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SHORT COMMUNICATION

Preliminary evaluation of the effect of partially hydrogenated 3-acetylisocoumarin derivatives on the cancer cells growth

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Abstract: In different conditions, three partial reduction products were obtained from 3-acetyl-1*H*-isochromen-1-one, namely 3-(1-hydroxyethyl)-1*H*-isochromen-1-one, 3-(1-hydroxyethyl)isochroman-1-one, and 3-ethylisochroman-1-one. The influence of two products with an alcohol fragment, as well as 4-hydroxy-1,2,3,4-tetrahydro-6*H*-benzo[*c*]chromen-6-one, on the growth of cancer cells was investigated through One Doses Full NCI 60 Cell Panel Assay. The only one significant effect was found: 3-(1-hydroxyethyl)-1*H*-isochromen-1-one practically stops the growth of CCRF-CEM cell lines (Leukemia).

Keywords: 3-acetyl-1*H*-isochromen-1-one (3-acetylisocoumarin); reduction; cancer cells.

Introduction

The modification of bioactive natural compounds and the synthesis of their analogues remains an applicable and productive direction in the search for new medicines [1, 2]. Isocoumarins and their hydrogenated derivatives are among the many examples of natural compounds which are so interesting to drug developers. Thus, inspired by the structure of the natural antitumor antibiotic Cytogenin [3], the authors [4] created a number of isocoumarins and 3,4-dihydroisocoumarins with a polar group in position 3 (Figure 1a) and investigated their antiproliferative activity. The alcohol **1** showed the ability to inhibit the growth of cancer cells.

In another study [5], the structure of Cladosporin [6], known primarily for its antimalarial activity, was modified to improve medical properties. Among the diversity of modifications, the most useful for antimalarial activity was the addition of a hydroxyl group into the methylene linker (compound **2**, Figure 1b).

Molecular docking in the work [4] and X-ray study of the substances-enzyme complex in the work [5] showed that both the hydroxyl group and the sp^3-hybridization of the carbon in position 3 of isocoumarin are important for effective binding. Thus, 3,4-dihydroisocoumarins with a hydroxyalkyl fragment in position 3 can reasonably be considered a promising class of substances from the side of their biological activity.

There are no many approaches to the synthesis of 3-(hydroxyalkyl)-3,4-dihydroisocoumarins.

For example, in the study [4], substance **1** was synthesized by catalytic hydrogenation of the corresponding isocoumarin **3** (Scheme 1a), and the last one was obtained by the cyclization of an alkyne with an *ortho*-carboxyphenyl substituent (this method is very popular in the isocoumarins' chemistry). Another interesting reaction, i.e. the oxidation of the double bond and the subsequent closing of the isochromanone system (Scheme 1b), was used for the synthesis of one of the objects for biological research in the study [5].

Another obvious option for obtaining 3-(hydroxylalkyl)-3,4-dihydroisocoumarins through the stage of the corresponding isocoumarins is the reduction of.

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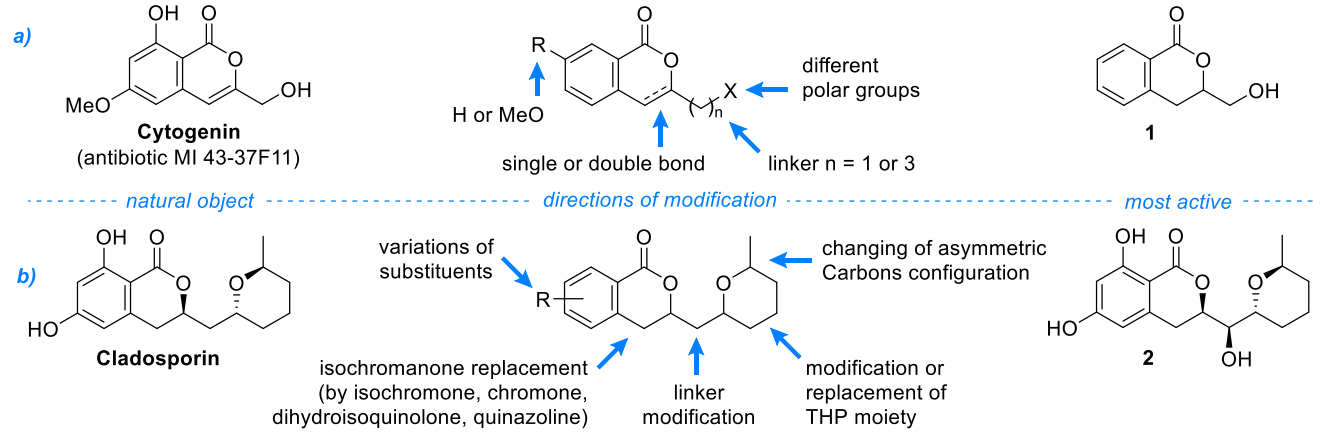
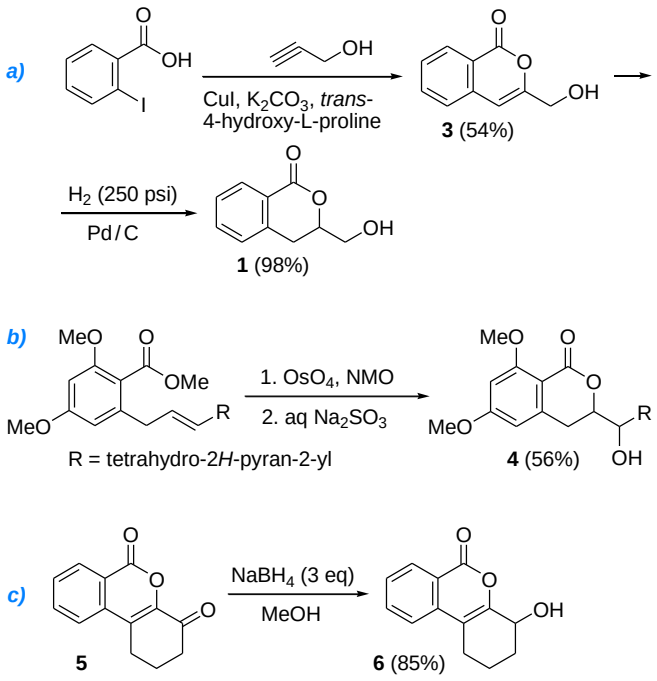


Figure 1. The examples of structural modification of natural isocoumarins and 3,4-dihydroisocoumarins for novel bioactive substances discovery.

3-acylisocoumarins. Surprisingly, there are not much information about 3-acylisocoumarins reduction in literature, and the most useful for us was the synthesis tricyclic condensed structure **6** with an alcohol group (Scheme 1c) [7].



Scheme 1. Approaches to the synthesis of 3-(hydroxyalkyl)-3,4-dihydroisocoumarins.

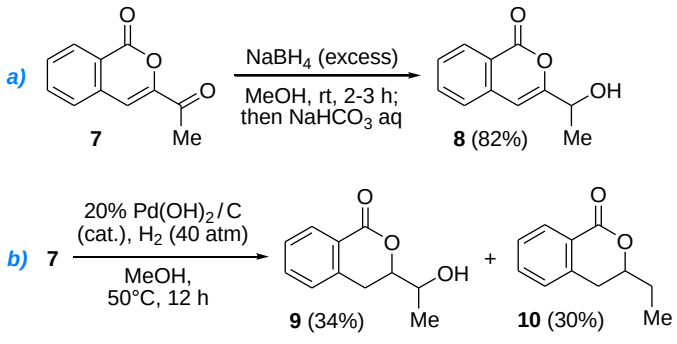
As demonstrated in literature [8], 3-acylisocoumarins are quite synthetically accessible substances, but their potential as substrates for further transformations has not been realized so far. This is also true for reduction reactions: 3-acylisocoumarins have at least three fragments capable of reduction, and it is possible to obtain a number of different products on the basis of one substrate; especially taking into account the possibility of different levels of reduction and both saving and opening of the cycle.

To understand the prospects of this direction, we aimed to investigate the effect of various reducing agents on 3-acetylisocoumarin and evaluate the impact of reduced derivatives on cancer cells.

Results and Discussion

Reduction of 3-acetylisocoumarin **7** with $NaBH_4$ under mild conditions, similar to those described in the literature for this reaction earlier [7], expectedly led to isocoumarin **8** (Scheme 2a). The synthesis of the tricyclic derivative **7** (Scheme 1c) was also reproduced using this technique with minor variations. Note that the structure of product **6** executes significant restrictions on the conformational mobility of the OH group compared to compound **8**.

A more forceful reduction of isocoumarin **7** is hydrogenation at high temperature and pressure with $Pd(OH)_2/C$ catalyst. This yields a mixture of compounds (Scheme 2b): 3,4-dihydroisocoumarin **9** with 1-hydroxyethyl fragment, which is a homologue of the bioactive primary alcohol **1** (see Figure 1a above), and 3,4-dihydroisocoumarin **10** with ethyl substituent. Products **9** and **10** were separated chromatographically, compound **9** with two asymmetric centres was isolated as 1:1 diastereomeric mixture.



Scheme 2. Reduction of 3-acetylisocoumarin under different conditions.

Three compounds were selected for research at the National Cancer Institute (NCI, Bethesda, Maryland, USA) (Table 1). A single-dose test of effect of racemic products **6,** **8** and diastereomeric mixture **9** on cancer cell growth

brought disappointing results: two out of three samples had no noteworthy effect on all cell lines. Table 1 shows their Mean Growth Percent (MGP) values for 60 cancer lines, and all of them are close to 100%. Moreover, the Delta values (the difference between the MGP and the GP value of the most inhibited cell line) of these compounds are also low, indicating the absence of any significant effect on the growth of all types of cancer cells. Only isocoumarin **8** showed the ability to inhibit the growth of one of the investigated leukemia lines selectively and almost completely (for CCRF-CEM cells the GP was 1.2%), while not causing a significant effect on the growth of other cell lines (Table 1).

The biggest surprise was the absence of activity in 3,4-dihydroisocoumarin **9**, because the difference in its structure from compound **1** isn't big. On the other hand: a previous study [4] used other parameters to evaluate the anticancer activity for compound **1**, and it was also inactive against some types of cancer cells.

Table 1. Percent of cancer cell growth in 1×10^{-5} M solution of the test compound compared to the control (according to One Doses Full NCI 60 Cell Panel Assay).

Com-pound	NSC Number	Mean Growth Percent, %	Delta, %
6	831590	103.0	13.7
8	831592	99.6	98.5 [a]
9	833505	112.3	19.3

[a] GP = 1.2 % for CCRF-CEM (Leukemia).

Conclusions

It can be concluded that the reductions of 3-acyl-isocoumarins really have a significant synthetic potential, because depending on the reaction conditions different degrees of saturation can be achieved and different types of products can be obtained. However, anticancer activity was recorded only for one substance to a single cancer line; even a substance similar in structure to the known anticancer agent did not show activity. Such a significant effect of small structural changes on bioactivity requires further research to obtain a larger number of products with various structural variations, and motivates us to continue researching the processes of isocoumarin derivatives reduction.

Experimental section

All reagents and solvents were purchased from Enamine Ltd. (www.enamine.net). TLC characterization was performed with pre-coated silica gel GF254 (0.2 mm). For column flash chromatography Silica gel 230-400 mesh was used. NMR spectra of the obtained products were recorded on a Varian Unity Plus 400 spectrometer (400 MHz for ^{1}H, 101 MHz for ^{13}C); ^{1}H NMR chemical shifts were calibrated using residual undeuterated DMSO (δ = 2.50 ppm) and $CDCl_3$ (δ = 7.26 ppm) signals; ^{13}C NMR chemical shifts are reported relative to the central DMSO (δ = 39.52 ppm) and $CDCl_3$ (δ = 77.16 ppm) signals. Melting points were measured on a MPA100 OptiMelt automated melting point system. Elemental analyses were performed at the Analytical Laboratory of the V.P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry of NAS of Ukraine and be in good agreement (±0.4%) with the calculated values. LC/MS spectra were recorded on an Agilent 1100 Series HPLC system equipped with a diode matrix with an Agilent LC/MS mass selective detector (chemical ionization).

Synthesis

3-Acetyl-1*H*-isochromen-1-one (**7**) was synthesized by condensation of 2-formylbenzoic acid with chloroacetone in MeCN solution with Et_3N as a base [9]. 2,3-Dihydro-1*H*-benzo[*c*]chromene-4,6-dione (**5**) was synthesized by interaction of benzenediazonium 2-carboxylate with cyclohexane-1,2-dione [7]. Spectral data of ketone **7** see [9], and ketone **5** see [10].

General method of reduction of the 3-acyl-1H-isochromen-1-ones.

The initial ketone (5 mmol) was suspended in methanol (25 mL), and $NaBH_4$ (191 mg, 5 mmol) was added in four portions at 0 °C with stirring in an half of hour. After finishing the reaction 50 mL of 8% aqueous solution of $NaHCO_3$ was added. The product was extracted with CH_2Cl_2 (3 × 20 mL); organic layer was dried over Na_2SO_4 and concentrated under reduced pressure.

*3-(1-Hydroxyethyl)-1H-isochromen-1-one (**8**).*

Spectral data are in agreement with Ref. [11], where compound **8** was obtained by Pd-catalyzed coupling of 2-iodobenzoic acid and but-3-yn-2-ol with following cyclization.

*4-Hydroxy-1,2,3,4-tetrahydro-6H-benzo[c]chromen-6-one (**6**).*

Yield: 924 mg, 86%; mp 175-176 °C (176-177 °C in Ref. [7]). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 (d, J = 7.8 Hz, 1H), 7.84 (t, J = 7.6 Hz, 1H), 7.63-7.51 (m, 2H), 5.54 (d, J = 5.6 Hz, 1H), 4.35 (d, J = 4.0 Hz, 1H), 2.64 (br. d, J = 16.5 Hz, 1H), 2.48-2.38 (m, 1H), 1.92-1.68 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.5, 152.3, 136.9, 135.1, 128.9, 128.2, 122.7, 120.3, 110.5, 63.2, 31.3, 22.4, 17.0. HPLC (CI) m/z $(M+H)^+$ 217.

*Synthesis of 3-(1-hydroxyethyl)isochroman-1-one (**9**) and 3-ethylisochroman-1-one (**10**).*

The ketone **7** (1.90 g, 10 mmol) was suspended in methanol (50 mL), and 0.1 g of $Pd(OH)_2$ / C (20 wt. %) was added. This mixture was heated (50 °C) and stirred in a hydrothermal autoclave at 40 atm H_2 for 12 h. After finishing the reaction, the catalyst was filtered off; the solvent was evaporated under reduced pressure. The products **9** and **10** were separated and purificated by column flash chromatography on silica gel using CH_2Cl_2 as eluent.

*3-(1-Hydroxyethyl)isochroman-1-one (**9**).*

Yield: 658 mg, 34%; mp 48-50 °C (1:1 diastereomeric mixture). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (ddd, J = 7.8, 3.4, 1.4 Hz, 1H), 7.51 (tt, J = 7.6, 1.8 Hz, 1H), 7.35 (td, J = 7.6, 3.8 Hz, 1H), 7.24 (t, J = 6.9 Hz, 1H), 4.39 (dt, J = 12.5, 3.5 Hz, 0.5H), 4.33 (ddd, J = 12.5, 5.5, 3.0 Hz, 0.5H), 4.13 (qd, J = 6.5, 3.8 Hz, 0.5H), 3.96 (p, J = 6.4 Hz, 0.5H), 3.18 (dd, J = 16.4, 12.3 Hz, 0.5H), 3.11 (dd, J = 16.2, 12.6 Hz, 0.5H), 2.89 (dd, J = 16.4, 3.1 Hz, 0.5H), 2.83 (dd, J = 16.2 Hz, 3.1 Hz, 0.5H), 1.31 (d, J = 6.4 Hz, 1.5H), 1.29 (d, J = 6.5 Hz, 1.5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.4 and 165.2, 139.2 and 138.9, 134.1 and 134.0, 130.4 and 130.3, 127.9 and 127.8, 127.7 and 127.7, 124.9 and 124.8, 82.5 and 82.2, 69.1 and 68.1, 29.6 and 27.2, 18.5 and 18.1. HPLC (CI) m/z $(M+H)^+$ 193.0.

*3-Ethylisochroman-1-one (**10**).*

Yield: 534 mg, 30%. Spectral and other data are in agreement with Ref. [12], where compound **10** was obtained by catalytic oxidation of 3-ethylisochroman.

Biological Assay

The anticancer activity of synthesized compounds was tested according to the International Program of the National Institutes of Health – DTP (Developmental Therapeutic Program) of the National Cancer Institute (NCI, Bethesda, Maryland, USA) on 60 cancer cell lines [13]; a description of the technique is also given in [14].

Notes

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Disclaimer. This material should not be interpreted as representing the viewpoint of the U.S. National Institutes of Health, or the National Cancer Institute.

The authors declare no conflict interest.

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Первинна оцінка впливу частково гідрованих похідних 3-ацетилізокумарину на ріст ракових клітин

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Резюме: Застосовуючи різні умови, із 3-ацетил-1*H*-ізохромен-1-ону отримано три продукти часткового відновлення: 3-(1-гідроксиетил)-1*H*-ізохромен-1-он, 3-(1-гідроксиетил)ізохроман-1-он, 3-етилізохроман-1-он. Досліджена дія двох продуктів зі спиртовим фрагментом, а також 4-гідрокси-1,2,3,4-тетрагідро-6*H*-бензо[*c*]хромен-6-ону, на ріст ракових клітин шляхом однодозового випробування на 60 лініях ракових клітин. Виявлено єдиний суттєвий вплив: 3-(1-гідроксиетил)-1*H*-ізохромен-1-он практично зупиняє ріст клітин лінії CCRF-CEM (Лейкемія).

Ключові слова: 3-ацетил-1*H*-ізохромен-1-он (3-ацетилізокумарин); відновлення; ракові клітини.