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IN VITRO EVALUATION OF ANTITUBULIN ACTIVITY OF TETRAHYDROPYRROLO[3,4-c]PYRROLE-1,3-DIONE DERIVATIVES

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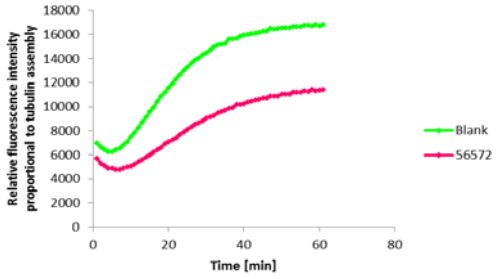
In a previous paper, the substituted tetrahydropyrrolo[3,4-c]pyrrole-1,3-dione (THPPD) has been presented as new potential colchicine site inhibitor discovered by *in silico* study.[1] In this short note, the results of preliminary *in vitro* study are presented. Six of seven evaluated THPPD derivatives inhibited *in vitro* tubulin assembly. Further, two structurally different molecules, also resulting from molecular docking study, have been evaluated and proved to exhibit antitubulin activity.



INTRODUCTION

Tubulin, a 100 kD heterodimer, is the major component of mitotic spindle.[2] Colchicine domain is one of the three binding sides of tubulin, and serves as a pocket for small organic molecules. Binding of a drug to colchicine domain prevents rapid tubulin assembly and thus causes mitotic arrest, quantitatively affecting more frequently diving cells. The colchicine side pharmacophore has been described already by Nguyen *et al.* to consist of two orthogonal aromatic systems.[3] *In silico* study described in previous paper revealed the (3*S*,3a'*R*,6a'*S*)-5'-benzyl-2',3',3a',6a'-tetrahydro-4'*H*-spiro[indoline-3,1'-pyrrolo[3,4-c]pyrrole]-2,4',6'(5'*H*)-trione (**1**) as new potential colchicine side lead.[1] Naphtalen-1-yl or phenyl could substitute the benzyl at the 5'-position, see Chart 1. The absolute configuration of THPPD derivative **1** seems to be crucial for binding, as the benzyl (naphtalen-1-yl, phenyl) and the indolin-2-one moiety create two orthogonal planes, one buried in the hydrophobic pocket of β-tubulin and the other positioned at the interface of α- and β-subunit. Substitution at the 3'-position of THPPD derivative **1** provides next chiral centrum. According to previous docking study, acetamide or propanamide 3'-substitution dominated among the best hits. The chirality at carbon 3' has no significant effect on the binding affinity. However, it determines the orientation of the ligand in the binding pocket, namely which of the two aromatic systems is buried in β-tubulin and which one is localized at the interface of α- and β-subunit.

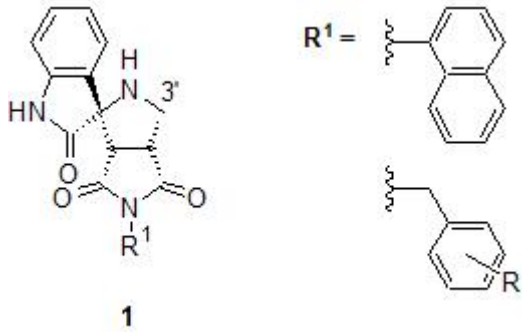


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Chart 1 – The absolute configuration of THPPD derivative **1** proposed by *in silico* study. Carbon 3', which can be further substituted, is depicted.

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This study provides the preliminary evaluation of antitubulin activity of seven structures bearing the motif **1** and two structurally unrelated molecules also resulted from previous docking study (Chart 2).

RESULTS AND DISCUSSION

Tubulin polymerizes to form microtubules. In the presence of fluorescent reporter, polymerization is followed by an increase in fluorescence emission. In the presence of inhibitor, the fluorescence intensity decreases proportionally to inhibition rate. Six of the seven evaluated THPPD structures inhibited tubulin assembly with inhibition rates ranging from 8 to 30% at 30 μM, and with compound **56572** being the most active (Figure 1). Compound **60694** was the only inactive THPDD derivative. It is also the only compound with ethyl substitution at the benzene ring of the 1,3-dihydro-2*H*-indol-2-one part of the molecule. The other evaluated THPPD derivatives are bearing either methyl or chlorine substitutions at this aromatic system. Thus, steric clash is probable explanation for the inactivity of derivative **60694**. Regrettably, the evaluated compounds are available only as mixtures of enantiomers without further data about the representation of each enantiomer. Assuming only the (3*S*,3a'*R*,6a'*S*)-5'-benzyl-2',3',3a',6a'-tetrahydro-4'*H*-spiro[indoline-3,1'-pyrrolo[3,4-c]pyrrole]-2,4',6'(5'*H*)-trione **1** derivatives to be active as predicted by docking study, higher activity of optically pure compounds shall be expected. The two other structurally unrelated molecules, **14752** and **74562**, showed similar modest inhibitory activity. These molecules were included in the experiment as they appeared among the best results of *in silico* study alongside with THPPD derivatives. They contain one chiral carbon and thus only two possible enantiomers exist. In contrast to THPPD derivatives, where the absolute configuration influences the overall molecular shape, the effects here are less clear, as the *R*- and *S*-enantiomer differs in a position of quite a small part of the molecule.

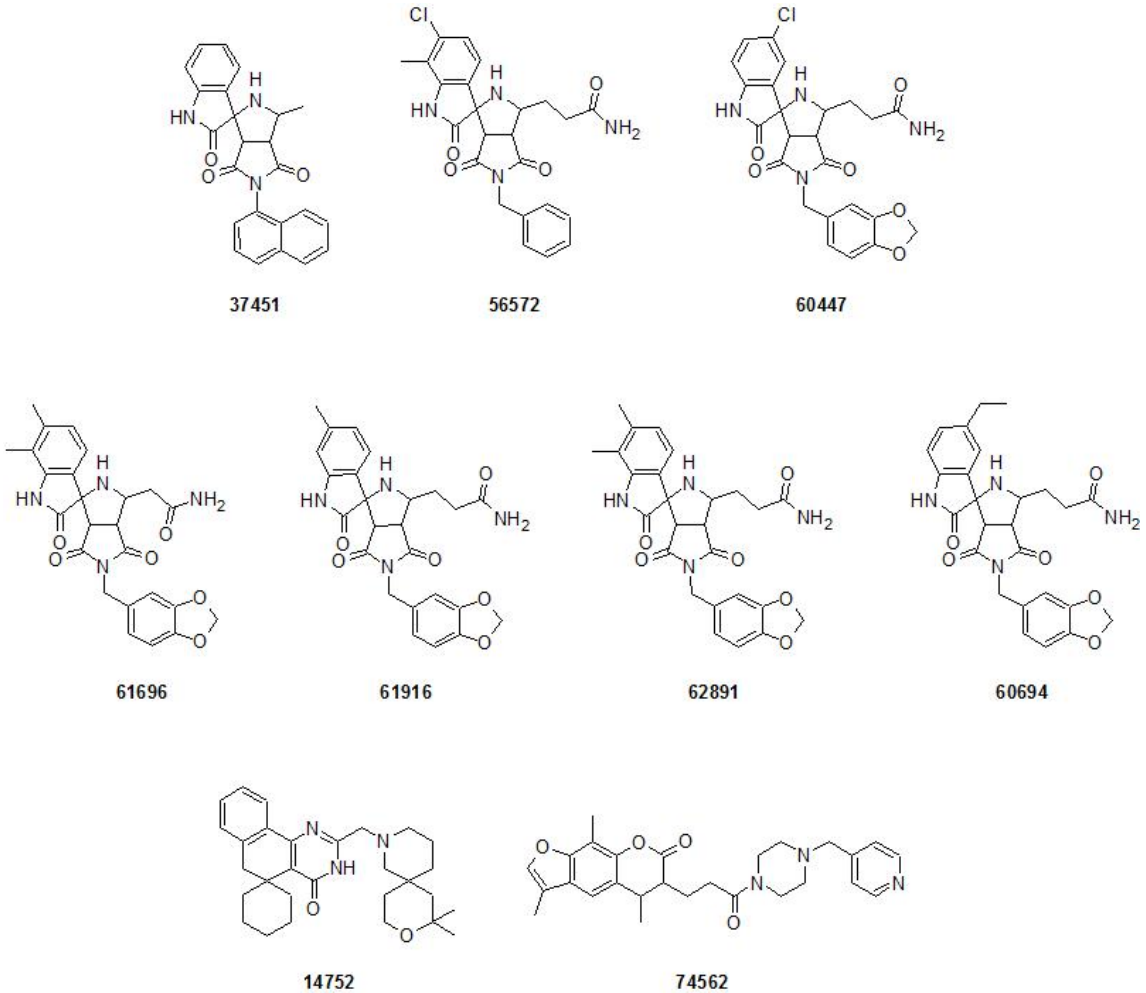

Chart 2 – Compounds evaluated for antitubulin activity (available as the mixtures of enantiomers).

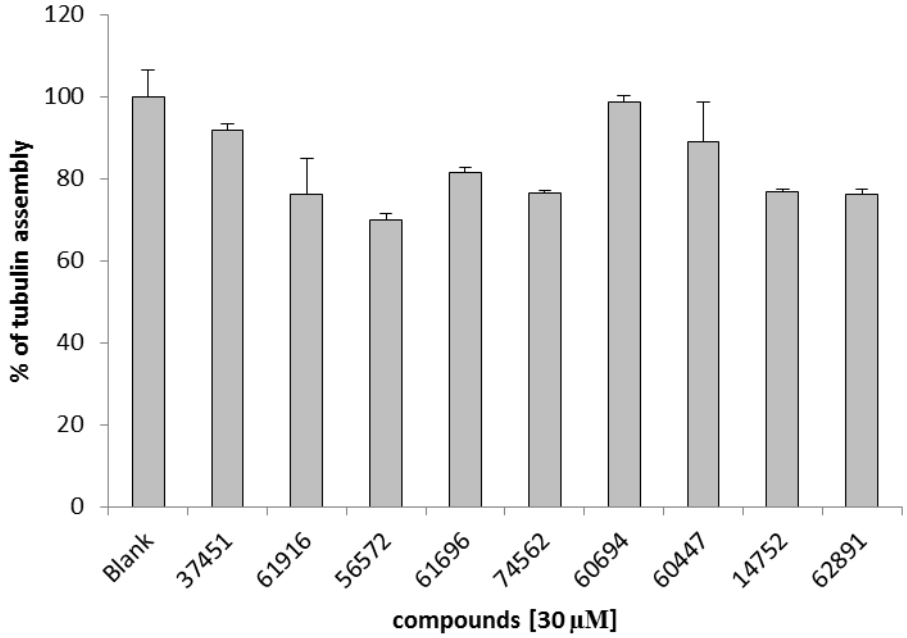

Fig. 1 – Antitubulin activity of evaluated compounds at concentration 30 μM expressed as the percentage of control tubulin assembly. Tubulin at total concentration 2mg/ml was incubated together with fluorecence reporter and inhibitor for one hour. The fluorescence, directly proportional to tubulin assembly, was recorded.

MATERIALS AND METHODS

Compounds were purchased (MolPort, Latvia) as mixtures of enantiomers. Tubulin assembly was determined using fluorescent based tubulin polymerization assay kit Cat. # BK011P (tebu-bio, France). The assay was performed in 96 well microtiter plate form according to standard assay protocol except of the total volumes reduced by 7.6 μL. Briefly, each well of the assay plate contained 4.4 μL of 10× strength compound which was warmed for 1 min to 37°C, and 43 μL of tubulin solution was pipetted into each well. Total tubulin concentration was 2mg/mL. The fluorescence intensity was recorded for one hour on Cytation™ 3 Cell Imaging Multi-Mode Reader (BioTek ® Instruments, Inc., Vermont, USA). Excitation was at 360 nm and emission at 420 nm.

CONCLUSION

The THPPD derivatives discovered by *in silico* study have been proved to show antitubulin activity *in vitro*. The activities of evaluated compounds were moderate. Based on *in silico* prediction, only one of eight possible enantiomers is supposed to be active, while undefined mixture of enantiomers is available commercially at this moment. This preliminary evaluation of activity can encourage chemists to synthesize or purify particular enantiomers for further *in vitro* evaluation. As the activity of the molecules bearing the lead structure has been proved, further modifications of the main skeleton can be proceeded in order to achieve better activity and overall suitable properties.

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