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Diarylheptanoids from the bark of black alder inhibit the growth of sensitive and multi-drug resistant non-small cell lung carcinoma cells

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ABSTRACT

An extended study of minor diarylheptanoids from the bark of black alder has resulted in the isolation of twenty diarylheptanoids, ten of which have not previously been reported (**14**–**18**, **20**–**24**). The structures and configurations of all compounds were elucidated by NMR, HRESIMS, UV, IR, and CD. The anti-cancer potency of twenty diarylheptanoids and four previously isolated compounds (**7**, **10**, **12**, **13**) was investigated in human non-small cell lung carcinoma cell lines (sensitive and multi-drug resistant variants) as well as in normal human keratinocytes. Diarylheptanoids with a *p*-coumaroyl group, **14** and **18**, platyphylloside (**1**), platyphyllonol-5-*O*-*β*-D-xylopyranoside (**2**), alnuside B (**4**) and hirsutenone (**9**) exhibited strong anti-cancer activity, considerably higher than diarylheptanoid curcumin, which served as a positive control. Compounds **4**, **9**, **14**, and **18** displayed significant selectivity towards the cancer cells. Structure/activity analysis of twenty-four closely related diarylheptanoids revealed a high dependence of cytotoxic action on the presence of a carbonyl group at C-3. Substitution of a heptane chain on C-5 and a number of hydroxyl groups in the aromatic rings also emerged as a significant structural feature that influenced their cytotoxic potential.

Introduction

Diarylheptanoids are naturally occurring phenolic compounds consisting of two aromatic rings joined by a heptane chain. There are acyclic and cyclic diarylheptanoids, of which the latter occur less frequently. They are derived from two C6-C3 blocks that are connected with one more carbon originating from malonyl CoA (Brand et al., 2006; Munde et al., 2012). These compounds are typical secondary metabolites in the genus *Alnus* Mill., and can also be found in other genera, such as *Zingiber*, *Curcuma*, *Alpinia* and *Betula*. The best known diarylheptanoid is curcumin which was isolated for the first time in the 19th century from *Curcuma longa* (Zingiberaceae). Curcumin possesses a wide range of biological activities (Aggarwal and Harikumar, 2009; Gupta et al., 2012). Other diarylheptanoids exhibit antioxidative (Matsuda et al., 1998; Tung et al., 2010a), anti-inflammatory (Lai et al., 2012), antiviral (Tung et al., 2010b), cytotoxic (Choi et al., 2008; Liu et al., 2008; Mshvildadze et al., 2007; Sun et al., 2008) and anti-cancer activities (Choi et al., 2008; Mshvildadze et al., 2007).

Only a few papers describe the secondary metabolites in the seeds and bark of *Alnus glutinosa* (Kumarasamy et al., 2006; Novaković et al., 2013; O'Rourke et al., 2005). In the previous study the chemoprotective activity of the most abundant diarylheptanoids from the bark of *A. glutinosa* (L.) Gaertn. in human lymphocytes DNA was examined (Novaković et al., 2013).

Herein we report on the isolation of twenty diarylheptanoids from the bark of black alder, and the structural elucidation of ten new compounds (**14**–**18**, **20**–**24**) which are present in small amounts. The anti-cancer potency in human non-small cell lung carcinoma (NSCLC) cell lines (sensitive and multi-drug resistant (MDR) variants) and the structure/activity relationship of twenty-four closely related diarylheptanoids (**1**–**24**) are described.

Results and discussion

From the chloroform/methanol (1:1) extract of the bark of *A. glutinosa*, twenty diarylheptanoids were isolated by a combination of silica gel column chromatography and semipreparative reversed phase HPLC. Ten of them are new compounds. All of the

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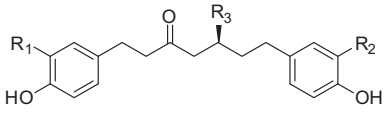
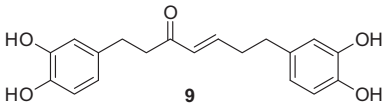
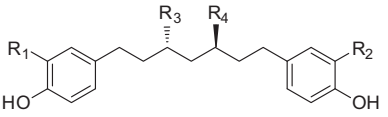
diarylheptanoids were identified according to their NMR (^{1}H, ^{13}C, HSQC, HMBC), UV, IR, CD, and mass spectra, and by a comparison with previously reported spectroscopic data. Following diarylheptanoids: platyphylloside (**1**) (Smite et al., 1993), platyphyllonol-5-*O*-*β*-D-xylopyranoside (**2**) (Chen et al., 2000), alnuside A (**3**), alnuside B (**4**), 5(*S*)-methylhirsutanonol (**6**), oregonin (**8**) (Kuroyanagi et al., 2005), hirsutanonol (**5**) (Gonzalez-Laredo et al., 1998), hirsutenone (**9**) (Ohta et al., 1984), 3(*R*)-1,7-di(3,4-dihydroxyphenyl)-3-hydroxyheptane (**11**), and 5(*S*)-1,7-di(3,4-dihydroxyphenyl)-5-O-*β*-D-[6-(*E*-3,4-dimethoxycinnamoylglucopyranosyl)] heptane-3-one (**19**) (Novaković et al., 2013) are known plant metabolites. Compounds: 5(*S*)-1,7-di(4-hydroxyphenyl)-5-O-*β*-D-[6-(*E*-*p*-coumaroylglucopyranosyl)]heptane-3-one (**14**), 5(*S*)-1,7-di (4-hydroxyphenyl)-5-O-*β*-D-[6-(*E*-3,4-dimethoxycinnamoylglucopyranosyl)]heptane-3-one (**15**), 5(*S*)-1-(4-hydroxyphenyl)-7-(3,4-dihydroxyphenyl)-5-O-*β*-D-[6-(*E*-3,4-dimethoxycinnamoylglucopyranosyl)]heptane-3-one (**16**), 5(*S*)-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)-5-O-*β*-D-[6-(*Z*-3,4-dimethoxycinnamoylglucopyranosyl)]heptane-3-one (**17**), 5(*S*)-1,7-di(3,4-dihydroxyphenyl)-5-O-*β*-D-[6-(*E*-*p*-coumaroylglucopyranosyl)]heptane-3-one (**18**), 5(*S*)-1,7-di(3,4-dihydroxyphenyl)-5-O-*β*-D-[6-(*Z*-3,4-dimethoxycinnamoylglucopyranosyl)]heptane-3-one (**20**), 5(*S*)-1,7-di(3,4-dihydroxyphenyl)-5-O-*β*-D-[6-(*E*-3,4,5-trimethoxycinnamoylglucopyranosyl)]heptane-3-one (**21**), 3(*R*)-1,7-di(3,4-dihydroxyphenyl)-3-O-*β*-D-[6-(*E*-3,4-dimethoxycinnamoylglucopyranosyl)]heptane (**22**), 3(*R*)-1,7-di(3,4-dihydroxyphenyl)-5-O-*β*-D-[6-(*Z*-3,4-dimethoxycinnamoylglucopyranosyl)]heptane (**23**) and 3(*R*)-1,7-di(3,4-dihydroxyphenyl)-5-O-*β*-D-[6-(*E*-3,4,5-trimethoxycinnamoy lglucopyranosyl)]heptane (**24**) are new. Their 1D and 2D NMR and HRESIMS spectra are provided in the Supporting information (Figs. S1–S71). Compounds 5(*S*)-hirsutanonol-5-*O*-*β*-D-glucopyranoside (**7**) (Ohta et al., 1984), aceroside VII (**10**) (Lee et al., 2006), rubranoside A (**12**) (Lee et al., 1998) and 1,7-di(3,4-dihydroxyphenyl)-5-hydroxy-heptane-3-*O*-*β*-D-xylopyranoside (**13**) (Kuroyanagi et al., 2005) are diarylheptanoids previously isolated from black alder (Novaković et al., 2013), and were included in the investigation of the biological activity in order to evaluate a more complete structure/activity relationship.



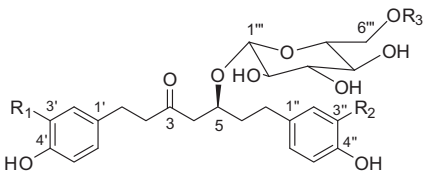
1: R_1=R_2=H; R_3=OGlc
2: R_1=R_2=H; R_3=OXyl
3: R_1=H; R_2=OH; R_3=OXyl
4: R_1=OH; R_2=H; R_3=OXyl
5: R_1=R_2=R_3=OH
6: R_1=R_2=OH; R_3=OMe
7: R_1=R_2=OH; R_3=OGlc
8: R_1=R_2=OH; R_3=OXyl

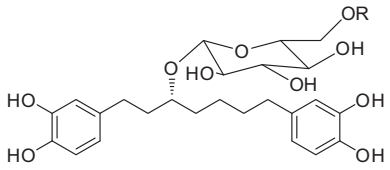


9



10: R_1=R_2=H; R_3=OGlc; R_4=H
11: R_1=R_2=OH; R_3=OH; R_4=H
12: R_1=R_2=OH; R_3=OGlc; R_4=H
13: R_1=R_2=R_3=OH; R_4=OXyl

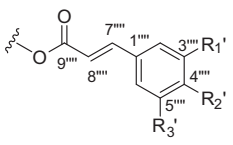




14: R_1=R_2=H; R_3=(*E*)-*p*C
15: R_1=R_2=H; R_3=(*E*)-DMC
16: R_1=H; R_2=OH; R_3=(*E*)-DMC
17: R_1=OH; R_2=H; R_3=(*Z*)-DMC
18: R_1=R_2=OH; R_3=(*E*)-*p*C
19: R_1=R_2=OH; R_3=(*E*)-DMC
20: R_1=R_2=OH; R_3=(*Z*)-DMC
21: R_1=R_2=OH; R_3=(*E*)-TMC

22: R=(*E*)-DMC
23: R=(*Z*)-DMC
24: R=(*E*)-TMC

(*E*)-*p*C: R_1'=R_3'=H; R_2'=OH
(*E*)-DMC: R_1'=R_2'=OCH_3; R_3'=H
(*E*)-TMC: R_1'=R_2'=R_3'=OCH_3



(*Z*)-DMCA: