Synthesis and antiproliferative activity of new 2-glyco-3-nitro-2*H*-chromenes

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Abstract

3-nitro-2*H*-chromenes and derivatives are compounds with diverse biological activity, among them, new 2-glyco-3-nitro-2*H*-chromenes have been prepared by *one-pot oxa*-Michael-Henry-dehydration reactions between carbohydrate-derived nitroalkenes and several salicylaldehydes, using a minimal amount of solvent and DBU as catalyst. The antiproliferative activity of these new compounds has been evaluated against a panel of six human solid tumor cell lines, and compared to pharmacological reference compounds, finding that their activities are in the low micromolar range and that some of them are more effective than the standards.

Keywords: 3-nitro-2*H*-chromenes, antiproliferative activity, structure-activity relationship, carbohydrates.

Introduction

3-Nitro-2*H*-chromene **1** (Figure 1) and its derivatives constitute an important group of oxygen heterocycles, being present this moiety in the structures of many natural flavonoids and anthocyanins, as well as in some members of the vitamin E family (tocopherols and tocotrienes) [1]. These compounds represent a class of organic compounds whose pharmacological properties [1], such as antihypertensive [2], antifungal [3], antitumoral [4] and anticoagulant [5], have been widely studied. They are also activators of potassium channels [6] and inhibitors of hypoxia-inducible factors (HIF) [7]. On the other hand, these compounds, and their analogues, have found application, due to their photophysical behaviour, in a wide variety of fields including laser dyes, organic light emitting devices (OLED´s) and optical brighteners, in addition to exhibiting photochromic properties [8]. Thus, due to their high availability and reactivity [9], these compounds are promising starting materials for the preparation of complex bioactive molecules.

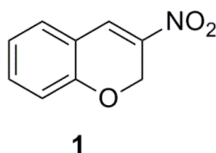


Figure 1. Structure of 3-nitro-2*H*-chromene **1**

Research on these compounds has considerably increased in recent years, although the first study on this type of heterocycles dates back to 1938. This increase in research is mainly due to the fact that new types of simple and ecological synthetic pathways have been described,

among which are those carried out in the absence of solvents, as the processes developed in this work, and which are part of what is known as "Green Chemistry". Another reason why 3-nitro-2*H*-chromenes are being studied is that these compounds are widely distributed in the plant kingdom and their activity as effective pesticides and pharmacophores has been recognised [10].

3-Nitro-2*H*-chromene derivatives have been recently identified as a new class of potent antitumor agents. Some of them show good inhibitory activity against mammal thioredoxin reductase (TrxRs) and inhibit the proliferation of A-549 (lung) cancer cells [11]. The skeleton of this molecule is the crucial pharmacophore to achieve good activity values, reporting Zhang et al. [12] that the halo-substitution at the 6-position of the chromene ring significantly decreases the GI_{50} values. In the same sense, Steinfelder et al. [13] studied which fragment of the molecule was necessary for exhibiting a pro-apoptotic effect, concluding that, in nitrostyrene derivatives, nitro group and the carbon-carbon double bond were the essential pro-apoptotic structural core, whereas the aromatic ring could be replaced by other aromatic heterocycles without affecting its activity.

Numerous synthetic pathways of 3-nitro-2*H*-chromenes derivatives have been described [14], being the most used the cascade reaction of salicylaldehydes or their corresponding imines with unsaturated nitrocompounds [15]. These preparation methods focus on asymmetric synthesis using chiral organocatalysts [16,17]. Conversely, to our knowledge, methods that introduce asymmetry using chiral reagents are very rare. On the other side, in spite of their numerous applications, there are few examples of 3-nitrochromenes containing carbohydrates moieties attached to this bicyclic system.

Many of the reactions in these procedures are catalyzed by a wide variety of bases in organic solvents, which can be toxic or harmful to the environment. In addition, these processes require very long reaction times and drastic conditions. For this reason, and due to the importance of this type of structures, we propose a general, rapid and easy methodology to prepare a wide variety of chiral derivatives of 3-nitro-2*H*-chromenes.

In this work we have employed 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as catalyst, together with a small amount of dichloromethane, to synthesize 3-nitro-*2H*-chromenes bearing an acyclic carbohydrate fragment in C2, whose inclusion, and because of their importance in diverse biological processes, is considered attractive in potential drug design. Consequently, the preparation of molecules containing carbohydrates moieties attached to the heterocyclic skeleton of chromene or any of its derivatives is a field of research of great interest. There are few examples of 3-nitro-2*H*-chromenes containing carbohydrate residues linked to this bicyclic system [18–20], although none of them being acyclic moieties. Hence, Tronchet et al. [19] have described that the cytotoxicity and chemotherapeutic properties of 3-nitro-2*H*-chromenes with carbohydrate chains bound to C-2 against *Polyoma* virus, *E. coli* and *B. subtilis* depend on the structure of these sugar chains. Finally, the antiproliferative activity of the new compounds against a panel of six human tumor cell lines has been evaluated.

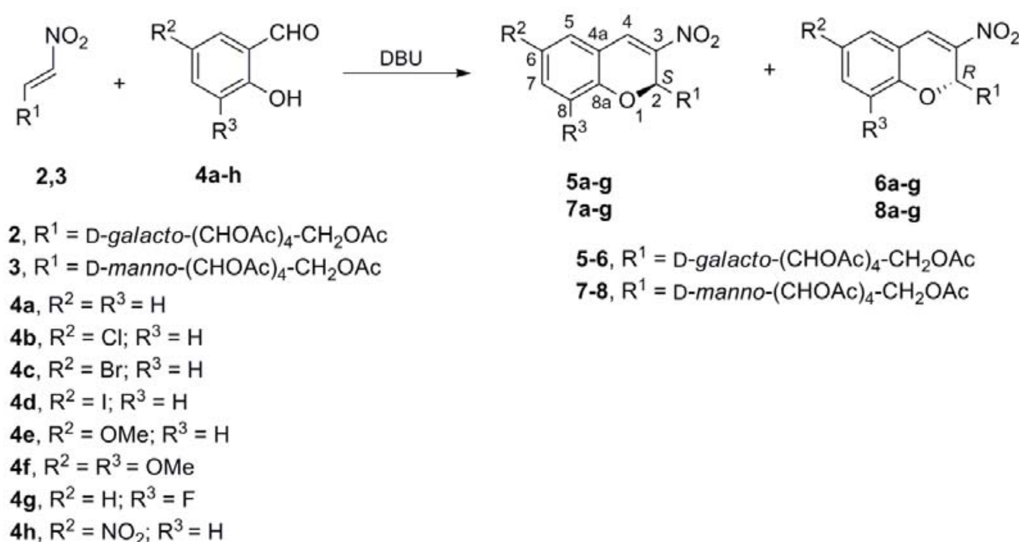
Results and Discussion

Chemistry

2-Glyco-3-nitro-2*H*-chromenes **5a-g** + **6a-g** and **7a-g** + **8a-g** were synthesized by *one-pot oxa*-Michael-Henry-dehydration reactions, at room temperature, between carbohydrate derived nitroalkenes **2**,**3** and salicylaldehydes with different substitution patterns (four halogenated, two donor an one acceptor group, **4a-h**), using DBU as catalyst and adding dichloromethane (3.12% mol) to homogenize the mixture (Scheme 1). Results are summarized in Table 1. Differences in diastereoselectivity of the processes could also be explained taking into account the different configurations of the first and the third chiral center of two carbohydrate moieties, used to evaluate their influence over the potential antiproliferative activity.

It is observed that the reaction times are shorter when more pronounced electron donor character of the substituents in the benzene ring, as this increases the nucleophilia of the hydroxyl group. For that reason, the shortest reaction time is for compound **4e**, since the methoxy group is strongly electron donor. Thus, the processes with the 5-halosubstituted salicylaldehydes **4b-d** are faster compared to the reaction carried out with unsubstituted salicylaldehyde **4a**. Finally, the reaction with 5-nitro-2-hydroxybenzaldehyde **4h** did not take place, probably due to the nitro group is a powerful electron withdrawing group.

The reactions between the substituted 3,5-dimethoxy salicylaldehyde **4f** and nitroolefins **2**,**3** did not occur, in spite of having two electron donor groups, possibly because of the steric hindrance caused by the methoxy group located at position 4 of the salicylaldehyde skeleton.



Scheme 1. Reaction between nitroalkenes **2,3** and salicylaldehydes **4a-h**.

Table 1. Reaction times, yields and products ratio for **5a-g** + **6a-g** and **7a-g** + **8a-g** products.

2					3				
R^2	R^3	t (min)	Yield (%)	Products (ratio) (*S*:*R*)	R^2	R^3	t (min)	Yield (%)	Products (ratio) (*R*:*S*)
H	H	90	32	1:0.6	H	H	30	31	1:0.25
OMe	H	50	55	1:0.75	OMe	H	30	75	1:0.3
Cl	H	75	63	1:0.62	Cl	H	120	60	1:0.88
Br	H	90	51	1:0.5	Br	H	90	56	1:0.35
I	H	70	50	1:0.63	I	H	80	37	1:0.73
H	F	90	—	1:0.27	H	F	90	—	0.76:1
OMe	OMe	--	--	--	OMe	OMe	--	--	--
NO_2	H	--	--	--	NO_2	H	--	--	--

Absolute configuration of C-2 in the 3-nitro-2*H*-chromene **5a** has been unambiguously determined by X-ray diffraction crystallography (Cambridge Crystallography Data Centre 1818887) (Figure 2). This has allowed us to confirm a correlation previously established by our group between the value of the coupling constant $J_{1',2}$ and the configuration at C-2 in structurally-related compounds bearing a peracetylated carbohydrate moiety, such as 3-nitro-1,2-dihydroquinolines [21] and 3-nitro-2*H*-thiochromenes [22]. Thus, for those carbohydrate chains-with D-*galacto*-configuration, $J_{1',2}$ is large (5-10 Hz) if C-2(*S*), and small (0-2 Hz) if C-2(*R*), and just the opposite for D-*manno*-configuration chains (Table 2). Configuration of C-2 of the rest of chromenes **5b-g** + **6a-g** and **7a-g** + **8a-g** was assigned based on this correlation.

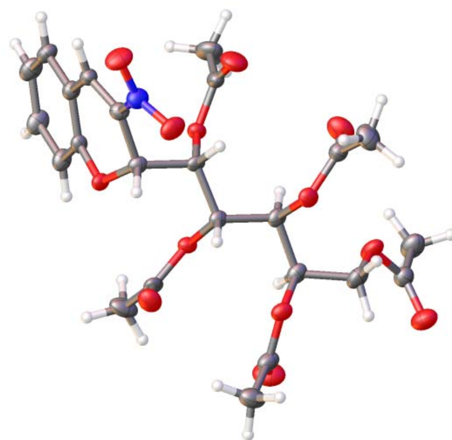
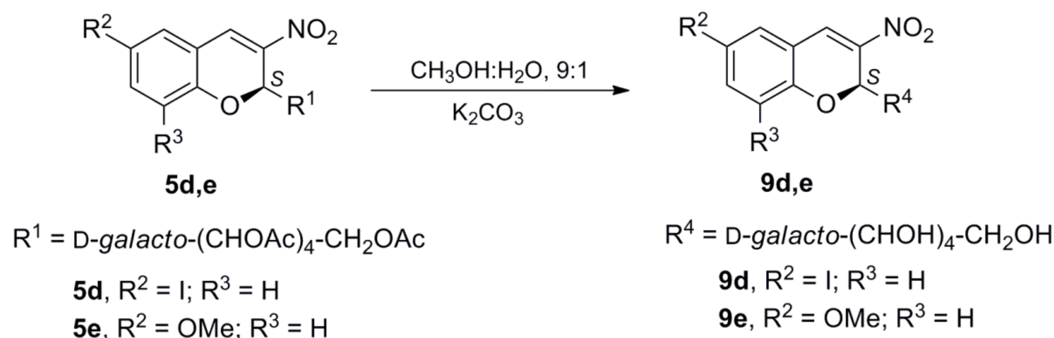


Figure 2. Thermal ellipsoids drawn at the 50% probability level for compound **5a**

Table 2 Correlation between C-2 configuration and value of $J_{1',2}$

2			3		
Compound	**$J_{1',2}$ (Hz)**	**C-2 configuration**	**Compound**	**$J_{1',2}$ (Hz)**	**C-2 configuration**
5a	9.0	*S*	**7a**	1.5	*S*
6a	<1.0	*R*	**8a**	7.0	*R*
5b	9.0	*S*	**7e**	1.5	*S*
5c	9.0	*S*	**7g**	1.2	*S*
5d	8.5	*S*	**8g**	8.0	*R*
5e	9.0	*S*			
5g	9.0	*S*			
6g	<1.0	*R*			

In order to test the antiproliferative activity of more soluble in aqueous media nitrochromenes than their precursors, the couple of deacetylated compounds **9d,e** were prepared by treatment of 3-nitro-2*H*-chromenes **5d**,**e** with potassium carbonate in methanol:water (9:1) for one hour (Scheme 2).



Scheme 2. Deacetylation of 3-nitro-2*H*-chromenes **5d,e**

Based on the quantitative structure-activity relationship studies carried out by González-Díaz et al. [23], from which it is concluded that more than 50% of the biological activity of the evaluated compounds lies in the *trans*-nitroolefin system, and confirmed by Steinfelder et al. [13] and our own group [21,22,24], structures of 3-nitro-2*H*-chromenes at positions 2, 6 and 8 were modified to evaluate how it affected the antiproliferative activity.

Antiproliferative activity

Antiproliferative activity of compounds **5a-g** + **6a-g** and **7a-g** + **8a-g** was evaluated against a panel of six human solid tumor cell lines: A549 (non-small cell lung), SW1573 (non-small cell lung), HBL-100 (breast), T-47D (breast), HeLa (cervix) and WiDr (colon) by using the protocol of the National Cancer Institute (NCI) of the United States [25], slightly modified [26]. GI_{50} values (concentration of compound required to inhibit a 50% of tumor cell growth) are depicted in Table 3. The standard anticancer drugs etoposide, cisplatin and 5-fluorouracil were used as positive controls.

C-6 substituted chromenes show, in general, better GI_{50} values than unsubstituted ones. It is observed that, in particular, the presence of a halogen atom in that position even improves the antiproliferative activity exhibited by the pharmacological standards used as reference (especially 5-fluorouracil). The low GI_{50} values of these compounds stand out against WiDr line,

considered as a multidrug resistant cell line (1.9, 1.7, 2.6 and 3.1 μM for **5b**, **5c**, **5d** and **8b**, respectively).

Table 3 Antiproliferative activity (GI_{50}) against human solid tumor cell lines.[a]

Compound	A549 (lung)	HBL-100 (breast)	HeLa (cervix)	SW1573 (lung)	T-47D (breast)	WiDr (colon)
			$GI_{50} \pm s_D$ (μM)			
5a	25 ± 9	39 ± 14	15 ± 6	35 ± 10	29 ± 8	32 ± 7
6a	9.2 ± 5.2	14 ± 5	14 ± 3	13 ± 3	16 ± 6	15 ± 4
5b	2.5 ± 0.9	2.9 ± 0.3	2.8 ± 0.4	2.4 ± 0.4	3.4 ± 0.2	1.9 ± 0.8
5c	3.0 ± 0.3	2.3 ± 0.1	2.8 ± 0.2	2.4 ± 0.3	3.5 ± 0.1	1.7 ± 0.4
5d	1.3 ± 0.4	2.0 ± 0.4	1.8 ± 0.7	1.0 ± 0.1	2.0 ± 0.5	2.6 ± 0.5
9d	63 ± 32	32 ± 3	28 ± 4	20 ± 1	43 ± 11	66 ± 30
5e	7.7 ± 2.4	11 ± 5	7.0 ± 0.6	4.3 ± 2.0	16 ± 3	21 ± 5
9e	>100	>100	>100	>100	>100	>100
5g	2.2 ± 0.9	3.4 ± 0.5	2.0 ± 1.0	2.0 ± 0.9	3.4 ± 1.0	2.3 ± 1.3
6g	39 ± 15	61 ± 16	42 ± 10	23 ± 4	>100	>100
5b+6b	2.2 ± 0.7	2.7 ± 0.5	2.4 ± 0.4	2.0 ± 0.8	3.2 ± 0.6	4.1 ± 0.9
5c+6c	1.5 ± 0.3	1.8 ± 0.2	1.5 ± 0.6	0.96 ± 0.06	2.3 ± 0.9	2.7 ± 0.9
5d+6d	1.5 ± 0.7	1.9 ± 0.5	1.5 ± 0.4	1.3 ± 0.6	2.1 ± 0.7	2.4 ± 0.8
7a	23 ± 5	27 ± 6	23 ± 4	23 ± 4	23 ± 7	28 ± 5
8a	32 ± 9	60 ± 28	26 ± 5	55 ± 16	32 ± 4	56 ± 13
8b	1.2 ± 0.2	2.0 ± 1.1	1.5 ± 0.2	1.2 ± 0.3	2.4 ± 0.7	3.1 ± 0.4
7e	2.4 ± 0.7	6.6 ± 3.1	2.7 ± 0.7	2.2 ± 0.3	5.2 ± 2.1	12 ± 2
7g	58 ± 34	>100	69 ± 8	44 ± 12	>100	>100
8g	22 ± 10	32 ± 4	24 ± 2	20 ± 3	18 ± 10	65 ± 21
Etoposide (VP-16)	0.7 ± 0.2	2.3 ± 0.9	3.0 ± 0.9	15 ± 2	22 ± 6	23 ± 2
5-fluorouracil	2.2 ± 0.3	5.5 ± 2.3	15 ± 5	4.3 ± 1.6	47 ± 18	49 ± 7
Cisplatin (CDDP)	2.1 ± 0.6	1.9 ± 0.2	2.0 ± 0.3	3.0 ± 0.4	15 ± 3	26 ± 6

[a]Values are mean of two to four experiments. n.t. = not tested

By other side, substitution with a methoxy group yields inconclusive results: the antiproliferative activity is better than the reference compounds for the HeLa, SW1573, T-47D and WiDr cell lines, but worse than for A549 and HBL-100. In the same way, 8-fluorosubstitution affects ambiguously, since only compound **5g** shows a comparable, and sometimes, better activity profile than the reference compounds for all cell lines evaluated, reaching the point that **6g** and **7g** are inactive against T-47D, WiDr and HBL-100. Lastly, it should also be noted that, for all cell lines, lower GI_{50} values have been found in the case of mixtures of 2*S* + 2*R* chromenes compared to pure 2*S* diastereoisomers, which could suggest a possible and mild synergistic effect.

From the data depicted in the Table 3, some conclusion can also be inferred about how the carbohydrate moiety affects antiproliferative activity. Thus, it is noteworthy the difference in GI_{50} values found in pairs **6a**-**8a** and **5g**-**7g**, being the latter inactive against three of the six cell lines tested (HBL-100, T-47D and WiDr). An important difference is also observed in the activity profiles of 3-nitrochromenes **5e** and **7e**, with GI_{50} values lower than the standards against HeLa, SW1573, T-47D and WiDr. Since chromenes with the same configuration in the new

chiral center generated, C-2, have been compared, these differences have to be exclusively attributed to the carbohydrate moiety.

In order to achieve chromenes soluble in aqueous media, compatible with the cellular tissue, deprotection of the carbohydrate moiety has been carried out, yielding the deacetylated derivatives **9d** and **9e**. Thus, it was observed that these compounds exhibited worse GI_{50} values than their acetylated precursors, being even **9e** inactive against all cell lines tested. These results are in line with those obtained for 3-nitro-1,2-dihydroquinolines with the same carbohydrate moiety, in which antiproliferative activity decreases after deacetylating. In this sense, it can be concluded that peracetylation of the carbohydrate chain likely to modulates the activity of these heterocycles.

It is also interesting to study how the carbohydrate fragment on C-2 affects the GI_{50} values of chromenes. Thus, for the A-549 cell line it is observed that the presence of D-*manno* configuration carbohydrate moiety on C-2 in 3-nitro-2*H*-chromene drastically decreases antiproliferative activity, compared to reported GI_{50} value of unsubstituted 3-nitro-2*H*-chromene (5.8 μM) [11]. However, compounds **8b** and **7e** showed greater inhibition of cell growth than analogs without substituent on C-2 (1.2 and 2.4 μM versus 1.5 and 5.6 μM, respectively) [11]. The same occurs in the case of the mixture **5c** + **6c** (1.5 versus 1.9 μM).

In comparison of GI_{50} range plot of tested compounds with reference anticancer drugs (Figure 3), it can be observed that GI_{50} ranges are larger in the pharmacological standards than in 2-glyco-3-nitro-2*H*-chromenes, which means that new compounds possess similar selectivity against the cell lines.

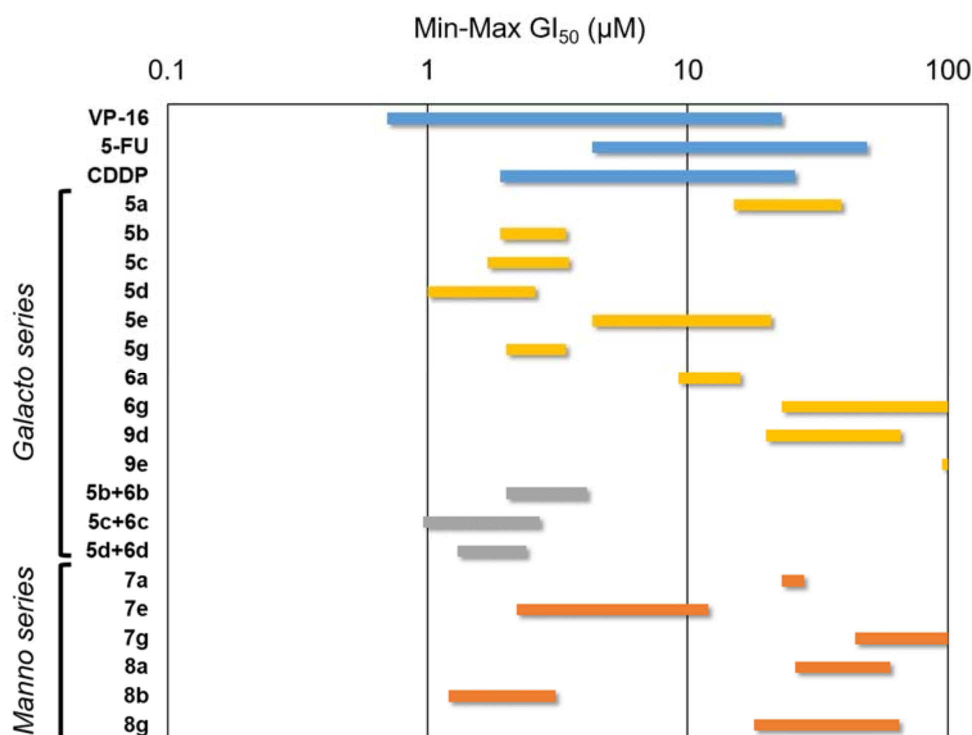


Figure 3. GI_{50} range plot of tested compounds.

Experimental

Materials and methods

All chemicals were purchased from commercial sources and used directly, without further purification. Preparative TLC was performed using silica gel (Merck 60 GF_{254}). TLC was performed on precoated Merck Kieselgel 60 GF_{254} aluminum backed plates; TLC spots were visualized by UV light. NMR spectra were recorded on a Bruker AC/PC instrument (500 MHz for ^{1}H and 125 MHz for ^{13}C) with tetramethylsilane as internal reference and deuterated chloroform or dimethylsulfoxide as solvents. Coupling constants were recorded in Hz. Characterization of NMR signals was based on homonuclear double-resonance and DEPT experiments. High resolution mass spectra were recorded on a Micromass Autoespec spectrometer, at the Centro de Investigación Tecnológica e Innovación (CITIUS) from the Universidad de Sevilla. Infrared spectra were recorded on an IR3000 Thermo Electron Corporation spectrophotometer in the range between 4000 and 600 cm^{-1}.
Evaluation of the antiproliferative activity was accomplished following the protocol of the NCI of the United States [25], slightly modified [26]. For each compound, the cells were exposed to serial decimal dilutions in the range of 0.001-100 µM for a period of 48 h. After exposure, the SRB method was applied to determine the optical density of each cell at 530 nm (main) and 620 nm (secondary). For each product concentration, the percentage of growth (PG) according to the NCI formulas was calculated.

Synthetic procedures

General procedure for 2-glyco-3-nitro-2*H*-chromenes 5a-g + 6a-g and **7a-g + 8a-g.** To a heterogeneous mixture of nitroalkenes **2,3** [27,28] (1 mmol) and the appropriate salicylaldehyde **4a-g** (1 mmol), DBU (0.1 mmol) and dichloromethane (0.2 mL, 3.12% mol) (to initially homogenize the mixture) were added. The mixture was stirred at room temperature until reaction completion (monitored by TLC, 1:3 hexane-diethyl ether) (Table 1). The crude was purified by flash chromatography on a silica gel column (1:3 hexane-diethyl ether). Chromenes were pure isolated by PTLC (1:3 petroleum ether-isopropyl ether, 3 elutions) or by crystallization in methanol.

General procedure for deacetylated products 9d,e. To a solution of **5d,e** (0.31 mmol) in methanol:water, 9:1 (4.6 mL), potassium carbonate (0.18 g) was added. The suspension was stirred at room temperature for 1 hour (TLC, 3:1, benzene-methanol). Crude reaction was acidified to pH ~ 6 with diluted HCl (if a solid appears) or Amberlite IR-120 (H^{+}), which was then filtered. Pure products crystallized from methanol.

Conclusions

New 3-nitro-2*H*-chromenes bearing an acyclic carbohydrate moiety at C-2 have been synthesized, and their antiproliferative activity against a panel of six human solid tumor cell lines has been assessed. Compounds **5d**, **8b** and mixtures of **5c**+**6c** and **5d**+**6d** were found to possess the best activity profile toward the six tested lines, also being **5c**+**6c** much better than pharmacological reference compounds against SW1573 cell line. Furthermore, 6-halo-3-nitro-2*H*-chromenes displayed strong growth inhibition of the chemotherapeutical resistant cell line WiDr.

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