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Induction of erythroid differentiation of human K562 cells by 3-*O*-acyl-1,2-*O*-isopropylidene-D-glucofuranose derivatives

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Abstract: In this paper we report the synthesis of twelve 3-*O*-acyl-1,2-*O*-isopropylidene-D-glucofuranose derivatives and the results obtained on their effects in inducing erythroid differentiation of human leukemic K562 cells. The data obtained demonstrate that two of the newly synthetized compounds are able to induce erythroid differentiation of K562 cells. In addition, these same compounds potentiate K562 erythroid differentiation induced by cytosine arabinoside, retinoic acid and mithramycin. Inducers of erythroid differentiation stimulating fetal γ-globin synthesis could be considered for possible use in the experimental therapy of hematological diseases associated with a failure in the expression of adult β-globin genes. © 1999 Elsevier Science Ltd. All rights reserved.

The K562 cell line, isolated and characterized by Lozzio and Lozzio[1] from a patient with chronic myelogenous leukemia in blast crisis, has been proposed as a very useful *in vitro* model system (a) to study the molecular mechanism(s) regulating the expression of embryonic and fetal human globin genes[2] as well as (b) to determine the therapeutic potential of new differentiating compounds[3]. K562 cells exhibit a low proportion of hemoglobin-synthetizing cells under standard cell growth conditions, but are capable to undergo erythroid differentiation when treated with a variety of compounds, including hemin, cytosine arabinoside (ara-C), butyric acid and 5-azacytidine[2-5]. Following erythroid induction of K562 cells, a sharp increase of cytoplasmic accumulation of Hb Portland ($\zeta_2\gamma_2$) and Hb Gower 1 ($\zeta_2\varepsilon_2$) is observed, accompained by an increase in the expression of human ε and γ globin genes[5].

Pharmacologically-mediated regulation of the expression of human γ-globin genes could be of interest in the search of potential therapeutic agents in hematological disorders; following the indications that increases in fetal Hb levels can be clinically beneficial in sickle cell disease[6] and β-thalassemia[7], a number of studies have been focused on the search of compounds able to stimulate γ-globin gene expression[8]. Among possible biological response modifiers, one of the most interesting classes of compounds are short fatty acids[3,9,10]. Accordingly, butyrates have been used in therapeutic trials to stimulate fetal hemoglobin gene expression in patients with β-globin hemoglobinopathies[11,12].

In this paper we report the synthesis of twelve 3-*O*-acyl-1,2-*O*-isopropylidene-D-glucofuranose derivatives, and we tested their effects in augmenting the proportion of benzidine-positive (hemoglobin-containing) cells in treated K562 cell populations. The results obtained demonstrate that two of the newly synthetized compounds are potent inducers of erythroid differentiation of K562 cells. In addition, these same

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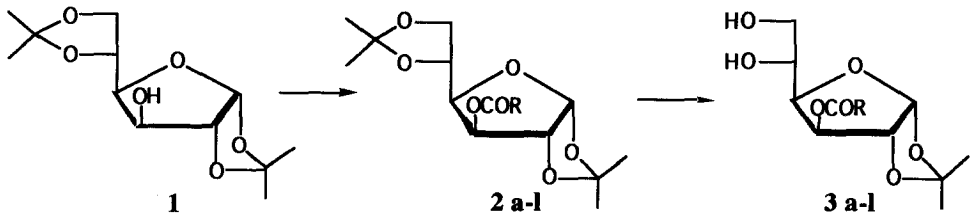
compounds potentiate erythroid induction of K562 cells treated with sub-optimal concentrations of cytosine arabinoside (ara-C), retinoic acid and mithramycin.

Chemistry

Twelve 3-*O*-acyl-1,2-*O*-isopropylidene-D-glucofuranose derivatives (**3a-l**) were prepared through the general procedure previously described for some members (**3a** and **3c**)[13,14], involving the esterification of commercially available **1** followed by the selective removal of the 5,6-*O*-isopropylidene protecting group (Scheme 1).

The preparation of the 3-*O*-acyl derivatives **2a-e**, having linear acyl residues, was easily performed in high yields according to the Villa protocol,[13] involving the treatment of **1** with the pertinent acyl chloride in the presence of an excess of triethylamine, followed by purification through flash chromatography. The same reaction with more sterically demanding acyl chlorides having α- or β-alkyl or aryl branching, caused an incomplete esterification of **1** even after very long reaction times; satisfactory high yield preparations of **2f-l** was however achieved when the esterifications were performed in pyridine. The subsequent removal of the 5,6-*O*-isopropylidene protecting groups from derivatives **2a-l** was achieved by a mild hydrolytic treatment with 80% aqueous acetic acid at 60-70 °C. The reactions were sufficiently rapid (20-90 min) and clean for most of the derivatives, and led, after purification by flash chromatography, to the pure derivatives[15] **3** in high isolated yields (90-97%). In the case of the derivatives **2d-g**, a reduction of the hydrolysis rate was observed. In the case of **2e**, the hydrolysis reaction was pushed to completion by warming at 100 °C for 2 h. However, a side-product had formed beside the expected derivative **3e** in an about 1:3 ratio. After chromatographic separation, NMR analysis showed that the side-product was 6-*O*-esanoyl-1,2-isopropylidene-D-glucofuranose, derived from the previously reported[13] acyl shift process from the secondary OH-3 to the primary OH-6. Neverthless, a satisfactory preparation of **3e** completely avoiding the acyl shift was achieved by subjecting **2e** to a selective deisopropylidenation according to a recent method with DDQ in CH_3CN/H_2O[16]. In the other cases, the expected derivatives **3d**, **3f** and **3g** were obtained in acceptable yields (56-78%) free from the respective acyl shift side-products by prolonged times of reaction (6-30 h) with 80% aqueous AcOH at 60 °C.

Scheme 1



1 **2 a-l** **3 a-l**

a: R= CH_3; **b**: R= CH_2CH_3; **c**: R= $(CH_2)_2CH_3$; **d**: R= $(CH_2)_3CH_3$; **e**: R= $(CH_2)_4CH_3$; **f**: R= $CH(CH_3)_2$; **g**: R= $C(CH_3)_3$; **h**: R= cyclopentyl; **i**: R= $CH_2CH(CH_3)_2$; **j**: R= $CH_2(CH_3)_3$; **k**: R= $CH_2CH(C_6H_5)CH_3$; **l**: R= $CH_2(C_6H_5)_2$

Biological studies

Human myeloid leukemia K562(S) cells[3] were maintained in RPMI 1640 (Gibco, BRL, Milan, Italy) in 10% fetal bovine serum (Gibco, BRL, Milan, Italy), 5% CO_2 supplemented with 50 units/ml penicillin, 50 μg/ml streptomycin[4]. Cell growth was studied by determining the cell number/ml after different days of *in vitro* cell culture[4].

Table 1 shows the percentage of benzidine-positive K562 cells after 7 days of cell culture in the absence or in the presence of the indicated concentrations of the studied 3-*O*-acyl-1,2-*O*-isopropylidene-D-glucofuranose derivatives, most of the corresponding fatty acids, ara-C and mithramycin. As clearly evident, compounds **3f** and **3g** showed capacity to induce erythroid differentiation of K562 cells, although to a lowerlevel when compared to ara-C and mithramycin[17]. By contrast, the other tested compounds showed lower

Table 1: Effects of 3-*O*-acyl-1,2-*O*-isopropylidene-D-glucofuranose derivatives on *in vitro* growth and erythroid differentiation of human leukemic K562 cells.

Compound	Cell proliferation IC_{50}	Erythroid induction* % of benzidine-positive cells (mM, nM)
1	2.8 mM	26 (3 mM); 18 (4 mM)
3a	3.8 mM	27 (4 mM)
3b	2.4 mM	21 (2 mM); 19.6 (3 mM); 25 (4 mM)
3c	0.8 mM	25.4 (2 mM)
3d	2 mM	24 (2 mM); 28 (3 mM); 25 (4 mM)
3e	1.2 mM	10.3 (1 mM); 21 (2 mM)
3f	2.5 mM	43 (3 mM); 54.2 (4 mM); 59 (5 mM)
3g	1.5 mM	51.7 (3 mM); 50.2 (4 mM)
3h	2.5 mM	7 (3 mM); 16.8 (4 mM); 8 (5 mM)
3i	1 mM	12 (2 mM)
3j	1.2 mM	20 (2 mM)
3k	0.5 mM	15 (1 mM)
3l	0.5 mM	14 (1 mM)
ara-C	500 nM	95 (250 nM)
mithramycin	10 nM	81 (20 nM)
propionic acid	4 mM	10 (4 mM)
butyric acid	2.4 mM	35 (3 mM)
isobutyric acid	4.8 mM	14 (5 mM)
pivalic acid	4.5 mM	12 (5 mM)

* Results are presented as maximum level of percentage of benzidine-positive (hemoglobin-containing) cells reached within 9 days induction period at the indicated concentrations of tested compounds.

Table 2: Effects of 3-*O*-acyl-1,2-*O*-isopropylidene-D-glucofuranose derivatives on erythroid differentiation of K562 cells.

Compound	Concentration	Erythroid induction*			
		day 3	day 5	day 7	day 9
-	-	5±1.5	7±2.1	7±2.5	5±1.8
3f	4 mM	20±4.2	35±7.2	56.8±9.1	50±11.1
3g	4 mM	22±7.1	25±8.5	50.7±11.4	59±9.7
isobutyric acid	4 mM	12±2.7	14±1.9	12±2.8	8±1.5
pivalic acid	4 mM	4±1.3	9±2.4	11±1.8	6±1.4
ara-C	500 nM	45±4.1	61.5±3.7	84.5±3.4	93.2±5.2
mithramycin	20 nM	15±2.2	55±5.2	75±5.2	80±4.7

* Results are expressed as proportion of benzidine-positive cells ± SD. In total, 9 independent experiments were performed, with the exception of experiments carryied on with isobutyric and pivalic acid (in this case n = 4). K562 cells were cultured for the indicated length of time without inducers (-) or in the presence of the indicated concentrations of **3f**, **3g**, ara-C, mithramycin, isobutyric acid and pivalic acid.

Table 3: Effects of sub-optimal concentrations of 3-*O*-acyl-1,2-*O*-isopropylidene-D-glucofuranose derivatives on erythroid differentiation of K562 cells cultured with sub-optimal concentrations of retinoic acid, ara-C or mithramycin.

Compound	Concentration	Erythroid induction*			
		-	retinoic acid 5 μM	ara-C 25 nM	mithramycin 5 nM
-		0.5	5-7	12.5	2.7
3f	2 mM	29	55.8	78	N.D.
3g	2 mM	15	67	90	34

* Results are expressed as proportion of benzidine-positive cells. N.D. = not done. K562 cells were cultured for 7 days without inducers (-) or in the presence of the indicated concentrations of **3f**, **3g**, retinoic acid, ara-C and mithramycin.

activity. Table 1 shows also that most of the newly synthetized 3-*O*-acyl-1,2-*O*-isopropylidene-D-glucofuranose derivatives cause decrease in the proliferation efficiency of K562 cells. 50% inhibition of cell growth (IC_{50}) occurs when K562 cells are cultured for 7 days in the presence of drugs concentrations ranging from 0.8 mM (**3c**) to 3.8 mM (**3a**). The values of IC_{50} of **3f** and **3g** were found to be 2.5 and 1.5 mM, respectively. This phenomenon is expected, as it is known that induction of erythroid differentiation of K562 cells is associated with a decrease of cell growth rates of differentiated cells[4]. The kinetic of K562 cell erythroid induction by **3f** and **3g** is shown in Table 2. Erythroid differentiation induced by **3f** and **3g** is a fairly

reproducible phenomenon, reaching a plateau level after 7 days of induction. The induction ability of **3f** and **3g** is significantly higher than that exhibited by isobutyric acid and pivalic acid.

Table 3 shows that when sub-optimal concentrations of ara-C, mithramycin and retinoic acid are administered to K562 cells together with sub-optimal concentrations of **3f** and **3g**, induction of K562 erythroid differentiation is obtained. These data suggest that **3f** and **3g** potentiate the effects of other known inducers of K562 cells erythroid differentiation.

Conclusions

The data presented in this paper demonstrate that the 3-*O*-acyl-1,2-*O*-isopropylidene-D-glucofuranose derivatives **3f** and **3g** could be considered as inducers of K562 cell erythroid differentiation exhibiting a biological activity much higher than that of the corresponding isobutyric acid and pivalic acid. In addition, according with Planchon *et al.*[18], 3-*O*-acyl-1,2-*O*-isopropylidene-D-glucofuranose derivatives are expected to exhibit increased *in vivo* stability when compared to butyric acid. Therefore, **3f** and **3g** could be proposed as possible agents to modulate production of embryo-fetal hemoglobins by human erythroid cells.

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15. All pure compounds were identified through the analysis of ^{1}H and ^{13}C NMR spectra; and, if new, by elemental analysis, values of C and H within ± 0.3% being always obtained. Physical data of compounds **3a** and **3c** were in accordance with the reported ones[11,12]; the following data were found for new derivatives (optical rotations measured at 20 ± 2 °C). **3b**: m.p. 117-119 °C (hexane/ethyl acetate), $[\alpha]_D$ + 10.5 (*c* 1.2, $CHCl_3$); **3d**: oil, Rf 0.33 (3:7 hexane/ethyl acetate), $[\alpha]_D$ + 12.9 (*c* 1.2, $CHCl_3$); **3e**: oil, Rf 0.31 (1:1 hexane/ethyl acetate), $[\alpha]_D$ + 9.6 (*c* 2.1, $CHCl_3$); **3f**: m.p. 78-80 °C (hexane/ethyl acetate), $[\alpha]_D$ +16.4 (*c* 1.2, $CHCl_3$); **3g**: 57 °C (hexane/diethyl ether), $[\alpha]_D$ +15.4 (*c* 0.9, $CHCl_3$); **3h**: oil, Rf 0.27 (1:1 hexane/ethyl acetate), $[\alpha]_D$ +18.6 (*c* 1.0, $CHCl_3$); **3i**: oil, Rf 0.25 (4:6 hexane/ethyl acetate), $[\alpha]_D$ + 15.0 (*c* 1.1, $CHCl_3$); **3j**: m.p. 42-45 °C (hexane), $[\alpha]_D$ + 17.1 (*c* 1.0, $CHCl_3$) ; **3k** (1:1 unseparable mixture of two diastereoisomers: m.p. 75-90 °C (hexane), $[\alpha]_D$ + 8.2 (*c* 1.2, $CHCl_3$); **3l**: m.p. 112-114 °C (hexane), $[\alpha]_D$ + 9.0 (*c* 1.0, $CHCl_3$).
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