Bioorganic & Medicinal Chemistry Letters, Vol.1, No.12, pp. 721-724, 1991
Printed in Great Britain

0960-894X/91 $3.00 + .00

DESIGN, SYNTHESIS AND CYTOTOXIC EVALUATION OF KETO-C-GLYCOSIDE FATTY ACID CONJUGATES.

Jean Herscovici*§, M. Idriss Bennani-Baiti†, Roland Montserret§, Charles Frayssinet†
and Kostas Antonakis§.
§Laboratoire de Chimie Organique Biologique et Spectroscopique. Institut de Recherches Scientifiques Sur le Cancer, CNRS, 94801 Villejuif, France.
†Laboratoire de Pathologie Cellulaire. Institut de Recherches Scientifiques Sur le Cancer, CNRS, 94801 Villejuif, France.

(*Received* 19 *September* 1991)

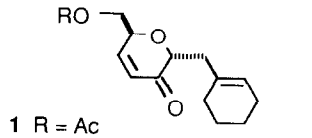
Abstract. Keto C-glycoside-fatty acid conjugates were synthesized from 6-hydroxy 2- and 4-keto unsaturated D-C-glycosides. These compounds were tested for cytotoxic activity against $LFCl_2A$ cells (Rat hepatocarcinoma cells). The introduction of a lipid chain to 2-keto C-glycosides induced a drop in the cyctotoxic activity of these compounds. On the other hand 4-keto unsaturated C-glycoside-fatty acid conjugates possessed IC_{50} values of 0.7-0.001 μM with **21** being the most potent.

Unsaturated keto-C-glycosides have attracted considerable interest recently with the discovery of their cytotoxic properties[1] as well as their use as building blocks for the synthesis of naturally occurring molecules[2].

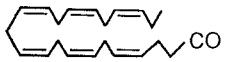
Our group has explored the possibility of improving the selectivity of antitumor drugs by their fatty acid conjugates on neoplastic cells expressing alpha fetoprotein (AFP)-receptors[3]. This approach is based on evidence that, contrary to normal resting cells, many tumor cells of varied origin express, *in vitro* and *in vivo*, specific cell surface receptors to AFP[4,5] and that these receptors, through their interaction with AFP, can be involved in the transfer of fatty acids into the cells[6].

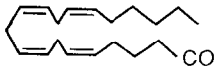
Taking into account that *erythro*-C-glycosides could be easily bound to fatty acid, and with the aim of enhancing the cytotoxic activity and selectivity of keto unsaturated C-glycosides, we have synthesized the first keto-C-glycoside fatty acid conjugates and all these compounds were evaluated against $LFCl_2A$ cells (Rat hepatocarcinoma cells).

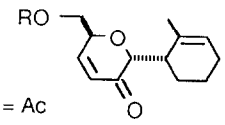
To explore the effect of the 6-O-acyl substituent on cytotoxic activity, enones with a 1-methyl cyclohexene or a methylene cyclohexene unit bond to the anomeric carbon, were prepared. First the synthesis of the keto alcohols **3**, **6**, **10** and **11** was carried out from the previously reported acetates prepared by methods developed in our laboratory[7,8]. Thus deacetylation of the 2-keto C-glycosides **1** and **2** using potassium carbonate afforded the keto alcohols **3** and **6** in 50% yield (scheme 1). The 4-keto alcohols **10** and **11** were prepared in two steps from the corresponding diacetates (scheme 2). The protected C-glycosides were first deacetylated using sodium methoxide. Then the allylic hydroxyls were selectively oxidized with MnO_2 (50%). Finally the keto-C-glycoside-fatty acid conjugates were obtained by coupling **3**,



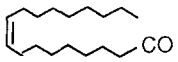
1 R = Ac
3 R = H

4 R = 

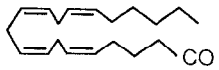
5 R = 

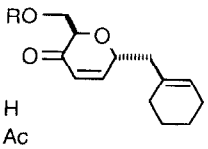


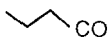
2 R = Ac
6 R = H

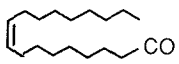
7 R = 

8 R = 

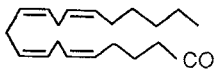
9 R = 

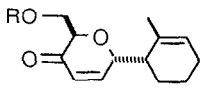


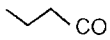
10 R = H
12 R = Ac
13 R = 

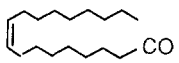
14 R = 

15 R = 

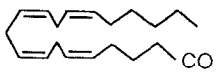
16 R = 

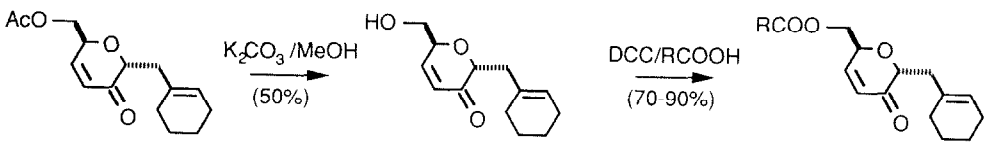


11 R = H
17 R = Ac
18 R = 

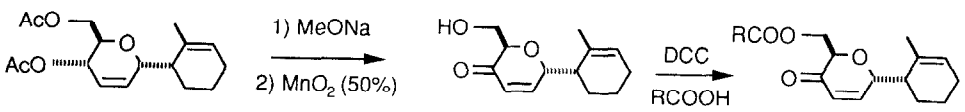
19 R = 

20 R = 

21 R = 



Scheme 1



Scheme 2

6, **10** and **11** with the carboxylic acid in the presence of dicyclohexylcarbodiimide (DCC)[9,10] (70-90%).

We determined the cytotoxicities of the unsaturated keto C-glycosides against LFCl2A cells, an established cell line derived from an hepatocarcinoma induced in the Commentry rat by dimethylamino azobenzene (DAB)[11-13]. All the results are summarized in Table 1.

Table 1 ***in vitro*** **cytotoxicity**

Entry	Compounds	IC_{50} (μM)
1	**1**	-
2	**2**	3
3	**3**	9.5
4	**4**	3×10^{1}
5	**5**	3.5×10^{1}
6	**6**	3.5
7	**7**	3.5×10^{1}
8	**8**	6.5×10^{1}
9	**9**	5
10	**10**	3.0
11	**12**	1.5×10^{-1}
12	**13**	1.5×10^{-1}
13	**14**	9×10^{-2}
14	**15**	8×10^{-2}
15	**16**	5×10^{-2}
16	**11**	7.0
17	**17**	7×10^{-1}
18	**18**	3.5×10^{-1}
19	**19**	5×10^{-2}
20	**20**	2×10^{-2}
21	**21**	10^{-3}
22	5-fluoro uracil	2.85×10^{1}

Examination of the data deserves further comments. Coupling the 2-keto C-glycosides **3** and **6** (IC_{50} 9.5 and 3.5) with a fatty acid led to the loss of the cytotoxic activity with IC_{50} ranging from 5 to 65 μM (entries 3, 4, 7-9). On the other hand we found 4-keto C-glycosides **10-16** and **11-21** to be cytotoxic with IC_{50} values of 7 to 0.001 μM (entries 12-15, 18-21). As expected the antitumor activity was dependent on the fatty acid. Table 1 revealed clearly that the keto C-glycosides-unsaturated fatty acid conjugates were more cytotoxic than the keto-C-glycosides saturated fatty acid conjugates. Among the unsaturated derivatives, arachidonates **16** and **21** were the more active. In addition **21** was the more cytotoxic keto unsaturated C-glycosides (IC_{50} = 0.001 μM). Finally it should be noted that introduction of fatty acids in the methylene cyclohexenyl series led to an increase of the cytotoxic properties by a factor 60 (**10** IC_{50} = 3 μM, **16** IC_{50} = 0.05 μM) and in the methyl cyclohexenyl series by a factor 7000 (**11** IC_{50} = 7 μM, **21** IC_{50} = 0.001 μM).

Acknowledgment. We gratefully acknowledge the financial support of the "Association pour la Recherche sur le Cancer" (ARC), Villejuif, France.

References and Notes.

1 Herscovici, J.; Bennani-Baiti, M. I.; Frayssinet, C.; Antonakis, K. *Biomed. Chem. Lett.;* in the press.

2 Herscovici, J.; Delatre, S; Antonakis, K. *Tetrahedron. Lett.*, **1991**, *32*, 1183.

3 Anel, A.; Halmos, T.; Torres, J. M.; Pineiro, A.; Antonakis, K.; Uriel, J. *Biochem. Pharmacol.*, **1990**, *40*, 1193.

4 Naval, J.; Villacampa, M.J.; Goguel, A.F.; Uriel, J. *Proc, Natl. Acad. Sci. USA.* ***1985***, *82*, 3301.

5 Uriel, J.; Laborda, J.; Naval, J.; Geuskens, M. *Biological Properties of Alpha-fetoprotein.* Mizejewski, G. J.; Jacobson, H. I. Eds. CRC Press, Boca Raton, 1989; Vol II, pp. 103-117.

6 Uriel, J.; Naval, J.; Laborda, J. *J. Biol. Chem.*, **1987**, *262*, 3579.

7 Herscovici, J.; Muleka, K.; Antonakis, K. *Tetrahedron. Lett.*, **1984**, *25*, 5653.

8 Herscovici, J.; Boumaïza, L.; Muleka, K.; Antonakis, K. *J. Chem. Soc. Perkin 1*, **1990**, 1995.

9 Hassner, A.; Alexanian, V. *Tetrahedron. Lett.*, **1978**, 4475.

10 All new compounds were fully characterized. Significant ^{1}H NMR data of **16** are the following: δ (300.13 MHz, $CDCl_3$) 0.88 (t, 3 H, *J* = 6.92 Hz), 1.26-1.36 (m, 6 H), 1.52-1.76 (m, 6 H), 1.96-2.16 (m, 8 H), 2.25 (dd, 1 H, *J* = 6.22 and 14.08 Hz), 2.34 (t, 2 H, *J* = 7.75 Hz), 2.44 (dd, 1 H, *J* = 7.75 and 14.08 Hz), 2.84 (m, 6 H), 4.3 (dd, 1 H, *J* = 2.53 and 11.41 Hz), 4.46 (dd, 1 H, *J* = 2.53 and 7.08 Hz), 4.56 (dd, 1 H, *J* = 7.08 and 11.41 Hz), 4.66 (dddd, 1 H, *J* = 2.01, 2.67, 6.22 and 7.75 Hz), 5.3-5.42 (m, 8 H), 5.55 (m, 1 H), 6.62 (dd, 1 H, *J* = 2.01 and 10.51 Hz), 7.05 (dd, 1 H, *J* = 2.67 and 10.51 Hz).

11 Lafarge-Frayssinet, C.; Garcette, M.; Emanoil-Ravier, R.; Morel-Chany, M.; Frayssinet, C. *Liver cells and drugs.* Guillouzo, A. Ed. Colloque INSERM, John Libbey Eurotext Ltd **1988**, *164*, 465.

12 Cassingena, R.; Lafarge-Frayssinet, C.; Painchault, V.; Estrade, S.; Nardeux, P.; Frayssinet, C. *Biol. Cell.* **1990**, *69*, 113.

13 LFC1_2A cells were grown in EMEM (Eagle'sMinimal Essential Medium, Eurobio) supplemented with 10% NCS (New born calf serum, 6 Flow Laboratories), 2 mM glutamine and antibiotics (100 UI penicillin/ml and 100 µg streptomycin/ml). They were usually seeded at 25,000 cells/ml medium in culture flask incubated at 37°C in a humidified incubator with an atmosphere of air/C02 of 95/5 and subcultured twice a week. Cell suspensions (200 µl) were dispensed in wells of microtest plates. The dilutions of keto-C-glycosides to be tested were performed in DMSO, 1 µl of each dilution was added to wells of microplates together with the cells suspensions. Cells were incubated with the cytotoxics for 72 hours. Twelve hours before the cells were harvested, 1 µCi tritiated thymidine was added to each well. Cultures were washed and collected with an automated sample harvester (Skatron) on glass fiber filters (Whatmann). The filters were dried and the radioactivity was counted in omnifluor in a liquid scintillation spectrometer. Each experiment was conducted in triplicate.