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Research paper

New natural amino acid-bearing prodrugs boost pterostilbene's oral pharmacokinetic and distribution profile

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ABSTRACT

The biomedical effects of the natural phenol pterostilbene are of great interest but its bioavailability is negatively affected by the phenolic group in position 4′ which is an ideal target for the conjugative enzymes of phase II metabolism. We report the synthesis and characterization of prodrugs in which the hydroxyl moiety is reversibly protected as a carbamate ester linked to the N-terminus of a natural amino acid. Prodrugs comprising amino acids with hydrophobic side chains were readily absorbed after intragastric administration to rats. The Area Under the Curve for pterostilbene in blood was optimal when prodrugs with isoleucine or β-alanine were used. The prodrug incorporating isoleucine was used for further studies to map distribution into major organs. When compared to pterostilbene itself, administration of the isoleucine prodrug afforded increased absorption, reduced metabolism and higher concentrations of pterostilbene, sustained for several hours, in most of the organs examined. Experiments using Caco-2 cells as an *in vitro* model for human intestinal absorption suggest that the prodrug could have promising absorption profiles also in humans; its uptake is partly due to passive diffusion, and partly mediated by H^+-dependent transporters expressed on the apical membrane of enterocytes, such as PepT1 and OATP.

1. Introduction

Resveratrol (3,4′,5-trihydroxystilbene), a natural polyphenol, has enormous biomedical potential. In addition to the many reports of positive effects on major syndromes such as cancer and metabolic disease (for recent reviews see [1,2]), benefits have also been observed or suggested against such conditions as, e.g., acne [3,4], caries [5], H. pylori-induced pathologies [6,7], osteoporosis [8,9], allergy [10]. The mechanisms of resveratrol action [11–14] include SIRT1 [15] and AMPK activation and downregulation of mTOR and NF-kB, significantly overlapping those proposed for berberine [16,17] and aspirin (salicylate) [18–22].

While most attention has been focused on resveratrol, other natural stilbenoids may actually possess analogous or even more marked bioactivities. Pterostilbene (3,5-dimethoxy-4′-hydroxystil bene) (revs.: [23–25]) (Fig. 1) may be the most interesting of these. It shows remarkable antineoplastic activity [26–30], it is anti-inflammatory [31,32], neuroprotective [33,34], it contrasts obesity [35] and it ameliorates the symptoms of metabolic syndrome [36]. Among the mechanisms underlying these effects are inhibition of P450 enzymes [37] and cyclooxygenases (especially COX-1) [38]. Pterostilbene has also been reported to induce autophagy [28,39], activate the Nrf2 pathway [40–42], activate eNOS [43] and upregulate PPARα [44]. Its higher lipophilicity may be a key factor in allowing it to reach higher brain levels than resveratrol [45]. In turn, these higher levels may explain the efficacy of pterostilbene

Abbreviations: AUC, Area Under the Curve; BCA, bicinchoninic acid; bis-PNPC, bis(4-nitrophenyl) carbonate; BSA, bovine serum albumin; DMEM, Dulbecco's Modified Eagle's medium; D-PBS, Dulbecco's phosphate buffered saline; E-3-S, estrone-3-sulfate; GlySar, glycyl-sarcosine; HATs, heterodimeric amino acid transporters; HBSS, Hanks' Balanced Salt Solution; HEPES, 4-(2-hydroxyethyl)-1-piper azineethanesulfonic acid; LAT1/2, L-type amino acid transporter 1/2; LOD, limit of detection; LOQ, limit of quantification; MES, 2-(N-morpholino)ethanesulfonic acid; OATPs, Organic Anion Transporting Polypeptides; PATs, proton amino acid co-transporters; PepT1, peptide transporter 1; SD, Standard Deviation; SEM, Standard Error of the Mean; TFA, trifluoroacetic acid; TLC, thin layer chromatography.

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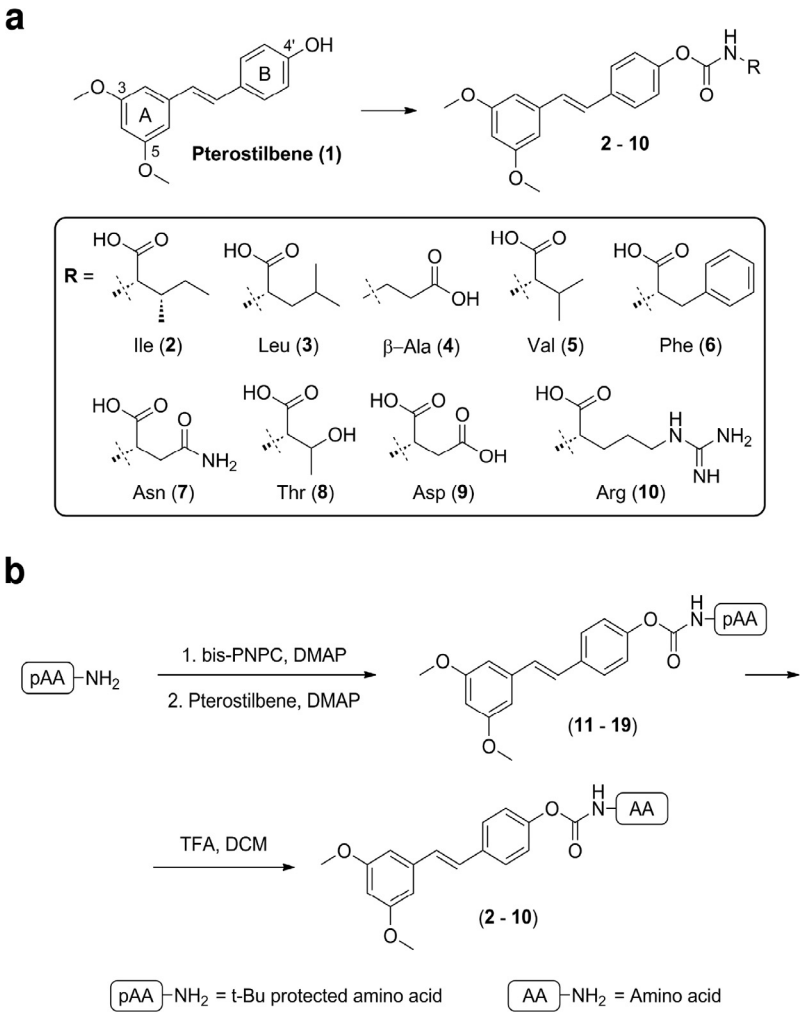

Fig. 1. N-monosubstituted carbamate amino acidic pterostilbene prodrugs (**2–10**): structure (a) and synthesis (b).

in ameliorating the symptoms of cognitive aging [46] and anxiety [47].

The efficacy of resveratrol and its analogues is however limited by the conjugative metabolism they undergo during absorption from the intestine and in the liver (e.g., for resveratrol: [48–52]; for pterostilbene: [53]). We are exploring one of the possible strategies to overcome this obstacle, namely the development of prodrugs [54–56]. A promising bond system for the linkage of promoieties is the carbamate ester, already utilized in various precursors of phenolic drugs [57–61]. N-monosubstituted carbamate esters proved suitable for utilization as pro-drugs [62] and we tested resveratrol derivatives bearing at each hydroxyl hydrophilic groups such as methoxy-oligo(ethylene glycol)s [63], galactosyl or glyceryl [64]. These constructs regenerated the parent compound with suitable kinetics, but their hydrophilicity limited absorption by diffusion through the intestinal wall. No evidence was found for an active transport mediated by intestinal sugar transporters [64]. In a quest for assistance by membrane carriers we turned to amino acid and peptide transport systems, and to natural amino acids as promoieties [65].

Amino acid transporters are a division of the superfamily of membrane transporters [66–68]. It includes heterodimeric amino acid transporters (HATs) and proton amino acid co-transporters (PATs), whose representatives in rat intestine include LAT1/2 (L-type amino acid transporter 1/2) [69] and PAT1/2 [70]. Both have relatively broad specificity, and have been exploited for the transport/absorption of prodrugs, with LAT1/2 being especially useful for permeation of the blood brain barrier.

We reasoned that help may be also (or alternatively) provided by relatively low-specificity peptide permeases of the intestinal wall, which have been classified into a few families [71,72]. Possibly the most relevant system may be the 4-member proton-coupled oligopeptide transporter family (SLC15A1-4) (revs.: [71–79]), predominantly expressed in the small intestine and kidneys, which mediates the uptake of di/tripeptides and of a variety of non-peptides. Some poorly absorbed drugs have been modified into peptidomimetic prodrugs targeting these transporters, in particular PepT1 (SLC15A1), to improve oral bioavailability (e.g.: [80–83]; revs: [84,85]).

Organic Anion Transporting Polypeptides (OATPs; SLCOs) [71,86,87] are a third transport system that might be exploited by amino acidic carbamoyl prodrugs for intestinal absorption. OATP-1A2 (SLCO1A2) and OATP-2B1 (SLCO2B1) are the OATP family members expressed in the apical membrane of enterocytes in human small intestine (orthologs are present in rats [88]); their substrates include bile acids, steroid conjugates, thyroid hormones, anionic peptides and drugs such as pravastatin or fexofenadine.

Amino acid derivatives of resveratrol had limited success: absorption of the prodrugs was unsatisfactory, possibly because of their hydrophilicity and of their extended structure [65]. Prodrugs carrying only one protective group of this sort would obviously be less ramified and bulky than the corresponding