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SQUALENE FUNCTIONALIZED WITH COUMARINES OR BENZENESULFONAMIDES AS HYBRID INHIBITORS FOR CARBONIC ANHYDRASE

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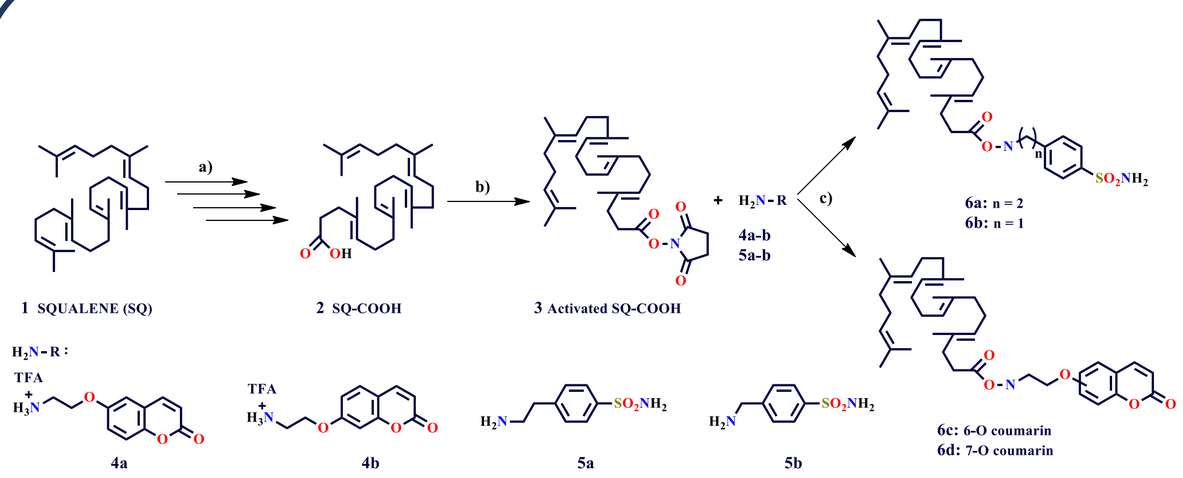
INTRODUCTION

Squalene is a natural lipid widespread in nature in different plants as well as in living organisms. In the human body, squalene is the biochemical precursor for cholesterol and can be found in skin tissue and liver.[1] Due to its unique polyunsaturated structure containing six isoprene units, squalene has the ability to self-assemble under different conditions conducing to improved biological properties and makes it a potential biocompatible candidate for drug and genes delivery applications involving the inhibition of carbonic anhydrases (CAs).[2,3] CAs are pervasive metalloenzymes in all the living organisms that have the property to catalyze the reverse hydration of carbon dioxide to generate the bicarbonate anion and the H^+ cation.[4,5] The human Carbonic Anhydrases (hCAs) are belonging to α-CA class, and can be found as 15 different isoforms which are distinct in terms of tissue distribution, cellular localization and kinetic characteristics.[6] These enzymes' mediated processes contribute to different biological pathways including respiration, pH and bicarbonate homeostasis, bone metabolism and tumorigenesis.[7,8] Furthermore, abnormal levels and/or activities have frequently been linked to a variety of human pathologies. In the last decades, CAs have emerged as an attractive target for the development of inhibitors or activators with unique, non-traditional medicinal uses.[9,10]

In this context, our studies were focused on the obtaining of new hybrid inhibitors for carbonic anhydrase isoforms based on squalene derivatives functionalized with zinc binding group such as the sulfonamide or coumarin moieties.[11] The addition of a sulfonamide moiety is thought to increase the selective inhibitory profile against the hCA II isoform, which is implicated in numerous diseases (i.e., glaucoma, epilepsy), meanwhile, the coumarines are highly efficient against tumor-associated hCA IX and XII isoforms.

SYNTHESIS



Scheme 1. *Synthesis pathway of the hybrid CAI-squalene derivatives. a) Four steps synthesis procedure (i. NBS, THF, 0 °C, 1.5h; ii. K_2CO_3, MeOH, RT, 2h; iii. HIO_4 x $2H_2O$, H_2O and 1,4-dioxane, RT, 2h; iv. $H_2Cr_2O_7$, H_2O and Et_2O, 0 °C, 2h); b) EDC·Cl, NHS, DMF dry, RT, 2–4 h; c) Et_3N, dry DMF, RT, 12–24 h.*

STRUCTURAL CHARACTERIZATION

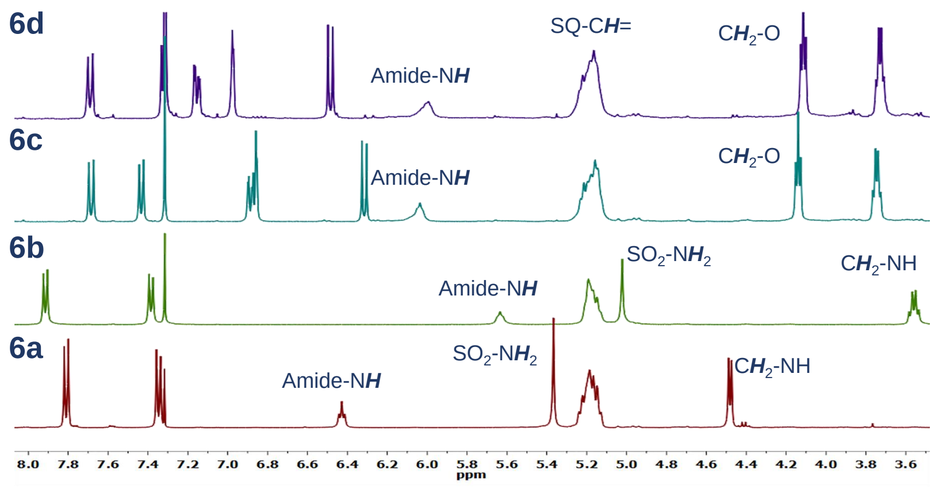


Figure 1. *^{1}H-NMR spectra of the hybrid CAI-squalene derivatives ($CDCl_3$, 400 MHz).*

ESI-MS: ***6a*** *calculated m/z: 582.39, found 583.5 [M+1];* ***6b*** *calculated m/z: 568.37, found 569.45 [M+1];* ***6c*** *and* ***6d*** *calculated m/z 587.40, found 588.51 and 588.48 [M+1].*

IN VITRO CAs INHIBITION ASSAY

Table 1. *Inhibition data and selectivity of human CA isoforms I, II, IX, and XII with compounds 6a-d and AAZ obtained by stopped-flow CO_2 hydrase assay.*

	K_i (nM)[*]				Selectivity report
Compound	hCA I	hCA II	hCA IX	hCA XII	(hCA II/IX)
6a	3516	62,3	1887	844	0,0330
6b	6842	5,0	3559	>10000	0,0014
6c	>10000	>10000	7215	>10000	>1,386
6d	>10000	>10000	8817	>10000	>1,134
AAZ	250	12,1	25,8	5,7	0,468

[*] Mean from 3 different assays, by a stopped-flow technique (errors were in the range of± 5-10 % of the reported values).

The cytosolic isoform hCA I was poorly inhibited by the hybrid 6a and 6b CAIs in the nanomolar concentration range (3516 nM and 6842 nM, respectively). On the other hand, high efficiency was obtained against hCA II, presenting K_i inhibition constants of 62.3 nM and 5 nM, respectively.

IN SILICO STUDIES

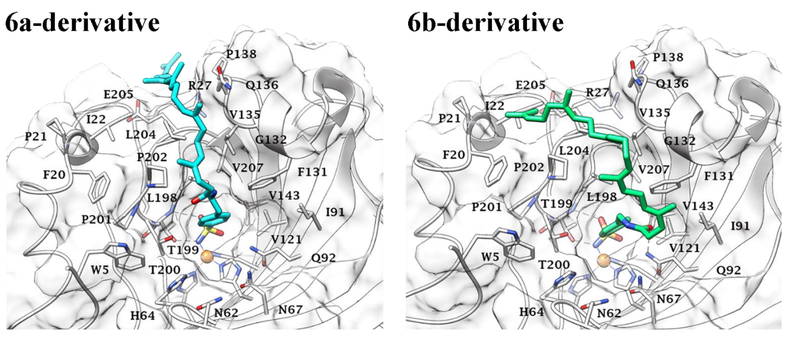


Figure 2. *In silico molecular modelling approach used to predict the binding mode of 6a and 6b derivatives into CA II active site.*

From the molecular modelling images, it was observed that the two squalene derivatives (6a and 6b) were positioned deep in the region of the active site by coordinating the zinc atom with the positively charged nitrogen atom of the sulfonamide group. The length of the methylene connector between the squalene-linked amide group and the sulfonamide-linked phenyl group leads to the formation of spaced hydrogen bonds between the amide group in the 6b-derivative and the NH_2 groups in the hCA II side chain, being correlated with better results obtained in the *in vitro* assay.

CONCLUSIONS

- By combining squalenic acid derivative with various zinc binding groups such as sulfonamides or coumarine derivatives, a series of hybrid squalene-based CAIs were synthetized.
- All the obtained compounds were fully characterized to confirm their chemical structure.
- Among the obtained hybrid CAIs, the squalene derivatives with benzensulfonamide moieties showed high selectivity and excellent inhibition profile of hCA II over the tumor-associate enzyme hCA IX and hCA XII, making them suitable candidates for preclinical evaluation in glaucoma or related diseases in which the hCA II is involved.

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