptor, all-trans-retinol retinyl ester

Establishing binding rationale

Docking of inhibitors and SAR

Selection of binding modes with similar ligand-protein contacts as lecitins

- -aliphatic rest in well-defined hydrophobic pocket surrounded by Phe25, Leu58, Leu97, Val101, Leu156
- - Positive charge in ionic interaction with Asp111
- - Phosphate moiety ionic interaction with Arg55



Results & Discussion:

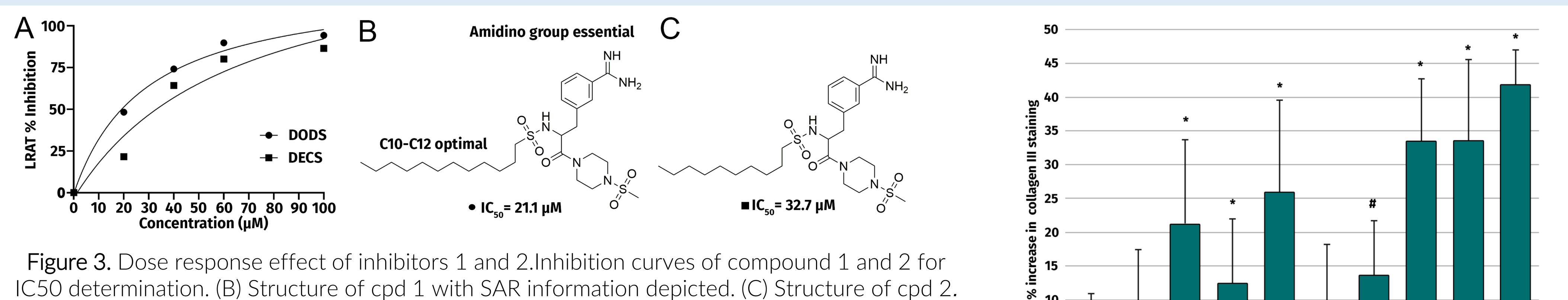


Figure 3. Dose response effect of inhibitors 1 and 2.Inhibition curves of compound 1 and 2 for IC50 determination. (B) Structure of cpd 1 with SAR information depicted. (C) Structure of cpd 2.

As no crystal structures are available for LRAT, a structural model had to be established and validated. The combination of the structural model obtained from AlphaFold together with the AutoDock Vina docking engine produced the most consistent results. Interestingly, the binding modes fulfilling the proximity of the reactive centers were highly similar for different phosphatidylcholine lipids, the binding mode of compound 1 (Figure 4A) showed that the C12 fatty acid nearly completely occupied the hydrophobic pocket. Hence, longer acyl moieties result in reduced inhibition likely due to steric limitations. The loss of inhibition by compounds without the amidino group could be explained by the lack of a potent ionic interaction in the active site. The mildly reduced inhibition resulting from the removal of the sulfomethyl moiety attached to the piperazine ring might be caused by the lack of hydrogen bonding interactions with Arg55 or Tyr118.

Figure 3. ex vivo collagen III immunostaining after topical application of test compounds IA/IB = cpd 1 or 2, ROH = retinol; statistics t-test # p<0.02, *p<0.01 versus vehicle control at day 8, control was normalized to level 0, error bars represent SD.

In the *ex-vivo* studies, ROH alone at 0.05% exerted as expected a moderate positive effect on collagen III synthesis. The LRAT inhibitors (IA and IB) were tested alone at 0.005% and 0.1% with a slight positive effect that was dose-dependent and the same inhibitors were tested in combination with 0.05% ROH. These combined tests revealed the strongest and also dose dependent increase on collagen III.

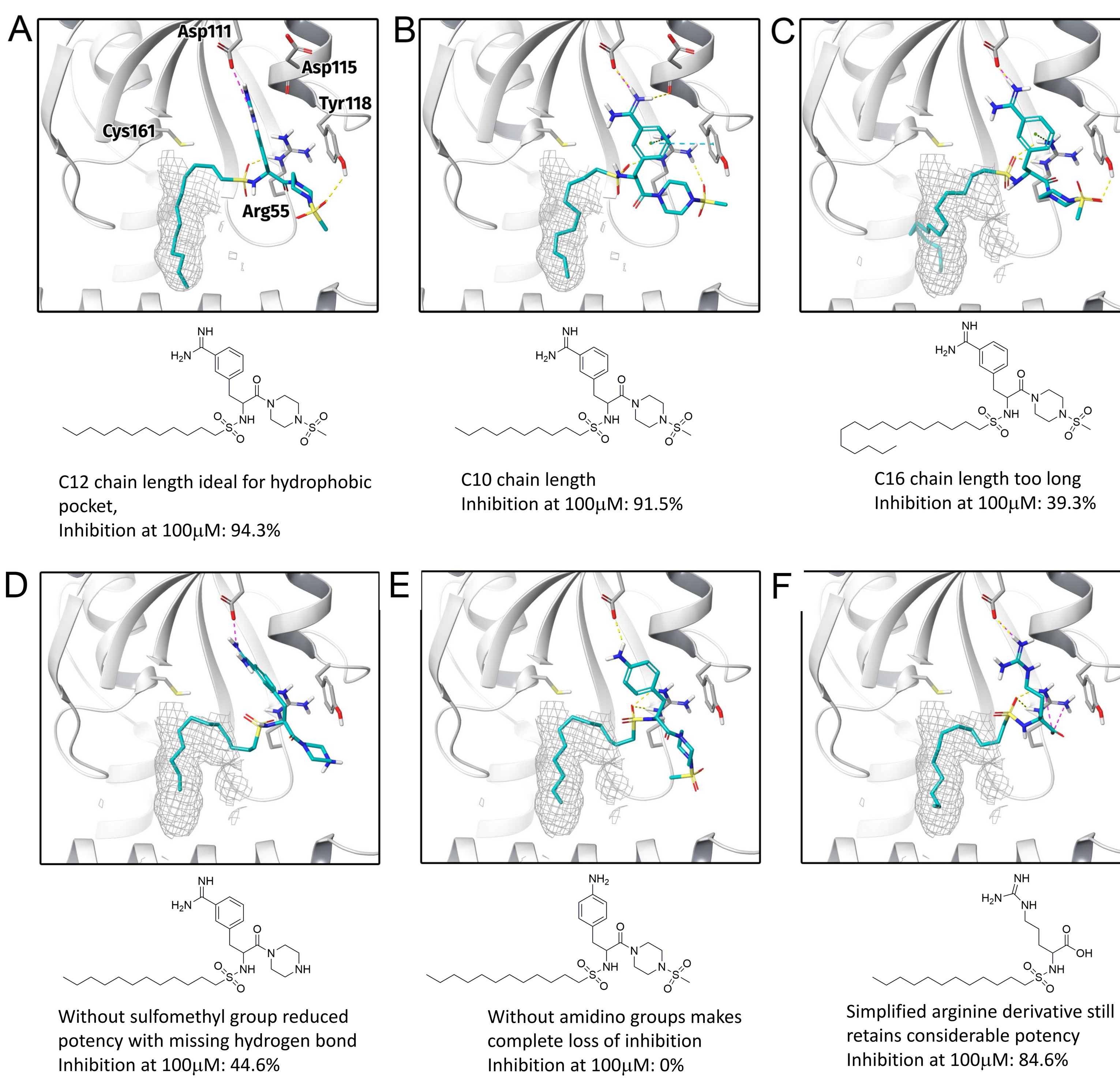


Figure 4. (to left side). Structural insights into the binding of LRAT inhibitors. Binding modes of (A) compound 1, (B) compound 2, (C) compound 3, (D) compound 4, (E) compound 5, (F) compound 6 determined by molecular docking. A hydrophobic cavity occupied by the aliphatic tails is shown in a surface representation.

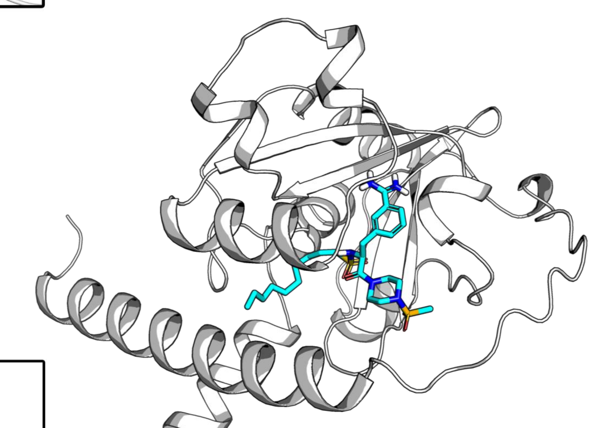


Figure 5. (above). Full strucute of LRAT enzymeincl. cpd2 generated by AlphaFold

Conclusions:

- The inhibition of LRAT enzyme involved in the esterification of ROH is a promising strategy to increase the effects of retinoids on the production of collagens for which the decrease is a hallmark in aged and photodamaged skin.
- Upon screening of several structures, we found that amidino substituted amino acid derivatives are highly effective LRAT inhibitors. Using molecular modeling, we could identify the SAR of this compound series by investigating key ligand-protein contacts such as the size of the hydrophobic pocket the acyl moieties reside in and an ionic interaction of the amidino group.
- On human skin we confirmed that the LRAT inhibitors combined with ROH upregulated collagen III content based in increased retinoid response that was higher than ROH alone.
- Overall, LRAT inhibition using the reported compounds is a very promising approach to further boost the efficacy of ROH and delivering superior anti-aging results.

Acknowledgements:

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