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Research paper

Oxadiazole-substituted naphtho[2,3-*b*]thiophene-4,9-diones as potent inhibitors of keratinocyte hyperproliferation. Structure–activity relationships of the tricyclic quinone skeleton and the oxadiazole substituent

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ABSTRACT

Novel analogues of oxadiazole-substituted naphtho[2,3-*b*]thiophene-4,9-diones were synthesized in which the tricyclic quinone skeleton was systematically replaced with simpler moieties, such as structures with fewer rings and open-chain forms, while the oxadiazole ring was maintained. In addition, variants of the original 1,2,4-oxadiazole ring were explored. Overall, the complete three-ring quinone was essential for potent suppression of human keratinocyte hyperproliferation, whereas analogous anthraquinones were inactive. Also, the oxadiazole ring per se was not sufficient to elicit activity. However, rearrangement of the heteroatom positions in the oxadiazole ring resulted in highly potent inhibitors with compound **24b** being the most potent analogue of this series showing an IC_{50} in the nanomolar range. Furthermore, experiments in isolated enzymatic assays as well as in the keratinocyte-based hyperproliferation assay did not support a major role of redox cycling in the mode of action of the compounds.

1. Introduction

Naphthoquinones constitute a class of naturally occurring compounds found in animals, plants and microorganisms [1]. Due to their biological and structural properties they are regarded as privileged structures in Medicinal Chemistry [2], and because of their demonstrated activity particularly against cancer cells these agents have been the subject of intensive research [3,4]. The exact molecular mechanism by which naphthoquinones exert their biological action still needs to be elucidated. At least in part, the leading theory holds that the quinone moiety reacts with two major enzymes such as NADPH-cytochrome P-450 oxidoreductase (CPR) and NAD(P)H:quinone oxidoreductase 1 (NQO-1), leading to redox cycling between the quinone and the semiquinone radical or hydroquinone forms (Fig. 1), thereby generating superoxide and even more reactive oxygen species (ROS) derived therefrom that could react with biological targets [5–11].

Prominent examples of bioactive naphthoquinones (Fig. 2) include ortho-quinoid β-lapachone (**1**) and the linearly anellated naphtho[2,3-*b*]furan-4,9-diones **2** (napabucasin) and **3**, which were isolated from the heartwood of the lapacho (Tabebuia) tree of the Bignoniaceae family together with a variety of other naphthoquinones and anthraquinones [12–14]. The inner bark, commonly known as “pau-d'arco”, is used as an analgesic, an antiinflammatory, and an antineoplastic by the native people in South America [15,16]. The biological significance of these quinones in cancer therapy has stimulated enormous research interest in this class of compounds [17–22]. Furthermore, we have previously reported that the lapacho tree provided some naphthoquinones with comparable activity against the growth of human keratinocytes relative to the antipsoriatic drug anthralin (**4**, Fig. 2) [23].

In our continuous interest in tricyclic antiproliferative agents

Abbreviations: 7-AAD, 7-aminoactinomycin D; CPR, NADPH-cytochrome P450 oxidoreductase; DCC, *N,N'*-dicyclohexylcarbodiimide; DHE, dihydroethidium; DMAP, 4-(dimethylamino)pyridine; DTPA, diethylenetriaminepentaacetic acid; NQO-1, NAD(P)H:quinone oxidoreductase 1; ROS, reactive oxygen species; rt, room temperature; SAR, structure–activity relationship; SOD, superoxide dismutase.

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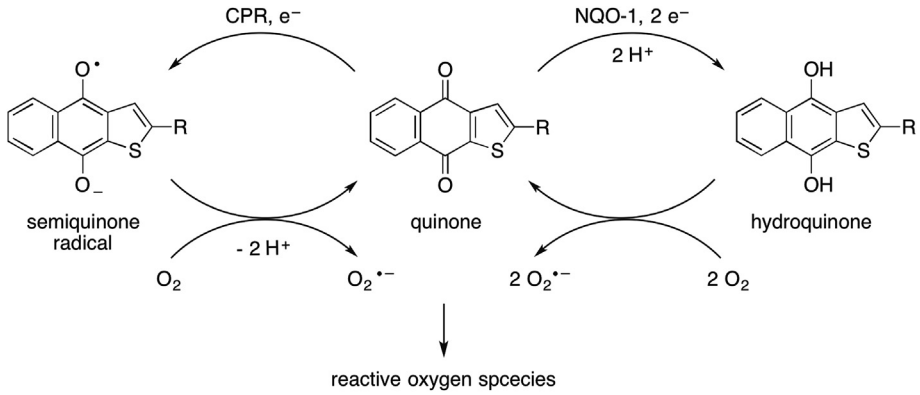

Fig. 1. Redox cycling of naphtho[2,3-*b*]thiophene-4,9-diones initiated through one-electron reduction by CPR/NADPH to the semiquinone radical and two-electron reduction by NQO-1/NADPH to the hydroquinone.

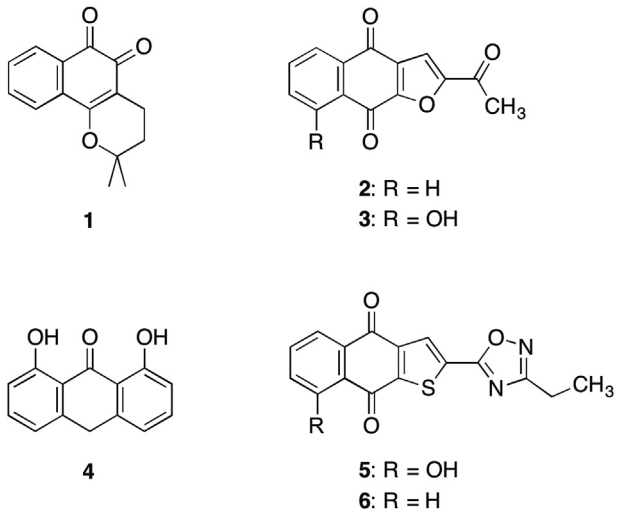

Fig. 2. Lapacho quinones (**1**–**3**), synthetic analogues (**5**, **6**) and anthralin (**4**).

such as anthracenones [24] naphtho[2,3-*b*]thiophen-4(9*H*)-ones [25], acridones [26], phenoxazines and phenothiazines [27] we prepared novel analogues of napabucasin (**2**) and studied the influence of side chain modification on the potency of these agents to suppress keratinocyte hyperproliferation [28]. Such a feature may be useful in the treatment of hyperproliferative skin disorders such as psoriasis, which is mainly characterized by excessive growth of keratinocytes [29]. In a subsequent study, we reported the synthesis and structure–activity relationships (SAR) for a series of compounds in which the furano-oxygen of the parent naphtho[2,3-*b*]furan-4,9-dione core was replaced by other heteroatoms [30]. Of these heterocyclic ring variants, the 8-hydroxynaphtho[2,3-*b*]thiophene-4,9-dione analogue bearing an oxadiazole ring on the 2-position of the tricyclic skeleton (**5**, Fig. 2) was the most potent inhibitor of keratinocyte hyperproliferation [30]. In this context, it is of interest to note that several compounds possessing an oxadiazole moiety have recently been reported as antiproliferative agents [31–35].

To further explore structure–activity relationships for the class of linearly anellated tricyclic quinones, we have now focused on identifying the minimum structural requirements for inhibitory action against keratinocyte hyperproliferation on the one hand, and, on the other, elucidating the role of the 2-oxadiazole substituent. Toward this goal, we compared the inhibitory potential of certain structural analogues in which the tricyclic quinone skeleton of naphtho[2,3-*b*]thiophene-4,9-dione **6** (Fig. 2) has been systematically replaced with simpler moieties while the 1,2,4-oxadiazole fragment was kept unchanged. We also report on the modification of the 1,2,4-oxadiazole ring of **6**, which resulted in a highly potent inhibitor of keratinocyte hyperproliferation. The biological testing procedures applied in this study were similar to those in our earlier work [28,30].

2. Chemistry

The syntheses of the desired substitution patterns and oxadiazole ring variants are shown in Schemes 1–6. Scheme 1 shows the preparation of **6** and its modified analogues **13a**–**13c** and **13l**. The required electron-rich 4,9-dimethoxynaphtho[2,3-*b*]thiophene (**8**) was prepared from unsubstituted quinone skeleton **7** by reductive methylation with sodium dithionite and dimethyl sulfate in the presence of tetrabutylammonium bromide. Regioselective 2-lithiation of the thiophene ring followed by carboxylation with dry ice yielded the activated carboxylic acid **9a**, which was then converted to 1,2,4-oxadiazole **13a** with *N*-hydroxypropionamidine in a one-pot reaction [36]. Oxidative deprotection of **13a** with diammonium cerium(IV) nitrate afforded quinone **6**. In a similar fashion, naphthothiophens **13b** and **13**c, which lack the methoxy group at the 9-position or both methoxy groups at the 4,9-positions, respectively, were prepared from precursors **10** and **12**. 4-Hydroxy analogue **13l** was obtained from its methoxy precursor **13b** by ether cleavage with boron tribromide.

The preparation of analogous compounds **13d**–**13f** and **13m**–**13u** (Fig. 3,5 and 6), in which the tricyclic quinoid system was downsized or replaced by other moieties, was also accomplished by reaction of the activated carboxylic acids and *N*-hydroxypropionamidine (Scheme 2). The requisite carboxylic acids were prepared as described in the Supplementary content or according to literature methods. Analogues comparable to **6** in which the 1,2,4-oxadiazole was appended to a quinone moiety (**13g**–**13k**, Fig. 4) were obtained from their corresponding para-dimethoxy aromatic precursors by oxidative demethylation as described above.

Scheme 3 outlines the synthesis of 5-ethyl-1,2,4-oxadiazole **19**, which is a substitution isomer of **6** where the 3,5-substituents at the oxadiazole ring have been exchanged with each other. Carbaldehyde **14** was transformed into the corresponding oxime **15** and dehydrated to nitrile **16**, which upon addition of hydroxylamine furnished amidoxime **17**. Ring closure with activated propionic acid in a one-pot reaction in pyridine following the method of Borg et al. [36] provided oxadiazole **18**, which was then converted to the