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Synthesis, structural characterization and biological activity of novel Knoevenagel condensates on DLD-1 human colon carcinoma

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ABSTRACT

Biologically active Knoevenagel condensates (**1**–**14**) of diarylheptanoids: 1,7-bis(3-methoxy-4-hydroxyphenyl)hepta-1,7-diene-3,5-dione and 1,7-bis(3-ethoxy-4-hydroxyphenyl)hepta-1,7-diene-3,5-dione, were synthesized and structurally characterized. Compounds **1**–**14** exhibited cytotoxicity against colon carcinoma cells, and their antiproliferative effect was associated with a significant decrease of multidrug resistance proteins. One of the underlying mechanisms of these effects is the reduction of intracellular and extracellular SOD enzymes by compounds **1**, **12** and **14**, which render the tumor cells more vulnerable to oxidative stress.

Introduction

Curcumin, a remarkable pharmacologically active product, exhibits low oral bioavailability a rapid first-pass metabolism and excretory system.[1] Therefore, new structurally modified derivatives of curcumin with better pharmacokinetic attributes are required. An affordable alternative are Knoevenagel condensates of curcumin, synthesized by reacting the curcuminoids with appropriate aldehydes. The products of condensations are non-enolisable compounds.[2] Previous studies have shown that Knoevenagel condensates of curcumin possess higher antibacterial activity than curcumin itself. Substituted benzylidene rings with electron releasing groups in the 2nd and 4th positions demonstrated extensive activity against *S. aureus* and *P. aeruginosa*.[3] Moreover, 4-arylidene curcumin analogues proved to have antiproliferative activity.[4,5] Several derivatives with various substituted benzylidene rings have showed significantly higher antitumor activity against 5 tested cancer cell lines (A549, HepG2, MCF-7, SW480 and CNE2) than their curcumin analogues.[6] Knoevenagel condensates are capable of complexation with metal ions through keto-groups.[2] Copper complexes of Knoevenagel condensates are more cytotoxic on H1299 and KBM-5 cell lines than curcumin alone.[7]

The present work is focused on the synthesis, structural characterization (NMR, IR, UV, HR-MS spectra) and biomedical activity of Knoevenagel condensates derived from 1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione and 1,7-bis(4-hydroxy-3-ethoxyphenyl)hepta-1,6-diene-3,5-dione, respectively. Compounds **1**–**14** were the subject of analytical ABTS and DPPH assays used to evaluate antioxidant capacity. All condensates were investigated *in vitro* on human colon carcinoma cell lines DLD-1, for the determination of cytotoxicity, SOD-mimetic activity (SOD1, SOD2, SOD3) and their capacity to oxidize glutathione (GSH).

Knoevenagel condensates were synthesized using a previously reported general procedure[6] (Scheme 1). The aromatic aldehyde (2 equiv.) and catalytic amounts of piperidine and acetic acid were added to a solution of a pure arylheptanoid (1 equiv.) in toluene. The reaction mixture was stirred overnight at 140 °C and the final product was isolated by column chromatography (ethyl acetate:

Abbreviations: ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); DPPH, di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium; MDR1, multidrug resistance gene 1; MRP1, multidrug resistance-associated protein 1; PBS, Phosphate-buffered saline; SOD, superoxide dismutase; GSH, glutathione; GSSG, glutathione disulfide.

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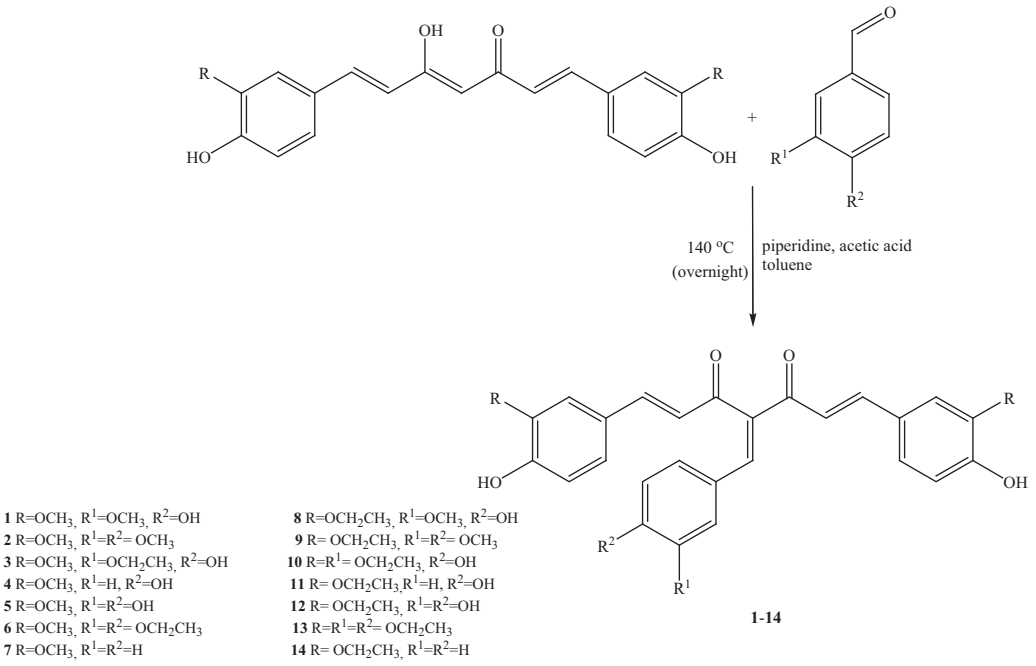

Scheme 1. Synthesis of Knoevenagel condensates **1**–**14**.

hexane, 2:1). Formerly reported condensates **1**, **2**, **4**, **5**, **7**[2,8,9] were obtained as yellow-orange powders with satisfactory yields (39–75%). The new derivatives **3**, **6** and **8**–**14**, were isolated as orange powders in relatively good yields (11–75%) (Supplementary information).

The antioxidant activity of Knoevenagel condensates **1**–**14** was determined in terms of radical scavenging activity using the stable radical DPPH. Condensates **1**–**14** and standards 1,7-bis(3-methoxy-4-hydroxyphenyl)hepta-1,7-diene-3,5-dione (**C1**) and 1,7-bis(3-ethoxy-4-hydroxyphenyl)hepta-1,7-diene-3,5-dione (**C2**) were tested as solutions in DMSO at a concentration of 1 mM. The DPPH assay shows that compound **5** has the highest activity among the fourteen synthesized conjugates and slightly higher antioxidant activity compared to the starting curcumin **C1**. When the antioxidant activity was followed by ABTS assay, compounds **1**, **5**, **8**, **12** exhibited higher activities compared to the other conjugates, **C1** and **C2**, respectively. (Fig. 1, Table S1-Supplementary information). These results follow the theory where the OH phenolic groups increase antioxidant activity. Based on the DPPH and ABTS values, the cytotoxicity of compounds **1**–**14** was determined in terms of a half maximal inhibitory concentration IC_{50}, (Table S2, Fig. S1-Supplementary information) calculated from the plotted graph of scavenging activity against the concentration of tested compounds.

The synthetic curcuminoids are known as compounds which are capable of modulating cell response to oxidative stress and the drug resistance mechanisms.[10] While the antioxidant activity of several curcumin structures in normal human cells is protective, in tumor cells it might be beneficial in the inhibition of antioxidant enzymes. Using the Knoevenagel condensation, we synthesized antiproliferative derivatives which target the redox signaling through the antioxidant enzymes. Knoevenagel condensates **1**–**14** have the capacity to inhibit DLD-1 colon cancer cells growth; their cytotoxicity was quantified[a] using the half maximal inhibitory concentration (IC_{50}), which is below 10 μM for **1**, **2**, **3**, **4**, **6**, **7**, **8**, **10**, **12**, **13** and **14**. With three exceptions (**5**, **9** and **11**) the Knoevenagel condensates cytotoxicity was higher than the standard platinum-based drug oxaliplatin. Compounds **5**, **9** and **11** displayed IC_{50} values under 100 μM; however, they are significantly higher in the whole series (Table 1).

The proliferation of the cells was measured quantitatively with fluorescence methods[b] at 4 h, 24 h, and 48 h, by exposing the DLD-1 cells to synthesized compounds **1**–**14**. As reference, we used the untreated cells which exhibit a high proliferation rate, as expected. (Table 1) The statistical significance of the changes in cell proliferation was quantified using the Hill slope deviation from 0, in the 95% confidence interval. Condensates **1**–**14** have the

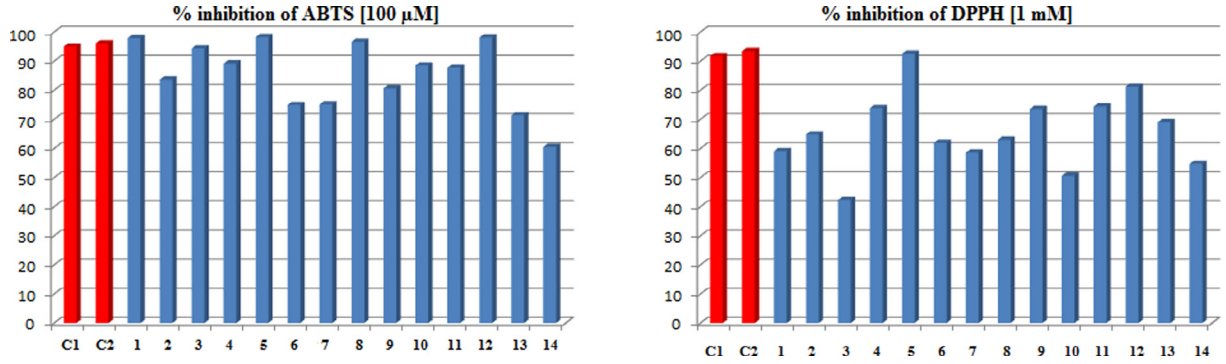

Fig. 1. Inhibition of ABTS at 100 μM and inhibition of DPPH at 1 mM for compounds **1**–**14** and standard arylheptanoids **C1** and **C2**.