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UNEXPECTED TRANSESTERIFICATION OF N-(TRIFLUOROACETYL) DOXORUBICIN WITH ACETYLSALICYLIC ACID: FORMATION OF 4'-O-ACETYL-N-(TRIFLUOROACETYL) DOXORUBICIN

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Abstract: Three new N-(trifluoroacetyl) doxorubicin analogues have been synthesized under mild reaction conditions. An unexpected transesterification reaction was observed when N-(trifluoroacetyl) doxorubicin was treated with acetylsalicylic acid under the same conditions.

The anthracycline antibiotics have attracted considerable interest because of their great therapeutic value in treating a number of human cancers.[1,2] Since its discovery, more than 2000 analogues of doxorubicin **1** have been synthesized and tested for biological activity.[3] Some of these analogues presented better activity and less toxicity than the parent drug. Among which, the cyanomorpholino doxorubicin derivative **3** and, more recently, the 2-pyrrolino doxorubicin derivative **4** are two of the most powerful anthracycline analogues known.[4,5] It was also observed that lipophilic anthracyclines were, generally, more potent than the hydrophilic anthracyclines. This was due to an increased accumulation of the lipophilic drug into the cells.[6] Moreover, it was demonstrated that lipophilic N-alkylanthracyclines possess inherent abilities to circumvent multidrug resistance *in vitro* and *in vivo*, possibly through alterations in normal intracellular drug trafficking.[7]

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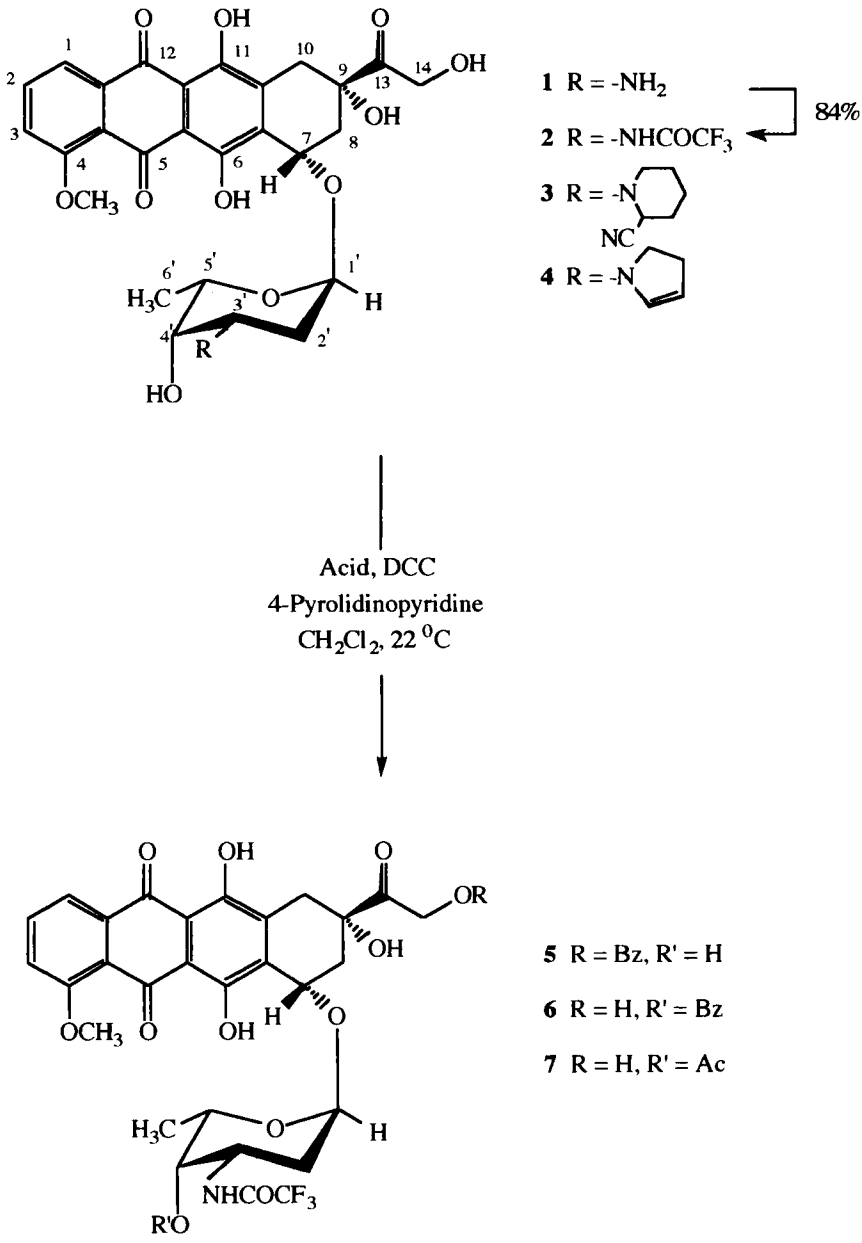
We have been trying to incorporate a non-toxic molecule (pro-moiety) to the N-(trifluoroacetyl) doxorubicin **2** nucleus in order to increase its lipophilicity. Theoretically, the non-toxic component would allow a greater accumulation into the cells and, consequently a greater cytotoxic activity. Benzoic acid and acetylsalicylic acid (2-acetoxybenzoic acid) were choosen based on both their own inherent lipophilic and non-toxic character. An ester bond was used to link the pro-moiety to the drug. It was anticipated that the possible hydrolysis of these component into the cells would simply release the parent drug (N-(trifluoroacetyl) doxorubicin) which would then freely intercalate DNA and produce its cytotoxic activity.[8] Esterification of the less hindered alcohols 14-OH and/or 4'-OH of N-(trifluoroacetyl) doxorubicin was probable.[9]

In this communication, we wish to report the synthesis and characterization of three new N-(trifluoroacetyl) doxorubicin analogues. Also, an unexpected transesterification product was observed when acetylsalicylic acid was used and a possible mechanism leading to its formation will be presented.

Synthesis and characterization of the new N-(trifluoroacetyl) doxorubicin analogues

As shown on scheme 1, the N-(trifluoacetyl) doxorubicin **2** was obtained from doxorubicin **1** upon treatment with S-ethyltrifluoroacetate as described previously.[10] Esterification of compound **2** with benzoic acid under mild reaction conditions using 1,3-dicyclohexylcarbodiimide in the presence of a catalytic amount of 4-pyrrolidinopyridine in dichloromethane led to two main products; the 14-benzoate derivative **5** (22%) and the 4'-benzoate derivative **6** (32%) as expected. Both derivatives present, on their ^{1}H NMR spectrum, additional signals in the aromatic region accounting for the phenyl group. Esterification at position 14 was confirmed by the presence of an AB system (located at 5.6 and 5.5 ppm) accounting for the C-14 methylene. In the starting material, the C-14 methylene was present as a singlet at position 4.7 ppm. A decrease in the free rotation of the bonds made the hydrogens at C-14 diastereotopic. Esterification at position 4' was confirmed by the presence of a singlet at 4.8 ppm for the C-14 methylene as found in the starting material. Also, the amide hydrogen, present as a doublet, was deshilded from 8.0 ppm for compound **2** to 8.68 ppm for compound **6**. Those signals confirmed the formation of the 4'-benzoate derivative **6**.



Scheme 1: Synthesis of doxorubicin analogues

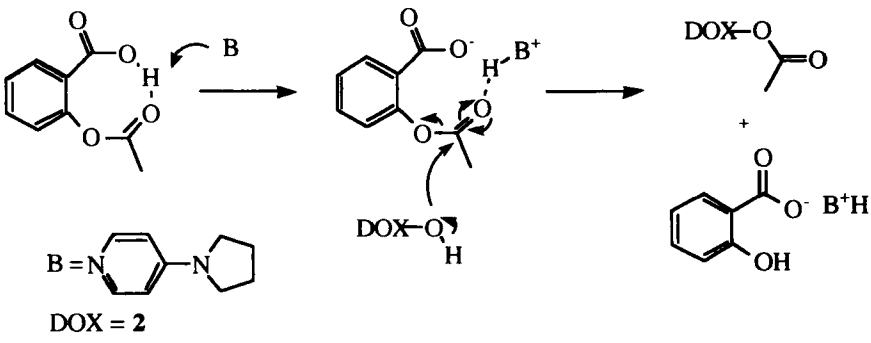
The esterification of compound **2** with acetylsalicylic acid, under the same reaction conditions as described above with benzoic acid, led mainly to the formation of the 4'-O-acetyl derivative **7** (40%) instead of the 14-acetylsalicylate and the 4'-acetylsalicylate initially expected. The ^{1}H NMR spectrum showed no additional aromatic protons, however, a singlet at 2.1 ppm (CH_3C=O) appeared. Moreover, a singlet at 4.8 ppm (CH_2-14) and a doublet at 8.4 ppm (-N*H*-(C=O)-CF_3) were detected. These signals were coherent with the formation of the transesterification product **7**. A complete description of IR and ^{1}H NMR (500 MHz) spectra for derivatives **2**, **5**, **6** and **7** is presented in the experimental section.

A possible mechanism explaining the formation of the 4'-O-acetyl derivative **7** is presented on scheme 2. Intramolecular catalytic activation of the acetate moiety on acetylsalicylic acid was sufficient to initiate the transesterification reaction. The 1,3-dicyclohexylcarbodiimide may not be involved during the process. This reaction will be further studied in our laboratory. Interestingly, the less hindered 14-OH was not esterified to a great extent. The 4'-OH, slightly more basic than the 14-OH, was the most reactive site. Consequently, the 4'-O-acetyl derivative **7** was formed predominantly. Thin layer chromatography analysis showed the presence of minors products presumably the 14-O-acetyl and the 4',14-di-O-acetyl derivatives which were not isolated.

In conclusion, three new N-(trifluoroacetyl) doxorubicin analogues were synthesized under mild reaction conditions. An unusual transesterefication reaction was observed while using acetylsalycilic acid and a mechanism explaining its formation was proposed. The biological activity of the new products will be evaluated in the future.

EXPERIMENTAL

Anhydrous reactions were performed under an inert atmosphere, the set-up assembled and cooled under dry nitrogen. The starting material, reactant, and solvents were obtained commercially and were used as such or purified and dried by standard means.[11] Organic solutions were dried over sodium sulfate (Na_2SO_4), and evaporated on a rotatory evaporator and under reduced pressure. All reactions were monitored by thin-layer chromatography (TLC). The plates were visualized



Scheme 2: Possible mechanism of transesterication

by UV fluorescence. Commercial TLC plates were Sigma T 6145 (polyester silica gel 60 Å, 0.25mm). Flash chromatography was performed according to the method of Still and co-workers on Merck grade 60 silica gel, 230-400 mesh.[12] All solvents used in chromatography had been distilled. Melting points (mp) were determined in capillary tubes with an Electrothermal apparatus and are uncorrected. The infrared spectra (IR) were taken on a Nicolet model 510P FT-IR spectrophotometer. Nuclear magnetic resonance (NMR) spectra were obtained in acetone-d_6 solution on a Bruker AMX-2 (500 MHz) instrument: chemical shifts were measured relative to tetramethylsilane (TMS, δ 0.0 ppm) for ^{1}H. Multiplicities are described by the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (double doublet), td (triple doublet). The NMR assignments were assisted by COSY 2-D spectra. Mass spectra (MS) were determined on a VG Micromass 7070 HS instrument using an ionization energy of 70 eV.

The preparation of N-(trifluoroacetyl) doxorubicin **2** was described in the literature,[10] however its IR and 500 MHz ^{1}H NMR spectra are presented below for comparison with the new derivatives. Compound **2** was purified by flash chromatography using a mixture of dichloromethane/methanol, 9/1.

Spectral data for N-(trifluoroacetyl) doxorubicin **2**: Rf = 0.53 (chloroform/methanol, 9/1); mp 173-175 °C (lit.[10] 172-174 °C); IR (KBr, υ_{max},

cm^{-1}): 3600-3200 (O-H and N-H), 1723 (C=O, C-13 ketone and trifluoroacetate), 1618 et 1580 (C=O, quinone), 1290-1100 (C-O); ^1H NMR (δ ppm, acetone-d_6): 14.16 (1H, s, 11-OH), 13.26 (1H, s, 6-OH), 8.02 (1H, broad d, 3'-NH), 7.94 (1H, d, J = 7.5 Hz, 1-H), 7.88 (1H, t, J = 8.0 Hz, 2-H), 7.61 (1H, d, J = 8.4 Hz, 3-H), 5.48 (1H, s, 1'-H), 5.32 (1H, s, 7-H), 4.81 (1H, d, J = 5.0 Hz, 4'-OH), 4.74 (2H, d, J = 5.0 Hz, 14-CH_2), 4.32 (1H, q, J = 6.3 Hz, 5'-H), 4.22 (1H, broad s, 3'-H), 4.21 (1H, s, 9-OH), 4.06 (3H, s, -OCH_3), 3.73 (1H, s, 4'-H), 3.62 (1H, t, J = 7.4 Hz, 14-OH), 3.17 (1H, d, J = 18.5 Hz, 10-H_β), 3.01 (1H, dd, J = 18.5 Hz, 5.0 Hz, 10-H_α), 2.47 (1H, d, J = 14.5 Hz, 8-H_β), 2.26 (1H, dd, J = 14.7 Hz, 4.3 Hz, 8-H_α), 2.14 (1H, td, J = 13.1 Hz, 3.7 Hz, 2'-H_β), 1.81 (1H, dd, J = 14.0 Hz, 3.7 Hz, 2'-H_α), 1.29 (3H, d, J = 5.8 Hz, -CH_3); HRMS calcd. for $C_{29}H_{28}F_3NO_{12}$: 639.1563; found: 639.1575.

Preparation of derivatives **5** and **6**: A solution of N-(trifluoroacetyl) doxorubicin **2** (50 mg, 0.078 mmol), benzoic acid (11.6 mg, 0.095 mmol), 1,3-dicyclohexylcarbodiimide (DCC, 19.35 mg, 0.094 mmol) and 4-pyrrolidinopyridine (4-PP, 1 mg, 6.6 x 10^{-6} mol) in dry dichloromethane (20 ml) was stirred at room temperature (22 ^{0}C) for 24 h. Afterwards, the reaction mixture was diluted with dichloromethane (100 ml) and ether (10 ml). The resulting solution was washed successively with sodium bicarbonate (2 x 20 ml, 5% aqueous) and with water (3 x 30 ml). The organic phase was dried, filtered and concentrated to a crude material. The residue was purified by preparative TLC (chloroform/methanol, 9/1) to give two main products; compound **5** (22%) (Rf = 0.65) and compound **6** (32%) (Rf = 0.75).

Spectral data for compound **5**: Rf = 0.65 (chloroform/methanol, 9/1); mp 141-143 ^{o}C; IR (KBr, υ_{max}, cm^{-1}): 3500-3200 (O-H and N-H), 1724 (C=O, C-13 ketone, ester and trifluoroacetate), 1626 and 1578 (C=O, quinone), 1290-1100 (C-O); ^1H NMR (δ ppm, acetone-d_6): 14.20 (1H, s, 11-OH), 13.30 (1H, s, 6-OH), 8.11 (2H, d, J = 7.8 Hz, H_a), 8.01 (1H, s, NH), 7.99 (1H, d, J = 7.5 Hz, 1-H), 7.90 (1H, t, J = 8.1 Hz, 2-H), 7.67 (1H, t, J = 7.2 Hz, H_c), 7.65 (1H, d, J = 8.3 Hz, 3-H), 7.56 (2H, t, J = 7.6 Hz, H_b), 5.64 and 5.50 (2H, 2 x d, J = 17.8 Hz, 18.3 Hz, 14-CH_2), 5.46 (1H, s, 1'-H), 5.29 (1H, s, 7-H), 5.10 (m, 1H, 4'-OH), 4.42 (1H, q, J = 6.4 Hz, 5'-H), 4.26 (1H, s, 3'-H), 4.24 (1H, s, 9-OH), 4.06 (3H, s, -OCH_3), 3.75 (1H, s, 4'-H), 3.28 (1H, d, J = 18.5 Hz, 10-H_β), 3.07

(1H, d, J = 18.5 Hz, 10-H_α), 2.70 (1H, d, J = 14.5 Hz, 8-H_β), 2.24 (1H, dd, J = 14.8 Hz, 4.4 Hz, 8-H_α), 2.20 (1H, td, J = 13.1 Hz, 3.8 Hz, 2'-H_β), 1.83 (2H, m, 2'-H_α), 1.34 (3H, d, J = 6.6 Hz -CH_3); HRMS calcd. for $C_{36}H_{32}F_3NO_{13}$: 743.1826; found: 743.1840.

Spectral data for compound **6**: Rf = 0.75 (chloroform/methanol, 9/1); mp 154-156 °C; IR (KBr, υ_{max}, cm^{-1}): 3500-3200 (O-H and N-H), 1726 (C=O, C-13 ketone, ester and trifluoroacetate), 1626 and 1578 (C=O, quinone), 1290-1100 (C-O); ^1H NMR (δ ppm, acetone-d_6): 14.25 (1H, s, 11-OH), 13.30 (1H, s, 6-OH), 8.58 (1H, d, J = 5.0 Hz, NH), 8.11 (2H, d, J = 7.9 Hz, H_a), 7.98 (1H, d, J = 7.7 Hz, 1-H), 7.91 (1H, t, J = 8.3 Hz, 2-H), 7.67 (1H, t, J = 7.4 Hz, H_c), 7.65 (1H, d, J = 8.2 Hz, 3-H), 7.56 (2H, t, J = 7.4 Hz, H_b), 5.67 (1H, broad s, 1'-H), 5.48 (1H, s, 4'-H), 5.30 (1H, s, 7-H), 4.85 (1H, s, 9-OH), 4.77 (2H, d, J = 5.3 Hz, 14-CH_2), 4.67 (1H, q, J = 6.2 Hz, 5'-H), 4.53 (1H, broad d, J = 18.5 Hz, 3'-H), 4.07 (3H, s, -OCH_3), 3.65 (1H, t, J = 7.4 Hz, 14-OH), 3.19 and 3.10 (1H, d, J = 18.5 Hz, 10-H), 2.53 (1H, d, J = 14.7 Hz, 8-H_β), 2.42 (1H, td, J = 13.0 Hz, 3.5 Hz, 2'-H_β) 2.37 (1H, dd, J = 14.7 Hz, 5.1 Hz, 8-H_α), 1.95 (1H, dd, J = 13.0 Hz, 3.8 Hz, 2'-H_α), 1.22 (3H, d, J = 5.7 Hz, -CH_3); HRMS calcd. for $C_{36}H_{32}F_3NO_{13}$: 743.1826; found: 743.1840.

Preparation of derivative **7**: The preparation of **7** was done under the same reaction conditions as described for the preparation of compounds **5** and **6**. The following quantities were used: N-(trifluoroacetyl) doxorubicin **2** (50 mg, 0.078 mmol), acetylsalicylic acid (17 mg, 0.095 mmol), DCC (19.35 mg, 0.094 mmol) and 4-PP (1 mg, 6.6 x 10^{-6} mol) and dichloromethane (20 ml).

Spectral data for compound **7**: Rf = 0.64 (chloroform/methanol, 9/1); mp 134-137 °C; IR (KBr, υ_{max}, cm^{-1}): 3500-3200 (O-H and N-H), 1730 (C=O, ester), 1724 (C=O, C-13 ketone and trifluoroacetate), 1626 and 1578 (C=O, quinone), 1290-1100 (C-O); ^1H NMR (δ ppm, acetone-d_6): 14.18 (1H, s, 11-OH), 13.27 (1H, s, 6-OH), 8.39 (1H, d, J = 5.0 Hz, NH), 7.96 (1H, d, J = 7.5 Hz, 1-H), 7.90 (1H, t, J = 7.6 Hz, 2-H), 7.64 (1H, s, J = 8.7 Hz, 3-H), 5.56 (1H, s, 1'-H), 5.24 (1H, s, 7-H), 5.19 (1H, s, 4'-H), 4.75 (2H, d, J = 3.0 Hz, 14-CH_2), 4.53 (1H, q, J = 6.6 Hz, 5'-H), 4.40 (1H, s, 9-OH), 4.38 (1H, broad s, 3'-H), 4.06 (3H, s, -OCH_3), 3.63 (1H, m, 14-OH), 3.17 (1H, d, J = 18.5 Hz, 10-H_β),

3.00 (1H, d, J = 18.5 Hz, 10-H_α), 2.48 (1H, d, J = 14.7 Hz, 8-H_β), 2.28 (1H, dd, J = 14.8 Hz, 5.1 Hz, 8-H_α), 2.19 (1H, td, J = 13.0 Hz, 3.4 Hz, 2'-H_β), 2.13 (3H, s, -$COCH_3$), 1.84 (1H, dd, J = 8.5 Hz, 3.0 Hz, 2'-H_α), 1.17 (3H, d, J = 7.0 Hz, -CH_3); HRMS calcd. for $C_{31}H_{30}F_3NO_{13}$: 681.1669; found: 681.1680.

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