Bioorganic & Medicinal Chemistry 22 (2014) 4935–4947

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Cytoprotective pyridinol antioxidants as potential therapeutic agents for neurodegenerative and mitochondrial diseases

Mohammad Parvez Alam, Omar M. Khdour, Pablo M. Arce, Yana Chen, Basab Roy, Walter G. Johnson, Sriloy Dey, Sidney M. Hecht *

Center for BioEnergetics, Biodesign Institute, and Department of Chemistry and Biochemistry, Arizona State University, Tempe, AZ 85287, United States

ARTICLE INFO

Article history:
Received 2 May 2014
Revised 13 June 2014
Accepted 19 June 2014
Available online 28 June 2014

Keywords:
Mitochondria
Cytoprotection
Lipid peroxidation
Reactive oxygen species
Microsomal stability

ABSTRACT

As part of our ongoing efforts to identify compounds having potential utility in treating neurodegenerative and mitochondrial disorders, a series of pyridinol analogues have been prepared. The synthetic route employed for the preparation of the new analogues is different, and considerably more efficient, than that used in previously reported studies. The new route yields a pair of pyridinol regioisomers that can be readily separated and evaluated. Their ability to quench lipid peroxidation and reactive oxygen species (ROS), and to preserve mitochondrial membrane potential ($\Delta\psi_m$) and support ATP synthesis is reported. The optimal side chain length was found to be 16 carbon atoms. The metabolic stability of those compounds having optimal biological activities was evaluated in vitro using bovine liver microsomes. The omission of any side chain hydroxyl group and introduction of an azetidine moiety at position 6 of the pyridinol redox core (**8** and **9**) increased their microsomal stability as compared to the exocyclic dimethylamino group. The favorable metabolic stability conferred by the azetidine moiety in compounds **8** and **9** makes these compounds excellent candidates for further evaluation.

1. Introduction

The survival of living organisms depends on the continuous availability of energy in the form of adenosine triphosphate (ATP). The mitochondrial electron transport chain, coupled to ATP synthesis, is responsible for conversion of the chemical energy of sugars, fatty acids, and amino acids into ATP.[1–4] The mitochondrial electron transport system accounts for 90% of the oxygen consumption in a cell, thereby producing reactive oxygen species (ROS) as a byproduct.[4–6] Under normal conditions, the effects of ROS are counteracted by a variety of antioxidants, involving both enzymatic and non-enzymatic mechanisms.[7,8] Reactive oxygen species produced by mitochondria cause oxidative damage that impairs the ability of mitochondria to make ATP and to carry out their metabolic functions. The decline in mitochondrial function is well recognized in aging, neurodegenerative diseases and many complex mitochondrial diseases.[9–13]

An actively pursued strategy for the treatment of mitochondrial and neurodegenerative diseases at the present time involves the use of antioxidants and coenzyme Q_{10} analogues to slow the progression of the mitochondrial degradation resulting from oxidative stress and bioenergetic deficiency. Earlier studies focused on a few nitrogen heterocyclic analogues of coenzyme Q_{10} and vitamin E have been reported.[14–21] Encouraging antioxidant activity has been shown in vitro, as judged by the ability of the tested compounds to scavenge free radicals, and preserve mitochondrial function, following the induction of oxidative stress in a spectrum of cell lines derived from patients with mitochondrial and neurodegenerative diseases.[14–20] Also, some of these analogues were able to augment ATP production in CoQ_{10} deficient cells.[19,20] These encouraging results suggested that further structural optimization might improve the potency and efficacy of lead compound **1**. Optimization of the oxidative metabolism of drug candidates to enhance their pharmacokinetic or toxicological properties is an important element of the drug discovery process. Compounds generally exhibit less desirable pharmacokinetic properties when subject to rapid metabolic clearance, which may compromise their in vivo pharmacological potency. Several functionalities susceptible to the attack of phase I enzymes in the liver are known; methylated nitrogen and oxygen atoms are examples. Presently, we address this issue by identifying pyridinol antioxidants with good efficacy but reduced liability due to oxidative metabolism.[22,23]

Described herein are the preparation and characterization of several new regioisomeric pairs of pyridinol derivatives. Compounds **1**–**9** were evaluated for their ability to suppress reactive oxygen

* Corresponding author. Tel.: +1 480 965 6625; fax: +1 480 965 0038.
E-mail address: sidney.hecht@asu.edu (S.M. Hecht).

http://dx.doi.org/10.1016/j.bmc.2014.06.040
0968-0896/

species and lipid peroxidation, for their effect on mitochondrial membrane potential, and for their ability to protect cultured FRDA and Leigh's lymphocyte cells from oxidative stress induced by glutathione depletion.[19] The compounds were also evaluated for their interaction with mitochondrial electron transport chain complexes, and for their ability to support ATP levels in CoQ_{10} deficient lymphocytes. Strategies involving protection against metabolic N-demethylation of the pyridinol ring were employed in this study; the pyridinol derivatives were evaluated for their stability in bovine liver microsomes. Compounds **2**–**7** have the same redox core as one described in an earlier study (compound **1**),[18] but have modified (10–19 carbon atoms) side chains lacking the hydroxyl group. Removal of the hydroxyl group from the side chain increased their antioxidant activity and metabolic stability, and decreased their inhibitory effects on the mitochondrial respiratory chain complexes (**2** and **3**). Increasing the side chain length to 16 carbon atom (**4** and **5**) significantly improved their biological activity. Compounds **8** and **9** were designed by using an optimal side chain length and replacing the dimethylamino moiety at position 6 of the pyridinol redox core with an azetidine group to increase their metabolic stability against phase I enzymes that mediate N-dealkylation.

2. Results and discussion

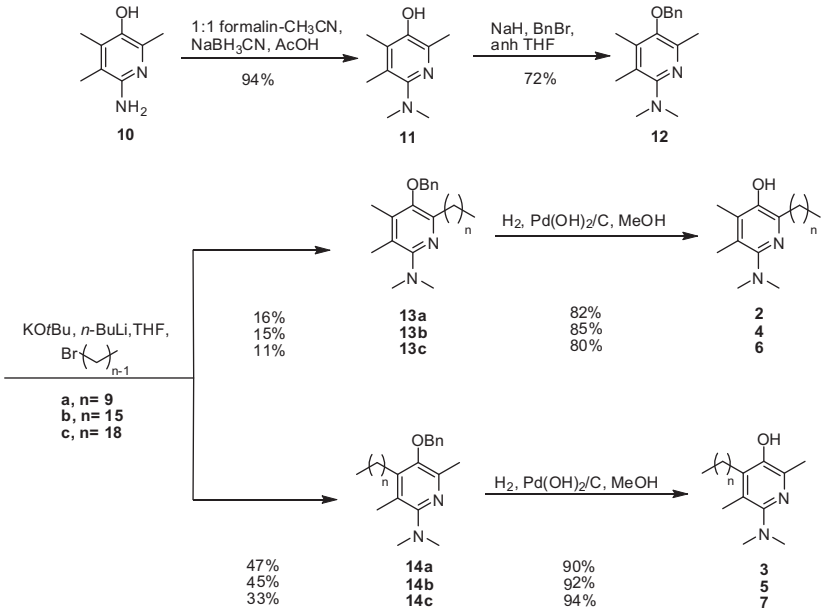
2.1. Design and synthesis of the new pyridinol analogues

The route used for synthesis of the desired pyridinol antioxidants is illustrated in Schemes 1 and 2. Accordingly, 6-amino-2,4,5-trimethylpyridin-3-ol (**10**) was synthesized by a method reported previously.[18,24] Reductive alkylation of **10** using formalin and sodium cyanoborohydride afforded **11** in 94% yield. The intermediate 3-pyridinol (**11**) was O-benzylated using benzyl bromide and NaH to afford O-benzylated core **12** in 72% yield. Aliphatic side chains were introduced by alkylation of **12** using Schlosser's super base (mixture of KO*t*Bu and *n*-BuLi) and the appropriate alkyl bromide.[25] Alkylation resulted in pairs of regioisomers which were readily separated by silica gel chromatography to afford **13a**–**13c** and **14a**–**14c**. The alkylated products were treated with Pearlman's catalyst and H_2 in MeOH to afford the desired pyridinol analogues **2**–**7**. A different synthetic route was employed to synthesize the pyridinol analogues containing an exocyclic azetidine ring. First, commercially available pyridoxine hydrochloride (vitamin B_6) was treated with $SOCl_2$ then with zinc dust in AcOH, followed by treatment with morpholine–iodine charge transfer complex (**15**) to afford iodopyridinol (**16**) in 58% yield.[24,26] Morpholine–iodine complex was synthesized by a reported method.[27,28] Briefly, morpholine and iodine were stirred in benzene in the dark to afford the charge transfer complex (**15**). O-Benzylation of **16** was achieved in 62% yield using K_2CO_3 and BnBr. Catalytic cross-coupling of halide **17** with azetidine was achieved using tris(dibenzylideneacetone)dipalladium(0) ($Pd_2(dba)_3$), 1,3-bis(2,6-diisopropylphenyl)imidazolium chloride ($ImPrPh_2{\cdot}HCl$) and KO*t*Bu to afford **18** in 71% yield.[29] The hexadecyl side chain was introduced using Schlosser's super base and bromopentadecane to afford **19** and **20** in 23% and 40% yields, respectively.[25] Finally, treatment of **19** and **20** with Pearlman's catalyst and H_2 in MeOH afforded analogues **8** and **9** in 85% and 94% yields, respectively.

2.2. Biochemical and biological evaluation

2.2.1. Mitochondrial electron transport chain function

Inhibition of any of the mitochondrial respiratory chain complexes can limit the potential therapeutic utility of CoQ_{10} analogues. Accordingly, it is desirable to prepare analogues that are minimally inhibitory to the respiratory chain. As an initial screen, we have studied the effects of the compounds on NADH oxidase activity, which encompasses the functions of mitochondrial complexes I, III and IV. We have recently reported the importance of side chain length on the interaction of coenzyme Q_{10} analogues with the mitochondrial respiratory chain to achieve improved bioenergetic and antioxidant activity.[17–20,30] In the light of these findings, we prepared analogues having different side chain lengths attached to the modified redox core (Fig. 1). The inhibitory effects of the test compounds on NADH oxidase (complexes I, III and IV) function were evaluated using submitochondrial particles (SMP). The results are presented in Table 1, and show that the inhibitory behaviors of these compounds were dose dependent. Compound **1**, having a 10 carbon atom side chain and a polar terminal hydroxyl group, strongly inhibited NADH oxidase, as shown before.[18,19] Removing the polar hydroxyl group (compounds **2** and **3**) resulted



Scheme 1.