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Synthesis of isoflavene-thiosemicarbazone hybrids and evaluation of their anti-tumor activity

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ABSTRACT

Phenoxodiol is an isoflavene with potent anti-tumor activity. In this study, a series of novel mono- and di-substituted phenoxodiol-thiosemicarbazone hybrids were synthesized via the condensation reaction between phenoxodiol with thiosemicarbazides. The *in vitro* anti-proliferative activities of the hybrids were evaluated against the neuroblastoma SKN-BE(2)C, the triple negative breast cancer MDA-MB-231, and the glioblastoma U87 cancer cell lines. The mono-substituted hybrids exhibited potent anti-proliferative activity against all three cancer cell lines, while the di-substituted hybrids were less active. Selected mono-substituted hybrids were further investigated for their cytotoxicity against normal MRC-5 human lung fibroblast cells, which identified two hybrids with superior selectivity for cancer cells over normal cells as compared to phenoxodiol. This suggests that mono-substituted phenoxodiol-thiosemicarbazone hybrids have promising potential for further development as anti-cancer agents.

Flavonoids are natural products found in foods such as soy or green tea, and they possess several biologically beneficial properties such as anti-tumor, anti-inflammatory, and anti-viral activities.[1] One of the most investigated flavonoids is genistein, due to its high abundance and high bioactivity compared to other flavonoids.[2] A synthetic derivative of genistein, phenoxodiol **1**, showed promising anti-proliferative activity and has been tested in clinical trials for drug resistant ovarian (NCT00382811) and prostate (NCT00557037) cancer.[3]

Phenoxodiol **1** can be further developed through molecular hybridization to enhance its anti-tumor activity. Molecular hybridization involves combining parts of bioactive compounds together to generate a hybrid molecule with improved biological activity.[4] Hybrids can exhibit greater specificity and efficacy, and may possess additional modes of action when compared with their parental molecules.[5–9] This was shown in a previous study where phenoxodiol **1** was hybridized with a 1-amino-2-propanol moiety present in the β-blocker propranolol and the analogues generated exhibited enhanced anti-proliferative and anti-angiogenic activities.[9] These results suggest that molecular hybridization is an effective strategy to further improve the biological properties of phenoxodiol **1**.

Thiosemicarbazones exhibit various biological properties such as anti-viral,[10] anti-bacterial[11–13] and anti-cancer[14–16] activities. The anti-tumor mechanism of thiosemicarbazones could be through the inhibition of DNA synthesis by blocking the reductive conversion of ribonucleotides to deoxyribonucleotides.[17] Furthermore, thiosemicarbazones based iron chelators have been shown to possess selective anti-tumor activity *in vitro* and *in vivo*.[18,19] These properties of thiosemicarbazones make it attractive for their use in molecular hybridization. It was anticipated that the phenoxodiol-thiosemicarbazone hybrids generated would show improved anti-proliferative activity and specificity compared to the parent molecules.

Two different classes of phenoxodiol-thiosemicarbazone hybrids were investigated in this study, namely mono- and di-substituted hybrids. To attach a thiosemicarbazone moiety, phenoxodiol **1** must first be functionalized with a ketone or aldehyde functionality. In a previous study, we have shown that C6 formylation of phenoxodiol **1** can be successfully achieved by reaction phenoxodiol **1** with paraformaldehyde in the presence of $MgCl_2$ and Et_3N.[20] Therefore, the thiosemicarbazone moiety was attached at the C6 position of phenoxodiol **1** to generate the mono-substituted hybrids. To generate di-substituted hybrids, ketone or aldehyde functionalities have to be installed at two different sites of phenoxodiol **1**. We have previously shown that the phenolic OH groups in phenoxodiol can be easily functionalized with alkyl halides to generate the corresponding ethers, and the attachment of the func-

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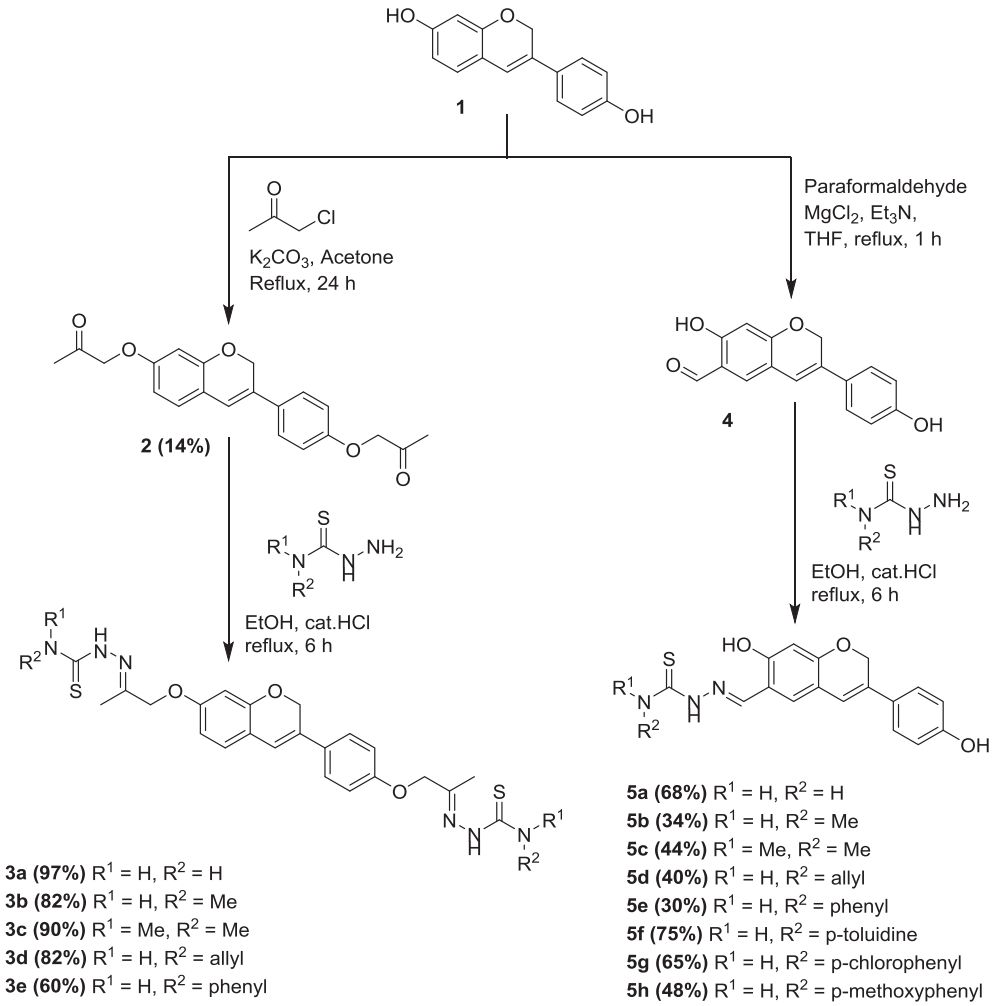

Scheme 1. Overall scheme for the synthesis of mono- and di-substituted phenoxodiol-thiosemicarbazone hybrids.

tional moieties at the 7 and 4′ positions of phenoxodiol **1** improved biological activity.[9] Hence, the thiosemicarbazone moiety was attached at the 7 and 4′ positions of phenoxodiol **1** to form the di-substituted hybrids.

The synthesis of the di-substituted hybrids was first attempted. To attach a ketone functionality, the 7 and 4′-OH groups of phenoxodiol **1** were reacted with chloroacetone in a substitution reaction according to a previously used method.[9] Refluxing phenoxodiol **1** with chloroacetone (4 eq.) in acetone with K_2CO_3 (4 eq.) for 48 h generated the desired di-ketone product **2** in 14% yield after column chromatography (Scheme 1). Nevertheless, these un-optimized reaction conditions generated sufficient quantity of the di-ketone **2** for the subsequent step.

The condensation reaction of the di-ketone **2** with a panel of substituted thiosemicarbazides was carried out in refluxing ethanol with catalytic amount of concentrated HCl for 6 h (Scheme 1). During this time, a precipitate formed in the reaction solution, which was filtered to give the desired 7,4′-di-substituted thiosemicarbazone hybrids **3a**–**e** in good yields of 60–97%.

For synthesis of the mono-substituted hybrids, the formylation of phenoxodiol **1** at the C6 position was first carried out by refluxing phenoxodiol **1** in THF with paraformaldehyde (3 eq.), $MgCl_2$ (2 eq.) and Et_3N (2 eq.) for 1 h. The reaction mixture was subsequently added to 2 M HCl and the desired C6-formyl phenoxodiol **4** precipitated as a yellow solid.[20] The C6-formyl phenoxodiol **4** was subsequently condensed with various thiosemicarbazides using the same method as before to generate the desired C6 mono-substituted thiosemicarbazone hybrids **5a**–**h** tumoin moderate to good yields of 30–75% (Scheme 1).

To determine if these new phenoxodiol-thiosemicarbazone hybrids possess any enhanced biological activity, their *in vitro* anti-proliferative activities were evaluated out against three different cancer cell lines, namely the neuroblastoma SKN-BE(2)C, the triple negative breast cancer MDA-MB-231, and the glioblastoma (brain cancer) U87 cell lines, represented as GI_{50} to measure the concentration at which 50% of the growth/proliferation of cancer cells are inhibited. Compounds that exhibited improved anti-proliferative activity against the cancer cell lines were selected for further cytotoxicity testing against the normal human fibroblast MRC-5 cell line, represented as IC_{50} to measure the concentration at which 50% of the normal cells are still alive. The compounds were tested up to a concentration of 100 μM against the cancer cell lines as any activity above 100 μM was deemed inactive.

The di-substituted hybrids **3** were first tested for their anti-proliferative activity against SKN-BE(2)C and MDA-MB-231. The results showed that only the *N*-dimethyl substituted compound **3c** showed anti-proliferative activity against SKN-BE(2)C (GI_{50}: 54 μM), while only the unsubstituted compound **3a** showed activity against MDA-MB-231 (GI_{50}: 25 μM) (Fig. 1 and Table 1). The remaining analogues showed GI_{50} values above 100 μM. As anti-proliferative activities of **3c** and **3a** were not better than the parent