Bioorganic Chemistry 64 (2016) 97–102

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Synthesis and tyrosinase inhibition activity of *trans*-stilbene derivatives

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ARTICLE INFO

Article history:
Received 25 October 2015
Revised 30 December 2015
Accepted 4 January 2016
Available online 4 January 2016

Keywords:
Stilbenoids
Tyrosinase inhibitors
Wittig reaction
Electron withdrawing groups
Resveratrol
Murine tyrosinase activity

ABSTRACT

Synthesis of a focussed library of *trans*-stilbene compounds through Wittig and other base catalysed condensation reactions is presented. The synthesized stilbenes were screened for their inhibitory potential against murine tyrosinase activity to explore the structure activity relationship (SAR). Presence of electron withdrawing group (–CN) at the double bond and hydroxyl group or halogen atom especially at para-position on the aromatic rings was found to significantly elevate the inhibitory activity. Among all the compounds screened, compounds **2**, **6**, **8**, **10**, **11**, **15** and **21** were found to exhibit appreciable inhibitory activity. Compound **21** ((*E*)-2,3-*bis*(4-Hydroxyphenyl)acryonitrile) was found to be the most active with an IC_{50} value of 5.06 μM which is less than half of the value 10.78 μM observed for resveratrol (common standard used in murine tyrosinase activity studies) under similar conditions. The results obtained from the present study reveal structural/functional group sensitivity for the tyrosinase inhibitory activity of stilbenoid moieties and are expected to be very helpful for the design and synthesis of novel, selective and effective tyrosinase inhibitors.

1. Introduction

Tyrosinase, an enzyme catalysing the rate-limiting step in the biosynthetic pathway of melanin pigments, is widely distributed in nature [1–3]. The enzyme participates in several important reactions of host defence, wound healing and sclerotization in insects and other arthropods [1,4–7]. Tyrosinase has also been found to be responsible for undesired enzymatic browning of farm products, such as bruised or cut fruits and vegetables, which subsequently leads to a significant decrease in their nutritional and market values [8–10]. In view of these different functions ascribed to tyrosinase, it seems that studies aimed at the design and development of novel tyrosinase inhibitors and a comprehensive understanding about their mode of action can prove very useful for understanding many life processes. Such studies besides providing sufficient insight into mechanism underlying the regulation of skin pigmentation in mammals are expected to help in a great way in search for proper cure to many dermatological disorders in mammals, design of alternative insect control agents and products useful to food technology and food processing. It is in this context that designing of novel tyrosinase inhibitors is receiving a considerable attention from food and animal scientists [10,11].

Realization about the importance of tyrosinase inhibitors for their impact on many physiological aspects especially in mammals and insects has stimulated an intense research activity aimed at designing of novel tyrosinase inhibitors and exploration of their mode of action. As an outcome of these studies, a huge library of tyrosinase inhibitors has been discovered from natural sources or synthesized in the laboratories [12–15]. However, various limitations have been reported about the use of these currently known tyrosinase inhibitors. While potentially active agents, such as kojic acid and arbutin, are yet to be demonstrated clinically efficient, others are associated with disadvantages like high cytotoxicity, insufficient penetrating power, low activity and low stability [16,17]. It is in this context that the use of traditional skin whitening products like hydroquinone, corticosteroids and mercury containing products has been prohibited, because these compounds have been found to be potentially mitogenic on account of their cytotoxicity to melanocytes [16,18–22]. Therefore, many alternative tyrosinase inhibitors with potential to suppress melanogenesis are being actively studied with the aim of developing safer preparations for

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http://dx.doi.org/10.1016/j.bioorg.2016.01.001
0045-2068/

the treatment of hyperpigmentation [23–25]. In light of these reports, currently there is a great demand for the development of natural product inspired skin whitening agents, which are free from harmful side effects. In this regard, phenolic compounds whose activity has been attributed to their structural resemblance to L-DOPA and tyrosine (the natural substrates of tyrosinase) [26,27], seem to be very potent agents. The present work is an outcome of our investigations aimed at designing of safe to use, natural product inspired potent tyrosinase inhibitors. Our literature survey exercise in this regard, motivated us for the synthesis and tyrosinase inhibition activity studies of stilbene based inhibitors which are presaged to be very promising but the mechanism behind their activity is yet to be fully understood [12,28].

For a molecular level understanding of the inhibitory action of stilbenoid moieties, we explored the structure-murine tyrosinase inhibition activity relation for variedly substituted stilbene compounds using resveratrol as a positive control. Since the already established SARs for this class of inhibitors indicate that the *trans*-olefin structure in the parent stilbene skeleton is essential for tyrosinase inhibition [29,30], we restricted our studies to only *trans* derivatives of stilbene compounds with a diverse substitution pattern on and around aromatic rings and olefinic bond.

2. Material and methods

2.1. Chemistry

All solvents were dried and freshly distilled prior to use. ^{1}H was recorded on a Bruker DPX 200 instrument in $CDCl_3$ using TMS as internal standard for protons. Mass spectra were recorded on ESI-esquire 3000 Bruker Daltonics instrument. Elemental analysis was carried out using Elemental Vario EL III elemental analyser. Chemical shift values are mentioned in δ (ppm) and coupling constants (*J*) are given in Hz. Mass-spectrometric (MS) data is reported in *m*/*z*. Elemental analysis data is reported in % standard. The progress of all reactions was monitored by TLC on 2 cm × 5 cm precoated silica gel 60 F254 plates of thickness 0.25 mm (Merck). The chromatograms were visualized under UV 254–366 nm and iodine. Melting points were determined on Buchi B-542 apparatus by an open capillary method and are uncorrected. Chemicals were purchased from M/s Aldrich Chemicals, Mumbai. Reagents and solvents used were mostly of LR grade.

2.1.1. Synthesis of stilbene derivatives

A library of twenty-two stilbene derivatives was synthesized in the present study. Wittig reaction between substituted aromatic aldehydes and Wittig-salts derived from simple/substituted benzyl chlorides (Scheme 1) was employed for the synthesis of compounds **1**–**15** (Table 1). *Trans* stilbenes were obtained from a mixture of *cis*- and *trans*-isomers by following reported procedures [31,32].

Base catalysed condensation reaction of substituted benzaldehydes with phenyl acetic acid (Scheme 2), was used for the synthesis of compounds **16**–**20** (Table 2). Phenyl acetaldehyde and phenyl methylacetate in presence of pyridine/acetic anhydride or sodium methoxide/methanol were used to achieve the substitution across olefinic bond; the reaction exclusively yields the *E*-isomer [33,34].

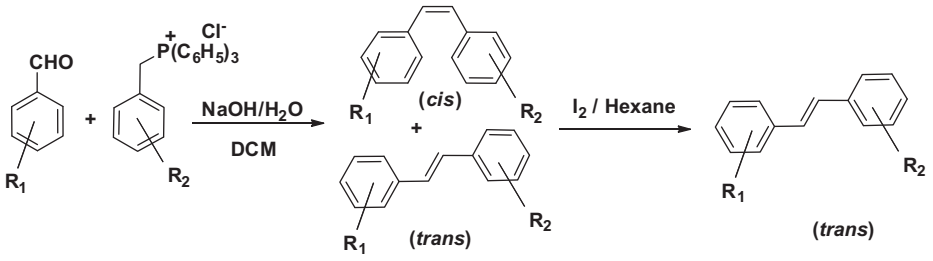
Table 1
Stilbenes synthesized using Wittig reaction.

Compound	R_1	R_2	Yield (%)[a]
1	H	H	89
2	4-F	H	88
3	4-Cl	H	88
4	4-Br	H	84
5	4-NO_2	H	81
6	4-CN	H	86
7	4-$OCOCH_3$	H	81
8	4-OH	H	82
9	4-OCH_3	H	86
10	4-F	4′-OCH_3	82
11	3-OCH_3, 4-Br	H	86
12	3,5-F	H	85
13	4-OCH_3	3′, 4′, 5′-OCH_3	85
14	2,3,4,5,6-F	H	84
15	–	One benzene ring replaced by anthracene	86

[a] Isolated yields after chromatographic purification.

Base (piperidine/pyridine) catalysed condensation of 4-hydroxyphenylacetonitrile with 4-hydroxybenzaldehyde and nicotinaldehyde respectively at 115 °C (Scheme 3), was followed for the synthesis of **21** and **22** in *E*-form (Table 3) [34,35]. The formation of *E*-isomers in all cases was established from the NMR spectra of the final products.

2.1.1.1. Synthesis of 1–15: representative procedure for synthesis of Trans-1,2-Diphenylethene (1). To a solution of benzyl chloride (0.126 g, 1.00 mmol) in dry toluene (7 mL), was added triphenylphosphine (0.313 g, 1.20 mmol) at ambient temperature. The reaction mixture was allowed to reflux for 18 h under N_2 atmosphere; during this time the Wittig salt precipitated out, it was filtered, washed and used for further reaction. To a solution of Wittig salt (0.08 g, 0.20 mmol) in CH_2Cl_2, was added NaOH solution (0.01 g, 0.25 mmol, 3 mL water) at ambient temperature. The solution turned orange red indicating the formation of Wittig ylide. To this solution was added benzaldehyde (0.018 g, 0.16 mmol) and the reaction mixture allowed to stir at ambient temperature for 3 h. The crude product formed was extracted with CH_2Cl_2, washed with brine and evaporated to dryness. After usual column chromatography (hexane: EtOAc; 9:1), the product obtained was a mixture of *cis* and *trans*-isomer, isolated in 87% yield. The above mixture was dissolved in hexane containing catalytic amount of iodine and allowed to reflux for 1 h. The reaction mixture was cooled down to ambient temperature, washed with $Na_2S_2O_5$ solution and the organic layer evaporated to give pure *trans*-isomer (**1**) in quantitative yield (89%).



Scheme 1. Synthesis of stilbenes (**1**–**15**) using Wittig reaction.