Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 20, pp. 2473-2476, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00457-X

SYNTHESIS AND BIOLOGICAL ACTIVITY OF 4'-AZIDO- AND 4'-TRIFLUORACETAMIDO-3'-CHLORO-3'-DEAMINO-4'-DEOXYDAUNORUBICIN.

Nectarios Aligiannis,[1] Nicole Pouli,[2] Panagiotis Marakos, [2] Alexios-Leandros Skaltsounis,[1] *

Stephane Leonce, [3]Alain Pierre ,[3] and Ghanem Atassi.[3]

University of Athens, Department of Pharmacy, Division of Pharmacognosy [1] and Division of Pharmaceutical Chemistry, [2] Panepistimiopolis-Zografou, Athens 15771 Greece.

Institut de Recherches SERVIER, Division de Cancerologie Experimentale, [3] 11, Rue des Moulineaux, 92150 Suresnes, France.

Abstract: The syntheses of 1-O-acetyl-4-azido-3-chloro-L-lyxo-hexopyranose and 1-O-acetyl-3-chloro-4-trifluoracetamido-L-lyxo-hexopyranose are described. These aminosugars were successfully employed for the synthesis of 4'-azido- 3'-chloro-3'-deamino-4'-deoxydaunorubicin and 3'-chloro-3'-deamino-4'-deoxy-4'-trifluoracetamidodaunorubicin. The cytotoxicity of the above-mentioned compounds is discussed.

The anthracycline glycosides daunorubicin and doxorubicin have been proved to be clinically important anticancer antibiotics.[1-3] However a dose cumulative cardiotoxicity and the development of resistance in chemosensitive tumors (MDR phenotype) have restricted their extensive use in chemotherapy.[4-6] As a consequence there is an interest in new derivatives that possess cytotoxicity against resistant tumors and reduced side effects. One of the most successful synthetic approaches in this field is the modification of the sugar part of anthracyclines, which is known to be a critical determinant for their pharmacological behaviour.[7]

Among numerous analogs are those in which positions 3- and 4- in the amino sugar have been modified, giving compounds with improved biological results. This is for example the case of 4'-aminoanthracyclines,[8] particularly isodoxorubicin,[9] which showed to be active both against leukemia and solid tumors and less cardiotoxic than doxorubicin itself.

Analogs having an amino group at C-3' replaced with an halogen (Cl or Br) have already been found to be active against resistant tumor cells .[10] It has recently been postulated that the increased lipophilicity of the sugar moiety of anthracyclines could be related to the ability to overcome MDR.[11,12]

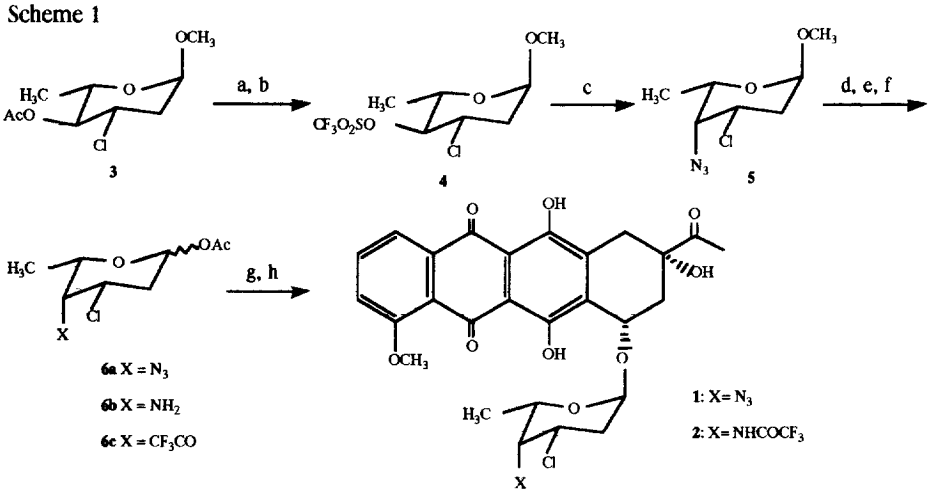
We describe here a facile synthesis of 4'-azido- and 4'-trifluoracetamido-3'-chloro-3'-deamino-4'-deoxydaunorubicin **1** and **2** respectively (Scheme 1), through the preparation of some new sugar analogs and their glycosidic coupling to daunomycinone.

Methyl-4-O-acetyl-3-chloro-2,3,6-trideoxy-α-L-arabino-hexopyranoside **3** has been prepared according to known procedures, starting from 3,4-di-O-acetyl-L-rhamnal.[13] Transesterification with sodium methoxide in methanol provided the corresponding hexopyranoside in 87% yield, which was then treated with trifluoromethanesulfonic anhydride [14] in DCE in the presence of DMAP to obtain the corresponding ester

* FAX: (301) 7238297. E-mail: askalts@atlas.uoa.ariadne-t.gr

4 in 62% yield. Treatment of **4** with sodium azide in anhydrous DMF afforded the azido analog **5** in 74% yield, [15] which was subsequently converted to the 1-O-acetyl derivative **6a** by acetolysis in 87% yield as a 9/1 mixture of the α and β anomers. The catalytic hydrogenation of **6a** proceeded quantitatively at atmospheric pressure over Pd/C to give the unstable amino sugar **6b**, which was immediately converted to

Scheme 1



a: CH_3ONa, CH_3OH, 4h, 0°C, b: $(CF_3SO_2)_2O$, DCE, DMAP, 2h, 0°C, c: NaN_3, DMF, 1h, 60°C ,d: $Ac_2O/AcOH/H_2SO_4$, 2h, rt, e: H_2, Pd/C, CH_3OH, 3h, rt, f: $(CF_3CO)_2O$, Et_2O, 2h, 0°C , g: HBr, C_6H_6, 15 min, 0°C , h: daunomycinone, HgO, $HgBr_2$, CH_2Cl_2 , 4h, rt.

the trifluoracetamide **6c** in 58% yield, [16] using trifluoracetic anhydride in dry ethyl ether. The reaction of **6c** with dry hydrogen bromide in benzene gave the corresponding glycosyl bromide, which was used immediately and in 2 fold excess for coupling with daunomycinone. Coupling was carried out in dichloromethane solution under modified Koenings-Knorr conditions (yellow mercury II oxide and mercury II bromide with 4Å molecular sieves). The reaction gave, after flash chromatography the α-anomer **2** in a good yield (67%).[17] Following an analogous procedure, **6a** [18] was converted to its 1-bromoderivative which was then coupled with daunomycinone under the above-mentioned conditions to afford the α-anomer **1** in 63% yield.[19]

The *in vitro* cytotoxic activity of the compounds **1** and **2** was evaluated using the murine leukemia L1210 cells obtained from the American Type Culture Collection (Rockville Pike, MD), the human epidermoid carcinoma cell line, KB-3-1 and also its 340-fold resistant to Adriamycin (ADR) subline KB-A1, kindly provided by Dr. M. Gottesman (Bethesda, MD). The cytotoxicity was measured by the Microculture Tetrazolium Assay as previously described.[20] Cells were exposed for ~ 4 doubling times (2 days for L1210, 4 days for KB-3-1 and KB-A1) to nine graded concentrations in triplicate of the cytotoxic drug. Results are expressed as IC_{50}, which is defined as the drug concentration inhibiting the proliferation by 50% with

respect to untreated cells. The resistance of the MDR cell line was expressed as the resistance factor (RF), which is the ratio between the IC_{50} on the resistant cells and the IC_{50} on the corresponding sensitive cells.

Concerning the cell cycle analysis, L1210 cells (2.5 10^5/ml) were incubated for 21 hours (approximately two doubling times) with various concentrations of cytotoxic drugs. Cells were then fixed by 70% ethanol, washed twice with phosphate-buffered saline (PBS) and incubated in PBS containing 100 μg/ml RNase and 25 μg/ml propidium iodide (PI) for 30 min. For each sample, 10,000 cells were analyzed on an Epics XL Coulter flow cytometer. The effect on the cellular cycle is that expected for a DNA interacting agent, i.e. a dose - dependent increase in the percentage of the L1210 cells accumulated in the G_2 + M phase.

The preliminary *in vitro* biological results are reported in Table 1. Both compounds were less potent than ADR or DNR when tested on the sensitive L1210 cells. Similarly, **1** and **2** exhibited a decreased cytotoxic activity in respect to ADR or DNR against the KB-3-1 cell line, but when tested on the KB A-1 cell line, which is higly resistant against ADR and DNR, **1** and **2** showed a clear advantage (RF value of 1.7 for **1** and 6.9 for **2**). These findings are comparable with those previously reported for the *in vitro* biological evaluation of 7-*O*-(4-*O*-acetyl-3-chloro-2,3,6-trideoxy-α,β-L-arabino-hexopyranosyl) daunomycinone.[10] However compounds **1** and **2** proved to be more active against the sensitive cells and this could indicate that the 4'-amino functionality of the molecule may be superior to the hydroxyl with respect to cytotoxic activity.

Table 1

Cell lines	IC_{50} (μM) and resistant factor			
	1	**2**	ADR	DNR
L1210	1.37±0.15	0.50±0.12	0.033±0.002	0.033±0.008
KB-3-1	1.61±0.29	0.55±0.005	0.021±0.003	0.007±0.002
KB-A1	2.76±0.88	3.79±0.76	7.01±0.27	2.07±0.34
RF	1.7	6.9	333.8	295.7

References and Notes

1. Young, R.C.; Ozols, R.F; Myers, C.E., *N. Eng. J. Med.*, **1981**, *305*, 139.
2. Arcamone, F. *Cancer Res.*, **1985**, *45*, 5995.
3. Weiss, R.B.; Sarosy, G.; Glagett-Carr, K.; Russo, M.; Leyland-Jones, B., *Cancer Chemother. Pharmacol.*, **1986**, *18*, 185.
4. Bristow, M.R. ; Billingham, M.E.; Mason, J.W.; Daniels, J.R., *Canser Treatment Report*, **1978**, *62*, 873.
5. Gottesman, M.M., *Cancer Res.*, **1993**, *53*, 747.
6. Kaye, S.B., *Br. J. Cancer*, **1988**, *58*, 691.
7. Arcamone, F., *Anticancer Agents Based on Natural Product Models.* Ed: J.M. Cassady and J.D. Douros, **1980** p 1. Academic Press New York.

8. Suarato, A.; Angelucci, F.; Bargiotti, A.; Caruso, M.; Faiardi, D.; Capolongo, L.; Geroni, C.; Ripamonti, M. ; Grandi, M., *Antracycline Antibiotics.* Ed: W. Priebe, **1993** ACS Symposium Series *574* , pp 142-155
9. Giuliani, F. ; Fiebig, H.H. ; Pezzoni, G. ; Bahari, B. ; Caruso, M. ; Bargiotti, A.; Suarato, A., *NCI-EORTC Symposium on New Drugs in Cancer Therapy*, Amserdam **1989**.
10. Demetzos, C. ; Skaltsounis, A.-L. ; Michel, S.; Tillequin, F. ; Koch, M.; Anstett, M.; Leonce, S.; Pierre, A. ; Atassi, G., *Anticancer Drug Design*, **1994**, *9*, 129.
11. Facchetti, I.; Grandi, M.; Cucchi, P.; Geroni, C.; Penco, S.; Vigevani, A., *Anticancer Drug Design*, **1991**, *5*, 385.
12. Coley, H.M. ; Twentyman, P.R. ; Workman, P., *Anticancer Drug Design*, **1992**, 7, 471.
13. Mitaku, S. ; Skaltsounis, A.-L.; Tillequin, F. ; Koch, M., *Synthesis* , **1992**, 1068.
14. Binkley, R.W. ; Ambrose, M.G., *J. Carbohydr. Chem.*, **1984**, *3*, 1.
15. We have also prepared the 4-tosyl- and the 4-mesyl- esters instead of **4**, but these compounds proved to be unsuitable as intermediates for the preparation of **5**, since after treatment of both esters with sodium azide even under drastic conditions, we could only isolate the starting material.
16 Data for compound **6c**, α-anomer (syrup). ^{1}H- NMR (200 MHz) δ(ppm) ($CDCl_3$): 6.73 (d, 2H, $J_{NH,4}$=12Hz, NH), 6.21 (d, 1H, $J_{1,2ax}$=4Hz, 1-H), 4.5 (m, 2H, 3,4-H), 4.25 (q, 1H, $J_{5,6}$= 6Hz, 5-H), 2.18 (m, 2H, 2-H_{ax}, 2-H_{eq}), 2.15 (s, 3H, $COCH_3$), 1.20 (d, 3H, J=6Hz, CH_3).
17 Data for compound **2**. (syrup) $[\alpha]_D$= +100^o. (c 0.055, $CHCl_3$), ^{1}H- NMR (200 MHz) δ(ppm) ($CDCl_3$): 14.04 (s, 1H, 6-OH), 13.26 (s, 1H, 11-OH), 8.05 (dd, 1H, $J_{1,2}$=8Hz, $J_{1,3}$=1Hz, 1-H), 7.79 (t, 1H, $J_{1,2}$=$J_{2,3}$=8Hz, 2-H), 7.40 (dd, 1H, $J_{2,3}$= 8Hz, $J_{1,3}$= 1Hz, 3-H), 6.49 (d, 1H, $J_{NH,4'}$=9.5Hz, NH) 5.53 (d, 1H, $J_{1',2'ax}$= 4Hz, 1'-H), 5.27 (t, 1H, $J_{7,8ax}$=1.5Hz, $J_{7,8eq}$=1.5Hz, 7-H), 4.36 (m, 3H, 3',4',5'-H), 4.20 (s, 1H, 9-OH), 4.08 (s, 3H, OCH_3), 3.27 (d, 1H, $J_{10eq,10ax}$=19Hz, 10-H_{eq}), 2.95(d, 1H, $J_{10ax,10eq}$=19Hz, 10-H_{ax}), 2.44 (s, 3H, $COCH_3$), 1.97-2.27 (m, 4H, 8-H, 2'-H), 1.24 (d, 3H, $J_{CH3,5'}$=6.5Hz, CH_3), DCIMS m/z $[M+NH_4]^+$= 659, $[M+H]^+$= 642.
18 Data for compound **6a**, α-anomer (syrup). ^{1}H- NMR (200 MHz) δ(ppm) ($CDCl_3$): 6.14 (dd, 1H, $J_{1,2eq}$=1Hz, $J_{1,2ax}$=3Hz, 1-H), 4.52 (ddd, 1H, $J_{2ax,3}$= 13Hz, $J_{2eq,3}$ =5Hz, $J_{3,4}$=3Hz, 3-H), 4.11 (qd, 1H, $J_{5,6}$=6Hz, $J_{4,5}$=2Hz, 5-H), 3.70 (dd, 1H, $J_{4,5}$=2Hz, $J_{4,3}$=3Hz, 4-H), 2.37 (td, 1H, $J_{2ax,2eq}$= 13Hz, $J_{1,2ax}$ =3Hz, $J_{3,2ax}$=13Hz, 2-H_{ax}), 2.1 (s, 3H, $COCH_3$), 2.05 (ddd, 1H, $J_{1,2eq}$=1Hz, $J_{3,2eq}$=5Hz, $J_{2ax,2eq}$=13Hz, 2-H_{eq}), 1.35 (d, 3H, J=6Hz, CH_3).
19 Data for compound **1**. (syrup) $[\alpha]_D$= +85^o (c 0.04, $CHCl_3$), ^{1}H- NMR (200 MHz) δ(ppm) ($CDCl_3$): 14.02 (s, 1H, 6-OH), 13.25 (s, 1H, 11-OH), 8.05 (dd, 1H, $J_{1,2}$=8Hz, $J_{1,3}$=1Hz, 1-H), 7.80 (t, 1H, $J_{1,2}$=$J_{2,3}$=8Hz, 2-H), 7.40 (dd, 1H, $J_{2,3}$=8Hz, $J_{1,3}$=1Hz, 3-H), 5.45 (d, 1H, $J_{1',2'ax}$= 4Hz, 1'-H), 5.27 (t, 1H, $J_{7,8ax}$=1.5Hz, $J_{7,8eq}$=1.5Hz, 7-H), 4.40 (m, 3H, 3',5'-H, 9-OH), 4.08 (s, 3H, OCH_3), 3.65 (br. s, 1H, 4'-H), 3.20 (d, 1H, $J_{10eq,10ax}$=19Hz, 10-H_{eq}), 2.90 (d, 1H, $J_{10ax,10eq}$=19Hz, 10-H_{ax}), 2.44 (s, 3H, $COCH_3$), 1.97-2.27 (m, 4H, 8-H, 2'-H), 1.35 (d, 3H, $J_{CH3,5'}$=6.5Hz, CH_3), DCIMS m/z $[M+H]^+$= 572.
20. Pierre, A. ; Dunn, T.A.; Kraus-Berthier, L.; Leonce, S.; Saint-Dizier, D.; Regnier, G.; Dhainaut, A.; Berlion, M.; Bizzari, J.-P.; Attassi, G., *Investigational New Drugs* **1992**, *10*, 137.

(*Received in Belgium* 29 *July* 1996; *accepted* 26 *September* 1996)