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2-Substituted 3-methylnaphtho[1,2-*b*]furan-4,5-diones as novel L-shaped *ortho*-quinone substrates for NAD(P)H:quinone oxidoreductase (NQO1)

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ABSTRACT

A series of L-shaped *ortho*-quinone analogs were designed by analyzing the binding mode with NQO1. Metabolic studies demonstrated that compounds **2m**, **2n** and **2q** exhibited higher metabolic rates than β-lapachone. The docking studies, which supported the rationalization of the metabolic studies, constituted a prospective rational basis for the development of optimized *ortho*-quinone analogs. Besides, good substrates (**2m**, **2n** and **2r**) for NQO1 showed higher selective toxicity than β-lapachone toward A549 (NQO1-rich) cancer cells versus H596 (NQO1-deficient) cells. Determination of superoxide ($O_2^{\bullet-}$) production and *in vitro* cytotoxicity evaluation in the presence of the NQO1 inhibitor dicoumarol confirmed that the *ortho*-quinones exerted their antitumor activity through NQO1-mediated ROS production by redox cycling. It was suggested that the L-shaped quinone substrates for NQO1 possessed better specificity and safety than β-lapachone.

1. Introduction

NAD(P)H:quinone oxidoreductase-1 (NQO1) is a dimeric flavoprotein that contains a non-covalently bound molecule of flavin adenine dinucleotide (FAD), and uses the reduced pyridine nucleotide NADH or NADPH as cofactor to catalyze the direct two-electron reduction of a wide variety of quinones [1,2]. Structural biology studies have revealed that NQO1 catalytically cycles using a ping-pong mechanism. In this mechanism, NAD(P)H binds to NQO1, reduces the FAD cofactor to $FADH_2$, and is then released in its oxidized form $NAD(P)^+$, allowing the quinone substrate to bind to the enzyme and to be reduced by $FADH_2$. NQO1-directed bioreduction can turn certain quinone substrates into potent cytotoxic compounds through the formation of unstable hydroquinones that are capable of either alkylating DNA or rapidly generating reactive oxygen species (ROS) through redox cycling [3]. Due to the dramatic elevation of NQO1 in many solid tumors and the little to no expression in normal tissues [4–7], quinone substrates bioreduced by NQO1 to unstable hydroquinones could be potent and selective antitumor agents, which identifies NQO1 as a promising therapeutic target for cancer therapy [8]. Several examples of NQO1 substrates with potent antitumor activity have already been reported in the literature, including the alkylators mitomycin C (MMC), EO9, RH1 and AZQ [9–12] and the redox cycling compounds streptonigrin (STN) [13,14], lavendamycin [15], deoxynyboquinone (DNQ) [12], β-lapachone (β-lap) [16] and tanshinone IIA (TSA) [17] (Fig. 1). Structurally, these substrates fall into two

Abbreviations: NQO1, NAD(P)H:quinone oxidoreductase-1; FAD, flavin adenine dinucleotide; ROS, reactive oxygen species; β-lap, β-lapachone; TSA, tanshinone IIA; MMC, mitomycin C; STN, streptonigrin; DNQ, deoxynyboquinone; PDB, protein data bank; equiv, equivalent; DMSO, dimethyl sulfoxide; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphe nyltetrazolium bromide; NSCLC, non-small cell lung cancer; DIC, dicoumarol; cyt *c*, cytochrome *c*; MOE, molecular operating environment; SAR, structure–activity relationship; RMSD, root mean square deviation.

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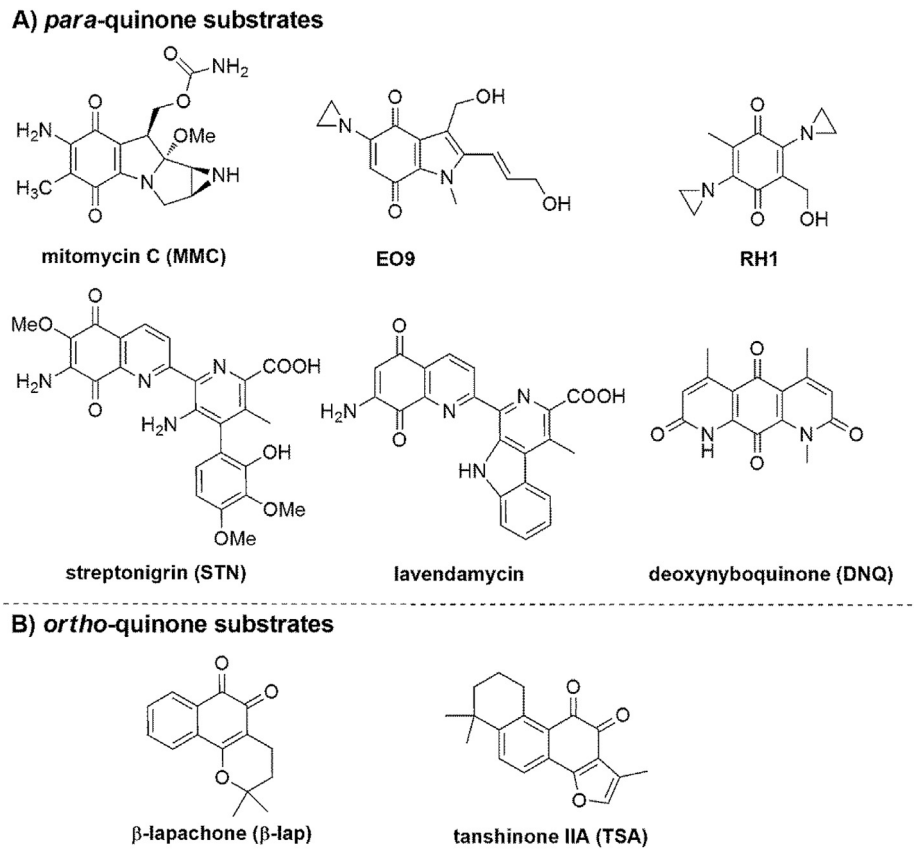

Fig. 1. Several examples of antitumor quinone substrates for NQO1.

main types: *para*-quinones (Fig. 1A) and *ortho*-quinones (Fig. 1B). Compared to the structurally diverse *para*-quinone substrates [9–15], the reported *ortho*-quinone substrates for NQO1 are limited to two natural products, β-lap and TSA. Thus, it is urgent to develop novel and efficient *ortho*-quinone substrates for NQO1 as potent NQO1-directed antitumor agents.

β-Lap (Fig. 1B) is a natural tetrahydropyran-fused *ortho*-naphanoquinone isolated from the *Bignoniaceae* family and is currently in multiple phase II clinical trials for the treatment of pancreatic adenocarcinoma [18]. Unlike conventional chemotherapeutic agents, β-lap has been reported to kill many human cancer cells selectively through rapid ROS generation mediated by NQO1 bioreduction [19]. However, the pyran ring in β-lap is not considered stable as it tends to be hydrolyzed to form ring-opening metabolic products [20], which could result in toxicity to normal tissues and cause side effects. Recently, TSA (Fig. 1B), another natural *ortho*-quinone isolated from *Salvia miltiorrhiza* (Danshen, in Chinese), has also been reported as the substrate for NQO1 [20]. Its structure features a relatively more stable aromatic furan ring fused to the *ortho*-naphanoquinone scaffold as compared to β-lap. In view of this, we initially merged the two natural NQO1 substrates into a new structure of 3-methylnaphtho[1,2-*b*]furan-4,5-dione (**1**) that retained the quinone pharmacophore for NQO1 metabolism (Fig. 2A). This planar structure **1** was then used as a starting point in the design of *ortho*-quinone analogs of β-lap as novel NQO1 substrates. Subsequently, by analyzing the X-ray crystal structure of human NQO1 in complex with its potent competitive inhibitor dicoumarol that obtained from the Protein Data Bank (PDB ID code: 2F1O) [21], we found that the binding site for NQO1 substrates is an L-shaped pocket. Notably, the bound ligand dicoumarol also has an L-type molecular shape that fits well with the L-shaped binding pocket of NQO1 (Fig. 2B). However, the reported *ortho*-quinone substrates and the merged compound **1** are all planar molecules. When docking compound **1** into the binding site of NQO1 using GOLD 5.1 software [22], it has been shown that this planar scaffold only occupied the bottom of the pocket parallel to FAD, but missed the side binding pocket that formed by Tyr128, Phe232, Phe236 and His194 residues (Fig. 2C). In view of this, based on the structural analysis of binding poses of **1** and dicoumarol, we are promoted to introduce nitrogen-containing side chains at the C2 position of **1** using methylene as a linker and to develop L-shaped *ortho*-quinone analogs **2a**–**2s** of β-lap as novel NQO1 substrates (Fig. 2A). The designed L-shaped substrates are expected to have higher specificity for NQO1 and better drug-like properties than the planar compound **1** and β-lap. Herein, we now report the details of a study to identify these L-shaped *ortho*-quinones **2a**–**2s** as efficient NQO1 substrates and examine the effects of the introduced side chains on the metabolism by NQO1 (Fig. 2A). Molecular docking has been applied to analysis the binding modes between the *ortho*-quinone substrates and NQO1, and thus to correlate their metabolic rates with predictions of the key interactions in the catalytic pocket of NQO1. These *ortho*-quinone analogs of β-lap have been tested *in vitro* for their antitumor activity against a pair of NQO1-rich and NQO1-deficient cancer cell lines. In addition, we attempted to confirm whether the *ortho*-quinone analogs exerted their antitumor activity through NQO1-dependent and ROS-mediated pathways.

2. Result and discussion

2.1. Chemistry

The designed 19 *ortho*-quinone target compounds **2a**–**2s** were synthesized in 4–5 steps from commercially available, inexpensive