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Original article

Structural factors affecting affinity of cytotoxic oxathiole-fused chalcones toward tubulin

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ABSTRACT

Synthesis, *in vitro* cytotoxic activity, and interaction with tubulin of (E)-1-(6-alkoxybenzo[*d*][1,3]oxathiol-5-yl)-3-phenylprop-2-en-1-one derivatives (**2**) are described. Some of the compounds demonstrated cytotoxic activity at submicromolar concentrations, and the activity could be related to interaction with tubulin at the colchicine binding site. Interaction of selected derivatives with tubulin was evaluated using molecular modeling, and two different modes of the interaction were identified. The proposed models demonstrate how particular structural fragments participate in binding to the tubulin and explain the importance of the fragments for cytotoxic activity. It was demonstrated that concerning binding to tubulin, the 6-alkoxybenzoxathiole ring can be considered as structural equivalent of trimethoxyphenyl motif of colchicine, podophyllotoxin or combretastatin A4. The observation opened new ways of rational modifications of several groups of tubulin binders.

1. Introduction

Tubulin polymerization disturbing agents occupy prominent place among anticancer drugs, with vinca alkaloids and taxanes serving as the most popular examples [1–4]. Three binding regions of tubulin are most frequently exploited in search for new inhibitors: vinblastine, taxol and colchicine binding sites. The last one serves as a target area for analogs of colchicine, combretastatin A4, podophyllotoxins and several others structural groups [5,6], including chalcones [7–9]. Despite numerous efforts, none of the antitumor chalcone analogs reached the market [10], yet this group is still among the most often studied [11–15], and understanding details of their mechanisms of action is important.

We have previously demonstrated that some derivatives of (*E*)-1-(benzo[*d*][1,3]oxathiol-6-yl)-3-phenylprop-2-en-1-one (**1**) (Fig. 1) exhibited very high, cytotoxic activity, at nanomolar level, and that the activity depended on a combined influence of three structural factors: (a) the presence of the heterocyclic ring, (b) the presence and structure of 5-OR group in ring A, and (c) substituents in ring B of chalcones. It was also found, that combination of the alkoxy group OR = OC_2H_5 with substituents *X* = (3-OH-4-OCH_3) or (3-F-4-OCH_3) was optimal, and that replacement of the sulfur atom in the heterocyclic ring by oxygen did not appreciably influence the cytotoxic activity [16].

The exceptionally high activity of chalcones **1** prompted us to further modify their structure, including a change of positions of the sulfur atom (structure **2**), and position of the heterocyclic ring (structure **3**), synthesis of compounds with polar OR substituents in ring A (**2**, OR = $OCH_2CH_2NR_2$ or OCH_2COOH), and compounds with hydrophobic substituents *X* in ring B (**2**, *X* = Cl or Br).

To explore mechanism of activity of the compounds, their influence on polymerization of tubulin, interaction with tubulin at the colchicine binding site, as well as on the cell cycle were tested.

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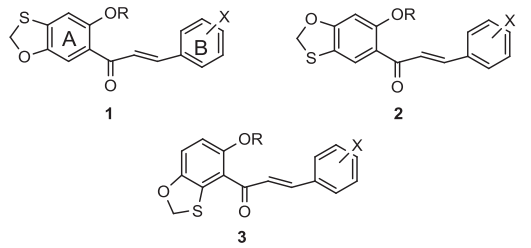
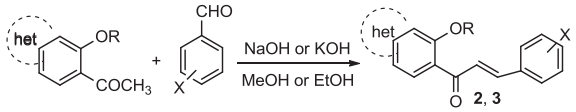


Fig. 1. Structures of the studied compounds.



Scheme 1. The synthetic route employed to access the described chalcones.

To clarify the topic further, interactions of the chalcones **2** with tubulin were approached by molecular modeling.

2. Results

2.1. Chemistry

Chalcones **2** and **3** were prepared by condensation of suitable acetophenones with benzaldehydes in alkaline alcoholic solution (Scheme 1).

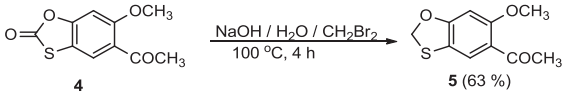
The starting 5-acetyl-6-methoxybenzo[*d*][1,3]oxathiole (**5**) was prepared from 5-acetyl-6-methoxybenzo[*d*][1,3]oxathiol-2-one (**4**) [17] by alkaline ring opening and recyclization with methylene bromide (Scheme 2).

Differently substituted 5-acetyl-6-alkoxybenzo[*d*][1,3]oxathioles (**8**–**10**) were prepared from 5-acetyl-6-hydroxybenzo[*d*][1,3]oxathiol-2-one (**6**) [17] by recyclization to oxathiole derivative **7**, and alkylation with suitable alkyl halide (Scheme 3).

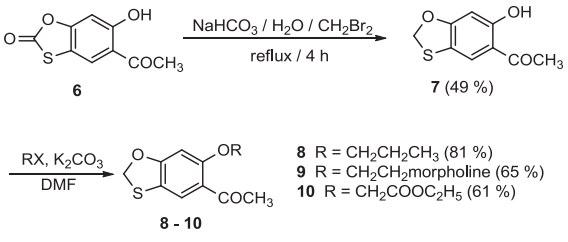
4-Acetyl-5-methoxybenzo[*d*][1,3]oxathiole (**12**) was prepared analogously to methoxy derivatives **5** from oxathiolone **11** [18] (Scheme 4).

2.2. Cytotoxic activity in vitro

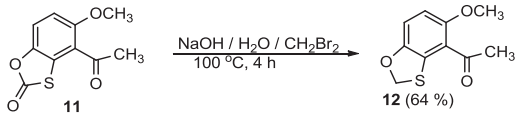
The cytotoxicity of the prepared compounds was evaluated against three tumor cell lines commonly applied to primary screening of potential anticancer compounds, using the MTT test



Scheme 2. Synthesis of 5-acetyl-6-methoxybenzo[*d*][1,3]oxathiole (**5**).



Scheme 3. Synthesis of 5-acetyl-6-alkoxybenzo[*d*][1,3]oxathioles (**8**–**10**).



Scheme 4. Synthesis of 4-acetyl-5-methoxybenzo[*d*][1,3]oxathiole (**12**).

(Tables 1 and 2). Based on the obtained values of inhibitory concentration, derivatives of benzoxathiole chalcone **2** were divided into four structural sub-groups. The first one included 6-alkoxy compounds with at least one alkoxy or hydroxy group in ring B (compounds **17**–**19**, **21**–**27**) (Table 1). Activity of these compounds was strongly influenced by the substitution pattern of ring B, and changed from 5.04 μM for 2,3-diOCH_3 derivative **22**, to 0.012 μM for 3-OH-4-OCH_3 derivative **27** (all IC_{50} values used in this discussion were determined for A549 cells). The second sub-group comprised unsubstituted and halogen-substituted compounds **13**–**16**, characterized by lack of hydrogen bond donor/acceptor in ring B and very similar activities, all being in the range 2–4 μM, regardless of position or nature of the halogen atom. The third sub-group included 6-aminoalkoxy compounds (compounds **27**–**32**), their activities also remained in a narrow range 1–4 μM, regardless of substitution pattern of ring B. The last sub-group included alkoxycarboxylic acids (compounds **20**, **33**–**35**) prepared to increase aqueous solubility through introduction of a COOH, however, the compounds were found to be biologically inactive. It was speculated, that the lack of cytotoxic activity might have been due to the poor penetration of the compounds through the cytoplasmic membrane under the MTT test conditions. Calculations performed for carboxylic acid **33** using the SPARC predictive modeling system [19] indicated that it was fully deprotonated at physiological pH (Fig. 2, panel A). Most probably, the negatively charged ion of **33** was unable to cross the membrane barrier. To verify this hypothesis, intracellular concentrations of carboxylic acid derivative **33** and related methoxy derivative **18** in A549 cells were determined after 2 h of incubation, and only marginal amounts of **33** were accumulated in living cells (Fig. 2, panel B).

Comparison of influence of substituents on activity of analogously substituted isomers **1** [16] and **2** (Fig. 1) indicated that position of the sulfur atom was not important, and isomeric pairs of compounds displayed very similar activity.

Concerning changes in the cytotoxic activity caused by location of the heterocyclic ring (isomer **2** *versus* **3**), the results depended on substitution of ring B. For unsubstituted and halogen-substituted compounds (**13**–**16** *versus* **36**–**39**, respectively) activities in both groups were very similar and remained in the narrow range 2–4 μM. The 4-OCH_3 substituted derivatives of isomer **3** (**40** – 10.6 μM; **43** – 9.43 μM) were 2–3 times less active than the unsubstituted compound (**36** – 3.66 μM), while for isomer **2** introduction of the 4-OCH_3 group resulted in 10 times increased cytotoxic activity, from 4.10 μM for **13**, to 0.352 μM and 0.320 μM for **18** and **23**, respectively. The opposite influence of substitution on activity indicated at different SAR for derivatives of isomers **2** and **3**.

2.3. Mechanisms of activity assays

To investigate whether the cytotoxic activity of the studied compounds was related to interaction with tubulin, the effect of compound **18** on polymerization of tubulin *in vitro* was followed by measuring changes in the optical density at 340 nm. With increasing concentrations of the compound a decrease in the signal was observed, with the IC_{50} value equal 5.80 μM (Table 3). Competition for the colchicine binding site test revealed that colchicine was effectively replaced by the compound **18** (Table 3).