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Evaluation of anti-pigmentary effect of synthetic sulfonylamino chalcone

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ABSTRACT

The 4′-(*p*-toluenesulfonylamino)-4-hydroxychalcone (TSAHC), which bears inhibitory chemotypes for both α-glucosidase and tyrosinase, was evaluated for tyrosinase activity and depigmenting ability relative to compounds designed to only target tyrosianse activity. TSAHC emerged to be a competitive reversible inhibitor of mushroom tyrosinase. More importantly, it was also able to return the melanin content of α-melanocyte stimulated by α-MSH to base levels unlike other inhibitors that only targeted tyrosinase. The Western blot for expression levels of proteins involved in melanogenesis showed that TSAHC significantly decreased three main tyrosinase related protein in melanin biosynthesis, tyrosinase, TRP-1 and TRP-2.

1. Introduction

Excessive/uneven pigmentation is the root cause of numerous skin problems including age spots, melasma and chloasma. These maladies can be initiated by ultraviolet light, chronic inflammation as well as abnormal levels of α-melanocyte stimulating hormone (α-MSH) which results in overproduction of melanin [1,2]. It is thus not unsurprising that many efforts have been devoted to screening both recognized and putative depigmenting agents in a bid to develop drugs to attenuate hyperpigmentation. Most of these screens have in some way targeted tyrosinase, which has been considered to be an effective approach to treat a variety of hyperpigmentary disorders [3]. Tyrosinase in *Homo sapiens* is a membrane-bound glycoprotein with an active site containing two copper ions. This enzyme catalyzes two different processes involving the oxidation of tyrosine, first to L-DOPA (monophenolase) and then to dopaquinone (diphenolase). Tyrosinase is thus a perfect target in many ways because it is produced only by melanocytic cells and to reach its fully active form it requires processing (glycosylation) in the ER and Golgi, after which time it is trafficked to specialized organelles, melamosomes [3]. Thus numerous highly tyrosinase specific processes can be targeted to effect inhibition of melanin production. In principle, tyrosinase activity can be attenuated by regulating the transcription of tyrosinase mRNA, tyrosinase related protein-1 (TRP-1), tyrosinase related protein-2 (TRP-2) [4] and its maturation via asparagine-liked oligosaccharide processing [5–7]. However, arguably the most direct route is to inhibit tryosinase (EC 1.14.18.1).

Numerous reports have focused on the inhibition of tyrosinase as the sole route to depigmentation of the epidermal layer [8,9]. Kojic acid is a representative tyrosinase inhibitor with an IC_{50} of 16 μM against mushroom tyrosinase. Its activity is ascribed to copper chelation. However, despite the wide knowledge garnered to date concerning depigmentation and the numerous ways to bring it about, we noticed that there has so far been no effort to target multiple proteins in the pathway to melanogenis in one inhibitor. For instance, one of the most widely employed strategies to effect a decrease in cellular tyrosinase activity which does not involve direct tyrosinase inhibition is the inhibition of enzymes involved in tyrosinase *N*-glycosylation [10]. This reduces tyrosinase

Abbreviations: IC_{50}, the inhibitor concentration leading to 50 % activity loss; K_i, inhibition constant; TRP-1, tyrosinase related protein-1; TRP-2, tyrosinase related protein-2; α-MSH, α-melanocyte stimulating hormone; TSAHC, 4′-(*p*-toluenesulfonylamino)-4-hydroxychalcone; NMR, nuclear magnetic resonance; TM4SF5, four-transmembrane L6 family member 5.

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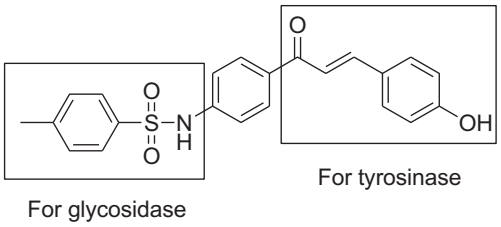


Fig. 1. Different chemotypes within TSAHC and their presumed targets.

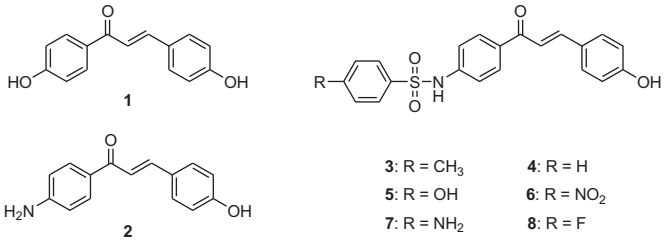


Fig. 2. Chemical structures of chalcone derivatives (**1–8**).

procesing and lowers tyrosinase activity because the tyrosinase can not reach its full maturity, and hence remains inactive. It thus occurred to us that a substance which can inhibit both tyrosinase and glycosidase may be a more promising depimenting agent.

Chalcones are natural products of widespread occurrence in plants. They have been reported to exhibit a wide range of pharmaceutical effects including antioncogenetic, anti-inflammatory, anti-ulcerative, antimalarial, antiviral, antifungal, and antibacterial activities [11]. Recently, they were highlighted as a new class of tyrosinase inhibitor in several publications. Nerya Ohad et al. reported that an OH group at the 4-position in B-ring of the chalcone is the major factor affecting inhibitory potency. This is presumably because these species have a phenol chemotype which the enzyme's native substrate, tyrosine, also contains [12]. In a previous communication, we showed that sulfonylamino chacones are potent α-glucosidase inhibitors with nanomolar IC_{50} values. Our structure activity relationship (SAR) studies unearthed some important requirements for α-glucosidase inhibitory activity (Fig. 1) [13,14]. In subsequent work, amino chalcones were able to reduce the tumorigenic proliferation induced by the transmembrane four L6 family member 5 (TM4SF5), the function of which is deeply related to *N*-glycosylation [15].

When all the above results were considered, we hypothesized that sulfonylamino hydroxy-chalcones (hybrids of tyrosinase inhibiting hydroxychalcones and glucosidase inhibiting sulfonamide chalcones) may have formidable antimelanogenic activity as they can inhibit both processing of tyrosinase as well as tyrosinase catalytic activity. Herein, we report that 4′-(*p*-toluenesulfonylamino)-4-hydroxychalcone (TSAHC) is an excellent depigmentary agent in a number of cell based assays through inhibition of tyrosinase's catalytic function and tyrosinase related proteins such as TRP-1 and TRP-2.

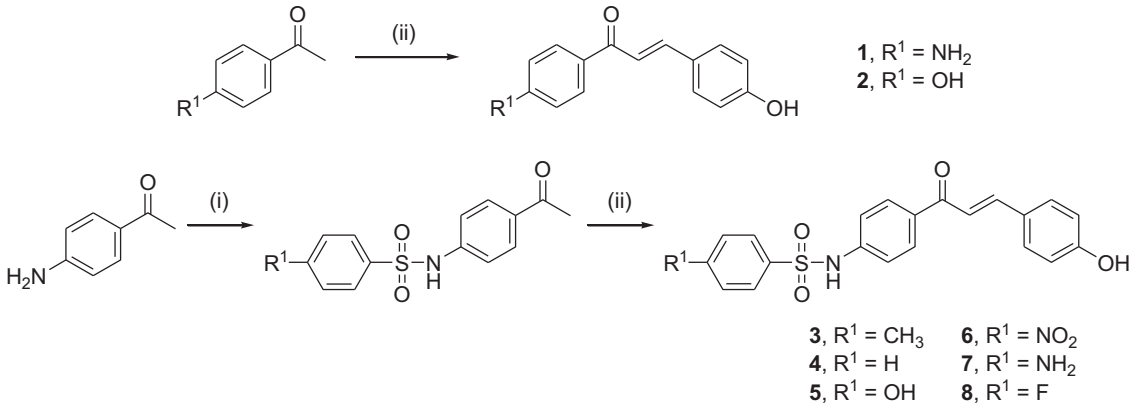
In these studies, we chose to focus our efforts on inhibitors bearing a *para*-hydroxy substituent in the B ring of the sulfonylamino chalcone. This is because it is well known that the corresponding catechol derived species are highly cytotoxic, being rapidly oxidized to *ortho*-quinones by tyrosinase [16]. As such they are not viable start points for *in vivo* screening of compounds which reduce pigmentation, which is the principal objective of this study. Furthermore, sulfonylamino chalcones bearing an unsubstituted phenyl unit on the ring B were not effective tyrosinase inhibitors *in vitro*, which is consistent with the general belief that a 4-hydroxy phenyl moiety on ring B of the chalcone is required for tyrosinase inhibitory activity.

2. Chemistry

Sulfonylamino chalcone derivatives were easily obtained through the Claisen–Schmidt condensation of hydroxybenzaldehyde and derivertized acetophenones using an acidic catalyst [13]. The 4′-(*p*-toluenesulfonylamino)-4-hydroxychalcone (TSAHC) was prepared from hydroxybenzaldehyde and *N*-sulfonylamino acetophenone using a catalytic amount of H_2SO_4 in MeOH (Scheme 1).

3. Results and discussion

The aim of the present work is to evaluate the anti-pigmentary effects of the bifunctional inhibitor TSAHC (**3**), which is adorned with functionalities for controlling both glycosidase and tyrosinase activities (Fig. 1). To allow us to draw meaningful comparisons we compare the efficacy of TSAHC with structurally similar inhibitors which only target tyrosinase. In our previous study, TSAHC inhibited α-glucosidase with 0.58 μM K_i value [13]. Molecular docking simulations revealed that sulfonylamino chalcones bind to the active site in a similar fashion to known inhibitors like acarbose and voglibose [14]. The sulfonylamino group plays the critical role in inhibitor/protein interaction. For example His111 and His348 within α-glucosidase can interact with the SO_2 function and indeed both these residues are crucial for an efficient enzyme/substrate binding interaction [14]. As shown in Fig. 2, the sulfonylamino chalcones (**3–8**) in this study also have a 4-hydroxychalcone moiety that was suggested to be instrumental in tyrosinase inhibiton by Nerya Ohad et al. [12].



Scheme 1. Synthesis of chalcone derivatives: (i) *p*-toluenesulfonyl chloride, pyridine, CH_2Cl_2, r.t, (ii) 4-Hydroxybenzaldhyde, H_2SO_4, MeOH, reflux.