Molecular Diversity (2020) 24:253–263
https://doi.org/10.1007/s11030-019-09948-1

ORIGINAL ARTICLE

Adamantyl pyran-4-one derivatives and their in vitro antiproliferative activity

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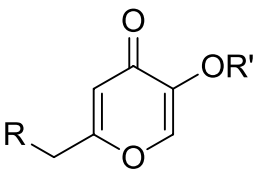
Received: 6 February 2019 / Accepted: 27 March 2019 / Published online: 5 April 2019

Abstract

Pyran-4-one (maltol, kojic acid and chlorokojic acid **1**) esters of adamantan-1-ylacetic acid were prepared through efficient synthetic routes in good yields and evaluated for their in vitro antiproliferative activity on four cancer cell lines: K562 (chronic myelogenous leukemia), HeLa (cervical cancer), Caco-2 (colorectal adenocarcinoma) and NCI-H358 (bronchioalveolar carcinoma). The results indicate that the presence and the position of the adamantyl acyl group or chlorine atom are the necessary requirement for antitumor activity of pyranone systems. Derivatives of kojic acid with either free (compounds **1** and **8**) or acylated 5-OH group (compounds **2** and **9**) have shown good-to-moderate activity (IC_{50} values ranging from 13.1 to 43.0 µM) on all cell lines. Adamantyl kojic acid derivative **5** with a free OH group on the position 2 showed activity only on the K562 cell line. It seems that removal of halogen or adamantyl unit from position 2 elicits antileukemic activity, as observed in compound **5**. The positive influence of the adamantyl unit was also observed on a 3-OH acylated derivative of maltol **I** which was also selectively active on the same cell line. 5-*O*-benzylated adamantyl compounds **6** and **7** and unmodified starting pyranones were found to be inactive. Antibacterial activity of compounds was also evaluated on *S. aureus* ATCC 13709, *M. catarrhalis* ATCC 23246, *E. faecalis* ATCC29212 and *E. coli* TolC-Tn10, but no activity was observed (MIC values 128–256 µg/mL).

Graphical abstract



Ad = adamantan-1-yl

Comp.	R	R'	K562 IC_{50} [µM]
2	Cl	$C(O)CH_2Ad$	13.1±1
5	OH	$C(O)CH_2Ad$	29.1±1.9
8	$OC(O)CH_2Ad$	H	16.3±2.7
9	$OC(O)CH_2Ad$	$C(O)CH_2Ad$	24.3±0.3

Keywords Kojic acid · Maltol · Adamantyl · Antiproliferation · Cancer

Introduction

Kojic acid (5-hydroxy-2-(hydroxymethyl)-4*H*-pyran-4-one, Fig. 1), a chelating compound and a common fungal metabolite isolated first from fungus *Aspergillus oryzae* in 1907, is known to inhibit the catecholase activity of tyrosinase, the rate-limiting essential enzyme in the biosynthesis of the skin pigment melanin [1]. Therefore, it is widely used primarily in the preparation of cosmetic biomaterials and skin

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11030-019-09948-1) contains supplementary material, which is available to authorized users.

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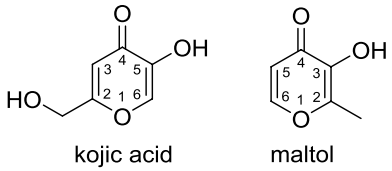


Fig. 1 Structural formula of the two naturally occurring pyran-4-one compounds—kojic acid and maltol

care products. Numerous studies also showed that kojic acid and its derivatives exhibit other various biological activities, such as antioxidant [2], antibacterial [1, 3, 4], antitumor [1, 5–8] anticonvulsant [9] and anti-inflammatory activity [10]. Various kojic acid derivatives were evaluated for their in vitro antileukemic activity on L1210 murine leukemia and K562 human leukemia cell lines [5] and displayed IC_{50} concentrations in low micromolar range.

Maltol (3-hydroxy-2-methyl-4*H*-pyran-4-one, Fig. 1) is a naturally occurring compound widely used as a flavoring agent. Similarly like kojic acid, maltol exhibits many practical applications primarily in food, cosmetic and pharmaceutical industry. Since it is also a metal iron chelator, it is often used in drugs such as vanadylmaltolate for the treatment of diabetes, and ferric trimaltol for the treatment of iron deficiency anemia [11]. It has been reported that maltol has a neuroprotective effect through its antioxidant properties and antiapoptotic effects in human neuroblastoma cells [11]. Furthermore, maltol suppressed the tumor growth of H22 transplanted tumor in vivo by improving the immune functions, inducing apoptosis and inhibiting angiogenesis [12].

Both maltol and kojic acid are often used as cheap starting material in the synthesis of many other biologically active compounds such as chlorokojic acid, allomaltol, pyromeconic acid and various pyridinone derivatives. They contain a promising polyfunctional heterocyclic skeleton with several important centers enabling additional reactions like oxidation, reduction, alkylation, acylation, nucleophilic and electrophilic substitution, Michael addition with a ring opening and chelation [13]. Thus, various moieties were introduced to their structures in an effort to improve their wide range of biological activities [13, 14].

A non-natural adamantane structure is often used to modulate physiological response of many biologically active compounds and drugs primarily by changing absorption, distribution, metabolism or excretion properties of a molecule. Adamantyl group can also bind to a hydrophobic site in an enzyme and to act as an inhibitor or affect ion channels by disrupting the transmembrane flow [15, 16]. We have already shown that the presence and adequate position of the adamantane moiety on the pyridinone skeleton in the adamantylated series of *N*-aryl-substituted pyridin-4-ones influence greatly their antitumor in vitro activities on several cancer cell lines [17] where four derivatives were singled out as the most potent compounds (IC_{50} values ranging from 0.3 to 7.0 μM). The starting material for the preparation of pyridinones is usually pyranone precursors, such as commercially available maltol and kojic acid. Adamantyl derivatives of maltol have not been yet reported to the best of our knowledge, but adamantyl derivatives of kojic acid have been mentioned in this decade as potential inhibitors of copper-containing enzyme, tyrosinase. Many inhibitors of tyrosinase have been synthesized and used as depigmenting agents for the treatment of hyperpigmentation and as antibrowning agents in vegetables and fruits. As already mentioned, kojic acid is a well-known tyrosinase inhibitor, but its inhibitory activity is not potent enough for the above purposes. Therefore, several kojic acid ester and thioether derivatives have been synthesized, usually by modification of the C-2 hydroxyl group for that purpose [10]. Some of the prepared derivatives also contained adamantyl moiety either directly connected to kojic acid through ester or thioether bond [18] or polyphenol units containing adamantane moieties were coupled with kojic acid [10]. Unfortunately, in the mushroom tyrosinase assay, adamantyl structural motifs were not responsible for inhibitory activity of compounds. The thioether linkage and flexible, simple alkyl and cycloalkyl groups appeared to be critical factors.

In view of our previous findings on the pyridinone derivatives [17] as well as our continuous investigation of their biological activity [19, 20], we wanted to extend our research and further explore the influence of the adamantyl moiety on the biological activities of pyranone systems as well.

Result and discussion

As pointed out, the presence and adequate position of the adamantyl moiety connected through the ester and/or amide bond on the pyridinone skeleton of *N*-aryl-substituted pyridin-4-ones influenced their antitumor in vitro activities [17]. Diadamantyl derivatives and their monoadamantyl analogues with the free iron chelation site were compounds with the highest activity on all investigated cancer cell lines (Fig. 2).

Thus, analogous pyran-4-one esters of adamantan-1-ylacetic acid needed for this study were prepared in several reaction steps starting from the commercially available pyranones, maltol and kojic acid. The key esterification step used for the preparation of monoadamantyl derivatives **I**, **2**, **4**, **7** and diadamantyl compound **9** was performed under Steglich conditions, using EDC × HCl/DMAP combination of reagents, in moderate-to-good yields (60–80%, Schemes 1 and 2). Chlorokojic derivative **1** was obtained in a very good yield (85%) from kojic acid. Out of many procedures known in the literature [9, 14, 18, 21–23] for the preparation of **1**,

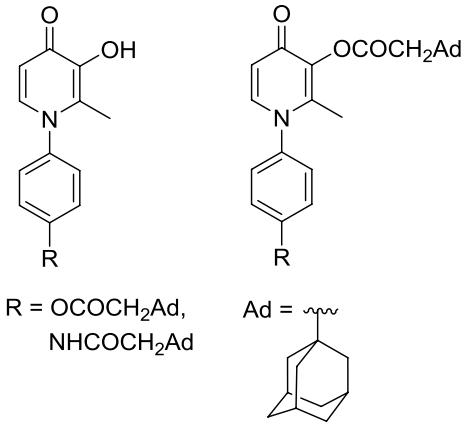
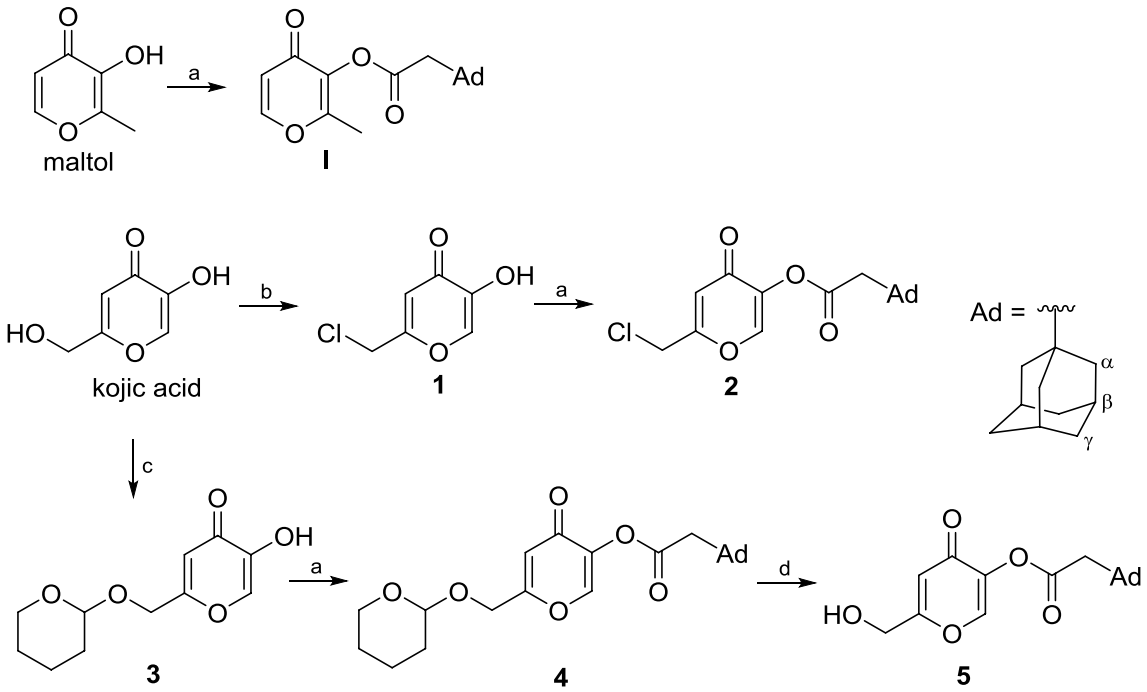


Fig. 2 Four most potent *N*-aryl-substituted pyridin-4-one derivatives with IC_{50} concentrations from 0.3 to 7.0 μM [17]

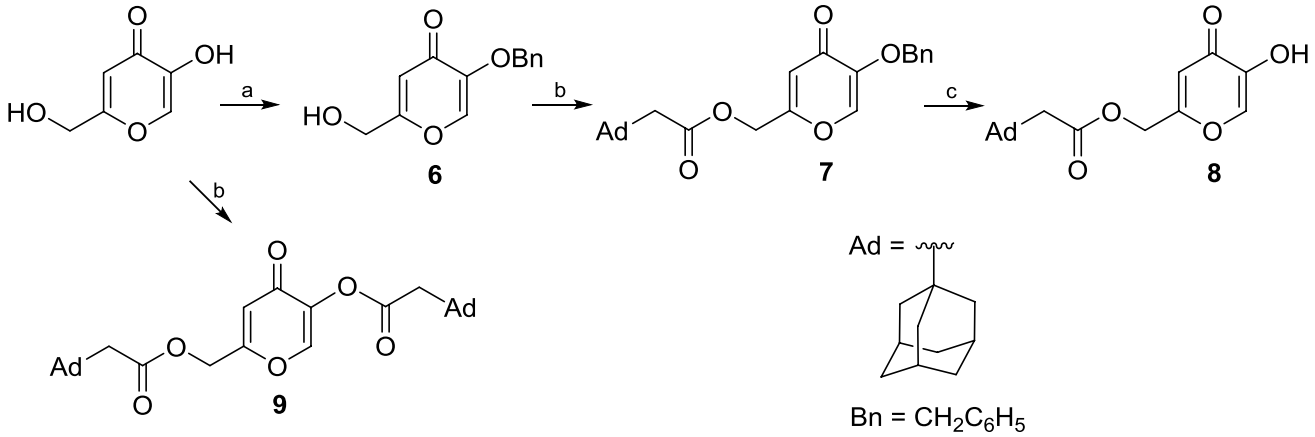
the ones which can be performed without the addition of organic solvent and in good yields were chosen [21–23]. In order to selectively prepare the monoadamantyl kojic acid derivatives **5** and **8**, with the acyl unit on the position 5 or 2 of the pyranone ring, respectively, protecting groups were used. Tetrahydropyranyl ether group was chosen for the selective protection of the primary hydroxyl group of the starting kojic acid, and it was introduced or removed accordingly using known and effective procedures [24–26]. Thus, 2-tetrahydropyranyloxymethyl derivative **3** was obtained with DHP and *p*-TsOH × H_2O as reagents, in moderate yield (47%), and protection was subsequently removed using *p*-TsOH × H_2O in order to prepare adamantyl derivative **5** (35%).

Benzyl protection was selectively introduced on the position 5 using classical Williamson ether synthesis, and 5-benzyloxy derivative **6** (84%, Scheme 2) was obtained from kojic acid and benzyl chloride (1.1 equiv.) in basic conditions [26]. The protection was removed after Steglich esterification step using a known dealkylating agent, strong Lewis acid boron tribromide. The reaction conditions in this step required careful tuning since used in excess or used in prolonged reaction times this reagent removes the acyl group as well. The hydrogenolysis procedure was also tested for the removal of the benzyl ether group in **7**, but the reaction resulted in overall hydrogenation of the whole conjugated pyranone system even in mild conditions and short reaction times (3.45×10^4 Pa, 10% Pd/C, 10 min).

Mannich bases of substituted piperazine with kojic and chlorokojic acid have been described as antimicrobial agents with significant activity against Gram-positive and Gram-negative bacteria [4, 21, 23]. There are several published data for the in vitro antibacterial activity on Gram-positive and Gram-negative strains for natural pyranone products, kojic acid and maltol [1, 3, 13, 27]. Since maltol is a known food flavoring agent, an extensive screening for its antifungal and antibacterial properties was already done and



Scheme 1 Reagents and conditions: (a) (i) $AdCH_2COOH$, DMAP, EDC × HCl, dry CH_2Cl_2 (for **I** and **4**) or mixture of dry solvents CH_2Cl_2/dioxane (for **2**), 0 °C, 30 min, (ii) rt, 24 h, 77% (**I**), 66% (**2**), 80% (**4**); (b) $SOCl_2$, Ar, 3 h, 85% (**1**); (c) DHP, *p*-TsOH × H_2O, dry CH_2Cl_2, 1 h, 47% (**3**); (d) *p*-TsOH × H_2O, CH_3OH, 0 °C, 1 h, 35% (**5**)



Scheme 2 Reagents and conditions: (a) BnCl, 11 M NaOH, CH_3OH, reflux, 24 h, 84% (**6**); (b) (i) $AdCH_2COOH$, DMAP, EDC×HCl, dry CH_2Cl_2, 0 °C, 30 min, (ii) rt, 24 h, 60% (**7**), 62% (**9**); (c) BBr_3, dry CH_2Cl_2, 75 min, 70% (**8**)

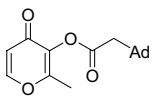
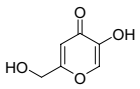
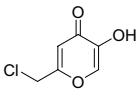
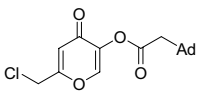
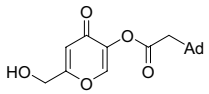
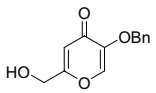
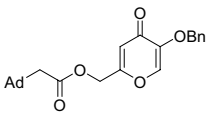
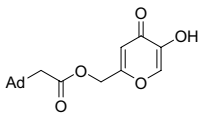
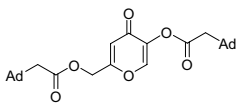
published in the literature in order to evaluate its toxicity for safe application in food industry. Similar screening was also done for kojic acid since this natural product is used as skin-whitening agent in cosmetics. Thus, other derivatives used in this study, adamantyl (**I**, **2**, **5**, **8**, **9**) and benzyl pyranone derivatives (**6** and **7**) as well as chlorokojic acid **1**, were investigated on two Gram-positive (*S. aureus* ATCC 13709 and *E. faecalis* ATCC29212) and two Gram-negative (*M. catarrhalis* ATCC 23246 and *E. coli* TolC-Tn10) strains for their antibacterial activity with azythromycin as referenced antibacterial agent. The weak activity of **1** was the only one observed (MIC values of 128 µg/mL on *E. faecalis* ATCC29212, *M. catarrhalis* ATCC 23246 and *E. coli* TolC-Tn10) the result which was comparable to that already reported in the literature for this compound on some of these strains [28].

Prepared pyran-4-one esters of adamantan-1-ylacetic acid were next evaluated for their in vitro antiproliferative activity (Table 1) on four cancer cell lines: K562 (chronic myelogenous leukemia, CML), HeLa (cervical cancer), Caco-2 (colorectal adenocarcinoma), NCI-H358 (bronchioalveolar carcinoma), as well as on normal cells (MDCK). The Lipinski rules serve as guidelines during drug discovery when a pharmacologically active structure is optimized stepwise to increase the activity and selectivity of the compound in order to maintain drug-like physicochemical properties. Ideally, according to those guidelines, a drug-like molecule should have logarithmic values of the partition coefficient (log *P*) from −0.4 to 5 to bypass the cell barrier with molecular weight between 180 and 500 [29]. There are several programs available today for the evaluation of the log *P* value of the compounds including ALOGPS 2.1 software which was used to assess the derivatives in this study [30].

Several attempts to improve the anticancer potential of kojic acid by introducing various chemical moieties to the compound have been already attempted by other researchers [5–8]. Bransova et al. [5] examined a number of halogenated (chloro, bromo and iodo), acylated (acetyl) and/or ether (methyl and benzyl) kojic acid derivatives for their antileukemic activity on L1210 murine leukemia cell growth. The most potent compounds were also evaluated on human leukemia K562 cell line, and the results were comparable. In general, the halogenated derivatives of kojic acid inhibited cell growth (IC_{50} values from 3.2 to 20.0 µM on L1210 murine leukemia cells). Kojic acid showed no effect on L1210 cell growth. Ether (benzyl or methyl group on the position 5) and acetyl derivatives of kojic acid in general showed no activity except for the 5-benzyloxy-2-chloromethylpyran-4-one which displayed IC_{50} value of 5.0 µM on L1210 murine leukemia cells. All results indicated the positive influence of halogen atom on the antileukemic activity of inspected derivatives.

We have chosen the K562 tumor cell line in this study so we could compare our results with the published data and furthermore compare the results obtained in our parallel ongoing structure–activity relationship study on pyridinone derivatives. In order to investigate the cell selectivity of prepared compounds, several other tumor lines were employed as well as a non-cancerous cell line. As expected, non-modified kojic acid showed no activity on all cell lines. Maltol, a new evaluated pyran-4-on skeleton, also showed no activity. On the other hand, kojic acid derivatives with either free (compounds **1** and **8**) or acylated 5-OH group (compounds **2**, **5** and **9**) showed good-to-moderate in vitro antiproliferative activity with IC_{50} ranging from 13.1 to 43.0 µM. Positive influence of the acyl (adamantan-1-ylacetyl) unit was also observed on 3-OH acylated derivative of maltol **I** which was selectively active only on the K562 cancer cell line (IC_{50} 33.9 ± 8.4 µM). Therefore, the presence of adamantyl group or chlorine atom is a prerequisite

Table 1 Compounds' calculated log *P* coefficients, molecular weight (MW) and IC_{50} values [μM] evaluated on four cancer cell lines (K562, chronic myelogenous leukemia; HeLa, cervical cancer; Caco-2, colorectal adenocarcinoma; and NCI-H358, bronchioalveolar carcinoma) and MDCK non-cancerous cell line as control

Compound	K562	HeLa	Caco-2	NCI-H358	MDCK	clog P^a	MW
 maltol	> 100	> 100	> 100	> 100	> 100	–0.24	126.11
 **1**	33.9 ± 8.4	> 100	> 100	> 100	> 100	3.58	302.36
 kojic acid	> 100	> 100	> 100	> 100	> 100	–1.02	142.11
 **1**	22.6 ± 5.1	34.9 ± 3.5	26.6 ± 2.8	36.1 ± 2.3	23 ± 2.7	0.28	160.56
 **2**	13.1 ± 1	32.2 ± 6.6	43 ± 5.4	29.7 ± 3.5	37.6 ± 2.3	3.95	336.81
 **5**	29.1 ± 1.9	> 100	> 100	> 100	> 100	2.93	318.36
 **6**	> 100	> 100	> 100	> 100	> 100	1.51	232.23
 **7**	70.5 ± 25.4	> 100	> 100	> 100	> 100	4.44	408.49
 **8**	16.3 ± 2.7	29.4 ± 4.8	18.4 ± 3.1	30.7 ± 4.1	20.4 ± 1.6	2.75	318.36
 **9**	24.3 ± 0.3	31.3 ± 4.5	40 ± 2.3	31.7 ± 5.6	33.8 ± 1.8	4.78	494.62

[a]Octanol–water partition coefficient as computed by ALOGPS 2.1 software [30]

for antitumor activity of pyranone systems since starting unmodified pyranones were found to be inactive. 5-*O*-benzylated adamantyl derivatives **6** and **7** showed complete inactivity on all cell lines similarly as 5-*O*-ether derivatives evaluated by Bransova et al. [5] which also indicates the importance of: (1) the position of the adamantan-1-ylacetyl group on the pyranone skeleton, (2) the type of acyl group incorporated since acetyl derivatives evaluated in previously published data [5] were found to be inactive and (3) the type of bond (ester *vs* ether) on the position 5 between the pyranone system and the incorporated moiety. Compound **5** with the adamantyl ester group on the position 5 was selectively active only on K562 tumor cell line (IC_{50} 29.1 ± 1.9 μM). It seems that removal of halogen and adamantyl unit from position 2 in structures **2** or **9** elicits selective activity, observed in compound **5**. The presence and the position of the adamantyl unit were also the important structural parameters that governed antiproliferative activity of previously investigated *N*-aryl-substituted pyridin-4-ones [17]. It should be also noted that the presence of the *N*-aryl part in such systems contributes additionally to their antiproliferative activities since their IC_{50} values on K562 cell line were lower compared to IC_{50} values of active compounds that are presented in this work and inspected on the same cell line. Nevertheless, selective antileukemic activity of compounds **I** and **5** which also possess good drug-like physicochemical properties makes them promising candidates for further structure optimization and fine-tuning which could result with even better biological response. Our primary objective, currently, is a 3D-QSAR study of all results obtained on pyridinone [17] and pyrone compounds which will, once finished, provide us with more precise guidelines in a design of even better leads.

Conclusion

In continuation of our ongoing research of *N*-aryl-substituted pyridinone derivatives, mostly in terms of their antiproliferative and antiadhesive activity, we have also investigated similar, naturally occurring pyranone systems which are usually used as a starting material in syntheses of mentioned pyridinone compounds. Thus, pyran-4-one esters of adamantan-1-ylacetic acid were successfully prepared using affordable and efficient methods in good yields and evaluated for their in vitro antiproliferative activity on four cancer cell lines (K562, HeLa, Caco-2 and NCI-H358) as well as on normal cells (MDCK). Compounds with either free (**1** and **8**) or acylated 5-OH group (**2** and **9**) showed good-to-moderate in vitro antiproliferative activity with IC_{50} ranging from 13.1 to 43.0 μM on all cell lines. Maltol derivative **I** and kojic acid derivative **5** were selectively active only on K562 tumor cell line. It seems that the presence and adequate position of the adamantyl acyl group or chlorine atom are a prerequisite for antitumor activity of pyranone systems. This is further substantiated by the complete inactivity of starting unmodified pyranones and ether 5-*O*-benzylated adamantyl derivatives **6** and **7**. The presence and the position of adamantyl acyl unit were also the important structural parameters that governed antiproliferative activity of our previously investigated *N*-aryl-substituted pyridin-4-one derivatives. Selective activity of compounds **I** and **5** which also possess satisfactory physicochemical properties according to Lipinski rules makes them promising candidates for further structure optimization in order to achieve better antileukemic response. Antibacterial activity of all pyranone adamantyl derivatives was evaluated on *S. aureus* ATCC 13709, *M. catarrhalis* ATCC 23246, *E. faecalis* ATCC29212 and *E. coli* TolC-Tn10, but compounds were found to be inactive.

Experimental section

General information

Reagents and solvents for the synthesis of compounds were obtained from commercial sources (mainly Sigma-Aldrich Corp., Taufkirchen, Germany, and Kemika, Zagreb, Croatia). When necessary solvents were further purified and/or dried using standard methods. Thin-layer chromatography (solvents and proportions are given in the text) was performed on Fluka silica gel (60 F 254) plates (0.25 mm), Steinheim, Germany. Visualization was achieved using UV light at 254 nm. Column chromatography (solvents and proportions are also given in the text) was performed on Merck silica gel 60 (70–230 mesh ASTM, Darmstadt, Germany). Melting points were determined in open capillaries using Büchi B-540 melting point apparatus (New Castle, DE, USA) and are uncorrected. ^{1}H and DEPTQ NMR spectra were recorded at room temperature on Bruker Avance III HD spectrometer at 400 and 100 MHz, respectively. Deuterated solvents are stated in the text. Chemical shifts (δ) are given in parts per million (ppm) downfield from tetramethylsilane as internal standard (s = singlet, br s = broad singlet, d = doublet, t = triplet and m = multiplet). Coupling constants (J) are quoted to the nearest 0.1 Hz. ESI–MS measurements in a positive ion mode were recorded on Agilent 6420 instrument (Palo Alto, CA, USA). High-resolution mass spectra (HRMS) were obtained by matrix-assisted laser desorption/ionization time-of-flight MALDI TOF/TOF mass spectrometer (4800 Plus MALDI TOF/TOF analyzer, Applied Biosystems Inc., Foster City, CA, USA) for all tested compounds.

Synthesis of 2-(chloromethyl)-5-hydroxy-4*H*-pyran-4-one (1) [21–23]

Kojic acid (0.500 g; 3.52 mmol) was placed in a round-bottom flask which was cooled in an ice bath, and freshly distilled $SOCl_2$ (7.5 equiv.) was slowly added next. Ice bath was removed, and the mixture placed under an argon atmosphere and stirred at room temperature for 3 h. The reaction was monitored by TLC (diethyl ether). Petroleum ether was added next, and the crude crystal residue was filtered off and washed thoroughly with the same solvent. The product was purified by column chromatography on silica gel (diethyl ether).

White solid (0.480 g, yield 85%), m.p. 165.5–166.5 °C, lit. [21–23] 166–167 °C.

^{1}H NMR (DMSO-d_6) δ/ppm: 4.65 (s, 2H, CH_2Cl), 6.57 (s, 1H, H-3), 8.12 (s, 1H, H-6), 9.30 (s, 1H, OH).

DEPTQ NMR (DMSO-d_6) δ/ppm: 41.20 (CH_2Cl), 113.19 (C-3), 140.10 (C-6), 145.97 (C-5), 161.64 (C-2), 173.68 (C=O).

ESI–MS: m/z 161.1 $[M+H]^+$.

HRMS Calcd for $C_6H_5ClO_3$ $[M+H]^+$: 161.0005. Found 160.9997.

Synthesis of 5-hydroxy-2-tetrahydropyranyloxymethyl-4*H*-pyran-4-one (3) [24, 26]

Kojic acid (0.500 g; 3.52 mmol) was suspended in dry CH_2Cl_2 (18 mL). 3,4-Dihydro-2*H*-pyran (DHP, 1.3 equiv.) and *p*-TsOH × H_2O (0.05 equiv.) were added next. Reaction mixture was stirred for 1 h at room temperature and monitored by TLC (ethyl acetate). It was extracted three times with 3% aqueous NaOH, and the combined aqueous layers were neutralized with 0.5 M NaH_2PO_4. After extraction with CH_2Cl_2, the organic layer was dried over anhydrous Na_2SO_4, filtrated and concentrated in vacuo.

White solid (0.378 g, yield 47%), m.p. 79.6 °C, lit. [26] 94.0 °C.

^{1}H NMR (DMSO-d_6) δ/ppm: 1.45–1.55 (m, 4H, 2 × CH_2, THP), 1.61–1.76 (m, 2H, CH_2, THP), 3.43–3.49 (m, 1H, CH_2O, THP), 3.70–3.76 (m, 1H, CH_2O, THP), 4.34 (d, 1H, J = 14.1 Hz, H_a, CH_aH_bO), 4.45 (d, 1H, J = 14.1 Hz, H_b, CH_aH_bO), 4.69 (t, 1H, J = 1.8 Hz, CH, THP), 6.40 (s, 1H, H-3), 8.06 (s, 1H, H-6), 9.15 (s, 1H, OH).

DEPTQ NMR (DMSO-d_6) δ/ppm: 18.68, 24.76, 29.79 (3 × CH_2, THP), 61.28 (CH_2O), 64.26 (CH_2O, THP), 97.74 (CH, THP), 111.66 (C-3), 139.61 (C-6), 145.82 (C-5), 164.07 (C-2), 173.70 (C=O).

ESI–MS m/z 249.2 $[M+Na]^+$.

Synthesis of 5-benzyloxy-2-hydroxymethyl-4*H*-pyran-4-one (6) [26]

Kojic acid (1 g, 7.04 mmol) was suspended in methanol (7 mL), and 11 M aqueous solution of sodium hydroxide was added next (1.05 mL, 11.55 mmol). To the resulting solution, benzyl chloride (1.34 mL, 11.70 mmol) was added dropwise and the solution was refluxed for 24 h and then cooled. The reaction was monitored by TLC (ethyl acetate/methanol, 5:1, *v*/*v*). After cooling, methanol was evaporated in vacuo and the crude product filtered off and washed excessively with combination of solvents diethyl ether/petroleum ether, 1:1, *v*/*v*. The product was finally purified by column chromatography on silica gel (ethyl acetate/methanol, 5:1, *v*/*v*).

Light yellow solid (1.38 g, yield 84%), m.p. 124.0–126.2 °C, lit. [26] 128–130 °C.

^{1}H NMR (DMSO-d_6) δ/ppm: 4.29 (d, 2H, J = 6.1 Hz, CH_2OH), 4.94 (s, 2H, CH_2, Bn), 5.68 (t, 1H, J = 6.1 Hz, OH), 6.32 (s, 1H, H-3), 7.35–7.42 (m, 5H, Ph), 8.17 (s, 1H, H-6).

DEPTQ NMR (DMSO-d_6) δ/ppm: 59.21 (CH_2OH), 70.49 (CH_2, Bn), 111.08 (C-3), 127.95, 128.02, 128.28 (5 CH, Ph), 136.06 (C, Ph), 141.21 (C-6), 146.48 (C-5), 167.88 (C-2), 173.08 (C=O).

ESI–MS: m/z 255.1 $[M+Na]^+$.

HRMS Calcd for $C_{13}H_{12}O_4$ $[M+H]^+$: 233.0814. Found 233.0810.

General procedure for the preparation of adamantyl pyran-4-one derivatives

An appropriate pyranone derivative (maltol, **1**, **3** or **6**, 0.200 g of each) was added to a solution of adamantan-1-ylacetic acid (1 equiv.) and DMAP (0.1 equiv.) in dry CH_2Cl_2 (6 mL for the preparation of **I**, **4** and **7**) or mixture of dry solvents CH_2Cl_2/dioxane (1:1, *v*/*v*, 6 mL for the preparation of **2**). The mixture was cooled down to 0 °C, and EDC × HCl (1.1 equiv.) was added next. The solution was stirred for 30 min at 0 °C and subsequently overnight at room temperature. The synthesis of diadamantyl derivative **9** from kojic acid (0.200 g) was carried out in an analogous manner using adamantan-1-ylacetic acid (2 equiv.), DMAP (0.2 equiv.) and EDC × HCl (2.2 equiv.) in dry CH_2Cl_2 (6 mL). All reactions were monitored by TLC (ethyl acetate/methanol, 5:1, *v*/*v*). Reaction mixture where combination of solvents was used was prior to extraction concentrated in vacuo. CH_2Cl_2 was added next, and the organic layer was washed twice with 0.2 M HCl, once with saturated aqueous $NaHCO_3$, once with water and finally dried over anhydrous Na_2SO_4. After filtration, the organic extract was concentrated in vacuo. The residues were purified by column

chromatography on silica gel (ethyl acetate/methanol, 5:1, *v*/*v*) giving the corresponding adamantyl derivatives **I**, **2**, **4**, **7** and **9**.

2-Methyl-4*H*-pyran-4-one-3-yl adamantan-1-ylacetate (I)

White solid (0.323 g, yield 77%), m.p. 107.0–108.3 °C.

^{1}H NMR (DMSO-d_6) δ/ppm: 1.62–1.69 (m, 12H, 6 Hα, 6 Hγ, Ad), 1.95 (br s, 3H, Hβ, Ad), 2.24 (s, 3H, CH_3), 2.31 (s, 2H, CH_2Ad), 6.40 (d, 1H, J = 5.8 Hz, H-5), 8.14 (d, 1H, J = 5.8 Hz, H-6).

DEPTQ NMR (DMSO-d_6) δ/ppm: 14.57 (CH_3), 27.87 (Cβ, Ad), 32.38 (C, Ad), 36.07 (Cγ, Ad), 41.34 (Cα, Ad), 47.18 (CH_2Ad), 115.78 (C-5), 137.65 (C-2), 155.70 (C-6), 158.84 (C-3), 167.49 (C=O, ester), 170.86 (C=O).

ESI–MS *m*/*z* 325.2 $[M+Na]^+$.

HRMS Calcd for $C_{18}H_{22}O_4$ $[M+Na]^+$: 325.1416. Found 325.1409.

2-(Chloromethyl)-4*H*-pyran-4-one-5-yl adamantan-1-ylacetate (2)

White solid (0.110 g, yield 66%), m.p. 155.5–157.0 °C.

^{1}H NMR (DMSO-d_6) δ/ppm: 1.61–1.67 (m, 12H, 6 Hα, 6 Hγ), 1.95 (br s, 3H, Hβ), 2.28 (s, 2H, CH_2Ad), 4.73, (s, 2H, CH_2Cl), 6.69 (s, 1H, H-3), 8.57 (s, 1H, H-6).

DEPTQ NMR (DMSO-d_6) δ/ppm: 27.90 (Cβ, Ad), 32.44 (C, Ad), 36.08 (Cγ, Ad), 40.89 (Cα, Ad), 41.31 (CH_2Cl), 47.18 (CH_2Ad), 115.45 (C-3), 140.32 (C-6), 149.68 (C-5), 162.96 (C-2), 167.78 (C=O, ester), 171.64 (C=O).

ESI–MS *m*/*z* 695.2 $[2M+Na]^+$.

HRMS Calcd for $C_{18}H_{21}ClO_4$ $[M+Na]^+$: 359.1026. Found 359.1019.

2-(Tetrahydropyranyloxymethyl)-4*H*-pyran-4-one-5-yl adamantan-1-ylacetate (4)

White solid (0.245 g, yield 80%), m.p. 102.5–103.8 °C.

^{1}H NMR (DMSO-d_6) δ/ppm: 1.47–1.57 (m, 4H, 2 × CH_2, THP), 1.61–1.70 (m, 14H, CH_2, THP; 6 Hα; 6 Hγ), 1.95 (br s, 3H, Hβ), 2.28 (s, 2H, CH_2Ad), 3.45–3.50 (m, 1H, CH_2, THP), 3.72–3.78 (m, 1H, CH_2, THP), 4.42 (d, 1H, J = 14.1 Hz, H_a, CH_aH_bO), 4.52 (d, 1H, J = 14.2 Hz, H_b, CH_aH_bO), 4.73 (t, 1H, J = 3.3 Hz, CH, THP), 6.51 (s, 1H, H-3), 8.49 (s, 1H, H-6).

DEPTQ NMR (DMSO-d_6) δ/ppm: 18.68, 24.74, 29.77 (3 CH_2, THP), 27.93 (Cβ, Ad), 32.43 (C, Ad), 36.09 (Cγ, Ad), 41.34 (Cα, Ad), 47.24 (CH_2Ad), 61.34 (CH_2O), 64.06 (CH_2O, THP), 97.89 (CH, THP), 111.88 (C-3), 140.29 (C-5), 149.24 (C-6), 165.04 (C-2), 167.84 (C=O, ester), 171.67 (C=O).

ESI–MS *m*/*z* 425.5 $[M+Na]^+$.

5-Benzyloxy-4*H*-pyran-4-one-2-ylmethyl adamantan-1-ylacetate (7)

White solid (0.211 g, yield 60%), m.p. 133.5–134.1 °C.

^{1}H NMR (DMSO-d_6) δ/ppm: 1.56–1.68 (m, 12H, 6 Hα, 6 Hγ, Ad), 1.92 (br s, 3H, Hβ, Ad), 2.14 (s, 2H, CH_2Ad), 4.95, 4.96 (2 × s, 4H, CH_2O and CH_2, Bn), 6.46 (s, 1H, H-3), 7.34–7.41 (m, 5H, Ph), 8.23 (s, 1H, H-6).

DEPTQ NMR (DMSO-d_6) δ/ppm: 27.81 (Cβ, Ad), 32.22 (C, Ad), 36.06 (Cγ, Ad), 41.50 (Cα, Ad), 47.50 (CH_2Ad), 60.74 (CH_2O), 70.49 (CH_2, Bn), 113.86 (C-3), 127.95, 128.07, 128,30 (5 CH, Ph), 135.91 (C, Ph), 141.48 (C-6), 146.74 (C-5), 161.59 (C-2), 169.91 (C=O, ester), 172.77 (C=O).

ESI–MS *m*/*z* 409.2 $[M+H]^+$.

HRMS Calcd for $C_{25}H_{28}O_5$ $[M+H]^+$: 409.2015. Found 409.2016.

5-Adamantan-1-ylacetyloxy-4*H*-pyran-4-one-2-ylmethyl adamantan-1-ylacetate (9)

White solid (0.362 g, yield 62%), m.p. 127.5–128.4 °C.

^{1}H NMR (DMSO-d_6) δ/ppm: δ 1.57–1.67 (m, 24H, Hγ, Hα, Ad), 1.94 (br s, 6H, Hβ, Ad), 2.16 (s, 2H, CH_2Ad), 2.27 (s, 2H, CH_2Ad), 5.00 (s, 2H, CH_2), 6.57 (s, 1H, H-3), 8.53 (s, 1H, H-6).

DEPTQ NMR (DMSO-d_6) δ/ppm: δ 28.41, 28.46 (Cβ, Ad), 32.85, 32.99 (C, Ad), 36.65 (Cγ, Ad), 41.88, 42.10 (Cα, Ad), 47.75, 48.08 (CH_2Ad), 61.28 (CH_2), 115.45 (C-3), 140.95 (C-5), 149.92 (C-6), 163.44, 168.31 (C=O, esters), 170.51 (C-2), 172.05 (C=O).

ESI–MS *m*/*z* 517.3 $[M+Na]^+$.

HRMS Calcd for $C_{30}H_{38}O_6$ $[M+H]^+$: 495.2747. Found 495.2741.

Synthesis of 2-hydroxymethyl-4*H*-pyran-4-one-5-yl adamantan-1-ylacetate (5)

Compound **4** (0.650 g, 0.50 mmol) was dissolved in dry methanol (20 mL), and the mixture was cooled in an ice bath to 0 °C.

p-TsOH × H_2O (1 equiv.) was added next, and reaction mixture was stirred for 1 h and monitored by TLC (ethyl acetate). It was concentrated in vacuo, and cold distilled water was added next (2–3 mL). Crude product was filtered off and dried.

White solid (0.180 g, yield 35%), m.p. 130.0–131.5 °C.

^{1}H NMR ($CDCl_3$) δ/ppm: 1.65–1.71 (m, 12H, 6 Hα, 6 Hγ), 2.00 (br s, 3H, Hβ), 2.32 (s, 2H, CH_2Ad), 3.49 (t, 1H, J = 6.6 Hz, OH), 4.94, (d, 2H, J = 6.4 Hz, CH_2O), 6.53 (s, 1H, H-3), 7.48 (s, 1H, H-6).

DEPTQ NMR ($CDCl_3$) δ/ppm: 28.58 (Cβ, Ad), 33.09 (C, Ad), 36.62 (Cγ, Ad), 42.12 (Cα, Ad), 48.07 (CH_2Ad), 60.62

(CH_2O), 113.09 (C-3), 140.94 (C-5), 147.87 (C-6), 168.43 (C-2), 169.70 (C=O, ester), 173.22 (C=O).

ESI–MS *m*/*z* 319.2 $[M+H]^+$.

HRMS Calcd for $C_{18}H_{22}O_5$ $[M+H]^+$: 319.1545. Found 319.1540.

Synthesis of 5-hydroxy-4*H*-pyran-4-one-2-ylmethyl adamantan-1-ylacetate (8)

Compound **7** (0.200 g, 0.49 mmol) was dissolved in dry CH_2Cl_2 (20 mL), and the mixture was cooled in an ice bath to 0 °C. Boron tribromide was added next (BBr_3, 1 equiv. of 1 M solution in dry CH_2Cl_2). The reaction mixture was stirred for 75 min and monitored by TLC (ethyl acetate/methanol, 5:1, *v*/*v*). It was stopped by the addition of distilled water (20 mL), and the mixture was stirred vigorously for additional 15 min. The two layers were separated, and the aqueous layer extracted once with CH_2Cl_2. Combined organic extracts were dried over anhydrous Na_2SO_4. After filtration, the solvent was concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate/methanol, 5:1, *v*/*v*), and the compound **8** was obtained.

White solid (0.108 g, yield 70%), m.p. 120.5–121.9 °C.

^{1}H NMR ($CDCl_3$) *δ*/ppm: 1.61–1.73 (m, 12H, 6 Hα, 6 Hγ), 1.98 (br s, 3H, Hβ), 2.17 (s, 2H, CH_2Ad), 4.91, (s, 2H, CH_2O), 6.52 (s, 1H, H-3), 7.85 (s, 1H, H-6).

DEPTQ NMR ($CDCl_3$) *δ*/ppm: 28.50 (Cβ, Ad), 32.93 (C, Ad), 36.59 (Cγ, Ad), 42.32 (Cα, Ad), 48.37 (CH_2Ad), 60.95 (CH_2O), 111.15 (C-3), 137.80 (C-6), 145.88 (C-5), 163.07 (C-2), 170.53 (C=O, ester), 173.95 (C=O).

ESI–MS *m*/*z* 319 $[M+H]^+$.

HRMS Calcd for $C_{18}H_{22}O_5$ $[M+H]^+$: 319.1545. Found 319.1533.

Cell culturing and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test

The experiments were carried out on four human cell lines, which are derived from four cancer types and one non-cancerous cell line (MDCK epithelial). The used cell lines have been variously sourced and maintained in our laboratory. All cell lines have been confirmed by phenotyping according to ATCC guidelines prior to use. The following cell lines were used: K562 (chronic myelogenous leukemia, CML), HeLa (cervical cancer), Caco-2 (colorectal adenocarcinoma) and NCI-H358 (bronchioalveolar carcinoma). Adherent cells were cultured as monolayers and maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin in a humidified atmosphere with 5% CO_2 at 37 °C. Suspension cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 1 mM sodium pyruvate, 10 mM HEPES, 100 U/mL penicillin and 100 µg/mL streptomycin in a humidified atmosphere with 5% CO_2 at 37 °C. Trypan blue dye exclusion method was used to assess cell viability prior to testing.

The growth inhibition activity was assessed as described previously [31, 32]. The cell lines were inoculated onto a series of standard 96-well microtiter plates on day 0, at 2×10^4 cells/mL (CML, HeLa, Caco-2 and MDCK). Suspension cells (K562) were seeded at 1×10^5 cells/mL. Test agents were then added in tenfold dilutions (10^{-8} to 10^{-4} M) and incubated for further 72 h. The solvent (DMSO) concentration never exceeded 0.25%. Working dilutions were freshly prepared on the day of testing. After 72 h of incubation, the cell growth rate was evaluated by performing the MTT assay, which detects dehydrogenase activity in viable cells. After 72 h of incubation with the tested compounds, MTT (Merck, Germany) was added. DMSO (Merck, Germany) was used to dissolve the formed MTT-formazan crystals. Absorbance was measured at 595 nm using a plate reader (iMark, Bio-Rad, Hercules, CA, USA). All experiments were performed in triplicate. The absorbance is directly proportional to the number of living, metabolically active cells. The percentage of growth (PG) of the cell lines was calculated according to one or the other of the following two expressions: If (mean A_{test}–mean A_{tzero}) ≥ 0, then PG = 100 × (mean A_{test}–mean A_{tzero})/(mean A_{ctrl}–mean A_{tzero}) or if (mean A_{test}–mean A_{tzero}) < 0, then PG = 100 × (mean A_{test}–mean A_{tzero})/A_{tzero}, where the mean A_{tzero} is the average of optical density measurements before exposure of cells to the test compound, the mean A_{test} is the average of optical density measurements after the desired period of time, and the mean A_{ctrl} is the average of optical density measurements after the desired period of time with no exposure of cells to the test compound. The results are expressed as IC_{50}, which is the concentration necessary for 50% of inhibition. The IC_{50} values for each compound are calculated from concentration–response curves using linear regression analysis by fitting the test concentrations that give PG values above and below the reference value (i.e., 50%). If, however, for all of the tested concentrations produce PGs exceeding the respective reference level of effect (e.g., PG value of 50), then the highest tested concentration is assigned as the default value, which is preceded by a ">" sign. Each test was performed in quadruplicate in at least two individual experiments.

Acknowledgements We wish to thank kindly Dr Hana Čipčić Paljetak and Dr Donatella Verbanac from the School of Medicine, University of

Zagreb, for investigating the antibacterial activity of compounds. We would also like to thank the Croatian Science Foundation for support of this work (Project IP-2014-09-7899).

Compliance with ethical standards

Conflict of interest We wish to confirm that there are no conflicts of interest associated with this publication.

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